Computational Studies on the Functional and Structural Impact of Pathogenic Mutations in Enzymes

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ABSTRACT

Enzymes are critical biological catalysts involved in maintaining the intricate balance of metabolic processes within living organisms. Mutations in enzymes can result in disruptions to their functionality, that may lead to a range of diseases. This review focuses on computational studies that investigate the effects of disease-associated mutations in various enzymes. Through molecular dynamics simulations, multiscale calculations, and machine learning approaches, computational studies provide detailed insights into how mutations impact enzyme structure, dynamics, and catalytic activity. This review emphasizes the increasing impact of computational simulations in understanding molecular mechanisms behind enzyme (dis)function by highlighting the application of key computational methodologies to selected enzyme examples, aiding in the prediction of mutation effects and the development of therapeutic strategies.

INTRODUCTION

Proteins are essential macromolecules that perform a vast array of functions in living organisms, from providing structural support to facilitating chemical reactions. Their significance in human biology cannot be overstated, as they play crucial roles in processes such as cell signaling, immune response, and tissue repair. Among these proteins, enzymes stand out as indispensable catalysts that accelerate biochemical reactions, enabling life-sustaining processes like DNA replication, metabolism, and cellular respiration.[1, 2] Without the precise action of enzymes, these processes would occur too slowly to sustain life, making them vital for maintaining cellular homeostasis and metabolic balance.

Given their central role in biological systems, enzymes are the focus of numerous studies. The International Union of Biochemistry and Molecular Biology (IUBMB) has classified enzymes into seven major categories based on the reactions they catalyze (**Figure 1**). Enzymes can be affected due to varied factors including changes in the environment[3], amino acid substitutions,[4-8] post translational modifications,[9] protein-protein interactions.[10] These alterations can result in significant changes in enzyme structure and function, leading to disruptions in catalysis, substrate binding, or protein folding, which can have profound biological consequences.

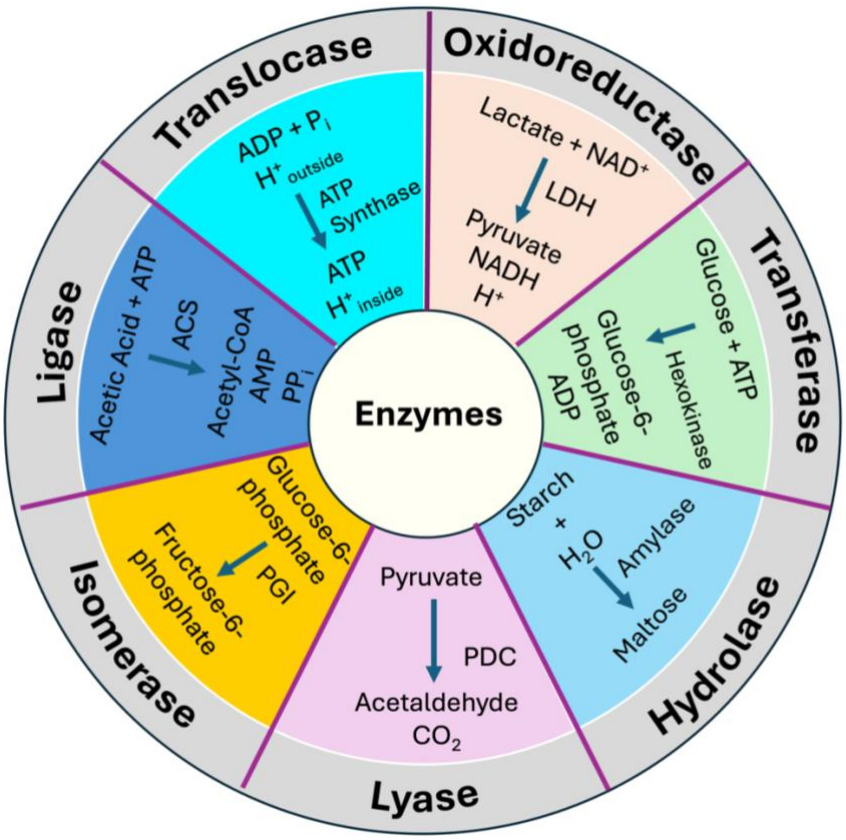


Figure 1. Classification of enzymes into their different families. These enzymes catalyze a plethora of reactions, and the ones shown are an example of, but not limited to, the type of reactions catalyzed by the said class of enzymes. Abbreviations used are as follows: LDH- Lactose Dehydrogenase, NAD- Nicotinamide Adenine Dinucleotide, ATP- Adenosine Triphosphate, ADP- Adenosine Diphosphate, PDC- Pyruvate Decarboxylate, PGI- Glucose-6-Phospho Isomerase, ACS-Acetyl-CoA Synthase.

Permanent alterations in the DNA sequence can arise due to a myriad reasons, from natural and spontaneous to anthropogenic[11-13], and give rise to mutations. Mutations can be of different types such as missense mutations, which changes an amino acid in the protein, a nonsense mutation that introduces a premature stop codon, or a silent mutation that has no effect on the protein. Insertions and deletions (indels) can add or remove nucleotides, potentially causing a frameshift that alters the entire downstream protein sequence, often resulting in nonfunctional proteins.[14] Other mutations include duplications, where a segment of DNA is copied, and splice site mutations, which affect mRNA splicing and may lead to incorrect protein production. While some mutations are harmful and can cause diseases, others may be neutral or even beneficial, contributing to genetic diversity and evolution.

Extensive experimental research has been conducted to understand the effects of disease-related mutations on enzymes. Through biochemical and structural analyses, scientists have gained valuable insights into the mechanisms by which these mutations disrupt enzyme function, providing the foundation for therapeutic interventions.[12-16] Mutations in enzymes have been linked to neurological disorders,[15-17] metabolic,[18-22] immune system disorders,[23, 24] and various forms of cancers.[25-30]

In recent years, computational simulations have provided a useful tools for the study of enzyme mutations, offering atomic-level insights into how these mutations affect enzyme structure and function.[31-35] Methods such as molecular dynamics (MD) simulations, protein structure prediction algorithms, coupled quantum mechanics/molecular mechanics (QM/MM) calculations, and machine learning (ML) tools have emerged as powerful approaches for modeling enzyme function and mutation effects. These tools enable researchers to simulate and predict the impact of mutations on enzymes, shedding light on their roles in disease.

Various MD simulation techniques, such as classical atomistic Molecular Dynamics (MD),[36-39] Accelerated MD (aMD),[40, 41] Coarse-Grained MD (CGMD),[42] Ab Initio MD (AIMD),[43] and Monte Carlo[44] allow detailed structural and dynamical analyses using force fields like OPLS,[45] AMBER,[46] GROMOS,[47] CHARMM,[48] AMOEBA,[49] GEM,[50] and others. Furthermore, coupled QM/MM methods offer a balance between accuracy and efficiency by applying quantum mechanical methods to enzyme active sites and molecular mechanics to the rest of the system.[51-53, 54] In conjunction with these tools, various visualization programs such as VMD,[55] UCSF Chimera,[56] PyMOL,[57] among others, are used for structural preparation, introducing mutations and certain analyses.

In addition to analyzing existing mutations, computational approaches have been developed to predict potential mutations and their effects. Energy Decomposition Analysis (EDA),[58-62] has also been utilized in mutation studies to reveal how mutations alter non-bonded interactions.[63-68] The combination of bioinformatics techniques and computational tools, such as Hypothesis-Driven-SNP-Search[69, 70] (HyDn-SNP-S) can be used to identify SNPs associated with missense mutations. A web-based pathogenicity prediction tool, PredictSNP,[71] is effective at predicting mutations. There are various other methods that have also been used for such predictions, and the recent development of machine learning methods have also been very effective. Methods such as RoseTTAFold,[72] Alphafold3,[73] ColabFold,[74] ESMFold,[75] OmegaFold[76] etc. are used to predict protein structure. Tools such as DDMut[77] have also been successfully used to predict the effects of mutations.[78]

This review highlights the use of computational methods and predictive tools to investigate the effects of disease-causing mutations on different enzyme classes, focusing on certain studies in detail.

DISCUSSION

OXIDOREDUCTASES

Oxidoreductases (classified as EC 1) are a superfamily of enzymes encompassing a vast group that catalyzes redox reactions, which are essential for various biochemical processes including oxygen incorporation, hydride transfer, and proton extraction. They play critical roles in numerous metabolic pathways, utilizing various cofactors like nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), or nicotinamide adenine dinucleotide phosphate (NADP). These enzymes are also used in various industrial applications[73-77] and play a pivotal role in diagnosing, prognosing, and treating various diseases.[79-81] Various computational studies have been performed on oxidoreductases to study different aspects.[64, 67, 82-97] A few studies highlighting pathogenic mutations are described below.

Some key members of this superfamily, notable for how mutations affect their function, have been studied computationally. P450 oxidoreductases (POR) are essential members of this superfamily in humans and are involved in various biochemical processes, including drug metabolism, steroidogenesis, and the breakdown of toxic substances. Deficiency or mutations in POR can lead to disruptions in steroid hormone production, causing various health issues, including disorders of sexual development, skeletal malformations, and, in milder cases, infertility. Kumar et al.,[82] Cui et al.,[83] Zhang et al.,[84] and Sahakitrungruang et al.[85] have employed various types of MD simulations, along with protein tunnel analysis, molecular mechanics-generalized Born surface area (MM-GBSA), and principal component analysis (PCA), to investigate how deficiency-related mutations in (POR and cytochrome P450 enzymes (CYP 17A1 and CYP 1A2) impact structural dynamics, ligand transport pathways, and enzymatic activity. Their computational findings, reinforced by experimental evidence from their studies, revealed that these mutations disrupt key interactions, diminish binding affinity, and impair enzyme function.

NADPH oxidases (NOX) are another member of the oxidoreductase superfamily primarily found in immune cells, such as neutrophils and macrophages, where they play a crucial role in producing reactive oxygen species (ROS) to defend against pathogens. Mutations or malfunctions in NADPH oxidases can impair immune function, contributing to conditions such as chronic granulomatous disease (CGD) and systemic lupus erythematosus (SLE). Researchers have

studied how mutations affect NADPH oxidase function using computational approaches such as mapping anchoring spots on protein surface ($ANCHORS_{MAP}$),[86] rigid body docking,[87] and protein-protein docking, supported by MD simulations.

Jacob et al.[88] investigated variants associated with systemic lupus erythematosus (SLE), identifying that the H389Q mutation weakens the interaction between Neutrophil Cytosolic Factor 2 (NCF2) and Vav1, leading to reduced ROS production. Similarly, Meijles et al.[89] demonstrated that phosphorylation of the p47phox subunit at Ser-379 acts as a molecular switch for enzyme activation, with the S379A mutation preventing key interactions and halting superoxide production. Together, these studies emphasize how mutations in NADPH oxidase disrupt its activity and contribute to immune-related diseases.

Human AlkB (AlkBH1–AlkBH9), and ten-eleven translocation (TET1, TET2, and TET3) enzymes belong to the Fe(II)/αKG-dependent dioxygenases. As key members of the oxidoreductase superfamily, these enzymes facilitate oxidation reactions in critical biochemical processes. AlkB is involved in DNA repair by oxidatively demethylating damaged nucleobases,[98, 99] while TET enzymes catalyze the oxidation of 5-methylcytosine (mC) to 5-hydroxymethylcytosine (hmC) and further derivatives, playing a crucial role in DNA demethylation and epigenetic regulation. Mutations in these enzymes can impair their redox function, disrupting electron transfer and substrate oxidation, leading to consequences such as increased DNA damage or cancer (in AlkB and TET mutations). These disruptions highlight the critical role of redox reactions in maintaining cellular function and tissue integrity.

Researchers have increasingly employed integrative computational-experimental approaches to interpret the biological functions of these mutations and their pathological consequences. By employing HyDn-SNP-S (Hypothesis Driven-SNP-Search), coupled with MD simulations and non-covalent interaction (NCI) analysis, Walker et al.[90] investigated a prostate cancer-associated SNP in AlkBH7, a key enzyme involved in fat metabolism and programmed necrosis. Their identified mutation, R191Q, was predicted to alter cofactor binding, which was confirmed through experimental validation within the same study.

Another important member of the oxidoreductase superfamily is the lysyl hydroxylase (LH1, LH2, and LH3) family. LH2 in the human body is essential for forming stable hydroxylysine aldehyde-derived collagen cross-links (HLCCs) via telopeptidyl lysine residues.[100, 101] This stability helps maintain the extracellular matrix, while excessive LH2 expression can cause fibrosis, sarcoma, and metastasis in lung and breast cancers.[102-105] Maghsoud et al.[91] and Lee et al.[92] collaboratively studied the inhibition of LH2 by employing molecular docking, polarizable-MD,

QM/MM, sequence alignment, protein tunnel analysis, and multiple experimental assays. They identified major oxygen-transporting tunnels in the apoenzyme containing several common residues. Through sequence alignment of the three LH enzymes in Homo sapiens with fourteen other organisms, they demonstrated that all the tunnel-defining residues (V653, Y655, L663, H666, D668, T673, N675, C690, H710, H718, E719, G720, I730, V732, and F734) are conserved across these species (see **Figure 2**). Through complimentary QM/MM computations and NCI analysis, they found several of these residues interact with the active site. These findings offer valuable insights into key factors for LH2 inhibition and provide a basis for future mutagenesis studies on the LH family, focusing on these conserved residues.

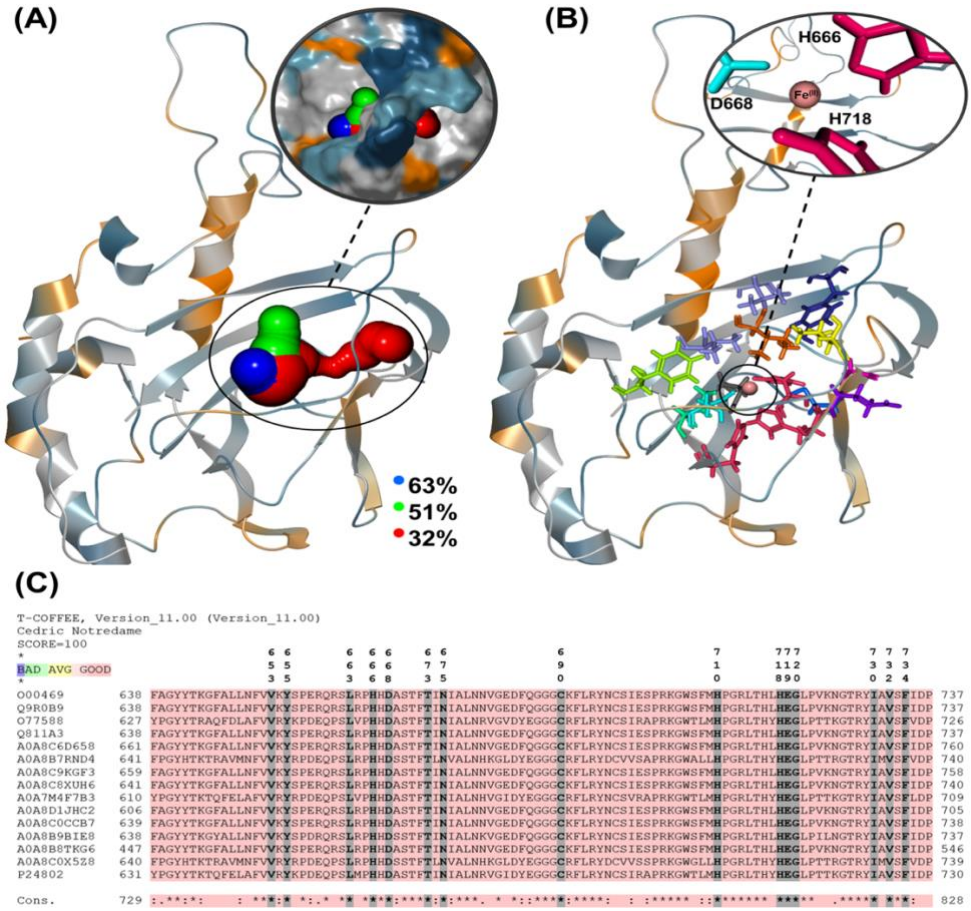


Figure 2. (A) O_2-transporti tunnels with the largest calculated percentages along the trajectory observed in the apoenzyme. **(B)** Close-up of the approximate positions of the tunnel-lining residues with more than ~80% presence in all calculated tunnels/representatives. **(C)** Condensed sequence alignment between human LH2 and selected members of the LH family. This figure has been reprinted, with permission, from reference (91), copyright (2023), American Chemical Society

Waheed et al.[106] performed computational studies on second sphere mutations on TET2, namely W1291A, N1387A, Y1902A, H1904R, and distal mutations including M1293A-Y1294A and K1299E-S1303N or a combination of both second-sphere and distal: S1290A-Y1295A. MD simulations were performed on the wild-type and all the variants. MM-GBSA analysis showed that the mutations affect substrate binding, which alters stability of protein-DNA complex. Hydrogen bond analysis shows that the mutants disrupt hydrogen bond networks. PCA analysis showed that the correlated movement between the Cys-rich N-terminal domain and the GS linker stabilizes the DNA-protein interactions in the WT more than the mutant. Additional QM/MM studies with ChemShell[107] package showed that the mutations cause a decrease in activity by increasing the reaction barrier. It is also seen that the mutants influence molecular orbital interactions. Electric field calculations, done using TITAN code,[108] showed that the mutations altered the electric field, which leads to higher barrier in some mutations.

TRANSFERASES

Transferases (classified as EC 2) are a diverse class of enzymes that catalyze the transfer of functional groups from a donor to an acceptor molecule. Functional groups can include methyl, glycosyl, phosphate etc. [109, 110] These enzymes play a fundamental role in various biochemical pathways such as cellular metabolism, signal transduction, detoxification and gene regulation, utilizing various divalent ions or cofactors such as ATP, NAD^+, etc.[111, 112] Transferases are crucial for maintaining cellular homeostasis, and hence, alterations and mutations in transferases can cause disruption of key functions and lead to various diseases such as cancer due to dysregulated pathways, liver diseases such hepatitis and cirrhosis, congenital disorders, among others.[113-117] Various studies on transferases using different computational methods have provided insight into the various aspects of transferases. [63 118-136] A few of them with a focus on pathogenic mutations are highlighted below.

Saxena et al. performed an *in silico* investigation on mutations on the glutamic-oxaloacetic transaminase 1 (GOT1), which is essential for encoding aspartate aminotransferase (AST), necessary for amino acid metabolism[118]. The study involved screening of 220 missense non-synonymous(ns) SNPs using sequence-based tools, such as PROVEAN[137], PANTHER[138], PolyPhen-2[139] among others for predicting the effect of protein structure and function, and SuSPect[140], PMut[141], SNAP[142] etc. to predict disease association. Further, deleterious mutations predicted by majority of these tools were studied using structure-based tools such as DynaMut[143], CUPSAT[143], iStable[144] etc. From the analysis, four highly deleterious mutations (L36R, Y159C,

W162C and L345P) were chosen for further investigation as they were predicted to decrease stability of the protein.

ConSurf[145] was further used to investigate the impact of these mutations depending on residue conservation. Based on this analysis, it was observed that L36R and Y159C were in high conservation residues, W162C was at a moderately conserved residue and L345P was in an extremely variable residue. However, multiple sequence alignment[146] (MSA) showed that all four of these residues are highly conserved. To analyze the phenotypic association of these mutations, FATHMM[147] was utilized, and it was seen that all of these mutations are dangerous and associated to central nervous system diseases and brain diseases. Proximal structural effect of the SNPs were studied using the HOPE[148] server, which shows that apart from L36R, the other mutations changed the secondary structure in its surroundings. Due to the changes in size and charge of the L36R mutation, this mutation might cause changes in interactions that are essential in binding.

MD simulations on the wild type (WT) and the variants were performed and the trajectories were analyzed for more understanding. It was revealed that the L36R and Y159C variants were less stable when compared to the WT, as evident by higher root mean square deviation (RMSD) values, along with all the four variants having higher flexibility, indicated by a higher root mean square fluctuation (RMSF). A radius of gyration (Rg) analysis showed that other than the W162C variant, the rest of the variants showed less compactness and higher flexibility than the WT. Analysis of the hydrogen bonds during the trajectory reveals that L36R and Y159C mutations show decreased number of hydrogen bonds, which conforms to the results of the HOPE server[148]. Secondary structure analysis also shows that these mutations cause changes in the formation of several secondary structures, due to changes in interactions. Principal component analysis (PCA) showed that all the four mutations altered the dynamics of the system and caused the system to have a more flexible motion when compared to the WT. Post-translational modification analysis of the protein predicts that the Y159C variant effects the most, due to it being present in the domain necessary for PTM, with L36R also being detrimental, due to its proximity. Overall, these studies show the deleterious effects of such mutations on the proteins, with L36R seen to be the most destabilizing. This study can be used to lay a cornerstone for future experimental studies on nsSNPs of this protein.

Polymerases are enzymes that catalyze the synthesis of long chains of polymers, such as polysaccharides, polyanhydrides, chitosan etc. or nucleic acids, such as DNA or RNA, and have proof reading and error correction capabilities.[149] Mutations in polymerases lead to a plethora of diseases[150] , including various cancer phenotypes.[151] Geronimo et al. investigated the effects of

8-oxoguanine (8OG) on the DNA damaging processing by polymerases[152]. MD simulations of the ternary structures of Pol μ and Pol β with 8OG incorporated into them were carried out, with metadynamics implemented to properly dock 8OG into the active site of the polymerases. It was seen that the Pol bound 8OG was in a higher energy conformation, whereas it adopted a lower energy conformation in isolated DNA. They speculated that this occurs due to the interactions of the phosphate group of 8OG with the polymerase residues, which was confirmed by free energy calculations. Further analysis of the effects of the R442 and R446 residues were studied using the R442A mutation and R442K/R446A double mutation in pol μ. It was seen that when these mutations were present, 8OG tends to adopt a low energy conformation. This study shows that by mutating the residues in the polymerase, the 8OG conformation can be regulated, and DNA damage can be repaired, which leads to prevention of diseases.

Parkash et al. investigated the most common cancer-associated mutation (P301R) in DNA Pol ε using both computational and experimental methods, X-Ray crystal structures were obtained for both the mutant and the WT of Pol ε exonuclease domain, and it was seen that they had minimal structural differences[127]. Examining the crystal structures and MD simulations of both the systems, both ternary and apo, it was revealed that R301 is largely positioned in an orientation that would cause steric clashes with the 3'-end of the DNA. This was validated by further population analysis of dihedral conformations. Due to being conformationally dynamic, the R301 residue might, at times, adopt a more favorable orientation for the DNA to bind to the active site. However, this incorporation of DNA is seen to cause change in catalytic orientation of the DNA, where the incoming nucleotide loses metal binding to one of the catalytic metals due to the displacement of one of the metals, which might hinder activity. Furthermore, a study of distances of the flexible regions of the exo loop to a more rigid residue shows that the loop is more flexible in the mutant than the WT, even though the conformation remains the same. The results might suggest the lack of exonuclease activity of the mutant to the steric hindrance, which is different from other commonly known cancer mutations to pol ε.

Swett et al. developed Hypothesis Driven-SNP-Search (HyDn-SNP-S) an approach that combines bioinformatics and atomistic simulations to uncover and characterize disease-associated mutations.[70] The application of HyDn-SNP-S to four cancer phenotypes (melanoma, lung, pancreatic and breast) on DNA polymerases yielded two previously unreported SNP on DNA Pol λ, which results in Pol λ R432W. MD simulations, correlation analysis and generalized masked Delaunay (GMD) analysis, revealed that the R432W mutation affects the dynamics of loop1. This loop has been experimentally reported to play an important role in fidelity.[149] The authors

hypothesized that the impact on the dynamics of this important loop could affect fidelity and thus be a factor on the observed breast cancer association. This mutation was validated by an experimental study a few years later, where they confirmed that this DNA polλ variant is associated with estrogen driven breast cancer.[153] HyDn-SNP-S has been subsequently extended to create the DNA repair cancer database comprising eight cancer phenotypes and twenty-two genes coding for DNA transaction enzymes. [69]

Along with structural and dynamic changes, computational analysis can also be used to study the change in activity. Maghsoud et al. reported a study on DNA Pol κ and its cancer-associated Y432S variant using MD simulations and QM/MM simulations.[63] Their results revealed that the mutation does not significantly affect the protein's overall structure and dynamics but causes considerable changes in the interactions. Additionally, it is seen that in the active site, the mutation changes the coordination of the metallic co-factors, causing certain structural changes, when compared to the WT. Energy decomposition analysis (EDA) of the two systems showed that the active site of the mutant is considerably more stabilized by interactions of the rest of the protein than that of the WT, suggesting the protein environment stabilizing the active site to counteract the changes in structure. QM/MM studies using Layered Interactive CHEmical Model (LICHEM)[53, 54] shed light on the lowered activity of the Y432S variant, where it was seen that the reaction was endoergic with a higher barrier in the mutation as compared to an exoergic reaction with a lower barrier in the wild-type (Figure 3). EDA of the transition state of both systems showed that the transition state of the wild-type was considerably more stable in terms of pair-wise interactions than the reactant, in contrast to the mutant where interactions in the transition state had a destabilizing effect. These results conform to experimental results by Stern et al., that showed the reduction of enzyme activity in presence of the mutation.[124]

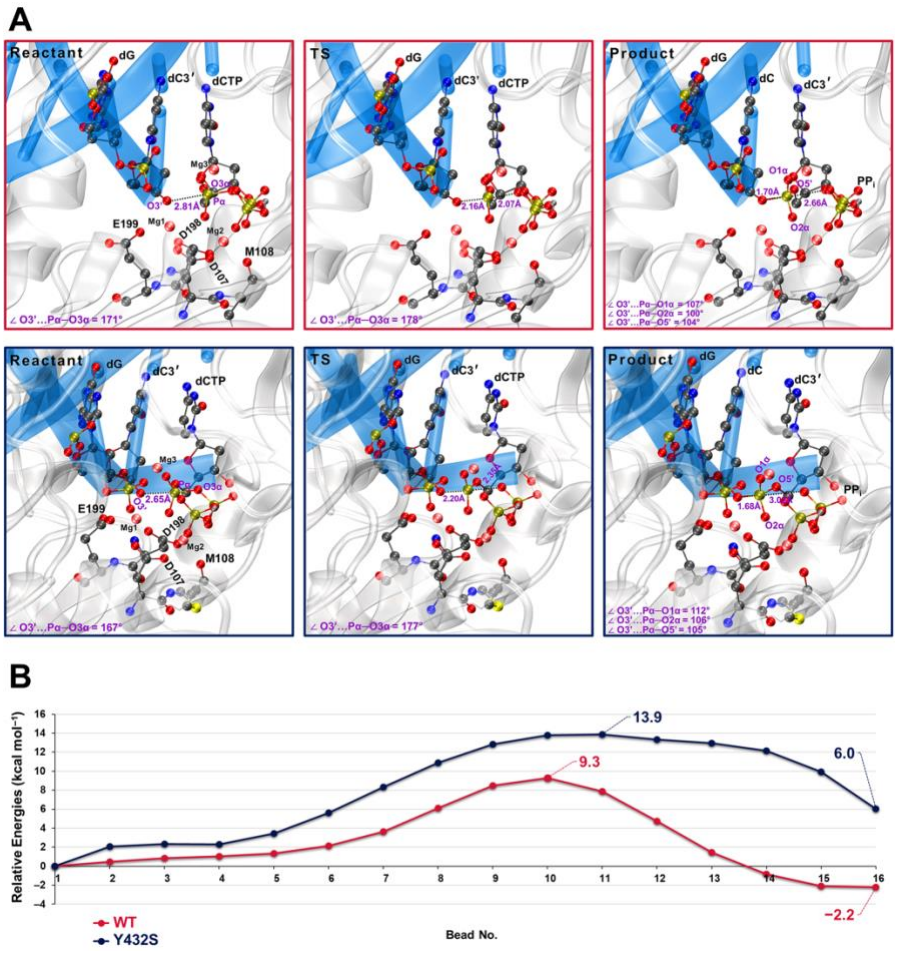


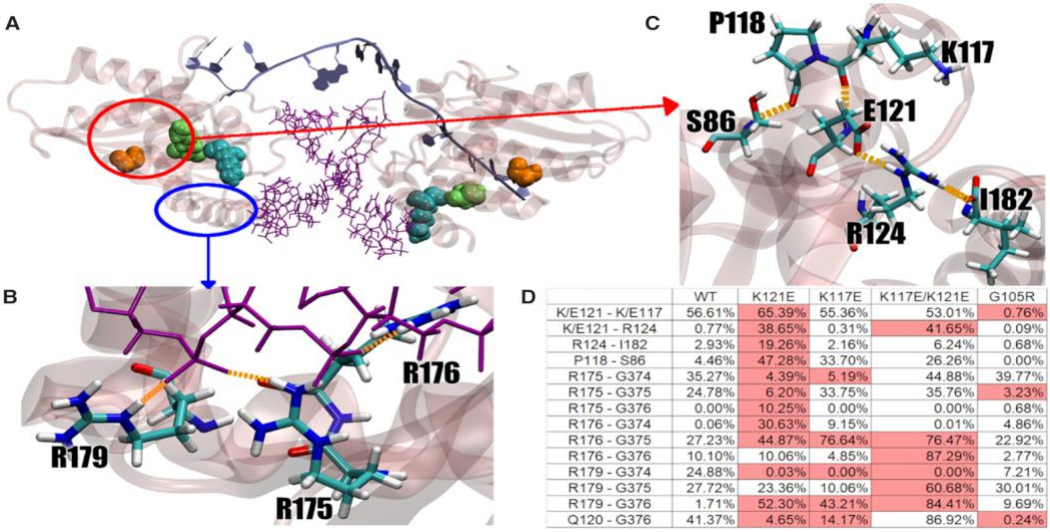
Figure 3. A. Optimized geometries for the reactant, product and transition states for the phosphoryl transfer in the active site for WT DNA Polκ (top) and Y432S variant (bottom). **B.** Minimum energy path for the phosphoryl transfer, showing a higher energy barrier and reaction energy for the Y432S variant (blue) than the WT (red). This figure has been reprinted, with permission, from reference (63), copyright (2024), American Chemical Society

HYDROLASES

Hydrolases (classified as EC 3) catalyze the breaking of chemical bonds using water. They are crucial to the working of the human body, as they are involved in efficient excretion, energy production, signaling and various synthetic cycles. Hydrolases are classified into thirteen categories, based on the types of bonds they cleave. Many computational methods are used to study mutations on these proteins[154-180]. A few are highlighted below.

Apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC) is a family of 7 members that catalyze RNA and DNA cytosine deamination, converting guanine to promutagenic uracil. The resulting lesions can cause mutations, making APOBEC a natural antiviral response

protein by disrupting viral replication. A combined computational and experimental study by Hix et al. investigated the effects of oncogenic mutations on APOBEC3H (A3H)[161]. Molecular Dynamics simulations (MD) were used to study five different systems: WT A3H, K121E cancer variant (associated to lung cancer, uncovered by HyDn-SNP-S), K117E/K121E, K117E and G105R. The simulation results revealed that the K121E variant results in new hydrogen bond interactions compared to the WT, leading to increased strain on the active site and dimer interface, which were predicted to affect activity and dimerization ability. (Figure 4). The K121E variant also showed increased flexibility in the rest of the protein other than the residues involved in changes in hydrogen bonding, as indicated by RMSF data. Further correlation and principal component analysis revealed altered correlation around the mutation site, along with constrained and higher correlated motion when compared to the WT.



	WT	K121E	K117E	K117E/K121E	G105R
K/E121 - K/E117	56.61%	65.39%	55.36%	53.01%	0.76%
K/E121 - R124	0.77%	38.65%	0.31%	41.65%	0.09%
R124 - I182	2.93%	19.26%	2.16%	6.24%	0.68%
P118 - S86	4.46%	47.28%	33.70%	26.26%	0.00%
R175 - G374	35.27%	4.39%	5.19%	44.88%	39.77%
R175 - G375	24.78%	6.20%	33.75%	35.76%	3.23%
R175 - G376	0.00%	10.25%	0.00%	0.00%	0.68%
R176 - G374	0.06%	30.63%	9.15%	0.01%	4.86%
R176 - G375	27.23%	44.87%	76.64%	76.47%	22.92%
R176 - G376	10.10%	10.06%	4.85%	87.29%	2.77%
R179 - G374	24.88%	0.03%	0.00%	0.00%	7.21%
R179 - G375	27.72%	23.36%	10.06%	60.68%	30.01%
R179 - G376	1.71%	52.30%	43.21%	84.41%	9.69%
Q120 - G376	41.37%	4.65%	14.17%	86.92%	0.24%

Figure 4. A. A3H Hap I (in pink) with RNA interface (purple) and DNA substrate (ice blue). The residues G105 (orange), K117 (cyan) and K121 (green) are also shown. **B.** Residues important for RNA binding. **C.** K121E mutations causes new hydrogen bond interactions. **D.** Table of largest HBond contributors, based on percentage simulation time. This figure has been reprinted, with permission, from reference (161), copyright(2020), Oxford University Press

The enhanced hydrogen bond networks were seen to involve the K117 residue, and hence it was predicted that mutating this residue would rescue the K121E variant. Analysis of the K117E/K121E variant shows that the hydrogen bond networks that are necessary for the protein stability and function are partially restored. It was also seen that the K117E/K121E variant shows similar motion to that of WT, with similar correlated motion, along with similar fluctuations of the loop. The predicted effects of the K121 cancer variants with respect to activity and dimerization, as well as the rescue phenotype of the K117E/K121E double mutant were all confirmed experimentally in the same study[161].

MutY DNA glycosylase (MUTYH) is involved in the repair of DNA in case of oxidative damage. It is involved in the first step of Base Excision Repair (BER) which is the deglycosylation of deoxyadenosine in 8-oxo-7,8-dihydroguanine (8OG):A mispair. Mutations in the MUTYH gene mainly cause MUTYH associated polyposis (MAP), an autosomal recessive disorder, that increases the risk of colorectal cancer. A study by Nikkel et al. focused on the Y126F and N146S variants in the wild-type MUTYH bacterial homologue. The N146S mutation is analogous to N224S mutation of MUTYH that is associated with MAP in humans[162]. They conducted MD simulations with AMBER and QM/MM (ONIOM) [181] calculations to study the structural and catalytic differences between WT and mutant systems. According to hydrogen bond analysis, they observed that there is a hydrogen bond between N146 and D144 which supported the hypothesis that N146 is important for positioning of D144 in the active site. In similar way, Y126 is proposed to be vital for positioning of E43 and a strong hydrogen bond was observed between Y126 and E43 during MD simulation. Y126F caused alterations in this interaction leading to structural destabilization of the active site. The N146S mutant disrupted hydrogen bond interactions that are crucial for cross-link formation, leading to a different catalytically incompetent structural conformation which D144 positions away from the active site.

Furthermore, QM/MM calculations revealed that Y126F shows an increase of the reaction barrier by 6.8 kJ/mol for the rate of hydrolysis reaction than WT. Another study by Trasviña-Arenas et al.[182] investigated two cancer-associated mutations in MUTYH; R241Q and N238S, that are important in allosteric network interactions between abasic (AP) site and [4Fe-4S] cluster, using both experimental and computational tools. Both variants showed a reduction in the local stability of the protein with total non-bonded inter-molecular interaction energy compared to WT, while R241Q has a larger impact on the stability of catalytic residue, D236 and AP site. A dynamic network analysis showed a connected network passing through R241 and N238 between the [4Fe-4S] cluster and the AP site, which is disrupted by both mutations. Additionally, the optimal path between AP site and [4Fe-4S] in WT is observed to go through R241 while this path is altered for all mutant systems. These results provided an explanation for the observed experimental phenotype in that that the mutations impact both the structural dynamics and the interconnections among the residues in the structural bridge between the two sites.

LYASES

Lyases (classified as EC 4) are enzymes that catalyze the breaking of chemical bonds by means other than hydrolysis and oxidation, often forming a new double bond or a new ring structure.[183-185] The lyase family includes fumarate hydratase, guanylate cyclase,

adenylosuccinate lyase, isocitrate lyase, lactoylglutathione lyase, among others and are crucial for certain bodily functions. Computational studies on various lyases have been carried out[186-192].

Fumarate Hydratase (FH) is an enzyme in the tricarboxylic acid cycle that converts fumarate to malate.[193] Mutations in FH have been linked to different diseases, including hereditary leiomyomatosis and renal cell cancer (HLRCC).[194, 195] Shorthouse et al. used MD simulations to investigate R233H FH[186]. This variant is known to impact FH function, but the mechanism was unknown. Structural analysis of angle of key domains showed that this mutation disrupts the hinge regions in the protein, resulting in conformational changes that occludes the catalytic site, reducing the enzyme's efficiency, thus categorizing the mutation as a loss-of-function (LOF). Additional study of the R268G mutant also showed conformational changes that influence catalytic activity.

Guanylin, encoded by the Guanylate Cyclase Activator 2A (GUCA2A) gene, is responsible for the activation of guanylate cyclase, consequently regulating water and electrolyte transport.[196, 197] Its structure is stabilized by two disulfide bonds, and disruptions in guanylin function are linked to gastrointestinal diseases, such as colon adenocarcinoma, adenoma and intestinal polyps.[187, 198, 199] Porto *et al*.[187] identified five deleterious SNPs in the GUCA2A gene (L6P, C104R, C112S, G114R, and C115Y), and MD simulations were used to study their effects. The L6P and G114R variants did not significantly alter the guanylin peptide structure, with G114R retaining the disulfide bonds. However, the C115Y variant exhibited structural characteristics with lower flexibility and higher values for radius of gyration (Rg) and solvent-accessible surface area. C104R and C112S disrupted a key disulfide bond, resulting in greater flexibility, though no significant changes in solvation potential energy were found. MD simulations suggest that C104R, C112S, and C115Y might be associated with diseases due to these structural disruptions. Additionally, two nonsense mutations at sites Glu89 and Tyr109 also showed truncated guanylin peptides, incapable of disulfide bonds.

Adenylosuccinate lyase (ADSL) is an enzyme in the purine salvage pathway that is required for the survival of Leishmania donovani, the pathogen that causes visceral leishmaniasis.[200-202] It catalyzes the conversion of adenylosuccinate into adenosine monophosphate (AMP) and fumarate, and its disruption can compromise the pathogen's ability for survival and replication. Bora et al. investigated two double mutants, N335Y/T367R (mutant I) and S321C/E334A (mutant II), and found that these mutations reduced AMP binding affinity, leading to decreased substrate binding.[188] MD simulations revealed that both mutants exhibited increased structural fluctuations compared to the native enzyme. MMPBSA calculations were performed to assess the binding

energy, indicating that these mutations negatively affected the enzyme's catalytic efficiency. The orientation of two important catalytic residues, His118 and His196, was changed by these mutations, which further decreased the enzyme's substrate specificity and activity. Mutant II showed slightly better efficacy at lowering functionality of the enzyme than mutant I, suggesting these mutations impair ADSL and could be targeted to inhibit pathogen survival.

Mycobacterium tuberculosis isocitrate lyase (MtbICL), an antituberculosis drug target, is a key enzyme in the glyoxylate cycle. The enzyme catalyzes the conversion of isocitrate into glyoxylate and succinate, aiding in carbon anaplerosis during the TCA cycle.[203] Shukla et al. investigated F345A, L418A, and H46A mutations in MtbICL(**Figure 5**), revealing significant impacts on enzyme structure and function despite their distance from the active site.[189 190 191] F345A and L418A mutations increased flexibility and disrupted structural stability, reducing enzyme activity. The H46A mutation (**Figure 5B**), though far from the active site, caused rigidity in the catalytic region, leading to a complete loss of activity. Principal component analysis (PCA) and cross-correlation analysis revealed that the H46A mutant enzyme had lost structural plasticity, resulting in decreased flexibility and increased rigidity in the catalytic region. Additionally, residue interaction network (RIN) analysis revealed that the mutation disturbed key residue interactions within the active site, further destabilizing its geometry. These findings highlight the importance of distal regions in maintaining enzyme function and suggest potential targets for anti-tuberculosis drug development.

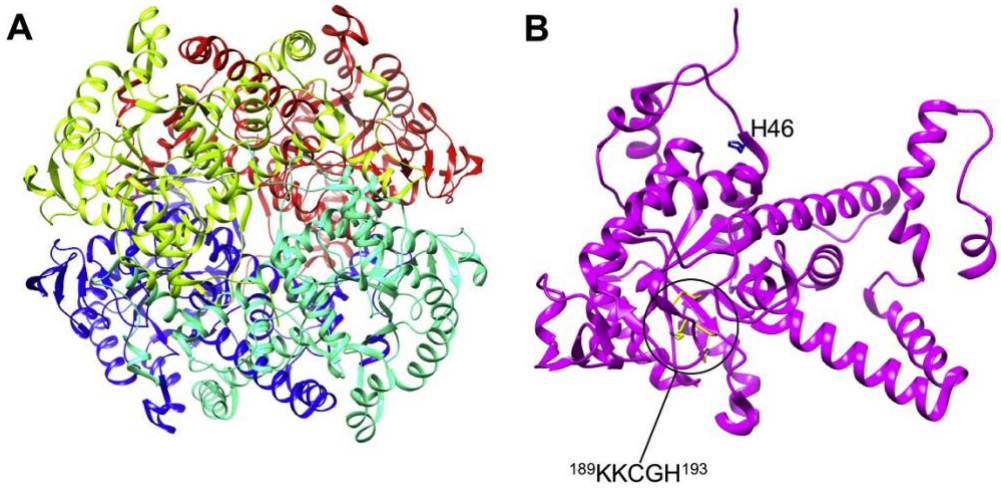


Figure 5**.** Structure of MtbICL. (A) Ribbon structure of the tetrameric MtbICL (PDB ID: 1F8I) with each subunit highlighted in a different color. (B) Chain A of MtbICL tetramer showing the position of His46 in blue stick and active site signature sequence (189KKCGH193) in yellow sticks. This figure has been reprinted, with permission, from reference (191), copyright (2018), Elsevier

Glyoxalase-1 (Glo-1) is an important enzyme in the Glyoxalase system that protects against dicarbonyl stress by detoxifying toxic byproducts like methylglyoxal (MGO).[204] Glo-1 is the major enzyme that neutralizes MGO. Lowered expression or activity of GLO1 has been seen to be associated with type 2 diabetes mellitus. Syed et al. investigated the structural impact of the R38Q missense mutation using molecular docking and MD simulations.[192] These simulations showed that the R38Q mutant protein had higher RMSD values, indicating reduced protein stability. The mutant also showed increased flexibility in specific residue locations and decreased compactness during simulation, as evidenced by the Rg analysis. Furthermore, hydrogen bond analysis revealed a loss of key interactions in the mutant, weakening ligand binding, which was further supported by MM/GBSA binding energy and center-of-mass (CoM) analyses. In conclusion, the R38Q mutation significantly lowers Glo-1 stability and compactness, affecting ligand-binding interactions and potentially contributing to disease through decreased enzyme function.

ISOMERASES

Isomerases (classified as EC 5) are a class of enzymes that catalyze the conversion of a molecule from one of its isomers to another. Isomers are a set of molecules that have the same molecular formula but differ in the orientation and position of the atoms, for example, glucose, fructose and galactose.[205, 206] They play a vital role in various metabolic pathways, such as carbohydrate metabolism, nucleotide biosynthesis, glycolysis and amino acid metabolism.[207] Mutations in isomerases lead to disruption in enzymatic functions. Deficiencies of isomerases due to certain mutations in the encoding gene can result in certain immune disorders, various autosomal disorders such as cardiac myopathy, chronic haemolytic anemia, among others, along with certain cancers and neurodegeneration.[208-210] Computational tools have been used to study various aspects of isomerases.[211-225] Some studies with a focus on disease-causing mutations are highlighted below.

Epimerases catalyze stereochemical changes within biological molecules and are involved in the metabolic breakdown of various amino acids and the conversion of galactose to glucose.[226] An important epimerase is UDP-galactose 4'-epimerase (GALE). This enzyme is responsible for galactose metabolism, and mutations in the epimerase gene lead to type III galactosemia.[227] McCorvie et al. [211] employed SNPeffect 4.0 server[228] to predict the biochemical effects of missense mutations on GALE. SNPeffect 4.0 server has been designed with four programs which has the ability to detect tendency to aggregate (TANGO)[229], form amyloids (WALTZ)[230], ability to bind chaperones (LIMBO), changes in stability (Fold-X)[231].They also implement I-Mutant 3.0[232] to detect the energetic change in experimental condition. These methods were applied to a set of

26 mutants, whose effects were unknown, of which three mutations (identified p.I14T, p.R184H and p.G302R) were seen to be severely impairing. Even though they found these mutations to structurally destabilize the system, it does not directly correlate to enzyme activity. They also suggest that mutations in the cofactor-substrate binding site may cause more disruption to enzyme activity.

Other important isomerases are the cis-trans isomerases, that catalyze the conversion between cis and trans isomers.[233, 234] These isomerases are important for protein folding, cell cycle progression and cancer regulation.[233, 235, 236] Rodriguez-Bussey et.al. investigated cyclophilin A (WT) and mutated analogues (V6I, V6T, V29L, and V29T), necessary for diverse biological processes and associated with various diseases using MD simulation.[215] The mutations perturbed active site dynamics along three distinct allosteric pathways, with two experimentally verified and one newly suggested by the investigators, complemented by residue-residue contact analysis. Additionally, a study by Wang et.al. on Protein interaction with NIMA1 (Pin1) enzyme investigated the effects of mutations on two conservative histidine residues through experiments and MD simulation.[215] It was observed that these mutations increased the flexibility of the PPIase domain, along with disrupting hydrogen bond networks in the protein system as observed from RMSD, RMSF, Rg, distance and hydrogen bond analysis.

Phosphoglucomutase (PGM) is an enzyme that catalyzes the transformation between glucose-1-phosphate and glucose-6-phosphate.[237, 238] Variants in PGM1 cause the inherited metabolic disease PGM1 deficiency, an autosomal recessive disorder that affects patients with hypoglycemia, hyperinsulinemia, heart, liver, and muscle pathologies. Gouliaev et al.studied the L516P mutation of PGM1[239]. Experimentally, they saw that this mutation causes reduced activity of the protein, most likely due to misfolding and proteasomal degradation. To further analyze such effects of mutations on PGM1, *in silico* techniques were employed on all possible single-site missense variants in human PGM1. A protein sequence alignment of 1468 distinct PGM1 orthologues was created, and then the Global Epistatic Model for predicting Mutational Effects (GEMME) [240] model was applied to it. This model identifies individual residues as well as residue pair conservation to determine variant effects and provides scores based on the effects of the mutations. This study revealed that neutral (WT-like) amino acid substitutions do not usually effect the stability of protein much, and have a score close to 0, The L516P variant was seen to have some negative effect on protein structure and function, with a score of -1.5. Furthermore, the Rapid Stability Prediction (RaSP) [241] model was utilized to predict the change in thermodynamic protein stability along with L516P, 18 other variants were compared for their stability to the WT.

The study showed that most of the SNPs to this protein were well tolerated, even though in most positions a proline substitution was detrimental to the stability of the protein. The L516P was also seen to be structurally unstable, conforming to the earlier claim. These results were further validated by comparative studies with previously submitted variant data in the gromAD database.

Currently 20 missense mutations of PGM1 have been characterized for PGM1 deficiency. They are either seen to affect the active site and catalytic efficiency or affect the protein folding and stability (**Figure 6**). Stiers et al. investigated a novel R422Q variant using different experimental procedure along with MD simulation. [217]The study revealed that this variant did not affect the catalytic activity but highly reduces the stability of the protein as observed from RMSD. Additionally, substrate binding to the variant further reduces protein stability, unlike the stabilizing effect seen in the WT. Another study by Stiers et al. [242] demonstrated the effects of eight mutations that are present in the highly conserved substrate-binding loop in domain 4 of PGM1 using experiments and MD simulation. In addition to RMSF, they utilized residence density, which represents a time-averaged distribution of atoms within a specific volumetric region throughout the simulation. It was observed that mutations at the R503 site and R515 site do not affect the protein structure directly but alter the flexible site of the D4 loop leading to enzyme disfunction. Investigation of the R503Q variant shows that the mutation causes conformational changes in the D4 loop, rendering it incapable of ligand binding. Other variants such as G506R, G511R, R515W and R515Q also have deleterious effects on PGM1, that were seen *in vitro*. Further computational analysis of these variants might provide more insight into their effects on the enzyme.

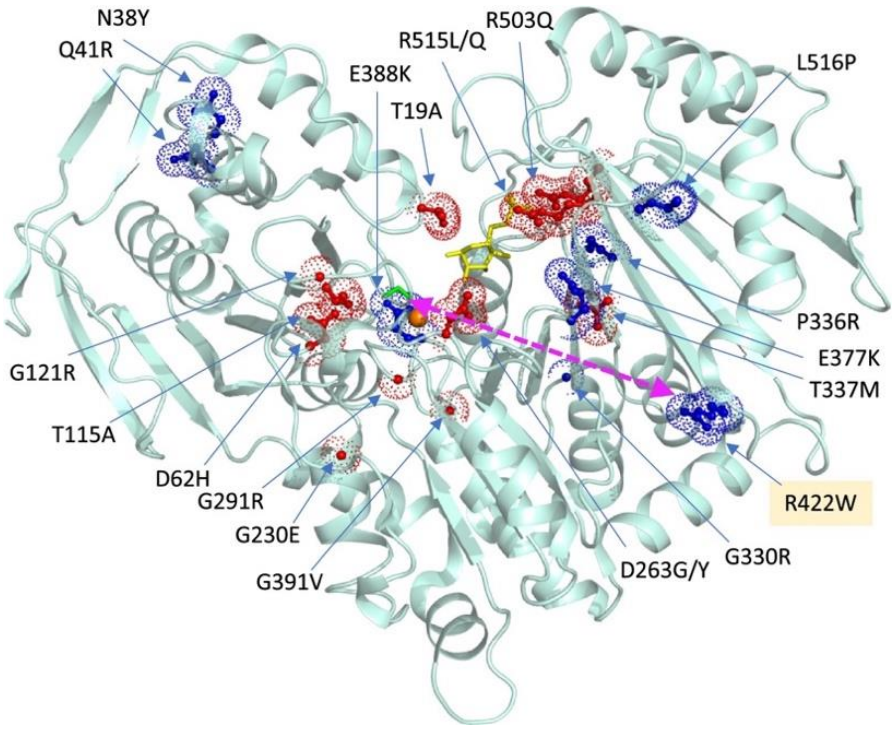


Figure 6. Crystal structure of human PGM1 highlighting carious missense mutations with different effects: red indicates effect on catalysis, whereas blue indicates effects on folding and/or stability. G6P is shown in yellow and Ser117 in green. The bound Mg^{2+} ion is shown as an orange sphere. This figure has been reprinted, with permission, from reference (217), copyright (2020), John Wiley and Sons

LIGASES

Ligases (classified as EC 6) are crucial enzymes that catalyze the formation of covalent bonds (e.g. C-N, C-O, C-S, phosphodiester etc.) between biomolecules, often using ATP for energy. They are classified into six subclasses based on the type of bond formed and are crucial to various biological applications such as DNA replication, repair and protein regulation, among others. Various computational studies have been done on ligases.[243-262] A few studies focusing on pathogenic mutations are highlighted below.

DNA ligases (LIG1, LIG2, LIG3, LIG4) catalyze the formation of phosphodiester bonds, which facilitate the joining of DNA strands. Both experimental and computational studies suggest that specific mutations (E566K, P529L, R641L, R771W, A624T) in LIG1 disrupt the enzyme's functionality, leading to diseases like leukopenia, neutropenia, lymphopenia, hypogamma, globulinemia, etc. Alajlan et al.[262] studied the A624T mutation on DNA LIG1 both experimentally and MD simulation using a coarse-grained force field. The superposition of protein structure dynamics between WT and A624T shows the difference in structural description mainly in three

loops (335–344, 640–646 and 853–865). They further computed residue interaction network (RIN) which predicts the connection among the protein residues and consequently build a network system based on covalent and non-covalent interactions (vander waals, H-bond, π-π etc). RIN analysis revealed the allosteric regulation in mutated system (missing in WT) associated with the LIG1 high-fidelity magnesium (335-344), as well as on DNA binding within the adenylation domain (640-646).

E3 ubiquitin ligases are crucial for determining substrate specificity and facilitating the transfer of ubiquitin from E2 enzymes to substrates in the ubiquitination system,.[254-256] While several studies have investigated mutations in E3 ligases, computational research has primarily focused on the BRCA1-BARD1 domain. The N-terminal RING domain of the Breast cancer type 1 susceptibility protein – BRCA1 Associated Ring domain (BRCA1-BARD1) heterodimer exhibits E3 ubiquitin ligase activity, aiding tumor suppression through DNA repair. During DNA damage, it localizes to the damaged site and transfers ubiquitin from the E2 enzyme (UBE2D3) to lysines 125, 127, and 129 on H2A, facilitating repair.[261, 263-266] Mutations in the RING domain of BRCA1-BARD1 abolish the interactions between them which subsequently hamper the E3 ubiquitin ligase activity increasing the chance of breast and ovarian cancers.[267-269]

Choudhury et al. investigated two cancer-predisposing mutations (A658C and I738V) in BARD1-BRCT using atomistic classical MD simulations.[246] They analyzed the trajectories of both WT and mutants through RMSD, RMSF, radius of gyration (R_g), SASA, hydrogen bond, covariance matrix principle component analysis (PCA) etc to predict the differences in dynamical structural change and flexibility of the systems. The I738V mutant showed greater flexibility and structural changes compared to the WT and R658C, leading to disruptions of interactions within the protein. It further suggested that the I738V mutation destabilizes the BARD1-BRCT domain, potentially affecting its stability and function. Further study examined mutations C645R, V695L, and S761N[244, 246, 251] in the BARD1-BRCT domain employing similar methodological approaches. These mutants showed greater structural shifts compared to the WT, with C645R and S761N causing local changes and V695L leading to global alterations. Hydrogen bonding patterns indicated reduced interactions for V695L and S761N as compared to WT and C645R. V695L displayed increased flexibility and differed significantly in interaction profiles as observed from MD analysis tools mentioned above.

Choudhury et al.‘s results suggested that substitution of valine, having extra –CH2– groups, in V695L increases flexibility while reducing stability and hindering functionality. Later they investigated the structural aspects of similar variants in BRCA1 and BARD1 domains to characterize the folding pattern of that region. Here also they adopted similar tools for trajectory

analysis which suggest that mutations don't perturb the structures significantly but exhibit region dependent flexibility. They predicted residue region 1798−180 of BRCA1 and 744 of BARD1 shows lower flexibility than WT. Their analysis concluded that the residues which are structurally aligned and identical between the two BRCT domains for WT and the mutants are important for functional characterization to reveal their potential role in health conditions and uncover their clinical significance.

A computational investigation by Kiewhuo et al.[247] analyzed sixteen BRCA1-BARD1 RING domain mutations to study their impact on the structure and function of the BRCA1-BARD1 heterodimer. They selected eleven BRCA1 mutations and five BARD1 mutations.[248-250] Along with RMSD, RMSF here they considered MM/PBSA to calculate the binding energy between two protein domains and how it is affected on mutations. Apart from that they used PIMA and PPcheck server to calculate the protein-protein interactions. They have also employed residue network analysis using NAPS and RING webservers to detect how mutation modulate the protein networks. They observed that the mutations caused local disruptions and higher structural fluctuations, particularly at Zn^{2+} binding sites and central helices, affecting heterodimer function. Although E3 ligase activity was impaired in some cases, the BRCA1-BARD1 dimer often remained intact and maintaining stability through hotspot residues (Trp34, Leu114 in BARD1 and BRCA1 Ile89) predicted by hotspot residue prediction servers. The study suggested that structural changes in mutant BRCA1 near the E2 binding site could hinder E2 interaction, reducing E3 ligase function and promoting cancer progression.[252] These results were consistent with experimental data showing that RING domain integrity is vital for E2 binding and ubiquitin transfer.

TRANSLOCASES

Translocases (classified as EC 7) are a class of enzymes that facilitate the transport of entities-such as ions, proteins, or other small compounds across cell membranes.[270-272] Translocases are further categorized into several subclasses based on the type of entities they transport and the mechanisms they employ. The translocation process is driven by reactions that are linked to oxidoreductase reactions, nucleoside triphosphate and diphosphate hydrolysis and decarboxylation reactions.[273] Translocases are essential for maintaining cellular homeostasis, metabolic regulation, and intracellular signaling. Many investigations on translocases employing diverse computational methods have shed light on how mutations influence different characteristics of these enzymes.[274-286]

ATPases[287, 288] are ATP-dependent transport proteins and are essential for various physiological processes.[289 290] Intracellular copper levels are regulated by Cu(I)-ATPases, [290]such as ATPase copper-transporting alpha (ATP7A) and ATPase copper-transporting beta (ATP7B).[291, 292] Mutations in these transporters can lead to copper imbalances, causing diseases like Menkes disease (copper deficiency) or Wilson disease (copper accumulation) in various tissues.[293-295] ATP7B protein is a transporter that loads Cu(I) onto newly formed cupro-enzymes in the trans-Golgi network (TGN) and removes excess copper by moving from TGN to the plasma membrane.

A study from Braiterman et al. [282] investigated seven mutations at the Ser653 site of ATP7B by varying the size, charge and the hydrophobicity. SASA calculations showed that S653 is buried within transmembrane (TM) segments. S653 in ATP7B was found to form a hydrogen bond with G710, and SWISS-MODEL structural bioinformatics server predicted that S653A, S653C, S653T mutations have minor structural changes while S653Y mutation causes the Y653 side chain to rotate outward, increasing SASA and disrupting the hydrogen bond with G710. Also, S653Y mutation decreased the exposure of neighboring residue Y713 in TM2 than WT. S653F had similar but less pronounced effect on Y713. Furthermore, they calculated electrostatic potential of the structures using adaptive Poisson-Boltzmann surface analysis (APBS) and identified a pocket near TM1 S653, TM2 G710 and TM2 helix. This pocket was less accessible with more neutral surface charges for S653Y. S653E and S653D caused significant changes in surface charge near the pocket while D653 increased negatively charged area and 653E showed less strong negative charge suggesting it is deeply buried within the protein. MD simulations using the generalized-born simple-switching approach and SASA calculations indicated that bulky substitutions and negative charge at Ser653 cause local distortions, which alter interaction between TM1 and TM2 and prevented the proteins from exiting the TGN. These findings were consistent with the experimental mutational analysis.

ABCB1 is one of the ATP binding cassette (ABC) genes which plays a key role in cellular homeostasis. [296, 297] Various immunosuppressive drugs have been introduced over the past two decades to ensure sustained graft survival for renal transplantation, among which Tacrolimus (TAC) is the most widely used and least toxic.[298, 299] Mallina et al.[283] conducted a study to investigate the effect of gene polymorphisms (S893T) in ABCB1 on the toxicity of TAC in renal transplantation patients. They studied the effect of the variant on protein stability using seven stability analysis tools including i- Mutant2.0[232], SDM[300], iStable[144], mCSM[301], MUpro[302], DUET[303], and ENCoM[304]. These tools calculated ΔΔG values to determine whether the variant stabilizes or destabilizes the protein. All tools, except i- Mutant2.0, predicted that the mutant destabilizes the

ABCB1 protein. Combining stability and amino acid interaction analysis, it was found that S893T reduces the flexibility of the protein.

The mitochondrial pyruvate carrier (MPC) protein is essential for transporting pyruvate, a key metabolic intermediate, from the intermembrane space into the mitochondrial matrix, where it enters the citric acid cycle.[305] Mutations in human MPC1 are associated with cancer, heart failure and neurodegeneration.[306] Xu et al.[286] developed de novo models of human MPC complexes, and the conformational dynamics of the MPC heterodimer (MPC1/2) were explored. Several distances of paired residues between MPC1 and MPC2 were calculated in WT and mutant systems to assess the conformational dynamics of the MPC1/2 heterodimer (**Figure 7**). The open or close conformation of the outward-facing side of MPC1/2 was determined by the distance between P48 of MPC1 and P63 of MPC2 and the inward-facing side of MPC1/2 was assessed by the distance between P75 of MPC1 and P91 of MPC2. A salt bridge was expected between D43 of MPC1 and R62 of MPC2 when there is minimal distance < 3.0 through the simulation. Another distance was calculated between two bulky hydrophobic residues which act as gating residues, Y62 of MPC1 and W82 of MPC2 to characterize the open or close conformation of the central path in MPC1/2. The results show that the functional MPC1/2 complex in WT prefers an inward-open conformation, with the carrier open toward the matrix, while outward-open states are less common. Consistent with experimental data, the MPC1 L79H mutation significantly disrupts the conformation of MPC1/2, impairing substrate transport, whereas the MPC1 R97W mutation retains transport activity.

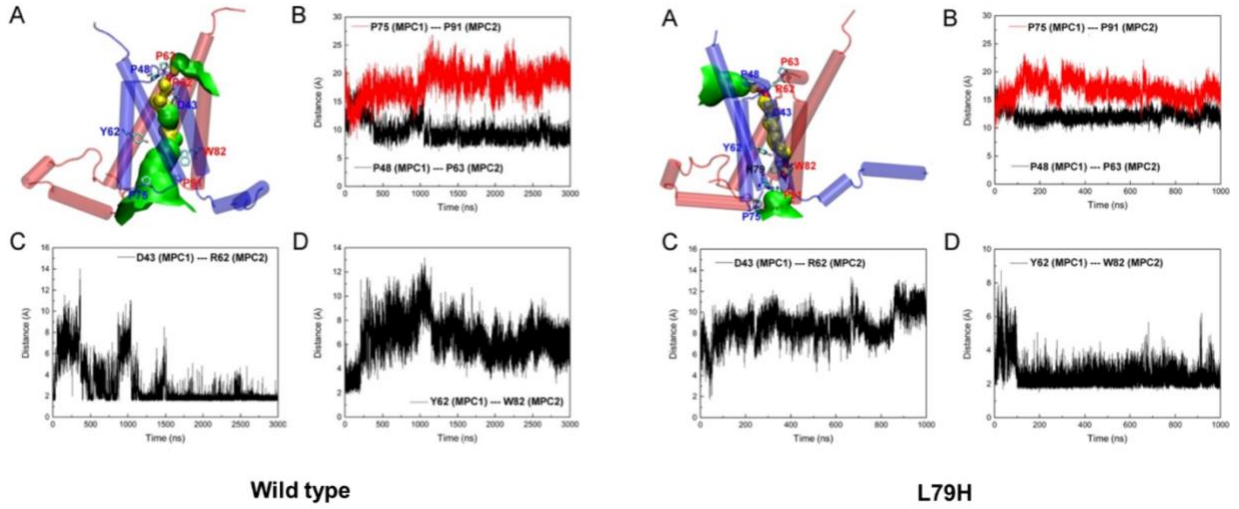


Figure 7. (A) Conformation representation of the MPC1/2 heterodimer in an inward-open state. Blue represents MPC1 and relevant residues, and red represents MPC2 and relevant residues. (B) Distance analysis over simulations between Pro48 of MPC1 and Pro63 of MPC2 and between Pro75 of MPC1 and Pro91 of MPC2. (C) Distance analysis between Asp43 of MPC1 and Arg62 of MPC2 over simulations. (D)

Distance analysis between gating residues Tyr62 of MPC1 and Trp82 of MPC2. This figure has been reprinted, with permission, from reference (286), copyright (2021), American Chemical Society

SUMMARY AND PERSPECTIVE

Computational analysis of disease-associated mutations in proteins has emerged as a powerful tool in understanding the molecular mechanisms that underlie the functional, structural and/or dynamical impacts of these mutations. Through methods such as molecular dynamics simulations, protein structure modeling, catalytic studies, and bioinformatics tools, researchers can predict how mutations affect enzyme stability, dynamics, and activity. These computational techniques allow for the identification of critical regions within enzyme structures that are particularly susceptible to mutations, providing valuable insights into how structural and functional alterations disrupt enzyme functionality. Such computational approaches are not only complementary to experimental studies but also offer atomistic detail that experimental methods might not capture. Moreover, they enable large-scale mutation screening, making these methods both efficient and cost-effective for exploring a wide variety of mutations.

Advances in bioinformatics and machine learning-based approaches, have further accelerated the prediction and analysis of enzyme mutations, enhancing our ability to anticipate their effects on catalysis, binding affinity, and overall stability. This integration of machine learning and computational tools provides a framework for identifying potential therapeutic targets, which is crucial for drug discovery and mutagenesis studies. Additionally, hybrid QM/MM methods provide deeper insights into the chemical and catalytic processes, improving the balance between computational efficiency and accuracy. These advancements allow researchers to explore the consequences of mutations not only on enzyme dynamics but also on their catalytic pathways and substrate interactions at a much finer resolution.

In addition to studying mutations, computational tools have also been effectively applied to explore other protein properties, such as substrate specificity, cofactor interactions, long-range allosteric effects, to name a few. This opens new avenues for research into enzyme engineering and the development of new biocatalysts, as well as applications in synthetic biology and industrial enzyme design. The continued refinement of these computational approaches, combined with experimental validation, holds great promise for advancing our understanding of protein-related diseases and their potential treatments.

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