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Screening for Potential G6PD Activator Using Molecular Docking and Enzyme Kinetics Analysis

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a widespread enzymatic disorder that increases susceptibility to oxidative stress-induced hemolysis. This study aimed to identify potential small-molecule activators for Class A G6PD mutants using a molecular docking approach. Seventeen chemically synthesized compounds (UTM1–UTM17) were evaluated *in silico* for binding affinity at the dimer interface of four clinically relevant G6PD variants: Nashville, Canton, Durham, and Alhambra. Compound UTM1–UTM5 showed promising affinity, drug-like properties and favourable ADME Toxicity profiles. UTM1 was selected for *in vitro* validation using recombinant G6PD variants expressed in E. coli and purified via immobilized metal affinity chromatography (IMAC). Kinetic assays revealed that UTM1 improved the catalytic efficiency of at least one mutant enzyme, highlighting its therapeutic potential. These findings support the feasibility of structure-based drug design in treating G6PD deficiency.

Keywords: G6PD deficiency; G6PD activator; molecular docking; enzyme kinetics; drug screening

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) plays a vital role in protecting red blood cells from oxidative damage by producing NADPH through the pentose phosphate pathway. G6PD deficiency, a common X-linked enzymatic disorder affecting over 400 million people worldwide (Nkhoma et al., 2009), leads to hemolytic anemia upon exposure to oxidative stressors (Garcia et al., 2021). Previous research has extensively characterized G6PD variants, including more than 230 mutations, in various geographical regions worldwide (Bancone et al., 2019). Research has demonstrated that the catalytic efficiency and protein stability of G6PD variants influence their clinical manifestations (Boonyuen et al., 2017). Notably, many mutations cluster around the structural NADP+ binding site and the dimer interface, which are critical for maintaining enzymatic activity (Wei et al., 2022). Despite its global prevalence and clinical implications, no pharmacological treatment exists to restore G6PD activity.

Research into potential small-molecule activators, such as AG1, is underway, aiming to enhance G6PD activity and provide a therapeutic avenue for affected individuals (Hwang et al., 2018; Raub et al., 2019; Zailani et al., 2023). Small-molecule activators, such as AG1, have shown promise in stabilizing mutant G6PD enzymes (Saddala et al., 2020). However, comprehensive evaluations of novel activators remain limited. This study aims to bridge that gap by employing molecular docking to screen AG1-derivative compounds, followed by in vitro enzymatic assays to validate their efficacy on recombinant Class A G6PD variants.

Materials and methods

Eighteen compounds (UTM1–UTM17) were sketched and optimized using Avogadro 1.2.0. Molecular docking was performed with AutoDock4 targeting the dimer interface of class A G6PD mutants: Nashville (R393H), Canton (R459L), Durham (K238R), and Alhambra (V394L). G6PD mutant enzyme structures were prepared using the mutagenesis wizard in PyMOL. Removal of water molecules, addition of polar hydrogens and Kollman charges were performed using the AutoDock Tools 1.5.7 program. Docking parameters of grid box dimensions were set around the dimer interface of AG1 binding site of G6PD at grid points X, Y, Z equivalent to 32.316, 56.453, 0.513 respectively with box dimensions of 40x40x40. The grid output file was then created using Autogrid4. Docking was performed using Autodock4 with the number of runs set at 50, population size at 150 and using the Lamarckian genetic algorithm. The results were output in .dlg file format, and the data (estimated inhibition constant, Ki and free binding energy values) were extracted. The best docking pose was selected based on the histogram, and the docking complex structure was saved as a .pdb file. Subsequently, for the absorption, distribution, metabolism, and excretion (ADME) properties of the compounds, canonical SMILES were input into the SwissADME website to obtain the prediction of ADMETox and drug-likeness properties.

The mutations were introduced via site-directed mutagenesis into *Escherichia coli* BL21 (DE3) cells using the pET-28a vector. Mutations were confirmed through Sanger sequencing, and the cells were cultured in LB Broth and induced to express the G6PD protein using IPTG. The expressed proteins were purified using cobalt-based IMAC and confirmed by SDS-PAGE.

The kinetic parameters (K_m, K_{cat}) of purified proteins were determined via UV-Vis spectrophotometry by measuring NADPH production at 340 nm in the presence of G6P and $NADP^+$. AG1 and UTM1 were tested for enzyme activation effects.

Results and discussion

Molecular docking simulations targeting the dimer interface of four clinically relevant G6PD mutants—Canton, Nashville, Durham, and Alhambra—demonstrated that several ligands from the UTM1 to UTM9 series demonstrated improved binding affinity compared to the reference compound AG1 (Table 1). The molecular docking data presented in Table 1 reveal distinct binding affinity profiles of 18 library compounds (AG1 and UTM1–UTM17) against four G6PD variants: Nashville, Canton, Durham, and Alhambra. Binding affinity, represented by binding free energy (ΔG) and estimated inhibition constant (K_i), varies among both ligands and variants. Overall, UTM1 to UTM11 demonstrated improved binding affinity compared to the reference compound AG1 in G6PD Durham and Alhambra. UTM11 exhibits the strongest binding affinity, particularly to the Nashville variant (ΔG = –9.00 kcal/mol, Ki = 0.25 μM), indicating a highly stable interaction, while AG1 shows higher affinity towards the Canton variant compared to the candidate compounds. UTM2 consistently binds well to all variants, suggesting it may be a broad-spectrum candidate. Several compounds, including UTM12, UTM13, UTM14, and UTM17, exhibit relatively poor binding across all variants (Ki > 25 μM), suggesting limited potential for further development. In contrast, UTM1 – UTM5 emerge as promising leads due to their favourable binding profiles and consistency across multiple G6PD variants. These findings support the potential of selected ligands for further investigation, particularly against G6PD variants that retain higher binding sensitivity. Superimposition analysis (Figure 1) also confirmed that the binding conformation of UTM1 – UTM 5 overlapped with that of AG1, validating its binding site.

Table 1: Binding affinity of library compounds on G6PD variants

Ligand	BINDING AFFINITY							
	Nashville		**Canton**		**Durham**		**Alhambra**	
	Binding Free Energy, ΔG (kcal/mol)	Estimated Ki (uM)	Binding Free Energy, ΔG (kcal/mol)	Estimated Ki (uM)	Binding Free Energy, ΔG (kcal/mol)	Estimated Ki (uM)	Binding Free Energy, ΔG (kcal/mol)	Estimated Ki (uM)
AG1	-7.63	2.54	-8.31	0.82	-6.29	24.40	-6.76	11.11
UTM1	-7.59	2.75	-7.58	2.78	-7.55	2.91	-7.62	2.62
UTM2	-7.89	1.64	-7.91	1.60	-7.90	1.62	-7.91	1.60
UTM3	-7.70	2.27	-7.64	2.51	-7.67	2.38	-7.72	2.18
UTM4	-7.46	3.41	-7.42	3.65	-7.44	3.51	-7.45	3.46
UTM5	-7.67	2.40	-7.56	2.90	-7.66	2.43	-7.72	2.21
UTM6	-7.18	5.46	-7.28	4.64	-7.19	5.33	-7.28	4.64
UTM7	-7.16	5.62	-7.00	7.38	-7.05	6.85	-7.22	5.08
UTM8	-7.51	3.12	-7.62	2.61	-7.56	2.87	-7.59	2.73
UTM9	-7.27	4.69	-7.45	3.47	-7.31	4.40	-6.30	24.26
UTM10	-7.03	7.07	-6.34	22.40	-7.58	2.80	-7.18	5.44
UTM11	-9.00	0.25	-7.78	1.97	-6.98	7.68	-6.28	25.06
UTM12	-6.55	15.92	-5.91	46.85	-7.59	2.71	-6.10	33.98
UTM13	-7.52	3.05	-6.48	17.76	-6.19	28.98	-6.18	29.36
UTM14	-6.16	30.50	-6.55	15.71	-5.97	41.87	-5.21	152.17
UTM15	-7.22	5.11	-6.82	10.04	-5.63	74.88	-7.31	4.39
UTM16	-6.16	30.56	-6.14	31.73	-5.64	72.97	-5.75	60.68
UTM17	-5.95	43.20	-6.15	30.86	-6.70	12.19	-7.06	6.64

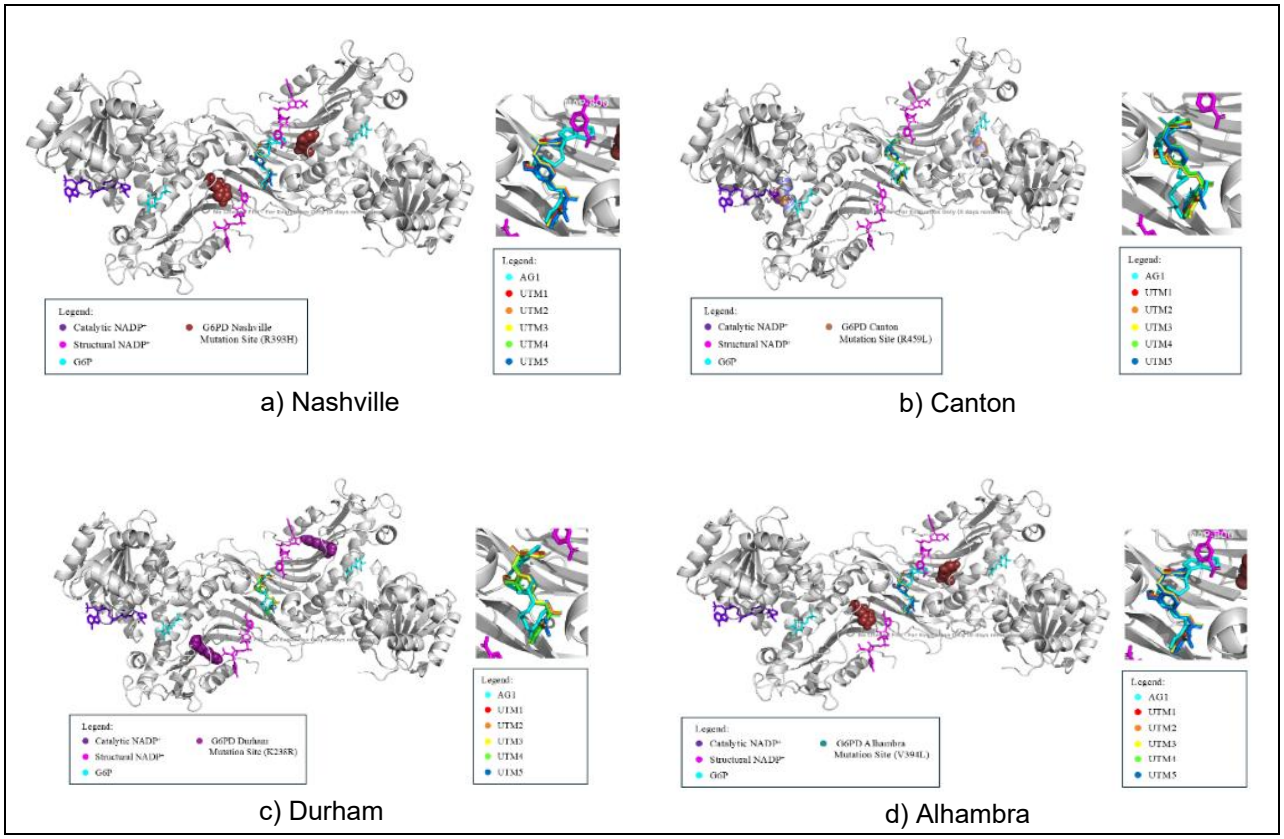

Figure 1 Superimposition of UTM1 – 5 at the dimer interface of G6PD variants.

The drug-likeness and ADME-related properties of compounds UTM1 to UTM17, as presented in Table 2, reveal that the majority of the ligands possess favourable physicochemical characteristics in line with Lipinski's Rule of Five. Most compounds have molecular weights (MW) below 500 g/mol, with the exception of UTM11, UTM13, UTM15, and UTM17, which exceed this threshold, potentially affecting their permeability and oral bioavailability. The hydrogen bond acceptor (HBA) to donor (HBD) ratios remain acceptable (ranging from 5:0 to 8:0), and total polar surface area (TPSA) values are generally within a favourable range for passive absorption. Notably, UTM5 through UTM8, with TPSA values of 123.87 Å², may face reduced permeability, as values above 120 Å² are often associated with limited oral absorption. Most compounds exhibit cLogP values between 3.95 and 5.87, indicating good lipophilicity, although UTM17 shows a notably high cLogP of 6.53, which may result in poor solubility or increased toxicity risk.

Lipinski's rule violations are minimal across the series; only UTM12, UTM15, and UTM17 show violations, with UTM17 violating two rules—likely due to its high molecular weight and lipophilicity. This compound also exhibits the lowest bioavailability score (0.17), suggesting limited potential for oral administration. In contrast, the remaining compounds share a uniform bioavailability score of 0.55, indicating moderate oral bioavailability potential. Overall, compounds UTM1 to UTM7, and UTM10 exhibit the most balanced drug-like profiles, complying fully with Lipinski's rules, maintaining reasonable molecular weights, lipophilicity, and polar surface areas, and are likely to have better pharmacokinetic profiles. These features support their prioritization for further development in drug discovery pipelines targeting G6PD variants.

Table 2: Drug-likeness properties of UTM1-UTM17

Compound	MW (g/mol)	HBA:HBD	TPSA(A)	cLogP	Lipinski violations	Bioavailability
UTM1	408.47	5:0	78.05	4.55	0	0.55
UTM2	422.49	5:0	78.05	4.88	0	0.55
UTM3	422.49	5:0	78.05	4.89	0	0.55
UTM4	422.49	5:0	78.05	4.91	0	0.55
UTM5	453.46	7:0	123.87	3.95	0	0.55
UTM6	453.46	7:0	123.87	4.00	0	0.55
UTM7	453.46	7:0	123.87	4.01	0	0.55
UTM8	426.46	6:0	78.05	4.87	1	0.55
UTM9	426.46	6:0	78.05	4.86	1	0.55
UTM10	432.49	5:0	93.98	4.92	0	0.55
UTM11	511.38	5:0	93.98	5.57	1	0.55
UTM12	446.51	5:0	93.98	5.20	0	0.55
UTM13	525.41	5:0	93.98	5.87	1	0.55
UTM14	460.54	5:0	93.98	5.55	0	0.55
UTM15	539.44	5:0	93.98	6.23	1	0.55
UTM16	474.57	5:0	93.98	5.87	0	0.55
UTM17	553.46	5:0	93.98	6.53	2	0.17

Following computational screening, UTM1 was selected for *in vitro* validation. Mutant G6PD enzymes were successfully expressed in *E. coli* BL21(DE3) and purified using immobilized metal affinity chromatography (IMAC). SDS-PAGE analysis (Figure 2) confirmed the presence of the His-tagged recombinant proteins at the expected molecular weight (~59 kDa). Protein yield and purity were sufficient (provide the estimated value of protein concentration) for subsequent enzymatic assays.

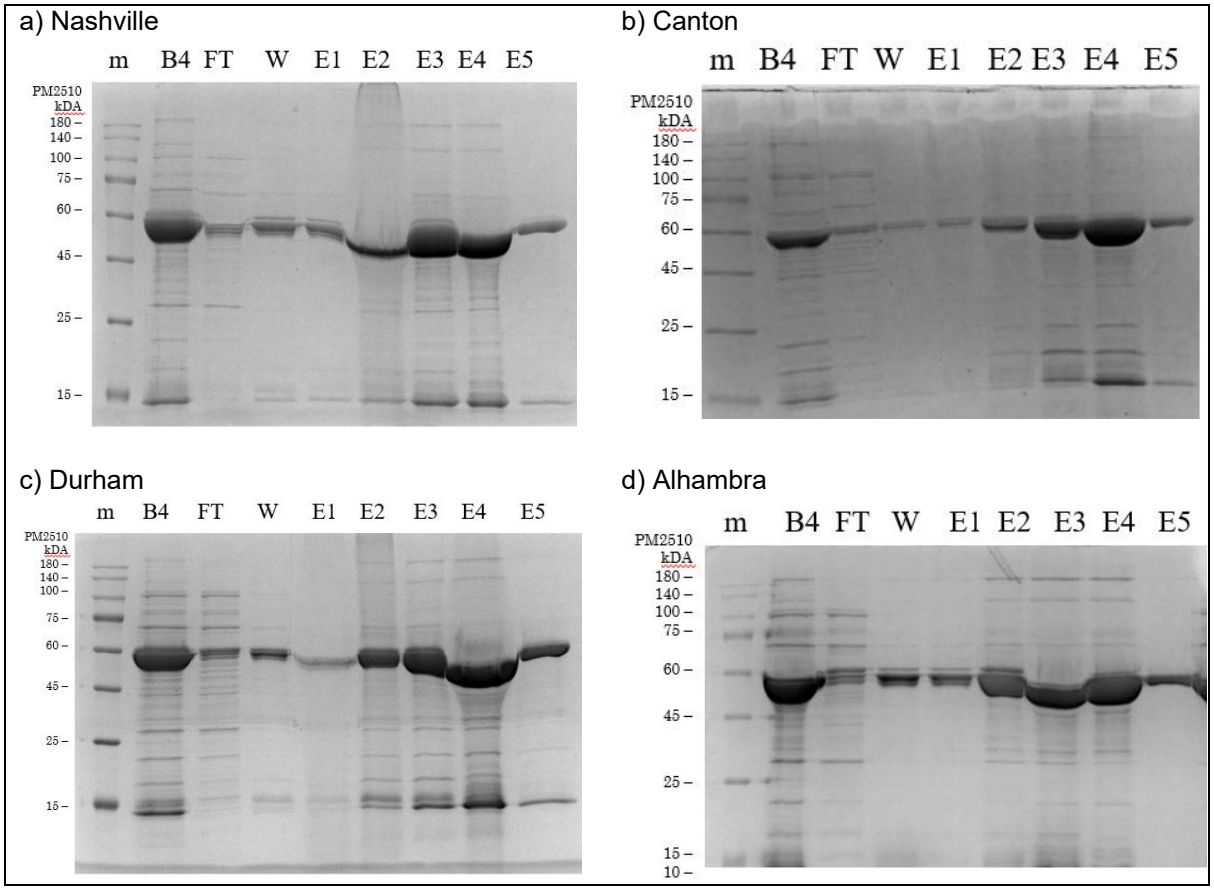

Figure 2 SDS-PAGE from expression of a) G6PD Canton, b) G6PD Nashville, c) G6PD Durham and d) G6PD Alhambra.

Enzyme kinetics of wild-type (WT) and mutant G6PD variants revealed reduced catalytic efficiency in all mutants compared to WT, as expected. Kinetic analysis (using Michaelis–Menten models) showed that G6PD Nashville had significantly increased K_m values for both G6P and $NADP^+$, indicating lower affinity. Figure 3 shows the effect of UTM1 and AG1 on enzyme activity compared to DMSO control (at concentration = 0μM). Notably, while AG1 showed modest activation in G6PD WT, Canton and Durham variants, UTM1 had a more pronounced effect in the Alhambra variant.

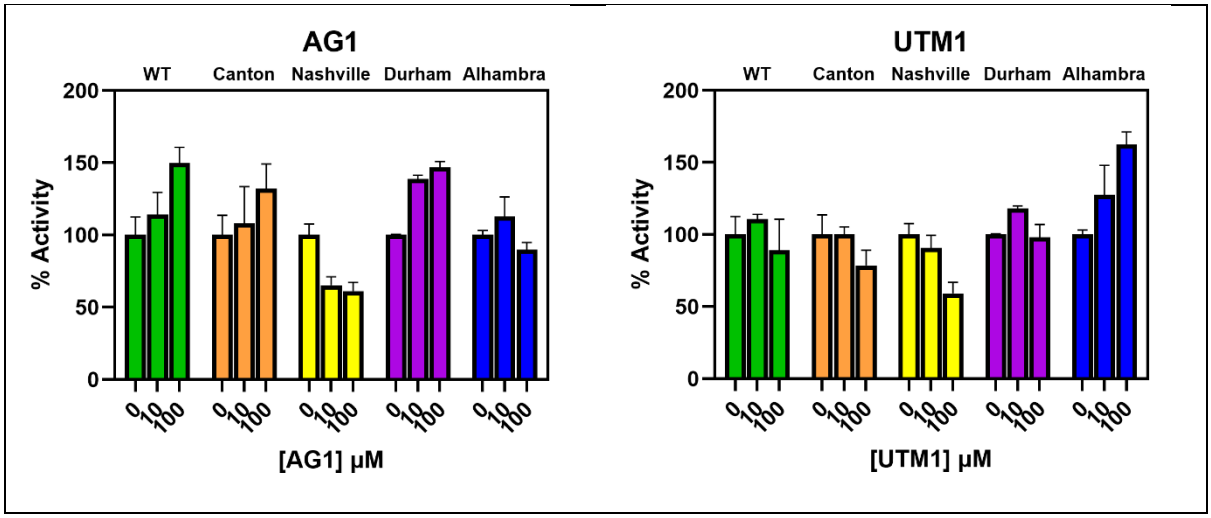

Figure 3 Effects of small molecule activators AG1 and UTM1 on G6PD WT and mutants

Conclusion

This study aimed to identify small molecules capable of binding with high affinity to the G6PD dimer interface and restoring enzymatic activity in recombinant Class A G6PD mutants. Molecular docking successfully identified five candidate compounds (UTM1–UTM5), exhibiting high affinity to selected G6PD variants, favourable drug-likeness and pharmacokinetic profiles. Four G6PD variants were expressed and characterized *in vitro*. Among the tested compounds, UTM1 demonstrated modest activation of the Alhambra variant, while AG1 showed limited efficacy, particularly in the WT, Canton, and Durham variants. Neither compound improved the activity of the Nashville variant, likely due to its more severe structural disruption. These findings support the feasibility of structure-guided drug discovery for G6PD deficiency and highlight the importance of continued compound screening, hit optimization, and structure-activity relationship studies to develop more potent and selective G6PD activators.

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References

Bancone, G., Chu, C. S., Somsakchaicharoen, R., Chowwiwat, N., Parker, D. M., & White, N. J. (2019). Characterization of G6PD mutations in populations of Southeast Asia and Oceania. *Wellcome Open Research*, 4, 15.

Boonyuen, U., Chamchoy, K., Swangsri, T., Junkree, T., Day, N., White, N., … & 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. https://doi.org/10.1016/j.ijbiomac.2017.06.002

Garcia, A., Koperniku, A., Ferreira, J., & Mochly-Rosen, D. (2021). Treatment strategies for glucose-6-phosphate dehydrogenase deficiency: past and future perspectives. *Trends in Pharmacological Sciences*, 42(10), 829-844. https://doi.org/10.1016/j.tips.2021.07.002

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

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, molecules & diseases*, 42(3), 267–278. https://doi.org/10.1016/j.bcmd.2008.12.005

Raub, A. G., Hwang, S., Horikoshi, N., Cunningham, A. D., Rahighi, S., Wakatsuki, S., & Mochly-Rosen, D. (2019). Small-Molecule Activators of Glucose-6-phosphate Dehydrogenase (G6PD) Bridging the Dimer Interface. *ChemMedChem*, *14*(14), 1321–1324. https://doi.org/10.1002/cmdc.201900341

Saddala, M., Lennikov, A., & Hu, H. (2020). Discovery of small-molecule activators for glucose-6-phosphate dehydrogenase (g6pd) using machine learning approaches. *International Journal of Molecular Sciences*, 21(4), 1523. https://doi.org/10.3390/ijms21041523

Wei, X., Kixmoeller, K., Baltrusaitis, E., Yang, X., & Marmorstein, R. (2022). Allosteric role of a structural NADP+ molecule in glucose-6-phosphate dehydrogenase activity. *Proceedings of the National Academy of Sciences*, 119(29), e2119695119.

Zailani, M. A. H., Raja Sabudin, R. Z. A., Ithnin, A., Alauddin, H., Sulaiman, S. A., Ismail, E., & Othman, A. (2023). Population screening for glucose-6-phosphate dehydrogenase deficiency using quantitative point-of-care tests: a systematic review. *Frontiers in Genetics*, 14, 1098828.