Journal of Medical Genetics and Clinical Biology
Homepage : https://journal.antispublisher.id/index.php/JMGCB
Email : admin@antispublisher.com

e-ISSN : 3032-1085
JMGCB, Vol. 3, No. 4, April 2026
Page 56-65

Genetic Mutations and Their Structural and Functional Impact on Enzymes: A Comprehensive Review

Mariam Jabbar Abboud[1], Iqbal Sahi Tuama[2]
[1,2]Wasit Education Directorate, Iraq

Check for updates OPEN ACCESS

DOI : https://doi.org/10.61796/jmgcb.v3i4.1704

Sections Info

Article history:
Submitted: January 05, 2026
Final Revised: January 30, 2026
Accepted: February 20, 2026
Published: March 03, 2026

Keywords:
Mutations
Enzymes
Biocatalysis

ABSTRACT

Objective: *Genetic mutations play a fundamental role in shaping enzyme structure, dynamics, and catalytic function, thereby influencing biological evolution, disease mechanisms, and biotechnological innovation. Understanding the relationship between genotype and enzymatic phenotype remains a major scientific challenge, particularly in predicting the functional consequences of missense and non-synonymous mutations.* ***Method:*** *This review provides a comprehensive examination of the structural and functional impact of genetic mutations on enzymes, emphasizing advances in computational and biophysical methodologies. Classical molecular dynamics simulations offer atomic-level insights into conformational flexibility and allosteric communication, while quantum mechanics/molecular mechanics (QM/MM) approaches elucidate catalytic mechanisms and electronic transitions during enzymatic reactions. Additionally, emerging machine learning strategies enable large-scale prediction of mutational effects and rational enzyme engineering by exploring complex sequence–structure–function relationships.* ***Results:*** *Variations ranging from single nucleotide substitutions to larger structural alterations may induce subtle or profound changes in protein folding, stability, substrate specificity, and reaction kinetics. The integration of physics-based simulations and data-driven models represents a transformative framework for understanding mutation-induced enzymatic alterations, accelerating enzyme design, improving predictive accuracy, and expanding applications in industrial biocatalysis and therapeutic development.* ***Novelty:*** *Such multidisciplinary approaches accelerate enzyme design, improve predictive accuracy, and expand applications in industrial biocatalysis and therapeutic development. Continued methodological innovation is essential to bridge existing gaps in correlating genetic variation with enzymatic performance.*

INTRODUCTION

Genetic mutations, which may be considered as changes in the nucleotide sequence in the genome of any organism, are intrinsic to the process of biological diversity and evolution [1]. Such changes may include single nucleotide polymorphism up to extensive chromosomal rearrangements that may have far-reaching consequences on the phenotype of an organism due to their effects on protein structure and function, especially in enzymes [2]. The complex relation between variations at the sequence level and the catalytic activity is an important issue to understand the evolution of the enzymes and establish new biotechnological applications [3]. Although most mutation is random, the fact that they become fixed by evolutionary timescales is often dictated by selective pressures which prefer variants that show better adaptation to a particular environmental niche [4]. As a result, the replacements of amino acids have the potential of increasing the efficiency and specificity of an enzyme and hence retaining the catalytic activity in a wide

range of conditions [4]. On the other hand, mutations on individual residues may disrupt protein folding stability, catalytic activity or even cause a total loss of functionality which in some cases can cause toxic effects which underlies genetic diseases [5]. Thus, the problem in deciphering the correlations between genotype and phenotype, particularly with disease is a major issue and as such, the currently predictive models are likely to give a good general solution but fail to predict individual mutations or particular phenotype [6]. In order to study these relationships in detail, it is necessary to thoroughly discuss the role of a mutation on the structure of the enzyme, ligand binding, and its catalytic mechanism [3]. This review examines the impact of genetic mutations on the structure and activity of enzymes as well as three major methodologies used in the characterization of these effects and their implications to enzyme engineering and medical intervention [7]. This will demand another study of how even small alterations in protein sequence can be propagated throughout the complicated structure of an enzyme, altering its dynamics as well as, ultimately, its biologic activity [8]. Other researches on connection between single nucleotide changes and functional sites, such as ligand binding sites are also significant in the investigation of how genetic variation may alter protein interactions and overall cell activities [9]. Nucleotide substitutions changing the amino acid sequence, particularly, missense and non-synonymous mutations are of much significance as they may alter protein stability, dynamics and interaction with other molecules leading to observable phenotypic changes [10], [11]. These modified changes can take the path of alteration in the kinetics of certain enzymes, alteration in the specificity of the substrate or even the ability to lose catalytic activity and the physiological consequences of such changes can be radical [12]. Such changes can be localized structural rearrangements of the active site, up to a complete change in the conformational changes to the entire enzyme that can eventually establish its processes of pathogenesis in various loss-of-function genetic diseases [8], [13]. This complexity of interactions is not determinable by simple computational and experimental means to characterize the effects of genetic alterations to enzyme dynamics and structural stability [14]. Despite this progress, large gaps still exist in the knowledge base of the precise mechanism of relation between enzyme structure and activity which is a factor contributing to the need to develop better methods of computation in order to simulate enzymatic processes and dynamical features with a greater degree of accuracy [15], [16].

Literature Review

Nevertheless, to this day, we still do not know the precise mechanism of enzyme structure and activity interaction with gaps, this is the reason why, in the development of better computational procedures, it is important to model properly enzymatic mechanism and dynamics. The design of the molecular dynamics simulations is also important in examining the effect of single amino acid substitution particularly that which occurs at non-active sites in triggering conformational changes which could spread to the active site hence altering the catalytic efficiency and substrate specificity [17], [18]. The lack of detailed quantitative studies of the effects of single nucleotide variants on the 3D protein structure, beyond the prediction field is indicative of a crucial gap to be filled

in future studies [10]. This gap becomes especially pronounced in the description of rare variants where weaknesses of the frequency present statistical challenges to associate them with particular phenotypes [9]. A viable prospect however is through biophysical methods to unravel the functional effects of these rare variants by shedding light on the underlying molecular processes, even in cases failure by population-based studies [19]. As an example, variants found in enzyme active sites, though comparatively uncommon, have been shown to have effects on catalytic activity and substrate binding, frequently of negative phenotypic effect [9]. This complexity of enzyme dynamics and the scale of conformational dynamics of many timescales results in the need to measure and understand the effect of mutations on enzyme activity in a combined experimental and computational experiment [14]. Even though crystallographic data will give us the static information, they are frequently unable to reflect on the dynamic nature of enzymes, and the computational methods can be used to clarify the functions of flexible parts and dynamic behavior of proteins in the catalysis [16]. Simulations at the molecular dynamics level, then, have specific benefits of offering atomic-level information about the motions of the enzyme on a wide range of timescales, and thus, interpolating the static structural models with dynamic landscapes of catalysis [14], [20]. This is an advanced modelling capability that enables the explanation of complex conformational changes that play a central role in enzymatic functionality and substrate recognition [21].

RESEARCH METHOD

Computational methods such as molecular dynamics simulations have become a crucial tool used to investigate the structure and dynamic consequences of mutations that enable them to determine the impact of the mutations on the protein function [22], [23]. The simulations could be used to uncover the effect of mutations on the residue flexibility and allosteric pathways and stability that would eventually lead to alterations in enzyme activity and substrate specificity [8], [24]. As an example, computational methods have demonstrated the capability of characterizing variations of unknown significance, providing information about their possible effect on protein stability and function, which is essential in genetic diagnosis and disease mechanisms [25]. Furthermore, this approach is applicable to the measurement of mechanistic effects on the structure and functioning of proteins through the comparison of mutated proteins with mutated proteins with known benign and pathogenic variants, as well as wild-type proteins [26]. This type of characterization can be performed by all-atom molecular dynamics simulations, which are capable of revealing even in tens of nanoseconds the conformational rearrangements caused by mutations and provide useful information about their molecular origins [27]. Those simulations, and more sophisticated methods, such as AI models enable the researchers to visualize enzyme-substrate interactions, examine how reactions work, and determine the enzyme stability under varying conditions, providing a dynamic view of the enzyme dynamics and conformational changes throughout the catalysis [28]. In particular, the classical molecular dynamics, although based on force fields, is extensively used to study the conformational flexibility of proteins, whereas more sophisticated

quantum mechanics/molecular mechanics (QM/MM) methods are invaluable in dissecting the electronic structure of enzyme-catalyzed reactions and clarification of catalytic reactions, transition states, and reaction pathways [29], [30].

RESULTS AND DISCUSSION

Results

Simulations using molecular dynamics have been specifically useful in explaining the effects of certain mutations on the dynamics of enzymes that play a key role in enzyme functioning [31]. These simulations play a crucial role in predicting how individual atom move over time, due to the interatomic interactions, and capturing an extensive variety of biomolecular processes such as conformational changes, ligand binding and protein folding at femtosecond temporal resolutions [32]. This has been used to investigate enzymes like CTX-M9, where allosteric mutants were more active in catalysis due to changes in their conformational shifts which could not be observed using the static structural analysis [33]. Likewise, umbrella simulations of sampling have been applied in constructing free energy profiles of conformational change, and a larger share of proposed mutations has been shown to change the conformational state of the equilibrium to particular states, thereby modifying the functions of enzymes [34]. These results highlight the role of dynamic structural studies over more static representations of enzyme functions and mutational effects. The changes in CTX-M9 were observed and the all atom RMSD values were 0.3 Å between the mutants and the wild type, showing slight but substantial structural changes affecting catalytic efficiency [35]. The prediction of how mutations will alter the reaction mechanism and rates limiting barriers made possible through computational tools are used to facilitate enhancement of catalytic efficiency in enzymes [36]. Also, computational techniques such as quantum mechanics /molecular mechanics (QM/MM) techniques have played an essential role in assigning the role of individual active site residues and confirming proposed reaction mechanisms, frequently clarifying much controversial information about enzymatic catalysis [30]. The classical molecular dynamics simulations are fundamental to the determination of binding sites and key interactions between enzymes and their substrates, but are not able to give mechanistic insights into enzyme-catalyzed reactions, requiring quantum-chemical simulations to fully understand them [37]. Nevertheless, they also have certain limitations: classical MD simulations cannot be used to study bond formation or breakage, which is one of the most important features of enzymatic reactions, and may consume large computational resources and other domain-specific knowledge to interpret the outcomes significantly, as they use short time steps and millions of interatomic interactions [38].

Discussion

Thus, regardless of the difficulties, a combination computational method that includes classical and quantum mechanical computational methods is necessary to have a complete understanding of the role of genetic mutation in the structure of the enzymes,

dynamics, and catalytic efficiency. The combined methodology enables the explanation of more subtle structural re-arrangements and the complex electronic changes that determine the functioning of the enzyme and provides a whole picture than possible in isolation [30]. These types of methodologies have greatly contributed to the field of computational enzymology, transforming our understanding of enzymatic function and catalytic processes, and providing powerful instruments in the area of enzyme design and optimization [39]. In fact, the growing number of computational capabilities used has provided a surge in successful predictive computational methods used in the field of enzyme engineering, such as structure-based and dynamics-based methods, which identify the most effective sites of mutagenesis to achieve desired properties by considering conformational ensembles [40]. An interesting case would be the Kemp eliminase HG3.17, which is designed to achieve 108-fold acceleration in the rate by optimally adjusting catalytically competent conformational ensembles at the cost of inactive ones, but requires the use of many molecular dynamics simulations to identify them [41]. In addition to these computational methods, machine learning is also starting to revolutionize enzyme design, by accelerating predictions as well as screening large datasets of potential biocatalysts thereby overcoming the scaling limitations regarding structure based approaches[29]. These machine learning and artificial intelligence models are adept in discovering complicated patterns and associations among large biological datasets, and provide a potent solution to difficulties including the non-linear sequence-structure-function relation and the vast combinatorial sequence space [42].

Synthesis and Future Perspectives

The extensive examination has shown the immense role of genetic mutations to the structure and functionality of enzymes and the significance of computerized methods in the resolution of such complex relations. Specifically, the molecular dynamics simulations and quantum mechanics/molecular mechanics models have permitted unprecedented insight into the mechanisms of catalysis and dynamic alterations due to mutations, which can be used to make the enzyme engineering and optimization more rational [28], [43]. Also, machine learning and deep learning strategies, based on data driven strategies are also being capitalized upon to advance the knowledge of sequence-structure-function interactions in enzymes to predict and design novel enzymes and enzyme variants to a vast range of applications [44]. Enzyme engineering is going to have a stronger physicist and machine learning interaction, where it is possible to develop much more effective and selective enzymes with individual properties in the industrial and therapeutic sector [29]. The ability to predict changes in binding affinity and activation free energy by these methods as EVB calculations, as well as more sophisticated machine learning to screen multipoint mutants is an enormous leap towards a more complicated enzyme design [45]. The integration will assist in sharpening our rational enzyme design skills which consist of identifying intricate patterns within big data sets and it will enable us to swiftly identify the optimal mutational pathways [46].

These two potent computational combined with machine learning methods have the promise of highly-speeding up the process of finding and optimizing enzymes, to be used in a wide range of biotechnological and medical uses, beyond the constraints of the traditional, labor intensive experimental methods [42], [47].

CONCLUSION

Fundamental Finding : Genetic mutations fundamentally shape enzyme structure, dynamics, and catalytic function, as nucleotide variations—especially missense and non-synonymous substitutions—induce structural rearrangements that alter protein stability, substrate binding, allosteric regulation, reaction mechanisms, and ultimately enzymatic efficiency or disease-related dysfunction. **Implication :** An integrative framework combining structural biology, biophysical experimentation, molecular dynamics simulations, QM/MM approaches, and machine learning–driven modeling is essential to bridge static structural data with dynamic catalytic behavior and to enable rational, scalable enzyme engineering across broad sequence spaces. **Limitation :** Current multiscale computational models and available experimental datasets remain constrained in accuracy, resolution, and coverage, limiting precise prediction of mutational outcomes and full mechanistic interpretation across diverse enzymatic systems. **Future Research :** Advancement in this field requires improving multiscale modeling precision, expanding high-quality experimental data, and strengthening interdisciplinary collaboration to enhance predictive power and accelerate enzyme optimization for biotechnology, medicine, and sustainable industrial applications.

REFERENCES

[1] A. T. S. Albanaz, C. H. M. Rodrigues, D. E. V. Pires, and D. B. Ascher, "Combating mutations in genetic disease and drug resistance: understanding molecular mechanisms to guide drug design," *Expert Opinion on Drug Discovery,* vol. 12, no. 6. Taylor & Francis, p. 553, May 11, 2017. doi: 10.1080/17460441.2017.1322579.

[2] J. Tian, C. D. McFarland, and J. Woodard, "Editorial: Structural understanding of the functional consequences of missense mutation," *Frontiers in Genetics,* vol. 14, Nov. 2023, doi: 10.3389/fgene.2023.1325326.

[3] A. J. M. Ribeiro, I. G. Riziotis, N. Borkakoti, and J. M. Thornton, "Enzyme function and evolution through the lens of bioinformatics," *Biochemical Journal,* vol. 480, no. 22. Portland Press, p. 1845, Nov. 22, 2023. doi: 10.1042/bcj20220405.

[4] P. Behzadi and N. Bernabò, *Computational Biology and Chemistry.* IntechOpen, 2019. doi: 10.5772/intechopen.83539.

[5] J.-Y. Xu *et al.,* "InstructPLM-mu: 1-Hour Fine-Tuning of ESM2 Beats ESM3 in Protein Mutation Predictions," *arXiv (Cornell University),* Oct. 2025, doi: 10.48550/arxiv.2510.03370.

[6] J. L. Pacheco-García, M. Cagiada, K. Tienne-Matos, E. Salido, K. Lindorff-Larsen, and Á. L. Pey, "Effect of naturally-occurring mutations on the stability and function of

cancer-associated NQO1: Comparison of experiments and computation," *Frontiers in Molecular Biosciences,* vol. 9, Nov. 2022, doi: 10.3389/fmolb.2022.1063620.

[7] A. Runthala, S. T. Selvan, and T. Kumaraguru, "Editorial: Enzyme design methodologies to enhance enzymatic yield," *Frontiers in Bioengineering and Biotechnology,* vol. 11, Aug. 2023, doi: 10.3389/fbioe.2023.1271980.

[8] J. E. Fuchs, I. G. Muñoz, D. J. Timson, and Á. L. Pey, "Experimental and computational evidence on conformational fluctuations as a source of catalytic defects in genetic diseases," *RSC Advances,* vol. 6, no. 63, p. 58604, Jan. 2016, doi: 10.1039/c6ra05499d.

[9] K. Yamada, H. Nishi, J. Nakata, and K. Kinoshita, "Structural characterization of single nucleotide variants at ligand binding sites and enzyme active sites of human proteins," *Biophysics and Physicobiology,* vol. 13, no., p. 157, Jan. 2016, doi: 10.2142/biophysico.13.0_157.

[10] R. Bhattacharya, P. W. Rose, S. K. Burley, and A. Prlić, "Impact of genetic variation on three dimensional structure and function of proteins," *PLoS ONE,* vol. 12, no. 3, Mar. 2017, doi: 10.1371/journal.pone.0171355.

[11] D. E. V. Pires, J. Chen, T. L. Blundell, and D. B. Ascher, "In silico functional dissection of saturation mutagenesis: Interpreting the relationship between phenotypes and changes in protein stability, interactions and activity," *Scientific Reports,* vol. 6, no. 1, Jan. 2016, doi: 10.1038/srep19848.

[12] C. J. Markin *et al.,* "Revealing enzyme functional architecture via high-throughput microfluidic enzyme kinetics," *Science,* vol. 373, no. 6553, Jul. 2021, doi: 10.1126/science.abf8761.

[13] E. Medina-Carmona *et al.,* "Site-to-site interdomain communication may mediate different loss-of-function mechanisms in a cancer-associated NQO1 polymorphism," *Scientific Reports,* vol. 7, no. 1, Mar. 2017, doi: 10.1038/srep44532.

[14] P. K. Agarwal, D. N. Bernard, K. Bafna, and N. Doucet, "Enzyme Dynamics: Looking Beyond a Single Structure," *ChemCatChem,* vol. 12, no. 19, p. 4704, Jun. 2020, doi: 10.1002/cctc.202000665.

[15] C. Jurich, Q. Shao, X. Ran, and Z. Yang, "Physics-based modeling in the new era of enzyme engineering," *Nature Computational Science,* vol. 5, no. 4. Nature Portfolio, p. 279, Apr. 24, 2025. doi: 10.1038/s43588-025-00788-8.

[16] Y. Ding, G. Pérez-Ortíz, J. Peate, and S. M. Barry, "Redesigning Enzymes for Biocatalysis: Exploiting Structural Understanding for Improved Selectivity," *Frontiers in Molecular Biosciences,* vol. 9. Frontiers Media, Jul. 22, 2022. doi: 10.3389/fmolb.2022.908285.

[17] E. Vallejos-Vidal *et al.,* "Single-Nucleotide Polymorphisms (SNP) Mining and Their Effect on the Tridimensional Protein Structure Prediction in a Set of Immunity-Related Expressed Sequence Tags (EST) in Atlantic Salmon (Salmo salar)," *Frontiers in Genetics,* vol. 10, Feb. 2020, doi: 10.3389/fgene.2019.01406.

[18] X. Wang, Q. Ma, J. Shen, B. Wang, X. Gao, and L. Zhao, "Application Fields, Positions, and Bioinformatic Mining of Non-active Sites: A Mini-Review," *Frontiers in Chemistry,* vol. 9. Frontiers Media, May 31, 2021. doi: 10.3389/fchem.2021.661008.

[19] M. Shirota and K. Kinoshita, "Current status and future perspectives of the evaluation of missense variants by using three-dimensional structures of proteins," *Biophysics and Physicobiology,* vol. 19, no., Jan. 2022, doi: 10.2142/biophysico.bppb-v19.0023.

[20] N. Mih, E. Brunk, A. Bordbar, and B. Ø. Palsson, "A Multi-scale Computational Platform to Mechanistically Assess the Effect of Genetic Variation on Drug Responses in Human Erythrocyte Metabolism," *PLoS Computational Biology,* vol. 12, no. 7, Jul. 2016, doi: 10.1371/journal.pcbi.1005039.

[21] D. Petrović and K. S. C. Lynn, "Molecular modeling of conformational dynamics and its role in enzyme evolution," *Current Opinion in Structural Biology,* vol. 52. Elsevier BV, p. 50, Sep. 08, 2018. doi: 10.1016/j.sbi.2018.08.004.

[22] M. McCoy, V. S. Shivakumar, S. Nimmagadda, M. S. Jafri, and S. Madhavan, "SNP2SIM: a modular workflow for standardizing molecular simulation and functional analysis of protein variants," *BMC Bioinformatics,* vol. 20, no. 1, Apr. 2019, doi: 10.1186/s12859-019-2774-9.

[23] C. G. Don and M. Smieško, "Microsecond MD simulations of human CYP2D6 wild-type and five allelic variants reveal mechanistic insights on the function," *PLoS ONE,* vol. 13, no. 8, Aug. 2018, doi: 10.1371/journal.pone.0202534.

[24] B. Tian *et al.,* "Enhancing Enzyme Activity with Mutation Combinations Guided by Few-shot Learning and Causal Inference," *Research Square (Research Square),* Nov. 2024, doi: 10.21203/rs.3.rs-5354708/v1.

[25] S. Jain *et al.,* "Evaluation of enzyme activity predictions for variants of unknown significance in Arylsulfatase A," *Carolina Digital Repository (University of North Carolina at Chapel Hill),* Mar. 2025, doi: 10.17615/m0qb-1843.

[26] D. Cocciadiferro *et al.,* "Exploiting in silico structural analysis to introduce emerging genotype–phenotype correlations in DHCR24-related sterol biosynthesis disorder: a case study," *Frontiers in Genetics,* vol. 14, Jan. 2024, doi: 10.3389/fgene.2023.1307934.

[27] Y. Karami, T. Bitard-Feildel, É. Laine, and A. Carbone, "'Infostery' analysis of short molecular dynamics simulations identifies highly sensitive residues and predicts deleterious mutations," *Scientific Reports,* vol. 8, no. 1, Oct. 2018, doi: 10.1038/s41598-018-34508-2.

[28] S. Kim *et al.,* "Multidisciplinary approaches for enzyme biocatalysis in pharmaceuticals: protein engineering, computational biology, and nanoarchitectonics," *EES Catalysis,* vol. 2, no. 1, p. 14, Oct. 2023, doi: 10.1039/d3ey00239j.

[29] J. Zhou and M. Huang, "Navigating the landscape of enzyme design: from molecular simulations to machine learning," *Chemical Society Reviews,* vol. 53, no. 16. Royal Society of Chemistry, p. 8202, Jan. 01, 2024. doi: 10.1039/d4cs00196f.

[30] A. Romero-Rivera, M. Garcia-Borràs, and S. Osuna, "Computational tools for the evaluation of laboratory-engineered biocatalysts," *Chemical Communications,* vol. 53, no. 2. Royal Society of Chemistry, p. 284, Sep. 06, 2016. doi: 10.1039/c6cc06055b.

[31] S. Gardiner *et al.,* "Resolution of physics and deep learning-based protein engineering filters: A case study with a lipase for industrial substrate hydrolysis," *PLoS ONE,* vol. 20, no. 9, Sep. 2025, doi: 10.1371/journal.pone.0332409.

[32] I. G. Sudarmanto, M. S. Rohman, and W. Artama, "THE EFFECT OF THE D185 MUTATION ON THE STABILITY AND FUNCTIONALITY OF RUBISCO LIKE PROTEIN (RLP) FROM CHROMOHALOBACTER SALEXIGEN BKL 5," *Jurnal Kimia,* p. 1, Jan. 2024, doi: 10.24843/jchem.2024.v18.i01.p01.

[33] U. Perricone *et al.,* "An overview of recent molecular dynamics applications as medicinal chemistry tools for the undruggable site challenge," *MedChemComm,* vol. 9, no. 6. Royal Society of Chemistry, p. 920, Jan. 01, 2018. doi: 10.1039/c8md00166a.

[34] M. C. Childers and V. Daggett, "Insights from molecular dynamics simulations for computational protein design," *Molecular Systems Design & Engineering,* vol. 2, no. 1, p. 9, Jan. 2017, doi: 10.1039/c6me00083e.

[35] M. J. Latallo, G. A. Cortina, S. Faham, R. K. Nakamoto, and P. M. Kasson, "Predicting allosteric mutants that increase activity of a major antibiotic resistance enzyme," *Chemical Science,* vol. 8, no. 9, p. 6484, Jan. 2017, doi: 10.1039/c7sc02676e.

[36] C. Jerves, R. P. P. Neves, S. L. da Silva, M. J. Ramos, and P. A. Fernandes, "Rate-enhancing PETase mutations determined through DFT/MM molecular dynamics simulations," *New Journal of Chemistry,* vol. 48, no. 1, p. 45, Nov. 2023, doi: 10.1039/d3nj04204a.

[37] P. Bowling, S. Dasgupta, and J. M. Herbert, "Eliminating Imaginary Vibrational Frequencies in Quantum-Chemical Cluster Models of Enzymatic Active Sites," *Journal of Chemical Information and Modeling,* vol. 64, no. 9, p. 3912, Apr. 2024, doi: 10.1021/acs.jcim.4c00221.

[38] A. J. Zavala, "Structure-function studies of β-lactamases," *HAL (Le Centre pour la Communication Scientifique Directe),* Dec. 2018, Accessed: Jun. 2025. [Online]. Available: https://theses.hal.science/tel-02895645

[39] K. Zinovjev, J. J. Ruiz-Pernía, and I. Tuñón, "The quantum revolution in enzymatic chemistry: combining quantum and classical mechanics to understand biochemical processes," *Pure and Applied Chemistry,* vol. 97, no. 10, p. 1223, Jul. 2025, doi: 10.1515/pac-2025-0500.

[40] P. Ferreira, P. A. Fernandes, and M. J. Ramos, "Modern computational methods for rational enzyme engineering," *Chem Catalysis,* vol. 2, no. 10, p. 2481, Oct. 2022, doi: 10.1016/j.checat.2022.09.036.

[41] F. Casilli, M. Canyelles-Niño, G. Roelfes, and L. Alonso-Cotchico, "Computation-guided engineering of distal mutations in an artificial enzyme," *Faraday Discussions,* vol. 252, no., p. 262, Jan. 2024, doi: 10.1039/d4fd00069b.

[42] M. F. Khan and Mohd. T. Khan, "AI-Driven Enzyme Engineering: Emerging Models and Next-Generation Biotechnological Applications," *Molecules*, vol. 31, no. 1, p. 45, Dec. 2025, doi: 10.3390/molecules31010045.

[43] S. Osuna, "The challenge of predicting distal active site mutations in computational enzyme design," *Wiley Interdisciplinary Reviews Computational Molecular Science*, vol. 11, no. 3, Aug. 2020, doi: 10.1002/wcms.1502.

[44] Y. Jiang, X. Ran, and Z. Yang, "Data-driven enzyme engineering to identify function-enhancing enzymes," *Protein Engineering Design and Selection*, vol. 36. Oxford University Press, Oct. 10, 2022. doi: 10.1093/protein/gzac009.

[45] M. Scherer, S. J. Fleishman, P. R. Jones, T. Dandekar, and E. Bencúrová, "Computational Enzyme Engineering Pipelines for Optimized Production of Renewable Chemicals," *Frontiers in Bioengineering and Biotechnology*, vol. 9. Frontiers Media, Jun. 15, 2021. doi: 10.3389/fbioe.2021.673005.

[46] S. S. Chaturvedi, D. Bím, C. Christov, and A. N. Alexandrova, "From random to rational: improving enzyme design through electric fields, second coordination sphere interactions, and conformational dynamics," *Chemical Science*, vol. 14, no. 40. Royal Society of Chemistry, p. 10997, Jan. 01, 2023. doi: 10.1039/d3sc02982d.

[47] Z. Song, Q. Zhang, W. Wu, Z. Pu, and H. Yu, "Rational design of enzyme activity and enantioselectivity," *Frontiers in Bioengineering and Biotechnology*, vol. 11. Frontiers Media, Jan. 24, 2023. doi: 10.3389/fbioe.2023.1129149.

***Mariam Jabbar Abboud (Corresponding Author)**
Wasit Education Directorate, Iraq
Email: mariam.jabbar1983@gmail.com

Iqbal Sahi Tuama
Wasit Education Directorate, Iraq
Email: Aqbal10121981@gmail.com