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STRUCTURAL PREDICTION AND COPPER(1) BINDING ANALYSES OF CsoR-LIKE HYPOTHETICAL PROTEIN OF Geobacillus zalihae

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By

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August 2016

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Copper is a transition metal which is essentially needed in living things. It plays various roles such as cofactor for enzymes which are important in cellular processes. However, high concentration of copper will lead to toxicity. Living organisms have metal homeostasis mechanisms in order to overcome such adversities caused by metals. In bacteria, such mechanisms help to stabilize cellular copper concentrations. To date, nine classes of copper regulation have been documented and classified based on the organisms from which they have been found. CsoR copper regulation is the most recently discovered mechanism. To date, only three CsoR proteins (from *Thermus thermophilus, Mycobacterium tuberculosis* and *Streptomyces lividans*) are structurally and functionally characterized. Hence, the effort of finding and characterizing more of these proteins from other bacterial strains are important to gain more significant comparisons of this protein across different bacterial taxa. A good platform to find and study in greater detail for such candidates would be within the pool of hypothetical proteins (HPs). HPs constitute approximately 30-40% of any given genome. They are often referred as "orphan" proteins due to their unknown functions stemming from their low sequence and/or structural homology to other known, well-characterized proteins. In this study, a scan on the complete genome of a locally isolated *Geobacillus zalihae* thermophile revealed the presence of a CsoR-like ($Csor_{Gz}$) hypothetical protein which contained CsoR-like_DUF156 domain and highly conserved Cys-His-Cys residues important for copper binding, similar to well characterized CsoR proteins. However, it only shares 30-38% sequence identity to well characterized CsoR proteins. 3-D structural prediction of $Csor_{Gz}$-like protein via threading predicted that the protein contains three helices per monomer. Its putative, conserved copper-binding residues, Cys46-His71-Cys75, were also found to be located on α2 helix of the predicted structure similar to other CsoR proteins. Amplification of the $csor_{Gz}$-like open reading frame from the genome of *G. zalihae* was achieved and the amplicon was cloned into pET-28b expression vector for heterologous production of the protein in *Escherichia coli* BL21 Star ® (DE3). Native-PAGE analysis of the purified recombinant $Csor_{Gz}$-like protein revealed that it is a dimeric protein. Secondary structure analysis of the protein with circular dichroism revealed that it comprises of mainly α-helices, similar to well

characterized CsoR proteins. Titration of Cu(I)Cl to the purified recombinant $Csor_{Gz}$-like protein revealed interaction of Cu(I) with the protein as revealed by UV/Vis, circular dichroism and fluorescence spectroscopy analyses, with a K_d of 6.4 x 10^{-5} M. It can be concluded that $Csor_{Gz}$-like protein, despite being a HP, contains the Cu(I) binding property thus gives more insights on copper regulator proteins in bacterial diversity.

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RAMALAN STRUKTUR DAN ANALISIS PENGIKATAN KUPRUM(1) TERHADAP PROTEIN HIPOTETIKAL MIRIP CsoR DARIPADA *Geobacillus zalihae*

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Kuprum merupakan sejenis logam peralihan yang diperlukan oleh organisma hidup. Ia memainkan pelbagai peranan seperti kofaktor enzim di mana ia sangat penting dalam proses sel. Walaubagaimanapun, kepekatan kuprum yang tinggi boleh mengakibatkan ketoksikan. Organisma hidup mempunyai mekanisme kawal atur logam untuk mengatasi kesan buruk sedemikian yang diakibatkan oleh logam. Di dalam bakteria, mekanisme kawal atur membantu untuk menyeimbangkan kepekatan kuprum. Sehingga kini, terdapat sembilan kelas pengawal atur kuprum direkod dan diklasifikasikan mengikut organisma di mana pengawal atur tersebut dijumpai. Pengawal atur kuprum *CsoR* ialah mekanisma kawal atur yang terbaharu dijumpai. Buat masa ini, hanya tiga protein CsoR (daripada *Thermus thermophilus, Mycobacterium tuberculosis* dan *Streptomyces lividans*) yang telah dicirikan struktur dan fungsinya. Oleh itu, usaha untuk mencari dan mengetahui lebih lanjut tentang protein ini daripada strain bakteria yang lain adalah penting untuk mendapatkan perbandingan yang signifikan merentasi taksa bakteria yang berbeza. Platform terbaik untuk mencari dan mengkaji lebih lanjut tentang protein tersebut adalah daripada protein hipotetikal (HP). HP merangkumi kira-kira 30-40% daripada sesebuah genom. HP juga sering digelar protein "yatim" kerana fungsinya yang tidak diketahui berpunca dari kesamaan struktur dan/atau jujukan yang rendah dengan protein-protein lain yang diketahui dan yang telah dicirikan dengan baik. Di dalam kajian ini, protein mirip-CsoR ($Csor_{Gz}$) telah ditemui di dalam genom bakteria termofil pencilan tempatan, *Geobacillus zalihae*. Protein hipotetikal ini mempunyai domain CsoR-like_DUF156 dan residu terpelihara Cys-His-Cys yang penting untuk pengikatan kuprum sepertimana protein CsoR lain yang telah dicirikan. Walaubagaimanapun, ia hanya berkongsi 30-38% identiti jujukan dengan protein CsoR yang telah dicirikan dengan baik. Ramalan struktur 3-D terhadap $Csor_{Gz}$ melalui kaedah *threading* meramalkan ia terdiri daripada tiga heliks per monomer. Residu terpelihara yang putatif untuk pengikatan kuprum, Cys46-His71-Cys75 juga ditemui pada heliks α2 struktur ramalan sama seperti protein CsoR yang lain. Amplifikasi rangka bacaan terbuka mirip-$csor_{Gz}$ daripada genom *G. zalihae* telah dicapai dan amplikon tersebut telah diklonkan ke dalam vektor pengekspresan pET-28b untuk penghasilan protein heterolog di dalam *Escherichia coli*

BL21 Star ® (DE3). Analisa Native-PAGE terhadap protein seakan-$Csor_{Gz}$ yang telah ditulenkan menunjukkan bahawa protein tersebut adalah protein dimer. Analisis struktur sekunder menggunakan *circular dichroism* pula menunjukkan bahawa protein tersebut terdiri daripada α-heliks sepertimana protein CsoR yang lain. Pentitratan Cu(I)Cl kepada protein rekombinan mirip-$Csor_{Gz}$ yang tulen menunjukkan interaksi antara Cu(I)Cl dengan protein tersebut seperti yang ditunjukkan oleh UV/Vis, *circular dichroism*, dan analisis spektrofotometer berpendarfluor, dengan K_d bersamaan 6.4 x 10^{-5} M. Ia boleh disimpulkan bahawa protein mirip-$Csor_{Gz}$,walaupun merupakan protein hipotetikal, memiliki ciri-ciri pengikatan Cu(I) sekaligus dapat memberi pandangan lebih mendalam tentang protein kawal atur kuprum di dalam diversiti bakteria.

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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:

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LIST OF ABBREVIATIONS

Ag^{+}	Silver ion
bp	Base pair
°C	Celcius
Cd^{2+}	Cadmium ion
cm	centimeter
Co^{2+}	Cobalt (II), cobaltous
CsoR	Copper sensing operon regulator
Cu^{+}	Copper (I), cuprous
Cu^{2+}	Copper (II), cupric
Cys	Cysteine
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
g	Gram
His	Histidine
kDa	Kilo dalton
L	Litre
µg	Microgram
mg	Milligram
µl	Microlitre
ml	Mililitre
µm	Micrometer
mM	Micromolar
MOPS	3-(N-morpholino)propanesulfonic acid
MWCO	Molecular weight cut-off
Ni^{2+}	Nickel (II), nickelous
nm	nanometer
NRMSD	Normalised root-mean-square deviation
OD	Optical density
%	Percent
Pb^{2+}	Lead (II), plumbous
PCR	Polymerase chain reaction
PAGE	Polyacrylamide gel electrophoresis
rpm	Rotation per minute
U	Unit
UV/Vis	Ultraviolet/Visible
Zn^{2+}	Zinc ion

CHAPTER I

INTRODUCTION

l.1 Introduction

Transition metals such as copper are essentially needed in living things. Copper plays so many roles such as cofactor for enzymes as well as in cellular processes (Rademacher and Masepohl, 2012). However, high concentration of copper will lead to toxicity via the formation of reactive oxygen species (ROS) and interference of protein-metal interaction by competing with other metals at the protein binding sites (Macomber and Imlay, 2009; Hiniker *et al*., 2005). In order to overcome toxicity, homeostasis mechanism is needed. Copper regulation in bacteria is one of the metal regulation mechanisms which help to stabilize cellular concentration of copper. To date, nine classes of copper regulation have been documented and classified based on the organisms they have been found in, namely CueR, CusRS, ComR (in *Escherichia coli*), CopL, CorE proteobacteria such as *Xanthomonas* sp. and *Myxococcus xanthus* respectively, BxmR in cyanobacteria and CopY, CsoR, YcnK in Gram-positive bacteria (Rademacher and Masepohl, 2012). Among these mechanisms, CsoR copper regulation is the most recently discovered mechanism. In this mechanism, CsoR, a copper sensor protein, acts as a regulator (repressor) in the expression of target genes involved in copper homeostasis in an operon. In excessive amount of Cu(I), CsoR binds to the metal ion, thus releasing itself from the regulator-binding site of the operon and finally allows the production of the proteins involved in copper regulation (Liu *et al*., 2007).

To date only three CsoR proteins (from *Thermus thermophilus* (Sakamoto *et al*., 2010), *Mycobacterium tuberculosis* (Liu *et al*., 2007) and *Streptomyces lividans* (Dwarakanath *et al*., 2012) are structurally and functionally characterized. Hence, the effort of finding and characterizing more of this protein from other bacterial strains are important to gain more significant comparisons of this protein among different bacteria. A good platform to find and study in greater detail for such candidates would be within the pool of hypothetical proteins (HPs). HPs significantly constitute approximately 30-40% of any given genome (Bork, 2000). They are often referred as "orphan" proteins due to their unknown functions as they have low sequence and/or structural homology to other known, well-characterized proteins (Hanson *et al*., 2010; Roberts, 2004).

In this study, a scan on the genome of a locally-isolated *Geobacillus zalihae* (Rahman *et al*., 2007) revealed the presence of a sequence encoding CsoR-like hypothetical protein ($CsoR_{Gz}$-like HP). This study is thus aimed to predict the structure and copper binding property of $CsoR_{Gz}$-like HP via multiple approaches ranging from *in silico* as well as biochemical and biophysical studies.

1.2 Research Objectives

1.2.1 To analyse the sequence and protein characteristics of *csoR*$_{Gz}$-like gene via bioinformatics analyses

1.2.2 To predict and determine the structural characteristics of CsoR$_{Gz}$-like HP via *in silico* and biophysical approach respectively

1.2.3 To determine Cu(I) binding ability and affinity of CsoR$_{Gz}$-like HP

REFERENCES

Agertt, F., Crippa, A.C., Lorenzoni, P.J., Scola, R.H., Bruck, I., Paola, L., Silvado, C.E. and Werneck, L.C. (2007). Menkes' disease: case report. *Arq Neuropsiquiatr* 65: 157–160.

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

Argüello, J.M., Eren, E. and González-Guerrero, M. (2007). The structure and function of heavy metal transport P_{1B}-ATPases. *Biometals* 20: 233–248.

Arredondo, M. and Núñez, M. T. (2005). Iron and copper metabolism. *Molecular Aspects of Medicine* 26: 313–327.

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. and Sherlock, G. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics* 25: 25–29.

Baker, J., Sengupta, M., Jayaswal, R.K. and Morrissey, J.A. (2011). The *Staphylococcus aureus* CsoR regulates both chromosomal and plasmid-encoded copper resistance mechanisms. *Environmental Microbiology* 13: 2495-2507.

Beaudoin, J., Ioannoni, R., Lopez-Maury, L., Bahler, J., Ait-Mohand, S., Guérin, B., Dodani, S.C., Chang, C.J. and Labbé, S. (2011). Mfc1 is a novel forespore membrane copper transporter in meiotic and sporulating cells. *The Journal of Biological Chemistry* 286: 34356–34372.

Benkert, P., Künzli, M. and Schwede, T. (2009). QMEAN server for protein model quality estimation. *Nucleic Acids Research* 37: W510–514.

Berezin, C., Glaser, F., Rosenberg, J., Paz, I., Pupko, T., Fariselli, P., Casadio, R. and Ben-Tal, N. (2004). ConSeq: the identification of functionally and structurally important residues in protein sequences. *Bioinformatics* 20: 1322–1324.

Berman, H.M. (2000). The Protein Data Bank. *Nucleic Acids Research* 28: 235–242.

Betts, M.J. and Russell, R.B. (2003) Amino acid properties and consequences of substitutions. *Bioinformatics Genetics* 4: 289–316.

Boal, A.K. and Rosenzweig, A.C. (2009). Structural biology of copper trafficking. *Chemical Reviews* 109: 4760–4779.

Böhm, H.J. (2003). Prediction of non-bonded interaction in drug design. In *Protein-Ligand Interactions: From Molecular Recognition to Drug Design*. Mörlenbach: Wiley-Verlag GmbH and Co.

Bork., P. (2000). Powers and pitfalls in sequence analysis: the 70% hurdle. *Genome Research* 10: 398-400.

Bornhorst, J.A. and Falke, J.J. (2000). Purification of proteins using polyhistidine affinity tags. *Methods in Enzymology* 326: 245-254.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.

Brown, K.R., Keller, G.L., Pickering, I.J., Harris, H.H., George, G.N. and Winge, D.R. (2002). Structures of the cuprous-thiolate clusters of the Mac1 and Ace1 transcriptional activators. *Biochemistry* 41: 6469-6476.

Brown, N.L., Stoyanov, J.V., Kidd, S.P. and Hobman, J.L. (2003). The MerR family of transcriptional regulators. *FEMS Microbiology Reviews* 27: 145–163.

Buchan, D.W., Minneci, F., Nugent, T.C.O., Bryson, K. and Jones, D.T. (2013). Scalable web services for the PSIPRED Protein Analysis Workbench. *Nucleic Acids Research* 41: W349–357.

Buxbaum, E. (2007). Protein structure. In *Fundamentals of Protein Structure and Function*. New York: Springer Science & Bussiness Media Inc.

Chang, F-M.J., Coyne, H.J., Cubillas,C., Vinuesa,P., Fang, X., Ma, Z., Ma, D., Helmann, J.D., Santos, A.G., Wang, Y-X., Dann, C.E. and Giedroc, D.P. (2014). Cu(I)-mediated allosteric switching in a copper-sensing operon repressor (CsoR). *The Journal of Biological Chemistry* 289: 19204–19217.

Chang, F-M. J., Martin, J.E. and Giedroc, D.P. (2015). Electrostatic occlusion and quaternary structural ion pairing are key determinants of Cu(I)-mediated allostery in the copper-sensing operon repressor (CsoR). *Biochemistry* 54: 2463-2472.

Changela, A., Chen, K., Xue, Y., Holschen, J., Outten, C.E., O'Halloran, T.V. and Mondragon, A. (2003). Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR. *Science* 301: 1383-1387.

Chaplin, A.K., Tan, B.G., Vijgenboom, E. and Worrall J.A.R. (2015). Copper trafficking in the CsoR regulon of *Streptomyces lividans. Metallomics* 7: 145-155.

Chen, K., Yuldasheva, S., Penner-Hahn, J.E. and O'Halloran, T.V. (2003). An atypical linear Cu(I)–S2 center constitutes the high-affinity metal-sensing site in the CueR metalloregulatory protein. *Journal of The American Chemical Society* 125: 12088–12089.

Chen, P.R. and He, C. (2008). Selective recognition of metal ions by metalloregulatory proteins. *Current Opinion in Chemical Biology* 12: 214-221.

Chillappagari, S., Miethke, M., Trip, H., Kuipers, O.P. and Marahiel, M.A. (2009). Copper acquisition is mediated by YcnJ and regulated by YcnK and CsoR in *Bacillus subtilis*. *Journal of Bacteriology* 191: 2362–2370.

Chivers, P.T. and Tahirov, T.H. (2005). Structure of *Pyrococcus horikoshii* NikR: Nickel sensing and implications for the regulation of DNA recognition. *Journal of Molecular Biology* 348: 597-607.

Clark, D.P. and Pazdernik, N.J. (2012). Recombinant DNA technology. In *Biotechnology: Academic Cell Update Edition*. London: Academic Press Publisher.

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

Corbett, D., Schuler, S., Glenn, S., Andrew, P.W., Cavet, J.S. and Roberts, I.S. (2011). The combined actions of the copper-responsive repressor CsoR and copper-metallochaperone CopZ modulate CopA mediated copper efflux in the intracellular pathogen *Listeria monocytogenes*. *Molecular Microbiology* 81: 457–472.

Corrêa, D.H.A. and Ramos, C.H.I. (2009). The use of circular dichroism spectroscopy to study protein folding, form and function. *African Journal of Biochemistry Research* 3: 164–173.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.M. and Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acid Research* 36: W465-9.

Desler, C., Suravajhala, P., Sanderhoff, M., Rasmussen, M. and Rasmussen, L.J. (2009). In silico screening for functional candidates amongst hypothetical proteins. *BMC Bioinformatics* 10: 289.

Donsante, A., Yi, L., Zerfas, P.M., Brinster, L.R., Sullivan, P., Goldstein, D.S., Prohaska, J., Centeno, J.A., Rushing, E. and Kaler, S.G. (2011). ATP7A gene addition to the choroid plexus results in long-term rescue of the lethal copper transport defect in a Menkes disease mouse model. *Molecular Therapy* doi: 10.1038/mt.2011.143.

Dwarakanath S, Chaplin, A.K., Hough, M.A., Rigali, S., Vijgenboom, E. and Worrall, J.A. (2012). Response to copper stress in *Streptomyces lividans* extends beyond genes under direct control of a copper-sensitive operon repressor protein (CsoR). *Journal of Biological Chemistry* 287: 17833–17847.

Festa, R.A. and Thiele, D.J. (2011). Copper: An essential metal in biology. *Current Biology* 21: R877-R883.

Festa, R.A., Jones, M.B., Butler-Wu, S., Sinsimer, D., Gerads, R., Bishai, W.R., Peterson, S.N. and Darwin, K.H. (2011). A novel copper-responsive regulon in *Mycobacterium tuberculosis*. *Molecular Microbiology* 79: 133–148.

Foster, A.W., Patterson, C.J., Pernil, R., Hess, C.R. and Robinson, N.J. (2012). A cytosolic Ni(II) sensor in cyanobacterium: Nickel-detection follows nickel affinity across four families of metal-sensors. *The Journal of Biological Chemistry* 287: 12142-12179.

Furst, P., Hu, S., Hackett, R. and Hamer, D. (1988). Copper activates metallothionein gene-transcription by altering the conformation of a specific DNA-binding protein. *Cell* 55: 705-717.

Galperin, M.Y. (2001). Conserved 'hypothetical' proteins: new hints and new puzzles. *Comparative and Functional Genomics* 2: 14–18.

Galperin, M.Y. and Koonin, E.V. (2004). 'Conserved hypothetical' proteins: prioritization of targets for experimental study. *Nucleic Acids Research* 32: 5452–5463.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M.R., Appel, R.D. and Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. In *The Proteomics Protocols Handbook*. (pp. 571-607). New York: Humana Press.

Ghosh, S., Rasheedi, S., Rahim, S.S., Banerjee, S., Choudhary, R.K., Chakhaiyar, P., Ehtesham, N.Z., Mukhopadhyay, S. and Hasnain, S.E. (2004). Method for enhancing solubility of the expressed recombinant proteins in *Escherichia coli*. *BioTechniques* 37: 418-423.

Giner-Lamia, J., López-Maury, L. and Florencio, F.J. (2015). CopM is a novel copper-binding protein involved in copper resistance in *Synechocystis* sp. PCC 6803. *MicrobiologyOpen* 4: 167–185.

Gómez-Santos, N., Pérez, J., Sánchez-Sutil, M. C., Moraleda- Muñoz, A. & Muñoz-Dorado, J. (2011). CorE from *Myxococcus xanthus* is a copper-dependent RNA polymerase sigma factor. *PLoS Genetics* 7: e1002106.

Graden, J.A., Posewitz, M.C., Simon, J.R., George, G.N., Pickering, I.J. and Winge, D.R. (1996). Presence of a copper(I)-thiolate regulatory domain in the copper-activated transcription factor Amt1. *Biochemistry* 35: 14583-14589.

Graden, J.A. and Winge, D.R. (1997). Copper-mediated repression of the activation domain in the yeast Mac1p transcription factor. *Proceedings of The National Academy of Sciences* 94: 5550-5555.

Grass, G., Rensing, C. and Solioz, M. (2011). Metallic copper as an antimicrobial surface. *Applied and Environmental Microbiology* 77: 1541–1547.

Greenfield, N.J. (2006) Using circular dichroism spectra to estimate protein secondary structure. *Nature Protocol* 1: 2876–2890.

Grossoehme, N., Kehl-Fie, T. E., Ma, Z., Adams, K. W., Cowart, D. M., Scott, R. A., Skaar, E. P. and Giedroc, D. P. (2011). Control of copper resistance and inorganic sulfur metabolism by paralogous regulators in *Staphylococcus aureus*. *Journal of Biological Chemistry* 286: 13522–13531.

Guruprasad, K., Reddy, B.V.B and Pandit, M.W. (1990). Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of aprotein from its primary sequence. *Protein Engineering* 4: 155-161.

Hanson, A.D., Pribat, A.,Waller, J.C. and Crécy-Lagard, V. (2010). "Unknown" proteins and "orphan" enzymes: the missing half of the engineering parts list--and how to find it. *Biochemical Journal* 425: 1–11.

Herring, C.D. and Blattner, F.R. (2004). Global transcriptional effects of suppressor tRNA and the inactivation of the regulator frmR. *Journal of Bacteriology* 186: 6714-6720.

Higgins, K.A. and Giedroc, D.P. (2014). Insights into protein allostery in the csor/rcnr family of transcriptional repressors. *Chemistry Letters* 43: 20–25.

Hiniker, A., Collet, J-F. and Bardwell, J.C. (2005) Copper stress causes an in vivo requirement for the *Escherichia coli* disulfide isomerase DsbC. *The Journal of Biological Chemistry* 280: 33785–33791.

Hirooka, K., Edahiro, T., Kimura, K. and Fujita, Y. (2012). Direct and indirect regulation of the *ycnKJI* operon involved in copper uptake through two transcriptional repressors, YcnK and CsoR, in *Bacillus subtilis. Journal of Bacteriology* 194: 5675–5687.

Hunter, S., Jones, P., Mitchell, A., Apweiler, R., Attwood, T.K., Bateman, A., Bernard, T., Binns, D., Bork, P., Burge, S., de Castro, E., Coggill, P., Corbett, M., Das, U., Daugherty, L., Duquenne, L., Finn, R.D., Fraser, M., Gough, J., Haft, D., Hulo, N., Kahn, D., Kelly, E., Letunic, I., Lonsdale, D., Lopez, R., Madera, M., Maslen, J., McAnulla, C., McDowall, J., McMenamin, C., Mi, H., Mutowo-Muellenet, P., Mulder, N., Natale, D., Orengo, C., Pesseat, S., Punta, M., Quinn, A.F., Rivoire, C., Sangrador-Vegas, A., Selengut, J.D., Sigrist, C.J., Scheremetjew, M., Tate, J., Thimmajanarthanan, M., Thomas, P.D., Wu, C.H., Yeats, C. and Yong, S.Y. (2012). InterPro in 2011: new developments in the family and domain prediction database. *Nucleic Acids Research* 40: D306-312.

Invitrogen. (2010). BL21 Star™ (DE3) *Chemically Competent Cells*. Retrieved 11 March 2015. From https://tools.lifetechnologies.com/content/sfs/manuals/oneshotbl21star_man.pdf.

Iwig, J.S., Rowe, J.L. and Chivers, P.T. (2006). Nickel homeostasis in *Escherichia coli*- the *rcnR-rcnA* efflux pathway and its linkage to NikR function. *Molecular Microbiology* 62: 252-262.

Jones, D.T. (1999). Protein secondary structure prediction based on position-specific scoring matrices. *Journal of Molecular Biology* 292: 195–202.

Karnachuk, O.V., Gavrilov, S.N., Avakyan, M.R., Podosokorskaya, O.A., Frank, Y.A., Bonch-Osmolovskaya, E.A. and Kublanov, I.V. (2015). Diversity of copper proteins and copper homeostasis systems in *Melioribacter roseus*, a

facultatively anaerobic thermophilic member of the new phylum *Ignavibacteriae*. *Microbiology* 84: 135-143.

Kelly, S.M., Jess, T.J. and Price, N.C. (2005). How to study proteins by circular dichroism. *Biochimica et Biophysica Acta* 1751: 119–139.

Kim, B.E., Nevitt, T. and Thiele, D.J. (2008). Mechanisms for copper acquisition, distribution and regulation. *Nature Chemical Biology* 4: 176–185.

Koonin, E.V. and Galperin, M.Y. (2003). Chapter 4: Principles and methods of sequence analysis. In *Sequence-Evolution-Function: Computational Approaches in Comparative Genomics*. Boston: Kluwer Academic.

Kreuder, J., Otten, A., Fuder, H., Tümer, Z., Tønnesen, T., Horn, N. and Dralle, D. (1993). Clinical and biochemical consequences of copper-histidine therapy in Menkes disease. *European Journal of Pediatrics* 152: 828–832.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.J. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.

Lehrer, S.S. (1971). Solute perturbation of protein fluorescence. Quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion. *Biochemistry* 10: 3254–3263.

Lesk, A.M. (2010). *Introduction to Protein Science: Architecture, Function, and Genomics*. New York: Oxford University Press Inc.

Liochev, S. I. and Fridovich, I. (2002). The Haber-Weiss cycle–70 years later: an alternative view. *Redox Report*. 7: 55–57.

Liu, T., Nakashima, S., Hirose, K., Shibasaka, M., Katsuhara, M., Ezaki, B., Giedroc, D. P. and Kasamo, K. (2004). A novel cyanobacterial SmtB/ArsR family repressor regulates the expression of a CPx-ATPase and a metallothionein in response to both Cu(I)/Ag(I) and Zn(II)/ Cd(II). *The Journal of Biological Chemistry* 279: 17810–17818.

Liu, T., Ramesh, A., Ma, Z., Ward, S.K., Zhang, L., George, G.N., Talaat, A.M., Sacchettini, J.C. and Giedroc, D.P. (2007). CsoR is a novel *Mycobacterium tuberculosis* copper-sensing transcriptional regulator. *Nature Chemical Biology* 3: 60–68.

Liu, T., Chen, X., Ma, Z., Shokes, J., Hemmingsen, L., Scott, R.A. and Geidroc, D.P. (2008). A Cu^{I}-sensing ArsR family metal sensor protein with a relaxed metal selectivity profile. *Biochemistry* 47: 10564-10575.

Lovell, S.C., Davis, I.W., Adrendall, W.B., De Bakker, P.I.W., Word, J.M., Prisant, M.G., Richardson, J.S. and Richardson, D.C. (2003). Structure validation by

Cα geometry: φ, ψ and Cβ deviation. *Proteins: Structure, Function, and Genetics* 50: 437–450.

Lubec, G., Afjehi-Sadat, L., Yang, J.W. and John, J.P. (2005). Searching for hypothetical proteins: theory and practice based upon original data and literature. *Progress in Neurobiology* 77: 90–127.

Lutsenko, S. (2010). Human copper homeostasis: a network of interconnected pathways. *Current Opinion in Chemical Biology* 14: 211–217.

Ma, Z., Cowart, D.M., Scott, R.A. and Giedroc, D.P. (2009a). Molecular insights into the metal selectivity of the Cu(I)-sensing repressor CsoR from *Bacillus subtilis*. *Biochemistry* 48: 3325–3334.

Ma,Z., Cowart, D.M., Ward, B.P., Arnold, R.J., Di Marchi, R.D., Zhang,L., George, G.N., Scott, R.A. and Giedroc, D.P. (2009b). Unnatural amino acid substitution as a probe of the allosteric coupling pathway in a mycobacterial Cu(I) sensor. *Journal of The American Chemical Society* 131: 18044–18045.

Ma,Z., Jacobsen,F.E. and Giedroc, D.P. (2009c). Coordination chemistry of bacterial metal transport and sensing. *Chemical Reviews* 109: 4644–4681.

Macomber, L. and Imlay, J. (2009) The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. *Proceedings of the National Academy of Science USA* 106: 8344–8349.

Marchler-Bauer, A. and Bryant, S.H. (2004). CD-Search: protein domain annotations on the fly. *Nucleic Acids Research* 32: W327–331.

Marley, J., Lu, M. and Bracken, C. (2001). A method for efficient isotopic labeling of recombinant proteins. *Journal of Biomolecular NMR* 20: 71-75.

Marrero, J., Auling, G., Coto, O. and Nies, D.H. (2007). High-level resistance to cobalt and nickel but probably no transenvelope efflux: Metal resistance in the Cuban Serratia marcescens strain C-1. *Microbial Ecology* 53: 123–33.

Mermod, M., Magnani, D., Solioz, M. and Stoyanov, J.V. (2012). The copper-inducible ComR (YcfQ) repressor regulates expression of ComC (YcfR), which affects copper permeability of the outer membrane of *Escherichia coli*. *Biometals* 25: 33–43.

Minion, F.C., Lefkowitz, E.J., Madsen, M.L., Cleary, B.J., Swartzell, S.M. and Mahairas, G.G. (2004). The genome sequence of *Mycoplasma hyopneumoniae* strain 232, the agent of swine mycoplasmosis. *Journal of Bacteriology* 186: 7123–7133.

Miyazawa, S. and Jernigan, R.L. (1996). Residue-residue potentials with a favourable contact pair term and an unfavorable high packing density term, for simulation and threading. *Journal of Molecular Biology* 256: 623–644.

Motlagh, H.N., Wrabl, J.O., Li, J. and Hilser, V.J. (2014). The ensemble nature of allostery. *Nature* 508: 331–339.

Nawapan, S., Charoenlap, N., Charoenwuttitam, A., Saenkham, P., Mongkolsuk, S. and Vattanaviboon, P. (2009). Functional and expression analyses of the cop operon, required for copper resistance in *Agrobacterium tumefaciens*. *Journal of Bacteriology* 191: 5159–5168.

Nazina, T.N., Tourova, T.P., Poltaraus, A.B., Novikova, E.V., Grigoryan, A.A., Ivanova, A.E., Lysenko, A.M., Petrunyaka, V.V., Osipov, G.A., Belyaev, S. S. and Ivanov, M.V. (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus, Bacillus thermocatenulatus, Bacillus thermoleovorans, Bacillus kaustophilus* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus, G. thermocatenulatus, G. thermoleovorans, G. kaustophilus, G. thermoglucosidasius* and *G. thermodenitrificans*. *International Journal of Systematic and Evolutionary Microbiology* 51: 433–446.

New England BioLabs. (2015). PCR Troubleshooting Guide. https://www.neb.com/tools-and-resources/troubleshooting-guides/pcr-troubleshooting-guide. Retrieved 27 February 2015.

Nose, Y., Kim, B.E. and Thiele, D.J. (2006). Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. *Cell Metabolism* 4: 235–244.

Ornstein, L. (1964) Disc Electrophoresis. I. Background And Theory. *Annals of the New York Academy of Sciences* 121: 321–349.

Osredkar, J. and Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *Journal of Clinical Toxicology* S3:001.

Outten, F.W., Outten, C.E., Hale, J. and O'Halloran, T.V. (2000). Transcriptional activation of an *Escherichia coli* copper efflux regulon by the chromosomal MerR homologue, CueR. *The Journal of Biological Chemistry* 275: 31024–31029.

Outten, F.W., Huffman, D.L., Hale, J.A. and O'Halloran, T.V. (2001). The independent *cue* and *cus* systems confer copper tolerance during aerobic and anaerobic growth in Escherichia coli. *Journal of Biological Chemistry* 276: 30670–30677.

Pace, C.N., Vajdos, F., Fee, L., Grimsley, G. and Gray, T. (1995). How to measure and predict the molar absorption coefficient of a protein. *Protein Science* 4: 2411-2423.

Pawlowski, K. (2008) Uncharacterized/hypothetical proteins in biomedical 'omics' experiments: is novelty being swept under the carpet?. *Briefings In Functional Genomics And Proteomics* 7: 283-290.

Petsko, G.A. and Ringe, D. (2004). From sequence to structure. In *Protein Structure and Function*. London: New Science Press Ltd.

Provencher, S.W. and Glöckner, J. (1981). Estimation of globular protein secondary structure from circular dichroism. *Biochemistry* 20: 33–37.

Puig, S., Andres-Colas, N., Garcia-Molina, A. and Penarrubia, L. (2007). Copper and iron homeostasis in Arabidopsis: responses to metal deficiencies, interactions and biotechnological applications. *Plant, Cell & Environment* 30: 271–290.

Punta, M., Coggill, P.C., Eberhardt, R.Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G., Clements, J., Heger, A., Holm, L., Sonnhammer, E.L., Eddy, S.R., Bateman, A. and Finn, R.D. (2012). The Pfam protein families database. *Nucleic Acids Research* 40: D290–301.

Quintanar, L. and Rivillas-Acevedo, L. (2013). Studying metal ion-protein interactions: electronic absorption, circular dichroism, and electron paramagnetic resonance. In *Methods in Molecular Biology*. London: SpringerLink Protocols.

Rademacher., C. and Masepohl, B. (2012). Copper-responsive gene regulation in bacteria. *Microbiology* 158: 2451–2464.

Rahman, R.N.Z.R.A., Leow, T.C., Salleh, A.B. and Basri, M. (2007). *Geobacillus zalihae* sp. nov., a thermophilic lipolytic bacterium isolated from palm oil mill effluent in Malaysia. *BMC Microbiology* 7: 77.

Reffuveille, F., Serror, P., Chevalier, S., Budin-Verneuil, A., Ladjouzi, R., Bernay, B., Auffray, Y. and Rince, A. (2012). The prolipoprotein diacylglyceryl transferase (Lgt) of *Enterococcus faecalis* contributes to virulence. *Microbiology* 158: 816–825.

Ridge, P.G., Zhang, Y. and Gladyshev, V.N. (2008). Comparative genomic analyses of copper transporters and cuproproteomes reveal evolutionary dynamics of copper utilization and its link to oxygen. *PLoS One* 3: e1378.

Rigo, A., Corazza, A., di Paolo, M.L., Rossetto, M., Ugolini, R. and Scarpa, M. (2004). Interaction of copper with cysteine: stability of cuprous complexes and catalytic role of cupric ions in anaerobic thiol oxidation. *Journal of Inorganic Biochemistry* 98: 1495-1501.

Roberts,R.J. (2004) Identifying protein function—a call for community action. *PLoS Biology* 2: E42.

Sakamoto, K., Agari, Y., Agari, K., Kuramitsu, S. and Shinkai, A. (2010) Structural and functional characterization of the transcriptional repressor CsoR from *Thermus thermophilus* HB8. *Microbiology.* 156: 1993–2005.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press.

Scheiber, I.F., Mercer, J.F. and Dringen, R. (2014). Metabolism and functions of copper in brain. *Progress in Neurobiology* 116: 33-57.

Schmid, F-X. (2007). Biological macromolecules: UV/visible spectrophotometry. In *Handbook of Proteins: Structure, Function and Methods*. West Sussex: John Wiley and Sons Ltd.

Shafeeq, S., Yesilkaya, H., Kloosterman, T.G., Narayanan, G., Wandel, M., Andrew, P.W., Kuipers, O.P. and Morrissey, J.A. (2011). The cop operon is required for copper homeostasis and contributes to virulence in *Streptococcus pneumoniae*. *Molecular Microbiology* 81: 1255–1270.

Sheehan, D. (2009). *Physical Biochemistry: Principles and Applications*. West Sussex: John Wiley and Sons.

Shmuely, H., Dinitz, E., Dahan, I., Eichler, J., Fischer, D. and Shaanan, B. (2004). Poorly conserved ORFs in the genome of the archaea Halobacterium sp NRC-1 correspond to expressed proteins. *Bioinformatics* 20: 1248–1253.

Siew, N. and Fischer, D. (2003a). Analysis of singleton ORFans in fully sequenced microbial genomes. *Proteins* 53: 241–251.

Siew, N. and Fischer, D. (2003b). Twenty thousand ORFan microbial protein families for the biologist? *Structure* 11: 7–9.

Sinden, R.R. (1994). *DNA Structure and Function*. California: Academic Press.

Smaldone, G. T. and Helmann, J. D. (2007). CsoR regulates the copper efflux operon copZA in *Bacillus subtilis*. *Microbiology* 153: 4123–4128.

So, P.T.C. and Dong, C.Y. (2007). Fluorescence spectrophotometry. In *Handbook of Proteins: Structure, Function and Methods*. West Sussex: John Wiley and Sons Ltd.

Sreerama, N., Venyaminov, S.Y. and Woody, R.W. (1999). Estimation of the number of alpha-helical and beta-strand segments in proteins using circular dichroism spectroscopy. *Protein Science* 8: 370–380.

Sreerama, N. and Woody, R.W. (1993). A self-consistent method for the analysis of protein secondary structure from circular dichroism. *Analytical Biochemistry* 209: 32–44.

Studier, F.W. (2005). Protein production by auto-induction in high-density shaking cultures. *Protein Expression and Purification* 41: 207-234.

Tan, B.G., Vijgenboom, E. and Worrall, J.R. (2014) Conformational and thermodynamic hallmarks of DNA operator site specificity in the copper sensitive operon repressor from *Streptomyces lividans*. *Nucleic Acids Research* 42: 1326–1340.

Taniguchi, N. (2005). Amino acids and proteins. In *Medical Biochemistry*. China: The Publisher.

Teramoto, H., Inui, M. and Yukawa, H. (2012). *Corynebacterium glutamicum* CsoR acts as transcriptional repressor of two copper/zinc-inducible P_{1B}-type ATPase operons. *Bioscience, Biotechnology, and Biochemistry* 76: 1952-1958.

Teramoto, H., Inui, M. and Yukawa, H. (2015). Copper homeostasis-related genes in three separate transcriptional units regulated by CsoR in *Corynebacterium glutamicum*. *Applied Genetics and Molecular Biotechnology* 99: 3505-3517.

Thaden, J.T., Lory, S. and Gardner, T.S. (2010). Quorum-sensing regulation of a copper toxicity system in *Pseudomonas aeruginosa*. *Journal of Bacteriology* 192: 2557–2568.

Tian, J., Wu, N., Li, J., Liu, Y., Guo, J., Yao, B. and Fan, Y. (2007). Nickel-resistant determinant from *Leptospirillum ferriphilum*. *Applied and Environmental Microbiology* 73: 2364–2368.

Tottey, S., Harvie, D.R. and Robinson, N.J. (2005). Understanding how cells allocate metals using metal sensors and metallochaperones. *Accounts of Chemical Research* 38: 775–783.

Upstone, S.L. (2013). Ultraviolet/Visible light absorption apectrophotometry in clinical chemistry. In *Encyclopedia of Analytical Chemistry*. London: Blackie Production and Associates.

Van Stokkum, I.H., Spoelder, H.J., Bloemendal. M., Van Grondelle, R. and Groen, F.C. (1990). Estimation of protein secondary structure and error analysis from circular dichroism spectra. *Analytical Biochemistry* 191: 110-118.

Vogel, C., Bashton, M., Kerrison, N.D., Chothia, C. and Teichmann, S.A. (2004). Structure, function and evolution of multidomain proteins. *Current Opinion in Structural Biology* 14: 208-216.

Vojdani, F. (1996). Solubility. In *Methods of Testing Protein Functionality*. London: Blackie Production and Associates.

Voloudakis, A.E., Reignier, T.M. and Cooksey, D.A. (2005). Regulation of resistance to copper in *Xanthomonas axonopodis* pv. *vesicatoria*. *Applied and Environmental Microbiology* 71: 782–789.

Waidner, B., Melchers, K., Stähler, F.N., Kist, M. and Bereswill, S. (2005). The *Helicobacter pylori* CrdRS two-component regulation system (HP1364/HP1365) is required for copper-mediated induction of the copper resistance determinant CrdA. *Journal of Bacteriology* 187: 4683–4688.

White, C., Lee, J., Kambe, T., Fritsche, K. and Petris, M.J. (2009). A role for the ATP7A copper-transporting ATPase in macrophage bactericidal activity. *The Journal of Biological Chemistry* 284: 33949–33956.

Whitmore, L. and Wallace, B. (2004). DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Research* 32: W668–73.

Wiedemann, C., Bellstedt, P. and Görlach, M. (2013). CAPITO--a web server-based analysis and plotting tool for circular dichroism data. *Bioinformatics* 29: 1750–1757.

Winge, D.R., Graden, J.A., Posewitz, M.C., Martins, L.J., Jensen, L.T. and Simon, J.R. (1997). Sensors that mediate copper-specific activationand repression of gene expression. *Journal of Biological Inorganic Chemistry* 2: 2-10.

Yi, S., Brickenden, A. and Choy, W-Y. (2008). A new protocol for high-yield purification of recombinant human prothymosin α expressed in *Escherichia coli* for NMR studies. *Protein Expression and Purification* 57: 1-8.

Yu, N.Y., Wagner, J.R., Laird, M.R., Melli, G., Rey, S., Lo, R., Dao, P., Sahinalp, S.C., Ester, M., Foster, L.J. and Brinkman, F.S.L. (2010) PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics* 26: 1608-1615.

Zeigler, D.R. (2014). The *Geobacillus* paradox: why is a thermophilic bacterial genus so prevalent on a mesophilic planet?. *Microbiology* 160: 1-11.

Zhang, Y. (2008). I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics* 9: 40.

Zhang, Y.Z., Zhou, B., Liu, Y.X., Zhou, C.X., Ding, X.L. and Liu, Y. (2008). Fluorescence study on the interaction of bovine serum albumin with p-aminoazobenzene. *Journal of Fluorescence* 18: 109–118.

Zhao, Z., Zhou, Z., Li, L., Xian, X., Ke, X., Chen, M. and Zhang, Y. (2014). A copper-responsive gene cluster is required for copper homeostasis and contributes to oxidative resistance in *Deinococcus radiodurans* R1. *Molecular BioSystems* 10: 2607-2616.

Zhou, P.B., Szczypka, M.S., Sosinowski, T. and Thiele, D.J. (1992). Expression of a yeast metallothionein gene family is activated by a single metalloregulatory transcription factor. *Molecular and Cell Biology* 12: 3766-3775.