Generating an Efficient Arginase Variant for Medical and Industrial Uses: in Silico Engineering

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Abstract

Human arginase is a multifaceted enzyme that can be utilized for various medical and industrial applications. However, no report explored the in-silico engineering of this novel enzyme. The crystal structure of human arginase 1 was downloaded from PDB and had its quality checked prior to further analysis. Afterwards, CUPSAT and DeepDDG webservers were employed to nominate the most stable variants which were prepared by UCSF Chimera v1.16 and refined by GalaxyRefine tool. Docking (to reference substrate and inhibitor), stability confirmation and dynamics simulations were conducted. G119L was the best in all the mentioned aspects which was afterwards in silico cloned as a final step. Accordingly, G119L is a valuable arginase mutant that deserves its experimental validation.

Keywords: Arginase, arginine, mutagenesis, enzyme engineering, in silico cloning

Introduction

L-Arginine is a basic semi-essential amino acid (AA) required for the synthesis of other AA, proteins and polyamines. Arginine is considered semi-essential AA given it can be synthesized *de novo* but cannot satisfy the required amount particularly during childhood. It plays important roles in detoxifying ammonia, nitric oxide synthesis, immunomodulation and hormonal secretion [1]. In the last decade, arginine has been used as a significant supplement for the muscle growth through nitric oxide-mediated vasodilation of endothelium and thus increases nutrient support to striated muscles and elimination of muscle metabolites after training [2]. Arginine is one of the metabolites in urea cycle purposed for ammonia detoxification. It is broken down via arginase enzyme to urea (the less toxic form of ammonia ready for excretion) and ornithine necessary for cellular growth stimulation. Arginase (EC 3.5.3.1) is a heterotrimeric metalloenzyme with Mn^{+2} is a cofactor in each subunit [3]. Arginase is a ubiquitous enzyme present in prokaryotes, yeasts, plants, invertebrates and vertebrates and contributes to critical division and development of those organisms [4]. In human, arginase has to isozymes, arginase 1 is 322 while arginase 2 is 354 AA residues encoded by different genes in distinct chromosomes. They share almost 50% sequence homology and this percentage is increased to 100% in the catalytic region [5].

Arginase has been demonstrated its potentiality for the application in distinct aspects. This include cardiovascular (CVS) and cancer therapy, as a replacement for enzyme-deficient patients and for the industrial production of ornithine [6]. In the general population, it was estimated that CVS deaths accounted for >50% in men and about 44% in women, compared with only nearly 10% and 8%, respectively, in the cancer cohorts [7]. Additionally, the survivors of cancers have medium- to long-term risk for developing CVS diseases compared to normal population [8]. Accumulating evidences suggested the implication of arginase in atherosclerosis [9], hypertension [10], and ischemic reperfusion (IR) [11]. Arginase was also reported to be upregulated in these conditions and certain types of cancer [12]. Conversely, arginase-2 protected myocardial IR injury in rats via blocking the inflammatory response through suppression of nuclear factor kappa B/tumor necrosis factor (NF-κB/TNF-α) pathway [13]. Arginase is administered to treat arginine auxotrophic tumors by depleting the highly demanded arginine and, hence, brakes the cancer tissue division and proliferation [14].

Concerning arginase deficiency, it is a rare genetic condition that results in hyperargininemia and the subsequent developmental retardation, intellectual disability, and mobility defects [15]. The current management is protein restriction, essential AA supplementation as well as ammonia scavengers. Despite these precautions, some children die before reaching adolescence due to severe metabolic decompensation [16]. Although liver transplant alleviates metabolic and biochemical problems, neurologic and mobility defects remain uncontrollable [17]. Indeed, no licensed therapeutic for this enzyme deficiency was approved to date albeit a clinical trial experimenting pegzilarginase as a replacement therapy is being undertaken [18]. This force medical care practitioners to focus only on controlling arginine levels for managing this rare condition [19].

Ornithine is a non-essential, non-protein AA that is found in meats, fish, dairy and eggs. Once ingested, its main role is participation in urea cycle. In addition, ornithine is known for its potency as an agonist for the AA receptor G-protein-coupled receptor family C group 6 subtype A (GPRC6A) and calcium-sensing receptors providing an extraordinary flavor action

[20,21]. L-Ornithine (but not D-isomer) was employed to "enhance" preference to sweet, salty, umami, and fat taste but not as a flavor [22]. This would substitute the commonly used one, i.e. monosodium glutamate (MSG) since many reports confirmed its adverse effects especially in excessive amounts [23,24]. Such a natural flavor is synthesized from arginine via arginase enzyme through metabolically engineered bacterial fermentation [25]. These facts prioritize the engineering of arginase enzyme preliminary through bioinformatics tools to be used for the mentioned applications and this is the goal of this study.

Methods

The planned strategy for the execution of this current work was summarized in Fig 1.

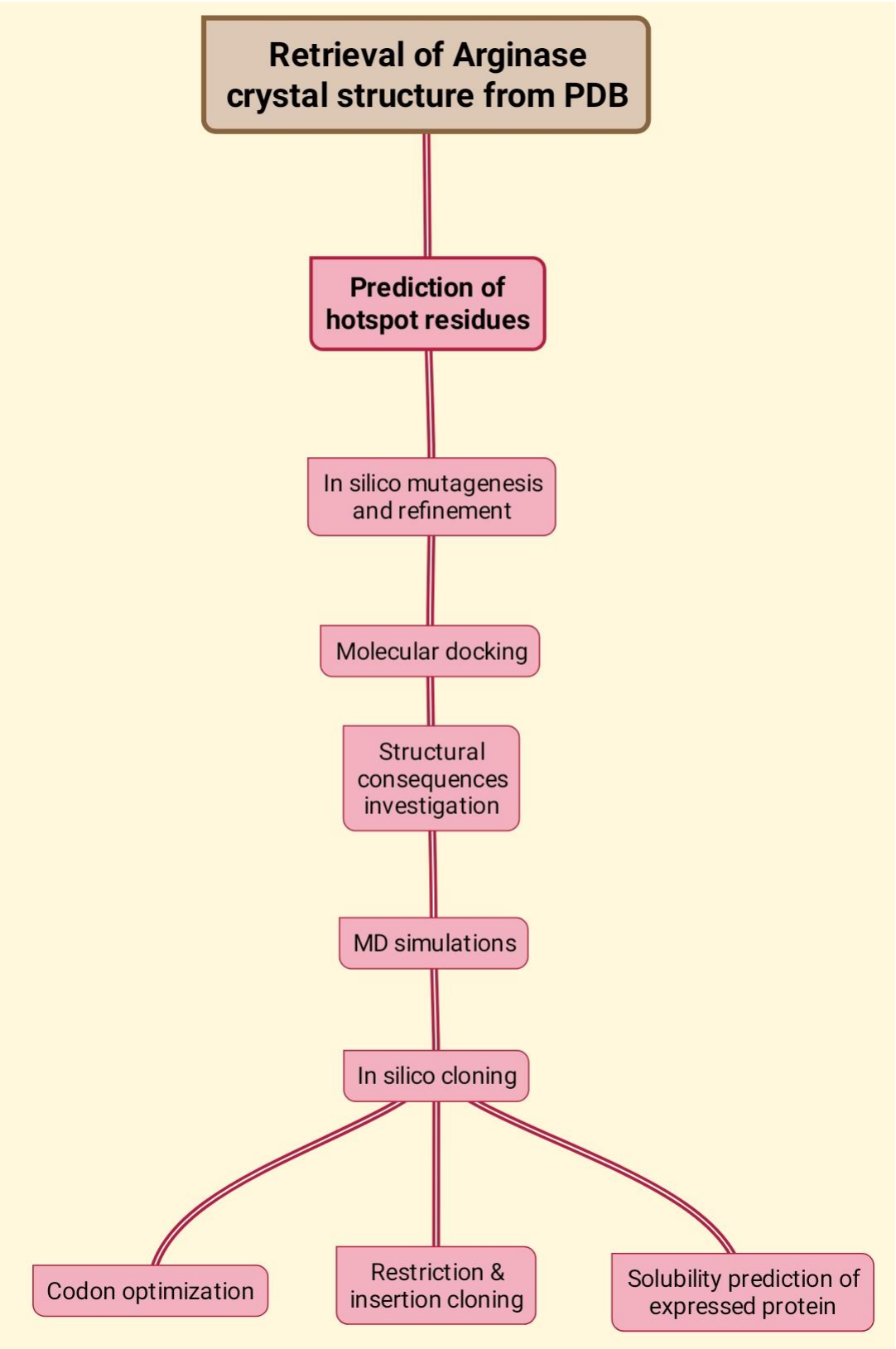


Figure 1 The planned outline of workflow to accomplish the current study

Retrieval and check of tertiary structure of arginase

Human arginase 1 was downloaded from protein data bank (PDB code: 2AEB). The crystal structure has a resolution of 1.29 Å [26]. All heteroatoms and water molecules were removed.

Only the chain A was considered for further exploration. The prepared protein tertiary structure has its quality assessed by SWISSMODEL [27] and ProsA tools [28].

Prediction of hotspot AA residues

CUPSAT [29] and DeepDDG webservers [30] were utilized for ranking the top stabilizing and enhancing mutations that would improves enzyme binding to its native substrate. In the two webservers, catalytic residues were excluded from in silico mutagenesis

In silico mutagenesis

UCSF Chimera v1.16 was employed for this purpose. The predicted hotspot residues and the corresponding mutants were prepared and the highest probable rotamers of the mutant conformers were chosen. This is followed by the energy minimization and refinement of the produced mutant via GalaxyRefine tool [31]. Models with lowest MolProbity, clashes and highest Ramachandran favored % were selected for the next step.

Molecular docking

The refined models of the generated mutants have been subjected to molecular docking through CB-Dock-2 tool [32]. They were docked to both reference substrate and inhibitor keeping all options as default. The pocket 1 was selected for all docking processes. The best docked complex was investigated for the type of interactions formed during docking using Discovery studio client 2021 program.

Structural effects of mutation

To evaluate the impact of the generated mutation on 3D architecture of the enzyme, PremPS server [33] was used. Besides revealing detailed interaction network of the mutants, PremPS server also predict the impact of such mutation on the overall stability of the enzyme structure.

MD simulations

MD simulations were conducted employing WebGro webserver [34]. GROMOS96 43a1 was the selected forcefield whereas the water model was SPC, box type was triclinic, salt type was NaCl, with neutralization and at 0.15 M concentration. The energy was minimized through steepest descent with 5000 steps. The equilibration type was NVT/NPT at 300 K, 1 bar. MD simulation was conducted for 50 ns to figure out the relative mean square deviation (RMSD), relative mean square fluctuations (RMSF), radius of gyration (Rg), and solvent-accessible surface area (SASA).

In silico cloning

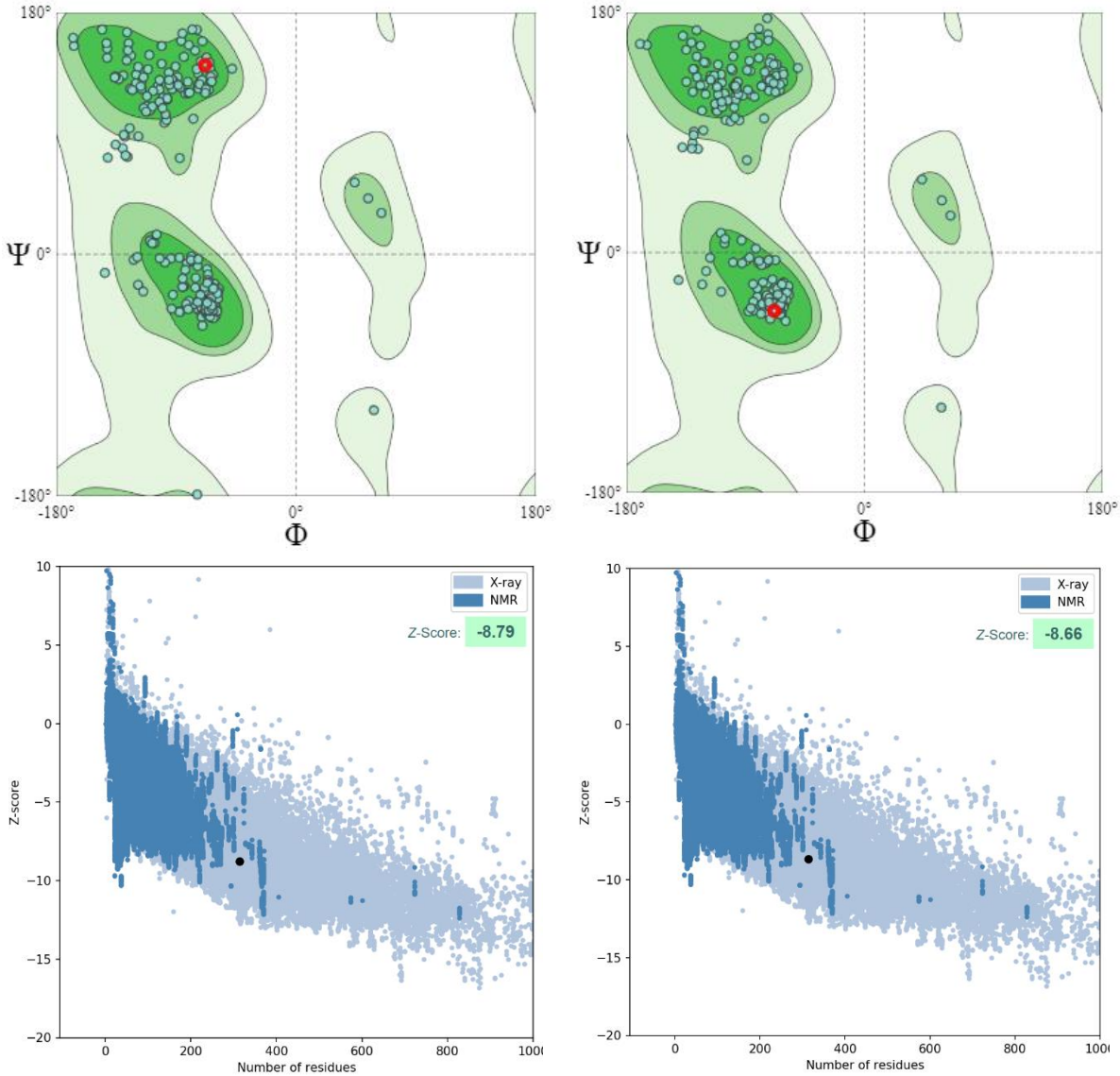
The mutant that gave best binding affinity toward arginine was chosen for the in silico cloning process. The protein sequence was backtranslated by backtranseq tool and then the sequence output was uploaded to Jcat tool [35]. This tool performs codon optimization based upon the expressing system in order to maximally increase the likelihood of gene expression in that system. Afterwards, the optimized sequence was inserted to pET-28a(+) vector through SnapGene 6.2 software . The gene was inserted between EcoRI and BamHI

restriction enzymes site. Finally, the solubility of the predicted gene to be translated was assessed via Protein-sol tool [36].

Results

Quality assessment

MolProbity and clash scores and of the WT and G119L tertiary structures were 1.21 and 1.30, 1.30 and 5.59 whereas Ramachandran favored percentage was the same (98.40%). Otherwise, Ramachandran plot, ProsA z scores (-8.79 versus -8.66) and energy profiles were not significantly different as elucidated in Fig 2.



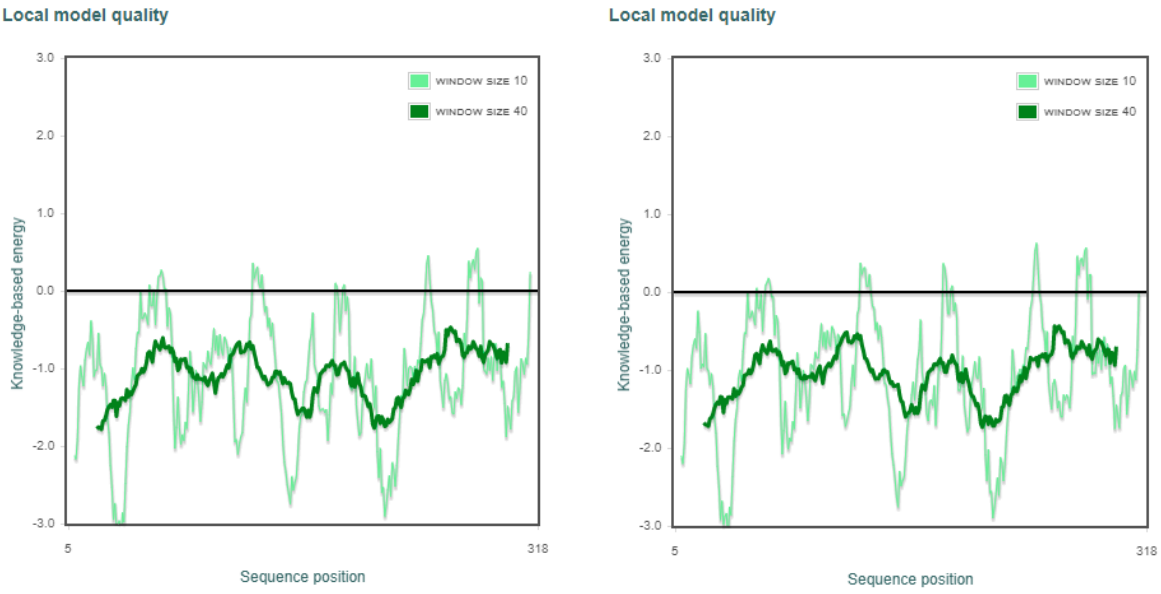


Figure 2 Quality assessment of wild-type arginase and G119L using Ramachandran plot and ProsA and energy plots

Molecular docking

Sixteen mutants were tested for their potentiality to enhance enzyme activity in terms of binding the native substrate relative to the WT version. Among all the top-ranked potential variants in CUPSAT and DeepDDG, G119L and, to a lesser extent, D274I have higher affinity toward the substrate arginine (-5.9 vs -5.6 kcal/mol). Furthermore, G119L is slightly had weaker binding affinity toward the reference inhibitor (Table 1). Therefore, G119L mutant was considered for further analysis.

Table 1 Docking scores to reference substrate and inhibitor

Enzyme	Substrate affinity	Inhibitor affinity
WT	-4.6	-5.6
G119L	-5.9	-5.4
G272L	-5.2	-5.3
K88L	-4.3	-5.6
S94P	-5.1	-5.6
D274I	-5.6	-5.9
D158T	-4.3	-5.5
G154V	-4.2	-5.2
I156R	-4.1	-5
K153E	-5.1	-5.2
P160W	-5.4	-5.2
S163N	-3.8	-4.7
V165H	-3.6	-5
G142L	-2.6	-2.9
P28L	-3.6	-4.3
P70L	-4.4	-4.1

S230C	-4.5	-3.7

WT: wild-type

Mutant impact on enzyme structure

As shown in Fig 3, it is obvious that the G119L mutant is no surprise to be more powerful than the WT as reflected by the numerous interactions (hydrophobic and polar H-bonds) have with the surrounding residues near the catalytic pocket. WT formed only two H-bonds with His 228 and Pro 226 while G119L variant formed two H-bonds with Leu 227 and His 228 in addition to the plenty hydrophobic interactions with Ile 121, Leu 219, Leu 220, Arg 225 and Leu 227. This is traced to the bulky side chain of Leu in comparison with Gly that has only hydrogen in its side chain. Concerning the deleteriousness of the mutation on enzyme stability, PremPS score was -0.12 indicating the stabilizing impact of G119L on the overall enzyme architecture.

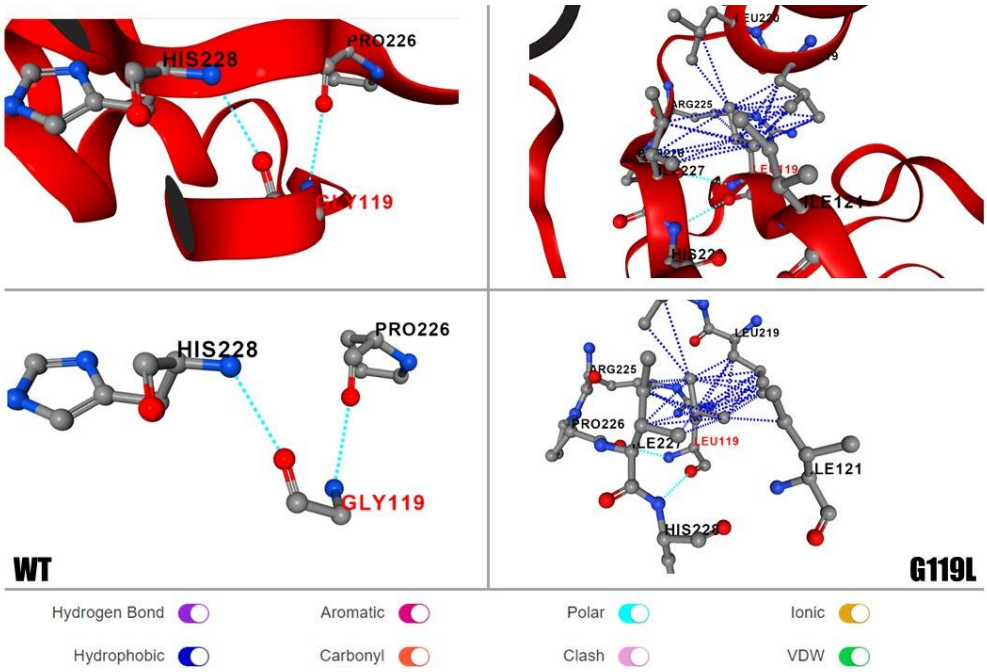
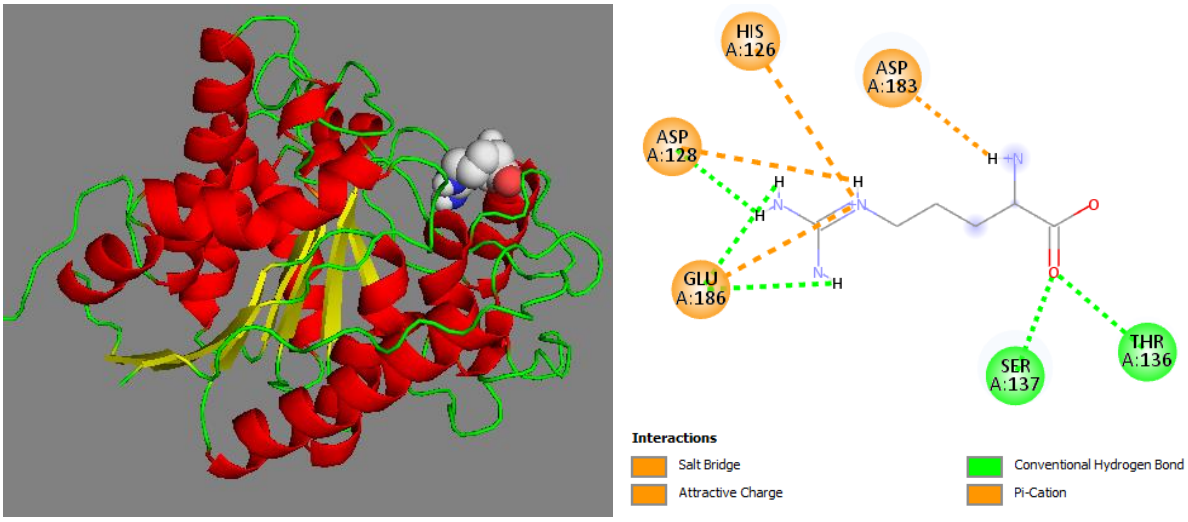


Figure 3 Structural consequence of the G119L mutant (Right) compared to WT enzyme (Left)

Protein-ligand interaction

The docked complex (G119L-arginine) is elucidated in Fig 4, which illustrates the fit of the substrate in the active center of the engineered enzyme. However, this binding is accounted for the five hydrogen bonds formed between Asp 128, Thr 136, Ser 137, and two bonds from Glu 186 and the native substrate. Additionally, salt bridges and Pi-Cation interactions were also significantly contributed to the strong bonding. These interpret the strong binding affinity of G119L variant to arginine (-5.9 kcal/mol).



(A) **(B)**

Figure 4 Elucidation of G119L-arginine interactions in 3D (A) and 2D (B) view

MD simulations

Data of MD simulations are shown in Fig 5. RMSD of the mutant form (G119L variant) exhibited more harmonic overall protein motion compared to WT. In addition, the RMSD values of G119L were less than the WT indicating the more stability of the mutant form. On the other hand, the WT of arginase manifested less fluctuations in during 50 ns simulation time in comparison with G119L mutant. The mutated Leu at 119 position had very low RMSF value (0.0894 Å) which means the relaxed state of the mutant Leu at this position. The more RMSF peaks shown can be traced to the conformational changes occurred to rearrange the interconnected AA in the tertiary structure so as to reach a more relaxed state after mutagenesis. The two structures exhibited roughly similar compactness as reflected by the convergent Rg. Likewise, the SASA of the WT had approximate values to G119L variant, i.e. started from 144, 140 nm^2 and ended at 131, 130 nm^2. These findings mirror the stability, flexibility and nearly comparable conformations of the G119L mutant and the WT.

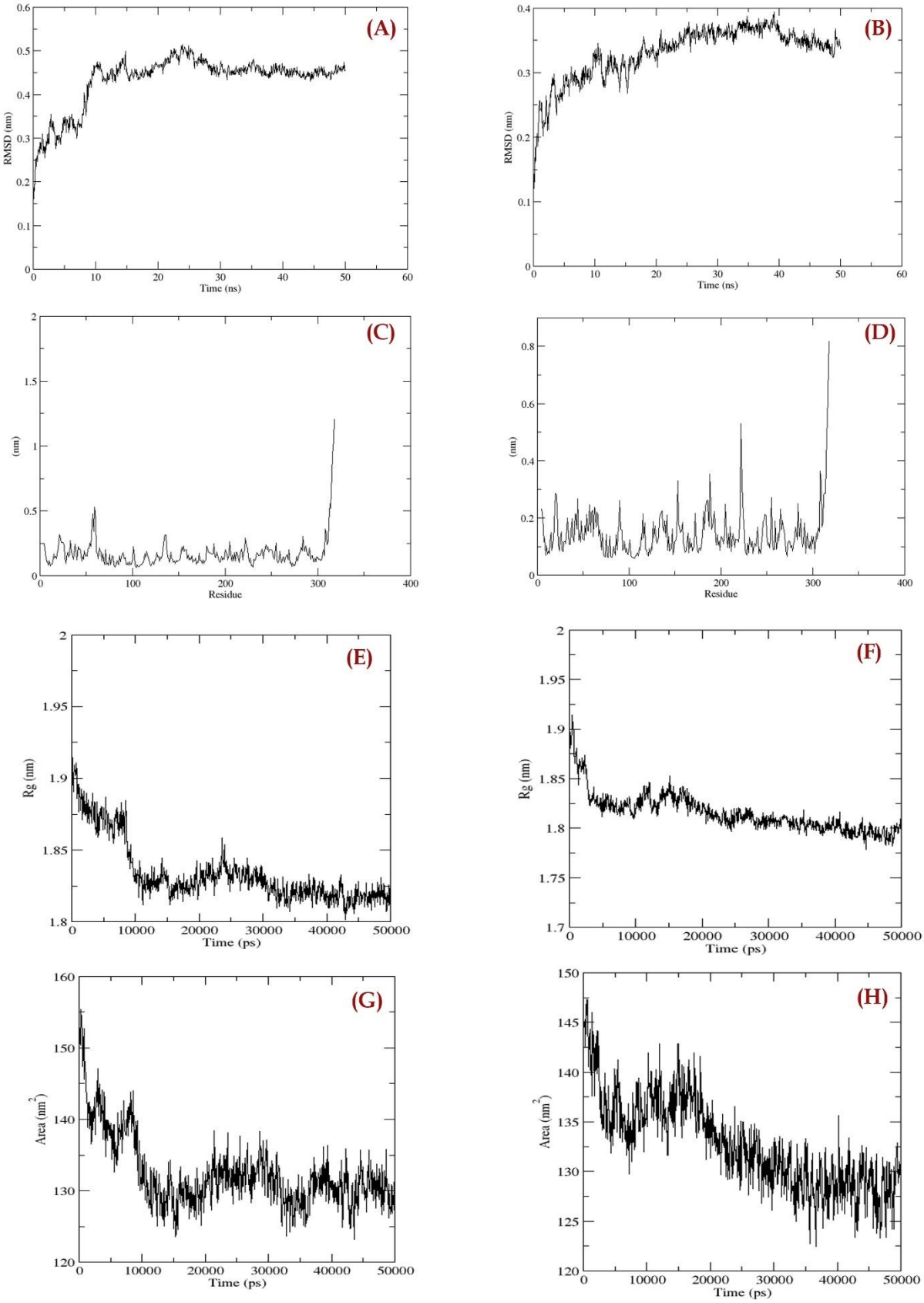
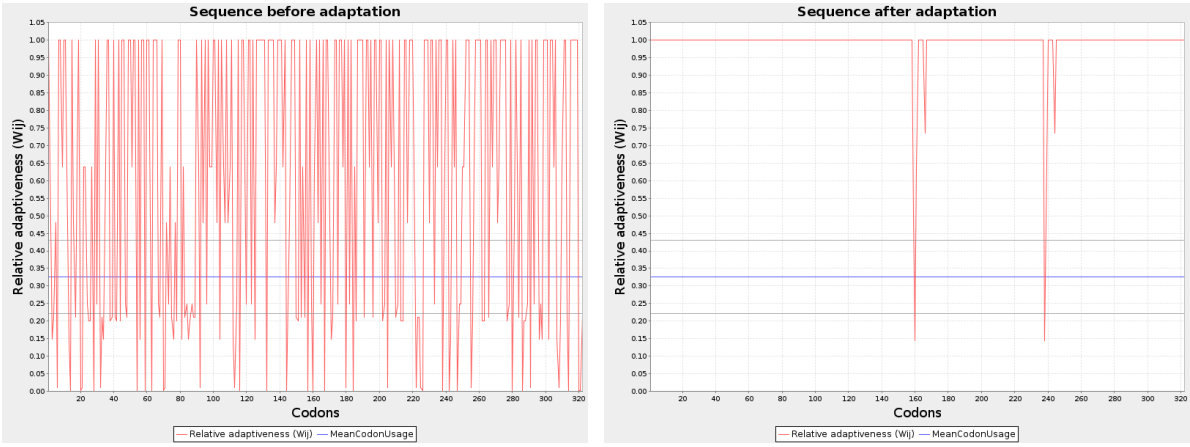


Figure 5 MD simulation of the WT and G119L mutant for 50 ns. RMSD, RMSF, Rg and SASA for WT (A, C, E and G) and G119L mutant (B, D, F and H) were shown.

In silico cloning

The CAI value of the backtranslated sequence from protein primary structure was 0.333 and the GC% was 66%. After the codon adaptation and optimization conducted by Jcat tool, CAI and GC% values were substantially improved, 0.980 and 51% respectively. The corresponding adaptiveness of the sequences are present in Fig 6.



(A) **(B)**

Figure 6 Relative adoptiveness of the arginase backtranslated sequence befor (A) and after (B) Jcat treatment

The codon-adopted sequence was 996 nucleotides in length and the whole vector is 6353 bp. 6X-His tag (six His residues) for affinity separation was embedded at the 3'-end of the inserted gene fragment. This sequence was inserted after introducing EcoRI and BamHI restrictions sites at the beginning and end of gene sequence. Then, restriction and insertion cloning was performed at the between the corresponding restriction sites as depicted in Fig 7.

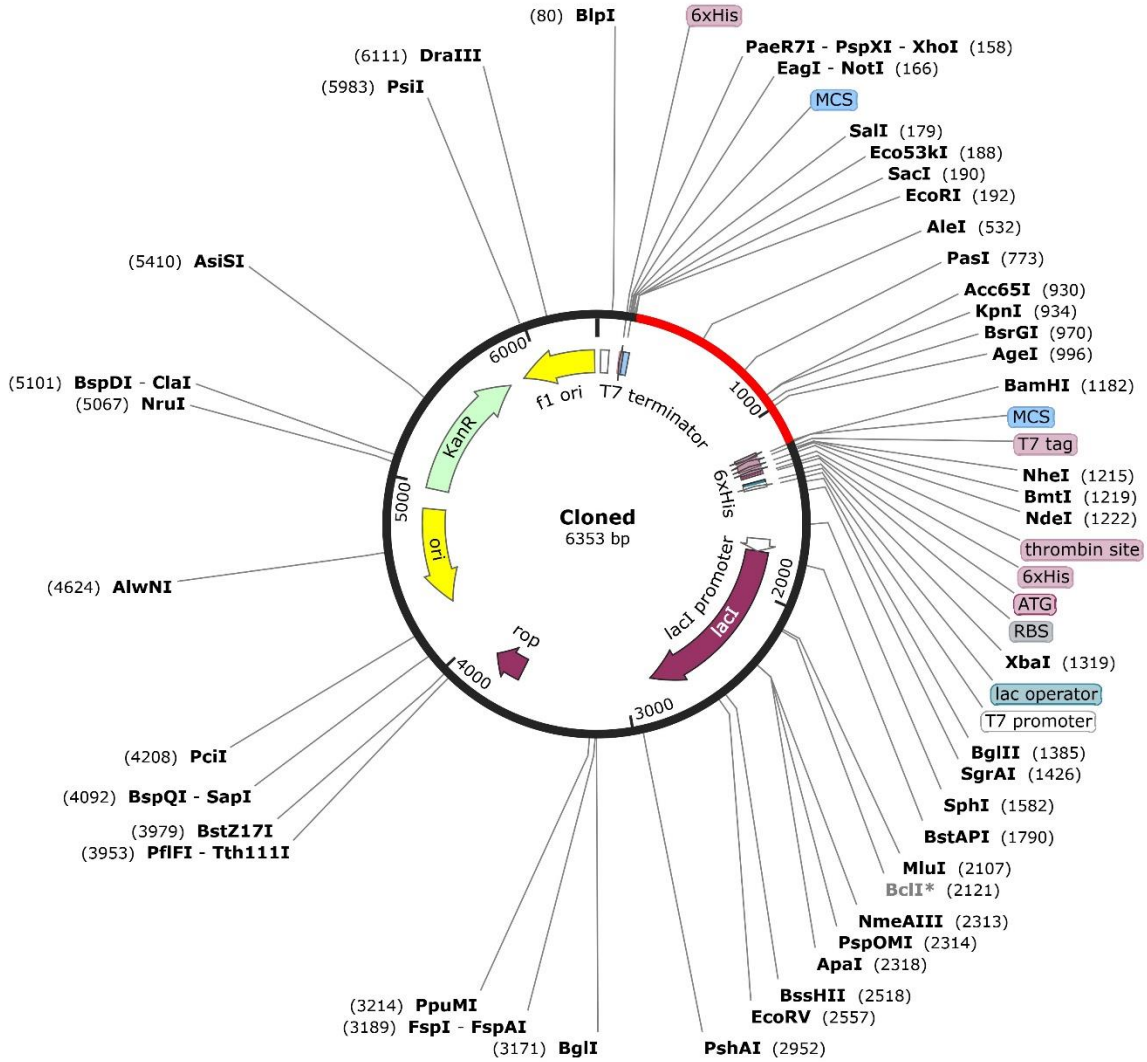


Figure 7 G119L-inserted pET-28(+)a plasmid. The inserted sequence was highlighted in red.

Protein-sol

The G119L mutant had an isoelectric point of 6.580 approaching neutral pH. Additionally, its solubility was estimated to be 0.521 which is greater than the solubility of average protein counterparts expressed in *E. coli* (Fig 8). This in turn would facilitate the separation process from the other proteins expressed in *E. coli* in addition to the His-tag added during cloning.

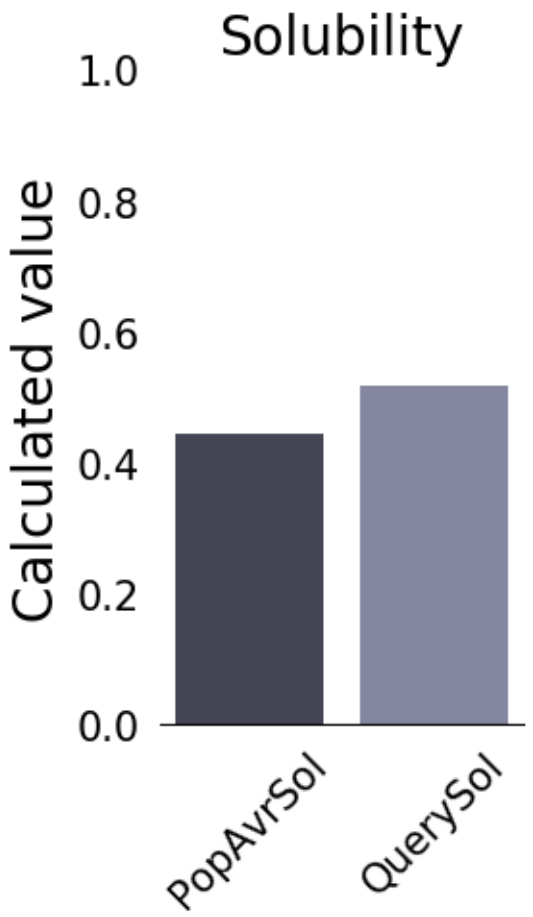


Figure8 Predicted solubility of the G119L mutant (QuerySol) in comparison with average proteins (PopAvrSol)

Discussion

Arginase is a hydrolase enzyme capable of degrading arginine into urea and ornithine. This reaction is of great importance rendered it a multifaceted enzyme that can be utilized for a variety of medical (to treat auxotrophic cancers [37] and arginase deficiency [38]) and industrial purposes (for manufacturing L-ornithine as food additive) . For arginase to satisfy such demands, it must have high activity and more stability to be recycled many times during the application process. Here where the significance of the current study comes into play. This in silico work aimed to engineer the arginase enzyme such that it has stronger binding affinity to the substrate, less inhibition, and more stability via various bioinformatics approaches.

The retrieved crystal structure of arginase from PDB proved its high quality as reflected by the low MolProbity score (1.21), high Ramachndran favored region occupancy and Z score of -8.79 in ProsA tools. This paved the way to commence in silico mutagenesis. Among the examined AA residues that are located outside the binding pocket (so not to interfere with catalytic function of the enzyme), G119L was the best mutant with highest binding affinity to the native substrate arginine (-5.9 kcal/mol) in comparison with the WT (-4.6 kcal/mol), i.e. 1.28-fold increase in theoretical activity. Furthermore, G119L had weaker inhibition by the

reference inhibitor compared to WT (-5.4 versus -5.6 kcal/mole). The strong binding is accounted for by the multiple H-bonds, electrostatics and hydrophobic interactions formed to arginine after mutation. The introduction of Leu in place of Gly at 119 position resulted in the formation of new H-bonds and hydrophobics near the active site as the Leu has a bulkier side chain than Gly. This interprets the -0.12 kcal/mole increase in stability after mutagenesis as given by PremPS server output. Similarly, the RMSD, RMSF, Rg, and SASA findings of the MD simulation for 50 ns reveled the stability, flexibility and comparable molecular motion of G119L mutant to the WT enzyme. This confirms the superior features of the mutant form which instigated me to provide an in-silico cloning as a preliminary step paving the way for its applicability in wet-lab setting. The protein sequence was backtranslated into nucleotide sequence and then optimized. The optimized sequence had CAI value and GC% of 0.980 and 51% compared to 0.333 and 65% before codon adaptation. Then, the optimized sequence was inserted to pET-28(+)a vector to be expressed in E. coli K2 strain. pET-28a is one of the most popular expression vectors in the biotech market (mentioned in >40,000 published articles). It has a copy number of 40, indicating the high yield as well as ease of downstream purification of the recombinant protein [39]. A His-tag (6X-His) was inserted to arginase sequence in order to ease its separation using Ni^{+2}-affinity columns after expression [40]. the solubility of the expressed protein was predicted to be 0.521, reflecting the potential high solubility since most E. coli expressed proteins had a value below 0.521 [36].

The reports regarding engineering of arginase is too scarce which raises the significance of this work. Cheng et al. [41] rationally screened variants of arginase in vitro for their potentiality to increase the attachment of poly-ethylene glycol (PEG) on its surface in order to improve its enzyme stability in vivo. Likewise, a novel single isoform of PEGylated human arginase was developed. This manifested potent cytotoxicity at sub-nM levels against cancer cell lines of breast, prostate or pancreatic origin given that arginine levels were diminished in a sustained fashion [42].

Conclusion

The current work investigated the utilization of various bioinformatics tools to generate engineered human arginase variants with stronger binding affinity to the substrate, less inhibition, and more stability. G119L was the best candidate that satisfied the mentioned criteria and exhibited comparable MD simulation pattern to WT. In silico cloning of this variant was also provided to be expressed in E. coli K2 and the resultant protein was designed to have His-tag for the facilitation of its downstream separation since its solubility had higher than average the expression system proteins. The extraordinary results of this theoretical work are recommended to be tested in the wet-lab setting.

References

[1] Morris SM. Arginine: beyond protein. Am J Clin Nutr 2006;83:508S-512S. https://doi.org/10.1093/ajcn/83.2.508S.

[2] Stefani GP, Marmett B, Alves JP, Möller GB, Heck TG, Frizzo MN, et al. Resistance training and L-arginine supplementation are determinant in genomic stability, cardiac contractility and muscle mass development in rats. PLoS One 2018;13:e0204858. https://doi.org/10.1371/journal.pone.0204858.

[3] Caldwell RB, Toque HA, Narayanan SP, Caldwell RW. Arginase: an old enzyme with new tricks. Trends Pharmacol Sci 2015;36:395–405. https://doi.org/10.1016/j.tips.2015.03.006.

[4] Dzik JM. Evolutionary roots of arginase expression and regulation. Front Immunol 2014;5:544. https://doi.org/10.3389/fimmu.2014.00544.

[5] Li Z, Wang L, Ren Y, Huang Y, Liu W, Lv Z, et al. Arginase: shedding light on the mechanisms and opportunities in cardiovascular diseases. Cell Death Discov 2022;8:1–14. https://doi.org/10.1038/s41420-022-01200-4.

[6] Caldwell RW, Rodriguez PC, Toque HA, Narayanan SP, Caldwell RB. Arginase: A Multifaceted Enzyme Important in Health and Disease. Physiol Rev 2018;98:641–65. https://doi.org/10.1152/physrev.00037.2016.

[7] Alam N, Wright AK, Ashcroft DM, Renehan AG. Cancer and cardiovascular disease. The Lancet 2020;395:1903–4. https://doi.org/10.1016/S0140-6736(20)30222-1.

[8] Strongman H, Gadd S, Matthews A, Mansfield KE, Stanway S, Lyon AR, et al. Medium and long-term risks of specific cardiovascular diseases in survivors of 20 adult cancers: a population-based cohort study using multiple linked UK electronic health records databases. Lancet 2019;394:1041–54. https://doi.org/10.1016/S0140-6736(19)31674-5.

[9] Al-Rufaie AHA, Yaseen AI, Al-Samarrai RRH. Evaluate the activity of arginase and some biochemical parameters in patients with atherosclerosis. AIP Conference Proceedings 2022;2450:020037. https://doi.org/10.1063/5.0094361.

[10] Arishe O, McKenzie J, Priviero F, Ebeigbe AB, Webb RC. L-arginase induces vascular dysfunction in old spontaneously hypertensive rats. J Afr Assoc Physiol Sci 2019;7:119–27.

[11] Tengbom J, Cederström S, Verouhis D, Böhm F, Eriksson P, Folkersen L, et al. Arginase 1 is upregulated at admission in patients with ST-elevation myocardial infarction. J Intern Med 2021;290:1061–70. https://doi.org/10.1111/joim.13358.

[12] Niu F, Yu Y, Li Z, Ren Y, Li Z, Ye Q, et al. Arginase: An emerging and promising therapeutic target for cancer treatment. Biomedicine & Pharmacotherapy 2022;149:112840. https://doi.org/10.1016/j.biopha.2022.112840.

[13] Huang X-W, Pan M-D, Du P-H, Wang L-X. Arginase-2 protects myocardial ischemia-reperfusion injury via NF-κB/TNF-α pathway. Eur Rev Med Pharmacol Sci 2018;22:6529–37. https://doi.org/10.26355/eurrev_201810_16067.

[14] Li M, Qin J, Xiong K, Jiang B, Zhang T. Review of arginase as a promising biocatalyst: characteristics, preparation, applications and future challenges. Critical Reviews in Biotechnology 2022;42:651–67. https://doi.org/10.1080/07388551.2021.1947962.

[15] Carvalho DR, Brum JM, Speck-Martins CE, Ventura FD, Navarro MMM, Coelho KEFA, et al. Clinical features and neurologic progression of hyperargininemia. Pediatr Neurol 2012;46:369–74. https://doi.org/10.1016/j.pediatrneurol.2012.03.016.

[16] Keshavan N, Wood M, Alderson LM, Cortina-Borja M, Skeath R, McSweeney M, et al. Clinical status, biochemical profile and management of a single cohort of patients with arginase deficiency. JIMD Rep 2022;63:123–30. https://doi.org/10.1002/jmd2.12266.
[17] Gomes Martins E, Santos Silva E, Vilarinho S, Saudubray JM, Vilarinho L. Neonatal cholestasis: an uncommon presentation of hyperargininemia. J Inherit Metab Dis 2010;33 Suppl 3:S503-506. https://doi.org/10.1007/s10545-010-9263-7.
[18] Diaz GA, Schulze A, McNutt MC, Leão-Teles E, Merritt JL, Enns GM, et al. Clinical effect and safety profile of pegzilarginase in patients with arginase 1 deficiency. J Inherit Metab Dis 2021;44:847–56. https://doi.org/10.1002/jimd.12343.
[19] Diaz GA, Bechter M, Cederbaum SD. The role and control of arginine levels in arginase 1 deficiency. Journal of Inherited Metabolic Disease 2023;46:3–14. https://doi.org/10.1002/jimd.12564.
[20] Oya M, Kitaguchi T, Pais R, Reimann F, Gribble F, Tsuboi T. The G protein-coupled receptor family C group 6 subtype A (GPRC6A) receptor is involved in amino acid-induced glucagon-like peptide-1 secretion from GLUTag cells. J Biol Chem 2013;288:4513–21. https://doi.org/10.1074/jbc.M112.402677.
[21] Shin S, Gombedza FC, Bandyopadhyay BC. l-ornithine activates Ca2+ signaling to exert its protective function on human proximal tubular cells. Cell Signal 2020;67:109484. https://doi.org/10.1016/j.cellsig.2019.109484.
[22] Mizuta H, Kumamoto N, Ugawa S, Yamamoto T. Additive Effects of L-Ornithine on Preferences to Basic Taste Solutions in Mice. Nutrients 2021;13:3749. https://doi.org/10.3390/nu13113749.
[23] Niaz K, Zaplatic E, Spoor J. Extensive use of monosodium glutamate: A threat to public health? EXCLI J 2018;17:273–8. https://doi.org/10.17179/excli2018-1092.
[24] Zanfirescu A, Ungurianu A, Tsatsakis AM, Nițulescu GM, Kouretas D, Veskoukis A, et al. A review of the alleged health hazards of monosodium glutamate. Compr Rev Food Sci Food Saf 2019;18:1111–34. https://doi.org/10.1111/1541-4337.12448.
[25] Wu X-Y, Guo X-Y, Zhang B, Jiang Y, Ye B-C. Recent Advances of L-ornithine Biosynthesis in Metabolically Engineered Corynebacterium glutamicum. Frontiers in Bioengineering and Biotechnology 2020;7.
[26] Di Costanzo L, Sabio G, Mora A, Rodriguez PC, Ochoa AC, Centeno F, et al. Crystal structure of human arginase I at 1.29-A resolution and exploration of inhibition in the immune response. Proc Natl Acad Sci U S A 2005;102:13058–63. https://doi.org/10.1073/pnas.0504027102.
[27] Bragina ME, Daina A, Perez MA, Michielin O, Zoete V. The SwissSimilarity 2021 Web Tool: Novel Chemical Libraries and Additional Methods for an Enhanced Ligand-Based Virtual Screening Experience. International Journal of Molecular Sciences 2022;23:811.
[28] Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Research 2007;35:W407–10.
[29] Parthiban V, Gromiha MM, Schomburg D. CUPSAT: prediction of protein stability upon point mutations. Nucleic Acids Research 2006;34:W239–42.
[30] Cao H, Wang J, He L, Qi Y, Zhang JZ. DeepDDG: predicting the stability change of protein point mutations using neural networks. Journal of Chemical Information and Modeling 2019;59:1508–14.
[31] Heo L, Park H, Seok C. GalaxyRefine: Protein structure refinement driven by side-chain repacking. Nucleic Acids Research 2013;41:W384–8.
[32] Liu Y, Yang X, Gan J, Chen S, Xiao Z-X, Cao Y. CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Research 2022.

[33] Chen Y, Lu H, Zhang N, Zhu Z, Wang S, Li M. PremPS: Predicting the impact of missense mutations on protein stability. PLoS Comput Biol 2020;16:e1008543. https://doi.org/10.1371/journal.pcbi.1008543.
[34] WebGro | UAMS n.d. https://simlab.uams.edu/index.php (accessed February 4, 2023).
[35] Grote A, Hiller K, Scheer M, Münch R, Nörtemann B, Hempel DC, et al. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Research 2005;33:W526–31.
[36] Hebditch M, Carballo-Amador MA, Charonis S, Curtis R, Warwicker J. Protein–Sol: a web tool for predicting protein solubility from sequence. Bioinformatics 2017;33:3098–100.
[37] Du Z, Li T, Huang J, Chen Y, Chen C. Arginase: Mechanisms and Clinical Application in Hematologic Malignancy. Frontiers in Oncology 2022;12.
[38] Diez-Fernandez C, Rüfenacht V, Gemperle C, Fingerhut R, Häberle J. Mutations and common variants in the human arginase 1 (ARG1) gene: Impact on patients, diagnostics, and protein structure considerations. Human Mutation 2018;39:1029–50. https://doi.org/10.1002/humu.23545.
[39] Shilling PJ, Mirzadeh K, Cumming AJ, Widesheim M, Köck Z, Daley DO. Improved designs for pET expression plasmids increase protein production yield in Escherichia coli. Commun Biol 2020;3:1–8. https://doi.org/10.1038/s42003-020-0939-8.
[40] Plavša JJ, Řezáčová P, Kugler M, Pachl P, Brynda J, Voburka Z, et al. In situ proteolysis of an N-terminal His tag with thrombin improves the diffraction quality of human aldo-keto reductase 1C3 crystals. Acta Cryst F 2018;74:300–6. https://doi.org/10.1107/S2053230X18005721.
[41] Cheng F, Yang J, Schwaneberg U, Zhu L. Rational surface engineering of an arginine deiminase (an antitumor enzyme) for increased PEGylation efficiency. Biotechnology and Bioengineering 2019;116:2156–66. https://doi.org/10.1002/bit.27011.
[42] Yu K-M, Pang TP, Cutler M, Tian M, Huang L, Lau JY-N, et al. Rational design, engineer, and characterization of a novel pegylated single isomer human arginase for arginine depriving anti-cancer treatment. Life Sciences 2021;264:118674. https://doi.org/10.1016/j.lfs.2020.118674.