MOLECULAR DYNAMICS SIMULATIONS ON STRUCTURAL DIFFERENCES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY VARIANTS AMONG THE ASIAN POPULATION

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DEDICATION

I would like to dedicate the completion of this thesis to my family, Mr Richard Louis, Mrs Vimala Devi Shanmugam, Ms Yanitha Meena Louis and Mr Ramanan TK. It was my family who encouraged and persuaded me to pursue my Masters. They believed in me even when I did not. I would like to thank my uncle Mr Rajah Jeganathen for being a guarantor for my M.Phil.

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) is responsible for red blood cell protection against free radicals. There are over 186 G6PD deficient variants which adversely affected the enzyme activity. In its active state, G6PD exists in dynamic equilibrium as dimer and tetramer, influenced by its ligands. A human G6PD monomer has three ligands, the glucose-6-phosphate (G6P) substrate, a catalytic nicotinamide adenine dinucleotide phosphate (NADP) cofactor, and structural NADP. Ligands like G6P disrupt dimer formation, whereas NADP favour tetramer formation. G6PD enzyme activity is dependent on the structural integrity of the dimer interface. The mechanism of mutation-induced structural instability and the physiological significance of ligands on G6PD structure and function remains unclear till date. More than 400 G6PD variants exists, only 10 per cent of mutations were analysed in depth, none of which includes variants common to Asian population. In this study, ten common Asian variants (G410D, K275N, R387C, V291M, L128P, R459L, V431M, H32R, G163S, and G131V) were chosen for analysis using molecular dynamics simulation (MDS). Since G6PD dimerization is crucial for basic activity, a G6PD dimer with ligands was constructed using molecular docking and simulated using GROMACS for 100 ns. The simulated trajectories of the variants against the wild type (WT) were used to evaluate changes at the mutation site, and the dimer and tetramer interfaces. Alterations in protein-ligand affinities were evaluated by analysing the molecular binding profile coupled with free binding energy calculations. The wild type and variants with high enzyme activity such as G131V and G163S, showed high structural integrity at the dimer interface characterized by intermolecular hydrogen bonds between Asp 421-Asp 421 and Glu 419-Thr 423 at βN, and salt bridges between Glu 206-Lys 407. The bonds spanned over both monomeric subunits, resulting in compact dimer indicated by low radius of gyration (Rg) values. The G6PD structures with low Rg exhibited increased distance between the βI–βJ loop, thus exposing the tetramer interface and tetramer salt bridge residues. The high solvent accessible surface area (SASA) characteristic indicates a high dimer-dimer affinity in tetrameric state. The βE–αe loop responsible for positioning G6P and the catalytic NADP for G6PD catalysis was retained in variants with stable dimer structures. Ligand interplay between the G6P and the structural NADP was evident; G6P trajectory frames showing high affinity toward G6PD, led to a low or total loss affinity of NADP. High NADP binding pocket occupancy contributed to a low Rg of the structures. This was the first G6PD MDS study to relate in-silico findings with existing biochemical and kinetic data. In short, findings from this study would be beneficial for variant assessment, prognostic marker identification and drug development. This MDS study was successful in validating empirical observations from previous biochemical and structural studies such as the loss of αn-αe interhelical interactions for R459L, impaired tetramerization for K275N and R459L, and protein-ligand affinities for the G410D, R387C, V291M, R459L, and G163S variants towards G6P and NADP.

ABSTRAK

Glucose-6-phosphate dehydrogenase (G6PD) bertanggungjawab untuk melindungi sel darah merah daripada radikal bebas. Terdapat 186 bilangan varian kerkurangan G6PD yang menjejaskan aktiviti enzim. Dalam keadaan aktif, G6PD wujud dalam keseimbangan dinamik sebagai dimer dan tetramer dipengaruhi oleh berberapa ligan. Monomer G6PD manusia mempunyai tiga ligan, substrat G6P, kofaktor nikotinamida adenine dinukleotida fosfat (NADP) pemangkin dan NADP struktur. Ligan seperti G6P mengganggu pembentukan dimer, manakala NADP menyokong pembentukan tetramer. Aktiviti enzim G6PD bergantung kepada integriti struktur antara muka dimer. Mekanisme mutasi menyebabkan ketidakstabilan struktur dan kepentingan fisiologi ligan pada struktur dan fungsi G6PD masih samar sehingga kini. Terdapat lebih 400 varian G6PD yang telah dikaji dan hanya 10% sahaja yang dianalisa secara mendalam, tetapi tiada satu pun varian yang lazim dalam populasi Asia. Dalam kajian ini, sepuluh varian G6PD lazim di Asia (G410D, K275N, R387C, V291M, L128P, R459L, V431M, H32R, G163S dan G163V) telah dipilih untuk kajian simulasi molekul dinamik (MDS). Memandangkan pendimeran G6PD adalah penting untuk aktiviti asas enzim, dimer G6PD dengan ligan telah dibina secara mengedok molekul dan disimulasi menggunakan GROMACS selama 100 ns. Trajektori simulasi varian terhadap jenis asal (WT) digunakan untuk menilai perubahan di tapak mutasi serta antara muka dimer dan tetramer. Perubahan dalam pertalian protein-ligan dinilai dengan menganalisis profil pengikatan molekul beserta pengiraan tenaga pengikat bebas. Jenis liar (WT) dan varian dengan aktiviti enzim tinggi seperti G131V dan G163S menunjukkan integriti struktur yang tinggi pada antara muka dimer yang dicirikan oleh ikatan hidrogen antara molekul antara Asp 421-Asp 421, Glu 419-Thr 423 pada βN dan jambatan garam antara Glu 206-Lys 407. Ikatan merentangi kedua-dua subunit monomer menjadikan dimer yang padat ditunjukkan oleh nilai jejari legaran (Rg) yang rendah. Struktur G6PD dengan Rg rendah menunjukkan peningkatan jarak antara gelung βI-βJ lantas mendedahkan antara muka tetramer dan residu jambatan garam tetramer. Kawasan permukaan boleh diakses pelarut yang tinggi (SASA) menunjukkan keafinan dimer-dimer yang tinggi dalam keadaan tetramerik. Gelung βE–αe yang bertanggungjawab untuk menetududukkan G6P dan NADP pemangkin bagi pemangkinan G6PD dikekalkan dalam varian dengan struktur dimer yang stabil. Interaksi ligan antara G6P dan NADP struktur adalah jelas, di mana bingkai trajektori G6P menunjukkan keafinan ikatan yang tinggi terhadap G6PD, manakala ikatan terhadap NADP adalah rendah atau terus tiada. Pengikatan NADP yang tinggi pada poket pula menyumbang kepada nilai Rg yang rendah. Kajian MDS G6PD ini adalah yang pertama mengaitkan penemuan siliko dengan data biokimia dan kinetik sedia ada. Penemuan kajian ini akan bermanfaat untuk penilaian varian, pengenalpastian penanda prognostik dan pembangunan ubatan. Kajian MDS ini berjaya mengesahkan pemerhatian empirikal daripada kajian biokimia dan struktur terdahulu seperti kehilangan interaksi antara helik αn-αe untuk R459L, pentetrameran terjejas untuk K275N dan R459L, serta pertalian ligan protein untuk varian G410D, R387C, V291M, R459L dan varian G163S dengan G6P dan NADP.

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LIST OF ABBREVIATIONS

G6PD	-	Glucose-6-phosphate-dehydrogenase
WHO	-	World Health Organization
RBC	-	Red blood cells
G6P	-	Glucose-6-phosphate
NADPH	-	Reduced nicotinamide adenine dinucleotide phosphate
NADP	-	Nicotinamide adenine dinucleotide phosphate
c.NADP		Catalytic nicotinamide adenine dinucleotide phosphate
s.NADP	-	Structural nicotinamide adenine dinucleotide phosphate
MDS	-	Molecular dynamics simulation
G6PDD	-	Glucose-6-phosphate-dehydrogenase deficiency
MDS	-	Molecular dynamics simulation
SEA	-	South East Asia
RMSD	-	Root-mean-square deviation
RMSF	-	Root mean square fluctuation
Rg	-	Radius of gyration
SASA	-	Solvent accessible surface
PCA	-	Principal component analysis
MMPBSA	-	Molecular mechanics Poisson-Boltzmann surface area
NVT	-	constant Number of particles, Volume, and Temperature
NPT	-	constant Number of particles, Pressure, and Temperature
App	-	Appendix

LIST OF SYMBOLS

$\rightleftharpoons$	-	Equilibrium
α	-	Alpha helix
β	-	Beta sheet
K	-	Kelvin - unit of temperature
$K_{cal}\ mol^{-1}$	-	Kilocalorie per mole – unit of energy
nm	-	Nanometer
nm^2	-	Newton per square Meter
ns	-	Nanoseconds
Å	-	Angstrom
K_{cat}	-	Turnover number
K_m	-	Michaelis constant
ns	-	Nanoseconds
$kJ \cdot mol^{-1}$	-	kilojoules per mole

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme responsible for red blood cell (RBC) protection against free radicals (Jinyoung Lee et al., 2018). G6PD produces the anti-oxidative component namely, the reduced nicotinamide adenine dinucleotide phosphate (NADPH) by catalysing a redox reaction involving the oxidation of its substrate glucose-6-phosphate (G6P) and the reduction of its cofactor NADP. These reactions generate an NADPH supply required to combat oxidative stressors (Horikoshi et al., 2021). Impaired G6PD catalysis hinders NADPH production, and may lead to redox dyshomeostasis, which is implicated with poor counter mechanisms to oxidative stressors. This leads to a number of issues which include free radical-induced cell lysis, impaired cell signalling, detoxification and apoptosis mechanisms, and the inability to detect and eradicate xenobiotics from the body efficiently (Ayer et al., 2014).

The human G6PD monomer has a G6P substrate, a catalytic NADP (c.NADP) cofactor, and a structural NADP (s.NADP) responsible for structural stability (Kotaka et al., 2005). G6PD exists in dynamic equilibrium of monomer ⇌ dimer ⇌ tetramer, depending on its environment which is influenced by ligands. G6PD tetramers tend to be formed in the presence of NADP ligands, whereas dimers tend to be disrupted by ligands like G6P (Au et al., 2000). Protein multimerization, namely dimerization and tetramerization play an important role in G6PD catalysis, where dimerization is crucial for basic enzyme activity, and tetramerization allows for a more structurally stable version of the protein (Cunningham et al., 2017; Kotaka et al., 2005). However, the mechanism and physiological significance of ligand dependent G6PD multimerization associated with mutations remain ambiguous.

Previous studies have by Horikoshi et al (2021) have attempted to understand the physiological significance of ligands coupled with the effects of class I mutations by using structure based mutagenesis and kinetic analysis. Results from the study report that class I mutations lose affinity towards s.NADP which result in disorientation of the C-terminal tail and αf helix, impairing G6P binding and overall enzyme activity (Horikoshi et al., 2021). However, insights on the mechanism of protein-ligand alterations due to mutations for other classes of variants remain elusive.

G6PD deficiency (G6PDD) is an X-linked recessive disorder that leads to low levels of the G6PD enzyme. Mutations on the gene encoding for G6PD alters its protein structure and multimerization capabilities, by altering amino acid side chains which consequently changes their polarity, charges, surface area, and intermolecular interactions (Doss et al., 2016; Hwang et al., 2018). G6PDD is the most common enzymopathy affecting over 400 million individuals worldwide. More than 186 G6PD variants are shown to be associated with G6PDD, with decreased activity or stability of the enzyme (Jinyoung Lee et al., 2018).

Depending on the enzyme activity and clinical phenotype for different G6PD mutations, they have been grouped into five classes (I, II, III, IV and V) by the World Health Organization (WHO). Class I (< 1% enzyme activity), class II (<10% enzyme activity) and class III (10 – 60 % enzyme activity) are the most severe mutations because they express low enzyme levels which leads to anemia. Classes IV (60-90% normal activity) and V (>110% increased activity) variants tend to have less damaging effects and are asymptomatic (Gautam, 2016). G6PD variants manifesting different clinical phenotypes complicate comprehending the mechanism of the disease. Moreover, since many mutations are distributed throughout the protein structure, understanding the structural-functional relationship for G6PD variants can be challenging (Cunningham et al., 2017). Despite numerous structural and biochemical studies performed on G6PD mutations, less than 10% of known variants have been studied in detail which relate their clinical manifestation to their unique mutations (Gómez-Manzo et al., 2017).

The deleterious effects of variants decrease in the order of I > II > III (Gautam, 2016). Class I variants tend to clustered at the dimer interface and s.NADP binding site, whereas class II and III tend to be clustered at the tetramer interface and catalytic domain respectively (Cunningham et al., 2017). Therefore, in a structural and functional context, high structural integrity at the dimer interface is crucial for basic G6PD enzyme activity. Recently discovered G6PD agonists were able to elevate low enzyme activity in variants by promoting dimer formation. By employing the use of gel electrophoresis, Hwang et al (2018) identified increased molecular weight of the G6PD protein due to an equilibrium shift of monomer to dimer in the presence of AG1 (G6PD agonist). The agonist was successful in increasing enzyme activity for selected variants by promoting dimeric states of G6PD. Hence, it is evident that G6PD enzyme activity is influenced by the structural integrity of the dimer interface and dependent on the ability to dimerize. Unravelling the deleterious effects of harmful G6PD variants on protein structure to establish a structural - functional link and understand how they affect enzyme activity would be useful for variant assessment and prognostic marker identification.

Molecular dynamics simulation (MDS) is known for its effectiveness in establishing structural and functional relationships for macromolecules and predicting the nature of protein-protein and protein-ligand interactions (Hospital et al., 2015). Therefore, simulating the G6PD protein would provide invaluable insights. There have been previous G6PD MDS studies, which evaluated the structural aberrations of G6PD variants common to the Arab, USA and German population. The study by H.Nguyen et al (2016) was successful in evaluating alterations in protein-ligand affinity for G6PD variants using free binding energy calculations using the molecular mechanic Poisson-Boltzmann surface area (MM-PBSA) approach and by computing the number hydrogen bonds between protein and ligand. Results from the MM-PBSA showed that the wild-type had a greater affinity towards the ligands than the variants. The study by Doss et al (2016) was successful in determining the effects of mutations by the analysing changes in chemical characteristics of the mutated residues coupled with an array of analyses such as root-mean-square deviation (RMSD), root-mean square fluctuation (RMSF), Intermolecular hydrogen bond analyses, solvent accessible surface area (SASA). These analyses allowed a structural comparison of the simulated variants against the wild-type.

Since GROMACS allows computing the presence and distance of intermolecular interactions, it would have been possible to evaluate the integrity of the dimer and tetramer interfaces by checking for the presence of salt bridges and hydrogen bonds which are crucial for the stability of both multimeric interfaces. However, both studies involved simulating G6PD monomers and dimers without ligands (Doss et al., 2016; H. Nguyen et al., 2016), therefore did not provide insights on the enzyme in its active state which is highly influenced by ligands. Since ligands influences G6PD multimerization and enzyme activity, simulating a G6PD dimer in complex with ligands would be useful in understanding the physiological effects of G6P, c.NADP and s.NADP in an active state of the enzyme, and allow evaluating how different mutations affect G6PD function.

1.2 Problem Statement

G6PDD has been associated with a variety of metabolic and neurological disorders, making G6PD drug development the need of the hour. However, drug development demands a biophysical and biochemical knowledge of G6PD variants and the mechanism of how they affect the protein structural integrity and enzyme activity. Although more than 400 G6PD variants have been reported, less than 10 % have been analyzed in depth. Moreover, G6PD-related MDS studies have only focused on mutations common to the USA, German and Middle Eastern population. Despite 5 – 20 % of the global incidence of G6PDD is reported in Asia, there is still a lack of knowledge for G6PD variants originating from Asia and SEA in a structural context. Therefore, by employing the use of MDS, this study aims to simulate G6PD variants originating from Asia to understand their mutational effects.

The human G6PD enzyme, in its active state exists as dimers or tetramers depending on its environment, greatly influenced by ligands. However, in terms of protein multimerization, dimerization is crucial for basic G6PD enzymatic activity. There are several crystal structures of G6PD deposited in the Protein Data Bank (PDB), however, there are no structures of the human G6PD dimer in complex with ligands available till date, hence making it difficult to understand the structural and functional changes associated with ligand dependent dimerization.

This presses the need to construct a G6PD dimer with ligands using molecular docking approaches. Moreover, mutations on the G6PD-ligand complex would create further structural changes affecting the G6PD variants compared to the native dimeric variant, which remains unknown, especially in G6PDD of the SEA & Asian variants. Understanding such intermolecular & structural changes on the G6PD-ligand complex could yield valuable structural and functional relationships related to changes at the dimer interface, tetramer interface and ligand binding sites.

Previous G6PD-related MDS studies were not corroborated with existing structural and biochemical reports, therefore did not bridge their *in-silico* findings with existing *in-vitro* knowledge of G6PD variants. Moreover, previous studies only simulated monomers and dimers without ligands. This was the first MDS study of the G6PD dimer bound to all its ligands, therefore mimicking G6PD in its active state. The rationale to simulate a G6PD dimer was based on the fact that the most harmful mutations are clustered at the dimer interface, and G6PD enzyme activity is dependent on the structural integrity of the dimer. To overcome the forementioned gaps, this study sought to validate the *in-silico* analyses derived from MDS with existing literature that report enzyme activities (k_{cat}) and protein-ligand affinities (K_m) for G6PD Asian variants, thereby establishing a structural and functional relationship for the variants being analyzed.

Previous studies did not analyze alterations at the dimer and tetramer interfaces due to a particular mutation nor due to the affinity towards a ligand. This study hypothesizes that variants with high enzyme activity (high k_{cat}) have stable dimer interfaces (characterized by the presence of salt bridges and hydrogen bonds between each monomeric subunit of the dimer) and exhibit the ability to tetramerize (characterized by increased surface area at the tetramer interface). Variants with stable dimer interfaces should develop greater affinities towards NADP rather than G6P ligands, as the latter disassociates dimers into inactive monomers. Computing the protein -ligand affinity using hydrogen bond analyses should match the affinity based on K_m values from previous kinetic characterization studies of G6PD. This was the first study to provide insights on ligand interplay and their physiological significance on the dimer ⇌ tetramer interconversion.

1.3 Research Objectives

The objectives of the research are:

(a) To prepare a complete G6PD dimer structure with substrates and cofactors using molecular docking approaches.

(b) To compare the structural stability of G6PD Asian variants against the native dimeric protein using MDS.

(c) To identify and validate changes in protein - ligand affinity based on the molecular binding profile and molecular mechanic Poisson-Boltzmann surface area (MMPBSA) approach.

1.4 Research Significance

Establishing a structural and functional relationship for G6PD variants was possible by corelating the structural integrity at the dimer interface with their reported enzyme activities from biochemical reports. Furthermore, this study will act as a platform to provide genotype-phenotype information that might be useful for G6PD drug development and enable a better understanding of G6PD pathogenicity. Despite categorization of different variants into five classes by the WHO, discrepancies exist whereby class I variants exhibit more than 10 % (Martínez-Rosas et al., 2020) and class III variants exhibit less than 4% of enzyme activity (Chao et al., 1991). This study was successful in reasoning for such discrepancies by analyzing alterations at critical domains such as the dimer and tetramer interfaces and ligand binding sites.

This study was successful in unravelling the required structural dynamics for G6PD to achieve optimum enzyme activity. In the process, the structural defects due to ten different variants originating from Asia were carefully assessed and allowed identifying the required dynamics of the mutated protein structures to ameliorate impaired enzyme activity. This information would be invaluable for G6PD drug development.

Recently discovered G6PD agonists such as AG1 has been effective in increasing the enzyme activity for selected variants. Saddala et al (2020) have identified compounds with similar mode of action as AG1. Since the docking model and trajectory analyses from this study was successful in reproducing the structural characteristics of existing G6PD literature, subsequent docking of identified G6PD agonists by Saddala et al (2020) onto the G6PD model should evaluate its effects on whether it would increase the structural integrity of the dimer and tetramer interface, hence indicating amelioration of low enzyme activity. Therefore, it would be useful to evaluate efficacies for a broader range of variants to overcome AG1's selective nature (Saddala et al., 2020).

1.5 Scopes of the study

A complete G6PD dimer bound to all its ligands (two G6P, c.NADP and s.NADP) was constructed by employing molecular docking approaches using AutoDock 4.2 and AutoDock Vina. The protein - ligand docked complexes were validated by a conformation, and intermolecular hydrogen bond check against reference crystal structures 2BHL and 2BH9, which are bound to G6P and c.NADP-s.NADP respectively. Matching the docking pose, hydrogen bond network and distance of the docked ligand towards their crystal structure counterpart is crucial to ensure that the simulation mimics protein dynamics of G6PD in a cellular setting.

After docking, the native dimer and ten variants originating from SEA and Asia were simulated using GROMACS 2018.1 for 100 ns. The structural changes of variants with respect to the WT were compared using different parameters such as root-mean-square deviation (RMSD), root-mean square fluctuation (RMSF), radius of gyration (Rg), Intermolecular hydrogen bond analyses, solvent accessible surface area (SASA), and principal component analysis (PCA). Detailed structural analyses were performed by comparing the structural integrity of the dimer and tetramer interface for the variants against the WT.

Finally, a molecular binding profile evaluation was performed to inspect the protein-ligand affinity, coupled with electrostatic, van der Waals, polar solvation, SASA, and free binding energy calculations using the MMPBSA method. The number of hydrogen bonds between protein and ligand was verified with existing K_m values to validate the protein-ligand affinity for variants selected for this study. Ligand dependent multimerization was observed from this study, where high NADP binding pocket occupancy increased the structural stability of the dimer shifting it towards a tetrameric state, whereas high G6P affinity disassociated the dimer characterized by high distance and loss of hydrogen bonds between each monomeric subunit of the dimer. Overall, this study served to act as a link to understand how and why different classes of G6PD mutations exhibit structural disparities and present with different levels of enzyme activity.

REFERENCES

Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindahl, E. (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX, 1-2*, 19-25. doi:https://doi.org/10.1016/j.softx.2015.06.001

Aier, I., Varadwaj, P. K., & Raj, U. (2016). Structural insights into conformational stability of both wild-type and mutant EZH2 receptor. *Scientific reports, 6*(1), 34984. doi:10.1038/srep34984

Ainoon, O., Boo, N. Y., Yu, Y. H., Cheong, S. K., Hamidah, H. N., & Lim, J. H. (2004). Complete molecular characterisation of glucose-6-phosphate dehydrogenase (G6PD) deficiency in a group of Malaysian Chinese neonates. *Malays J Pathol, 26*(2), 89-98.

Aksoy, Y., Ogüs, I. H., & Oauzer, N. (2001). Purification and some properties of human placental glucose-6-phosphate dehydrogenase. *Protein Expr Purif, 21*(2), 286-292. doi:10.1006/prep.2000.1370

Alina, M. F., Azma, R. Z., Norunaluwar, J., Azlin, I., Darnina, A. J., Cheah, F. C., . . . Ainoon, O. (2020). Genotyping of Malaysian G6PD-deficient neonates by reverse dot blot flow-through hybridisation. *J Hum Genet, 65*(3), 263-270. doi:10.1038/s10038-019-0700-7

Anuar, N. F. S. K., Wahab, R. A., Huyop, F., Amran, S. I., Hamid, A. A. A., Halim, K. B. A., & Hood, M. H. M. (2021). Molecular docking and molecular dynamics simulations of a mutant *Acinetobacter haemolyticus* alkaline-stable lipase against tributyrin. *Journal of Biomolecular Structure and Dynamics, 39*(6), 2079-2091. doi:10.1080/07391102.2020.1743364

Au, S. W. N., Gover, S., Lam, V. M. S., & Adams, M. J. (2000). Human glucose-6-phosphate dehydrogenase: the crystal structure reveals a structural NADP+ molecule and provides insights into enzyme deficiency. *Structure, 8*(3), 293-303. doi:https://doi.org/10.1016/S0969-2126(00)00104-0

Ayer, A., Gourlay, C. W., & Dawes, I. W. (2014). Cellular redox homeostasis, reactive oxygen species and replicative ageing in Saccharomyces cerevisiae. *FEMS Yeast Research, 14*(1), 60-72. doi:10.1111/1567-1364.12114

Bitencourt-Ferreira, G., Veit-Acosta, M., & de Azevedo, W. F., Jr. (2019). Electrostatic Energy in Protein-Ligand Complexes. *Methods Mol Biol, 2053*, 67-77. doi:10.1007/978-1-4939-9752-7_5

Boonyuen, U., Chamchoy, K., Swangsri, T., Junkree, T., Day, N. P. J., White, N. J., & Imwong, M. (2017). A trade off between catalytic activity and protein stability determines the clinical manifestations of glucose-6-phosphate dehydrogenase (G6PD) deficiency. *International Journal of Biological Macromolecules, 104*, 145-156. doi:https://doi.org/10.1016/j.ijbiomac.2017.06.002

Chao, L. T., Du, C. S., Louie, E., Zuo, L., Chen, E., Lubin, B., & Chiu, D. T. (1991). A to G substitution identified in exon 2 of the G6PD gene among G6PD deficient Chinese. *Nucleic acids research, 19*(21), 6056-6056. doi:10.1093/nar/19.21.6056

Chen, J., Sawyer, N., & Regan, L. (2013). Protein-protein interactions: general trends in the relationship between binding affinity and interfacial buried surface area. *Protein science : a publication of the Protein Society, 22*(4), 510-515. doi:10.1002/pro.2230

Cunningham, A. D., & Mochly-Rosen, D. (2017). Structural analysis of clinically relevant pathogenic G6PD variants reveals the importance of tetramerization for G6PD activity. *Matters, 2017*, 10.19185/matters.201705000008. doi:10.19185/matters.201705000008

Daghestani, M., Purohit, R., Daghestani, M., Daghistani, M., & Warsy, A. (2019). Molecular dynamic (MD) studies on Gln233Arg (rs1137101) polymorphism of leptin receptor gene and associated variations in the anthropometric and metabolic profiles of Saudi women. *PloS one, 14*(2), e0211381-e0211381. doi:10.1371/journal.pone.0211381

Doss, C. G. P., Alasmar, D. R., Bux, R. I., Sneha, P., Bakhsh, F. D., Al-Azwani, I., . . . Zayed, H. (2016). Genetic Epidemiology of Glucose-6-Phosphate Dehydrogenase Deficiency in the Arab World. *Scientific reports, 6*(1), 37284. doi:10.1038/srep37284

Elyassi, A. R., & Rowshan, H. H. (2009). Perioperative management of the glucose-6-phosphate dehydrogenase deficient patient: a review of literature. *Anesth Prog, 56*(3), 86-91. doi:10.2344/0003-3006-56.3.86

Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. (2016). Computational protein–ligand docking and virtual drug screening with the AutoDock suite. *Nature protocols, 11*(5), 905-919. doi:10.1038/nprot.2016.051

Frank, J. E. (2005). Diagnosis and management of G6PD deficiency. *Am Fam Physician, 72*(7), 1277-1282.

Fu, C., Luo, S., Li, Q., Xie, B., Yang, Q., Geng, G., . . . Fan, X. (2018). Newborn screening of glucose-6-phosphate dehydrogenase deficiency in Guangxi, China: determination of optimal cutoff value to identify heterozygous female neonates. *Scientific reports, 8*(1), 833-833. doi:10.1038/s41598-017-17667-6

Ganczakowski, M., Town, M., Bowden, D. K., Vulliamy, T. J., Kaneko, A., Clegg, J. B., . . . Luzzatto, L. (1995). Multiple glucose 6-phosphate dehydrogenase-deficient variants correlate with malaria endemicity in the Vanuatu archipelago (southwestern Pacific). *Am J Hum Genet, 56*(1), 294-301.

Gautam, K. (2016). Glusoce-6-phosphate dehydrogenase- History and diagnosis. *Journal of Pathology of Nepal, 6*, 1034. doi:10.3126/jpn.v6i12.16260

Gómez-Manzo, S., Marcial-Quino, J., Vanoye-Carlo, A., Serrano-Posada, H., Ortega-Cuellar, D., González-Valdez, A., . . . Arreguin-Espinosa, R. (2016). Glucose-6-Phosphate Dehydrogenase: Update and Analysis of New Mutations around the World. *International journal of molecular sciences, 17*(12), 2069. doi:10.3390/ijms17122069

Gómez-Manzo, S., Quino, J., Ortega-Cuellar, D., Serrano-Posada, H., Gonzalez-Valdez, A., Vanoye Carlo, A., . . . Vivas, R. (2017). Functional and Biochemical Analysis of Glucose-6-Phosphate Dehydrogenase (G6PD) Variants: Elucidating the Molecular Basis of G6PD Deficiency. *Catalysts, 7*, 135. doi:10.3390/catal7050135

Gong, Z.-h., Tian, G.-l., Huang, Q.-w., Wang, Y.-m., & Xu, H.-p. (2017). Reduced glutathione and glutathione disulfide in the blood of glucose-6-phosphate dehydrogenase-deficient newborns. *BMC Pediatrics, 17*(1), 172. doi:10.1186/s12887-017-0920-y

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of cheminformatics, 4*(1), 17-17. doi:10.1186/1758-2946-4-17

Hirono, A., Miwa, S., Fujii, H., Ishida, F., Yamada, K., & Kubota, K. (1994). Molecular study of eight Japanese cases of glucose-6-phosphate dehydrogenase deficiency by non-radioisotopic single-strand conformation polymorphism (SSCP) analysis. *Blood, 83*, 3363-3368. doi:10.1182/blood.V83.11.3363.bloodjournal83113363

Horikoshi, N., Hwang, S., Gati, C., Matsui, T., Castillo-Orellana, C., Raub, A. G., . . . Wakatsuki, S. (2021). Long-range structural defects by pathogenic mutations in most severe glucose-6-phosphate dehydrogenase deficiency. *Proceedings of the National Academy of Sciences, 118*(4), e2022790118. doi:10.1073/pnas.2022790118

Hospital, A., Goñi, J. R., Orozco, M., & Gelpí, J. L. (2015). Molecular dynamics simulations: advances and applications. *Advances and applications in bioinformatics and chemistry : AABC, 8*, 37-47. doi:10.2147/AABC.S70333

Howes, R. E., Piel, F. B., Patil, A. P., Nyangiri, O. A., Gething, P. W., Dewi, M., . . . Hay, S. I. (2012). G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLoS medicine, 9*(11), e1001339-e1001339. doi:10.1371/journal.pmed.1001339

Huang, K., Luo, S., Cong, Y., Zhong, S., Zhang, J. Z. H., & Duan, L. (2020). An accurate free energy estimator: based on MM/PBSA combined with interaction entropy for protein–ligand binding affinity. *Nanoscale, 12*(19), 10737-10750. doi:10.1039/C9NR10638C

Huang, Y., Choi, M. Y., Au, S. W., Au, D. M., Lam, V. M., & Engel, P. C. (2008). Purification and detailed study of two clinically different human glucose 6-phosphate dehydrogenase variants, G6PD(Plymouth) and G6PD(Mahidol): Evidence for defective protein folding as the basis of disease. *Mol Genet Metab, 93*(1), 44-53. doi:10.1016/j.ymgme.2007.08.122

Hwang, S., Mruk, K., Rahighi, S., Raub, A. G., Chen, C.-H., Dorn, L. E., . . . Mochly-Rosen, D. (2018). Correcting glucose-6-phosphate dehydrogenase deficiency with a small-molecule activator. *Nature communications, 9*(1), 4045-4045. doi:10.1038/s41467-018-06447-z

Iwai, K., Hirono, A., Matsuoka, H., Kawamoto, F., Horie, T., Lin, K., . . . Ishii, A. (2001). Distribution of glucose-6-phosphate dehydrogenase mutations in Southeast Asia. *Hum Genet, 108*(6), 445-449. doi:10.1007/s004390100527

Jaghoori, M. M., Bleijlevens, B., & Olabarriaga, S. D. (2016). 1001 Ways to run AutoDock Vina for virtual screening. *Journal of Computer-Aided Molecular Design, 30*(3), 237-249. doi:10.1007/s10822-016-9900-9

Kotaka, M., Gover, S., Vandeputte-Rutten, L., Au, S. W., Lam, V. M., & Adams, M. J. (2005). Structural studies of glucose-6-phosphate and NADP+ binding to human glucose-6-phosphate dehydrogenase. *Acta Crystallogr D Biol Crystallogr, 61*(Pt 5), 495-504. doi:10.1107/s0907444905002350

Kumar, C. V., Swetha, R. G., Anbarasu, A., & Ramaiah, S. (2014). Computational Analysis Reveals the Association of Threonine 118 Methionine Mutation in PMP22 Resulting in CMT-1A. *Advances in bioinformatics, 2014*, 502618-502618. doi:10.1155/2014/502618

Kumari, R., Kumar, R., & Lynn, A. (2014). g_mmpbsa—A GROMACS Tool for High-Throughput MM-PBSA Calculations. *Journal of Chemical Information and Modeling, 54*(7), 1951-1962. doi:10.1021/ci500020m

Lang, P. T., Brozell, S. R., Mukherjee, S., Pettersen, E. F., Meng, E. C., Thomas, V., . . . Kuntz, I. D. (2009). DOCK 6: combining techniques to model RNA-small molecule complexes. *Rna, 15*(6), 1219-1230. doi:10.1261/rna.1563609

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J Chem Inf Model, 51*(10), 2778-2786. doi:10.1021/ci200227u

Lee, J., Kim, T. I., Kang, J.-M., Jun, H., Lê, H. G., Thái, T. L., . . . Na, B.-K. (2018). Prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency among malaria patients in Upper Myanmar. *BMC infectious diseases, 18*(1), 131-131. doi:10.1186/s12879-018-3031-y

Lee, J., Park, J., Choi, H., Kim, J., Kwon, A., Jang, W., . . . Cho, B. (2017). Genetic Profiles of Korean Patients With Glucose-6-Phosphate Dehydrogenase Deficiency. *Annals of laboratory medicine, 37*(2), 108-116. doi:10.3343/alm.2017.37.2.108

Li, Q., Yang, F., Liu, R., Luo, L., Yang, Y., Zhang, L., . . . He, Y. (2015). Prevalence and Molecular Characterization of Glucose-6-Phosphate Dehydrogenase Deficiency at the China-Myanmar Border. *PloS one, 10*(7), e0134593-e0134593. doi:10.1371/journal.pone.0134593

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews, 4*(8), 118-126. doi:10.4103/0973-7847.70902

Malde, A. K., Zuo, L., Breeze, M., Stroet, M., Poger, D., Nair, P. C., . . . Mark, A. E. (2011). An Automated Force Field Topology Builder (ATB) and Repository: Version 1.0. *J Chem Theory Comput, 7*(12), 4026-4037. doi:10.1021/ct200196m

Martínez-Rosas, V., Juárez-Cruz, M. V., Ramírez-Nava, E. J., Hernández-Ochoa, B., Morales-Luna, L., González-Valdez, A., . . . Gómez-Manzo, S. (2020). Effects of Single and Double Mutants in Human Glucose-6-Phosphate Dehydrogenase Variants Present in the Mexican Population: Biochemical and Structural Analysis. *International journal of molecular sciences, 21*(8), 2732. doi:10.3390/ijms21082732

Minucci, A., Moradkhani, K., Hwang, M. J., Zuppi, C., Giardina, B., & Capoluongo, E. (2012). Glucose-6-phosphate dehydrogenase (G6PD) mutations database: review of the "old" and update of the new mutations. *Blood Cells Mol Dis, 48*(3), 154-165. doi:10.1016/j.bcmd.2012.01.001

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem , 30*(16), 2785-2791. doi:10.1002/jcc.21256

Nguyen, H., Nguyen, T., & Le, L. (2016). Computational Study of Glucose-6-phosphate-dehydrogenase deficiencies using Molecular Dynamics Simulation. *Journal of Life Sciences, 4*, 32-39.

Nguyen, N. T., Nguyen, T. H., Pham, T. N. H., Huy, N. T., Bay, M. V., Pham, M. Q., . . . Ngo, S. T. (2020). Autodock Vina Adopts More Accurate Binding Poses but Autodock4 Forms Better Binding Affinity. *J Chem Inf Model, 60*(1), 204-211. doi:10.1021/acs.jcim.9b00778

Nkhoma, E. T., Poole, C., Vannappagari, V., Hall, S. A., & Beutler, E. (2009). The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells Mol Dis, 42*(3), 267-278. doi:10.1016/j.bcmd.2008.12.005

Nuchprayoon, I., Sanpavat, S., & Nuchprayoon, S. (2002). Glucose-6-phosphate dehydrogenase (G6PD) mutations in Thailand: G6PD Viangchan (871G>A) is the most common deficiency variant in the Thai population. *Hum Mutat, 19*(2), 185. doi:10.1002/humu.9010

Pereira, G. R. C., Vieira, B. d. A. A., & De Mesquita, J. F. (2021). Comprehensive in silico analysis and molecular dynamics of the superoxide dismutase 1 (SOD1) variants related to amyotrophic lateral sclerosis. *PloS one, 16*(2), e0247841-e0247841. doi:10.1371/journal.pone.0247841

PyMOL. Retrieved from https://www.pymol.org/

Ramírez, D., & Caballero, J. (2018). Is It Reliable to Take the Molecular Docking Top Scoring Position as the Best Solution without Considering Available Structural Data? *Molecules, 23*(5). doi:10.3390/molecules23051038

Richardson, S. R., & O'Malley, G. F. (2022). Glucose 6 phosphate dehydrogenase deficiency. In *StatPearls*. Treasure Island (FL): StatPearls Publishing.

Robinson, P. K. (2015). Enzymes: principles and biotechnological applications. *Essays in biochemistry, 59*, 1-41. doi:10.1042/bse0590001

Ross, C., Nizami, B., Glenister, M., Sheik Amamuddy, O., Atilgan, A. R., Atilgan, C., & Tastan Bishop, Ö. (2018). MODE-TASK: large-scale protein motion tools. *Bioinformatics, 34*(21), 3759-3763. doi:10.1093/bioinformatics/bty427

Saddala, M. S., Lennikov, A., & Huang, H. (2020). Discovery of Small-Molecule Activators for Glucose-6-Phosphate Dehydrogenase (G6PD) Using Machine Learning Approaches. *Int J Mol Sci, 21*(4). doi:10.3390/ijms21041523

Sarker, S. K., Islam, M. T., Eckhoff, G., Hossain, M. A., Qadri, S. K., Muraduzzaman, A. K. M., . . . Mannoor, K. (2016). Molecular Analysis of Glucose-6-Phosphate Dehydrogenase Gene Mutations in Bangladeshi Individuals. *PloS one, 11*(11), e0166977-e0166977. doi:10.1371/journal.pone.0166977

Schmid, N., Eichenberger, A. P., Choutko, A., Riniker, S., Winger, M., Mark, A. E., & van Gunsteren, W. F. (2011). Definition and testing of the GROMOS force-field versions 54A7 and 54B7. *Eur Biophys J, 40*(7), 843-856. doi:10.1007/s00249-011-0700-9

Stevens, D., Wanachiwanawin, W., Mason, P., Vulliamy, T., & Luzzatto, L. (1991). G6PD Canton a common deficient variant in South East Asia caused by a 459 Arg→Leu mutation. *Nucleic Acids Res, 18*, 7190. doi:10.1093/nar/18.23.7190

Tang, H.-Y., Ho, H.-Y., Wu, P.-R., Chen, S.-H., Kuypers, F. A., Cheng, M.-L., & Chiu, D. T.-Y. (2015). Inability to maintain GSH pool in G6PD-deficient red cells causes futile AMPK activation and irreversible metabolic disturbance. *Antioxidants & redox signaling, 22*(9), 744-759. doi:10.1089/ars.2014.6142

Tantular, I. S., Matsuoka, H., Kasahara, Y., Pusarawati, S., Kanbe, T., Tuda, J. S., . . . Kawamoto, F. (2010). Incidence and mutation analysis of glucose-6-phosphate dehydrogenase deficiency in eastern Indonesian populations. *Acta Med Okayama, 64*(6), 367-373. doi:10.18926/amo/41322

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry, 31*(2), 455-461. doi:10.1002/jcc.21334

Valencia, S. H., Ocampo, I. D., Arce-Plata, M. I., Recht, J., & Arévalo-Herrera, M. (2016). Glucose-6-phosphate dehydrogenase deficiency prevalence and genetic variants in malaria endemic areas of Colombia. *Malaria Journal, 15*(1), 291. doi:10.1186/s12936-016-1343-1

Zhang, Y., & Skolnick, J. (2005). TM-align: a protein structure alignment algorithm based on the TM-score. *Nucleic acids research, 33*(7), 2302-2309. doi:10.1093/nar/gki524

LIST OF PUBLICATIONS

1. Amran, S. I., Louis, N. E., and Jamalis. J. (2020) Chapter 10, In vitro and in vivo techniques with consideration of biological system. Publisher: Penerbit UTM Press. ISBN: 9789835217401 (Book Chapter – Published)

2. Louis, N. E., Diana Engku Baharuddin, P. N. S., Latif, N.A., Hamza, M.A., and Amran, S. I. Preliminary study of structural changes of glucose-6-phosphate dehydrogenase deficiency variants. Proceeding of 8th International Graduate Conference on Engineering, Science and Humanities (IGCESH2020). (pp. 211- 214), SPS UTM, eISSN 2735-055X. (Conference Proceeding – Published).

3. Louis, N.E., Hamza, M.A., Latif, N.A., Alonazi, M. A., Warsy, A., and Amran, S. I. Preliminary study of structural changes of glucose-6-phosphate dehydrogenase deficiency variants. 4th International Conference on Molecular Biology and Biotechnology (ICMBB 2021). Asia Pacific Journal of Molecular Biology and Biotechnology, Volume 29 (2) Supplementary June 2021 (p74), ISSN 0128-7451; eISSN 2672-7277 (Abstract – Published)

4. Louis, N. E., Hamza, M.A., Diana Engku Baharuddin, P. N. S., Chandran, S., Latif, N.A., Alonazi, M. A., Halim, K.B.A., Warsy, A., and Amran, S. I. (2022) Preliminary study of structural changes of glucose-6-phosphate dehydrogenase deficiency variants. BioMedicine: Vol. 12: Iss. 2, Article 1. (Journal Article – Accepted)