Probing the potentials of γ-globin genes for Hemoglobinopathies (Sickle Cell Anemia)

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Dedication

I dedicate this thesis to my beloved mother, **Nasreen Akhtar**, whose boundless love and sacrifices have been my source of strength. To my father, **Muhammad Anwar**, whose unwavering support and guidance have shaped my academic journey. To my brothers, **Muhammad Usama** and **Muhammad Haseeb**, for their encouragement and understanding during challenging times. Your belief in my abilities has been a source of inspiration, and I am grateful for your guidance. This work stands as a testament to the love, support, and inspiration I have received from each of you. Thank you for being my pillars of strength.

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Contents

Abstract 1
1. Introduction 3
1.1 Hemoglobinopathies 3
1.2 Sickle Cell Anemia 3
1.2.1 Symptoms of Sickle Cell Anemia **5**
1.3 Treatment 7
1.4 Challenges in curing Sickle Cell anemia 8
1.5 Adult Hemoglobin 9
1.6 Mutant (Sickled) Hemoglobin 11
1.7 Fetal Hemoglobin 13
1.8 Adults vs Fetal Hemoglobin 14
1.9 Objective 17
1.10 Problem Statement 18
2. Literature Review 21
2.1 Emergence of sickle cell anemia 21
2.2 Genetic Basis of Sickle Cell Anemia 22
2.3 Polymerization GLU6VAL 23
2.4 Hereditary Persistence of Fetal Hemoglobin (HPFH) 24
3. Methodology 27
3.1 Step 1: Collection of Protein structure 27
3.2 Step 2: Structure Preparation 29
3.2.1 Heme removal 30
3.2.2 Energy Minimization 30
3.3 Step 3: Molecular Dynamics Simulations 31
3.3.1 System Preparation 31
3.3.2 Force Field Selection 32
3.3.3 Energy Minimization 32
3.3.4 Equilibration 33
3.3.5 Production MD run 33
3.3.6 Analysis 34
4. Results 36

4.1 RMSD Graphs of Adult, Fetal, and Sickled Hemoglobin showing the stability of the structures 36
4.2 RMSF graphs Adult, Fetal, and Sickled Hemoglobin showing the stability of the structures. 40
4.3 Radius of Gyration of Adult Hemoglobin showing the compactness of the structure..... 46
4.4 Hydrogen Bonds Analysis of Fetal, Adult, and Sickled Hemoglobin 47
4.5 Different Conformation of Structures of HBA, HBF, HBS after MD simulation at different time frames 53
5. Discussion..... 56
6. Conclusion 60
7. References..... 63

List of Figures

Figure 1. Adult Hemoglobin structure having 4 chains along with Heme group 10

Figure 2. shows the conversion of Glutamic acid into Valine and then Round Red blood cells into sickle-shaped Hemoglobin [47]. 12

Figure 3. Beta Globin gene cluster on chromosome 11 [52]. 13

Figure 4. PDB ID 1FDH: shows the alpha and gamma subunits of fetal hemoglobin 15

Figure 5. PDB ID 4HHB: Shows the alpha(red) and beta(yellow) subunits of Adult hemoglobin 16

Figure 6. shows sickled hemoglobin cells disrupting the flow of Normal hemoglobin cells. Sickle cells are abnormally shaped erythrocytes that have the potential to obstruct the circulation of blood. Typical red blood cells are spherical and pliable, enabling them to smoothly circulate throughout blood arteries. The sickling of red blood cells may lead to vaso-occlusive crises, characterized by periods of pain, tissue damage, and organ failure [84]. 22

Figure 7. Sickle cell genotypes alter hemoglobin and red blood cells. Sickle cell trait, a disorder characterized by heterozygosity for the normal β-globin and βS genes, is often asymptomatic. HbS generation results from compound heterozygosity of βS and other β allele mutations. Under hypoxia, HbS polymerizes into stiff fibers within red blood cells (RBC) due to its decreased O2 affinity. Distortion, stiffness, and fragility from HbS polymerization cause RBC sickling. Initial sickling is reversible, alternating oxygenation and deoxygenation. Chronic oxy–deoxy cycles cause permanently sickled RBCs, a characteristic of sickle cell disease [91]. 23

Figure 8. The Crystal structure of 1FDH (Fetal Hemoglobin, HbF) retrieved from RCSB PDB [108]. 27

Figure 9. The Crystal structure of 1Si4 (Adult Hemoglobin, HbA) retrieved from RCSB PDB [111]. 28

Figure 10. shows the Crystal structure of 2HBS retrieved from RCSB PDB [113] 29

Figure 11. Workflow of Molecular Dynamics Simulation: From system setup and force field application to energy minimization, equilibration, and the production MD run, depicting the dynamic behavior of bimolecular systems at the atomic level over time. 31

Figure 12. RMSD plot of Adult, Fetal, and Sickled Hemoglobin structures: comparison of the stability of these structures over 200ns time for MD Simulation. 36
Figure 13. Root Mean Square Deviation graph of Adult Hemoglobin showing the stability of the structure..... 37
Figure 14. RMSD plot of fetal Hemoglobin HBF showing the stability of the structure. 38
Figure 15. RMSD plot of Mutant Hemoglobin Structure (Sickled Hemoglobin). 39
Figure 16. RMSF plot of Adult Hemoglobin HBA along with interactions of chain A(alpha) with Chain B (beta) 40
Figure 17. RMSF plot of fetal hemoglobin HBF along with interactions of Chain A(alpha) with Chain B (gamma) 41
Figure 18. The Root Mean Square Deviation of the mutant structure of Hemoglobin (HBS) along with Interactions between Chain A (alpha) and Chain B(Beta) 43
Figure 19. The graph depicts a progressive reduction in the radius of gyration of hemoglobin, indicating the unfolding of the protein. Rgx and Rgy represent elongation along the x and y axes, while Roz implies enhanced compactness along the z-axis..... 46
Figure 20. Figures a, b, c, d, e, and f depict distinct hydrogen bond interactions present in the structure of Adult Hemoglobin. Each subfigure specifically emphasizes a separate hydrogen bond, showcasing the Distances between the residues of chain A and chain B. 48
Figure 21. Figures a, b, c, d, e, and f depict distinct hydrogen bond interactions present in the structure of Mutant Hemoglobin (HBS). Each subfigure specifically emphasizes a separate hydrogen bond, showcasing the Distances between the residues of chain A and chain B. 49
Figure 22. Figure 20 Figures a, b, c, d, e, and f depict distinct hydrogen bond interactions present in the structure of Fetal Hemoglobin. Each subfigure specifically emphasizes a separate hydrogen bond, showcasing the Distances between the residues of chain 51
Figure 23. Shown are the dynamic structures of normal hemoglobin (HbA) and sickle cell hemoglobin (HbS) extracted at various time frames during Molecular Dynamics simulations. From the initial static configurations to stable states at the simulation's conclusion. 54

List of Tables

Table 1. Amino acid's one and three-letter codes 1

Table 2. Previous treatments are available for the treatment of SCD along with their limitations.7

Table 3. Represents the Residues of Chain A of Adult Hemoglobin that are forming Hydrogen bonds with Chain Residues of Chain B along with their Molecular Distance..... 47

Table 4. Represents the Residues of Chain A of Mutant Hemoglobin that are forming Hydrogen bonds with Chain Residues of Chain B along with their Molecular Distance..... 49

Table 5. Represents the Residues of Chain A of Fetal Hemoglobin that are forming Hydrogen bonds with Chain Residues of Chain B along with their Molecular Distance..... 50

Abbreviations

EM	Energy Minimization
Fe2+	Iron ion
HB	Hemoglobin
HBA	Hemoglobin Adult
HBB	Hemoglobin Sub Unit Beta
HBF	Hemoglobin Fetal
HBG	Hemoglobin Sub Unit gamma
HBS	Hemoglobin Sickled
HPFH	Hereditary persistence of fetal hemoglobin
LCR	Locus Control Region
MD	Molecular Dynamics
Ns	Nano second
NSAID	Non-steroidal anti-inflammatory drugs
PBD	Protein Data Bank
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
Rg	Radius of Gyration
SCD	Sickle Cell Disease
γ –globin	gamma globin

Table 1. Amino acids one and three-letter codes

Amino acid	Three letter code	One letter code
Alanine	ala	A
Arginine	arg	R
Asparagine	asn	N
Aspartic acid	asp	D
Cysteine	cys	C
Glutamic acid	glu	E
Glutamine	gln	Q
Glycine	gly	G
Histidine	his	H
Isoleucine	ile	I
Leucine	leu	L
Lysine	lys	K
Methionine	met	M
Phenylalanine	phe	F
Proline	pro	P
Serine	ser	S
Threonine	thr	T
Tryptophan	trp	W
Tyrosine	tyr	Y
Valine	val	V

Abstract

Hemoglobinopathies are a cluster of hereditary disorders that impact the structure and functionality of hemoglobin. These illnesses include a broad spectrum of irregularities, with sickle cell anemia being one of the more well-known forms. Sickle cell anemia arises from a genetic mutation GLU6VAL in the HBB gene that results in the synthesis of abnormal hemoglobin (HbS) and the characteristic deformation of red blood cells into a sickle shape. Sickle-shaped cells then ultimately block the flow of normal blood cells causing anemia, organ damage, pain crises, and other infections that lead to reduced life expectancy. The treatment of sickle cell anemia is complex because of its genetic origin, needing precise treatments that address the underlying mutation in the HBB gene.

The research used Molecular Dynamics simulations to investigate the molecular complexities of normal and fetal hemoglobin, to discover crucial interaction patterns that contribute to their anti-sickling abilities. Thus, the objective is to probe the binding pattern and the stability of the alpha-gamma chain complex of the fetal hemoglobin for the modulation of the beta chain in adults to rescue the mutated function of sickled hemoglobin.

The study used a Structural Bioinformatics approach to conduct Molecular Dynamics (MD) simulations for 200ns, aiming to examine the dynamic interactions occurring inside the alpha and beta chains of both wild-type sickled and normal hemoglobin Furthermore, the scope of the investigation was broadened to examine the patterns of alpha-gamma interaction within fetal hemoglobin.

The findings of the MD simulations not only enhanced understanding of the structural dynamics but also provided crucial insights into the modified interactions that underlie the manifestation of sickle cell anemia. Studying the alpha-gamma patterns in fetal hemoglobin has enhanced knowledge of the molecular characteristics associated with hemoglobinopathies. Thus, the identification of interaction patterns not only demonstrates the potential of computational methods in addressing complex genetic diseases at the molecular level but also offers a practical pathway for the identification of therapeutic target

Chapter 1
Introduction

1. Introduction

1.1 Hemoglobinopathies

This chapter provides an introduction to the complex research domain of hemoglobinopathies, which are a collection of genetic illnesses that impact the functioning of hemoglobin. Additionally, it establishes the foundation for exploring the possible therapeutic applications of γ-globin genes.

Hemoglobinopathies are hereditary conditions that either impair the structure of the hemoglobin molecule or the synthesis of it [1]. Hemoglobin molecules are made up of polypeptide chains, and the genetic code governs the chemical structure of these chains. Although different hemoglobin molecules are designated alphabetically in order of discovery (for example, A, B, and C), the first abnormal hemoglobin to be discovered, sickle cell hemoglobin, was given the name hemoglobin S[2]. This was because sickle cell hemoglobin was distinguished from other hemoglobin molecules by its electrophoretic mobility. The electrophoretic mobility of hemoglobin molecules is a technique utilized for determining both the speed and distance of their movement under the influence of an electric field in a given sample [3]. Employing this knowledge can assist experts in determining the underlying cause of the blood's condition.

Hemoglobinopathies refer to a heterogeneous collection of hereditary illnesses that give rise to various blood disorders. These conditions arise from abnormalities in the structure, production, or function of hemoglobin, the key protein involved in the transportation of oxygen within red blood cells. Researchers worldwide have shown significant interest in studying illnesses such as thalassemia, hemoglobin C disease, and hemoglobin D disorders, owing to their clinical importance and intricate genetic characteristics [4]. Within the complex realm of hematological illnesses, the primary emphasis of this research endeavor is centered on a disorder of notable significance: **sickle cell anemia**.

1.2 Sickle Cell Anemia

Sickle cell anemia has been shown to disrupt the movement of hemoglobin molecules within the bloodstream in various manners. The typical adult hemoglobin molecule, often known as Hb A, comprises two pairs of chains labeled alpha and beta, respectively [5]. Additionally, in a study, it was described that normal adult blood contains less than 1.4% hemoglobin F (fetal hemoglobin,

consisting of alpha and gamma chains; and less than 2.5% hemoglobin A2 (which are made up of alpha and delta chains) [6].

Sickle cell anemia is an inherited blood illness caused by a particular genetic mutation in the HBB gene, which causes the production of defective hemoglobin called hemoglobin S (HbS). This genetic aberration exhibits autosomal recessive inheritance, necessitating the acquisition of a mutant gene from both parents for the disorder to manifest. Hemoglobin is an essential protein found in red blood cells that plays a vital role in the transportation of oxygen throughout the body [7]. In sickle cell anemia, the modified structure of HbS leads to the red blood cells adopting a unique sickle or crescent shape. Contrary to regular, pliable disc-shaped red blood cells, sickle cells are inflexible and have less ability to traverse the circulatory system. An important result of this atypical form is the heightened probability of these sickle cells being trapped in narrow blood channels, causing obstructions and hindered blood circulation. This tendency is most evident at times of stress, dehydration, or hypoxia, which leads to vaso-occlusive crises [8]. These distressing occurrences, referred to as crises, may manifest in different regions of the body and are a distinctive characteristic of sickle cell anemia. The structural anomalies of sickle cells also lead to a reduced lifetime for these cells, resulting in hemolysis - the disintegration of red blood cells. Anemia is a disorder where the blood's capacity to transport oxygen is decreased due to the ongoing breakdown of red blood cells. Hence, persons afflicted with sickle cell anemia often encounter symptoms such as exhaustion, debility, and paleness. In addition to the difficulties related to anemia and vaso-occlusive crises, sickle cell anemia may lead to a range of consequences. Organ damage is a notable worry, impacting vital organs such as the spleen, liver, kidneys, and lungs [9]. Moreover, the heightened vulnerability to infections, especially in youngsters, is a noteworthy characteristic of the illness. Individuals diagnosed with sickle cell anemia have a heightened susceptibility to experiencing strokes, acute chest syndrome, and stunted development in juvenile instances. The management of sickle cell anemia includes the mitigation of symptoms, the prevention of complications, and the enhancement of overall quality of life. Presently, the available treatments include pain control, the administration of Hydroxyurea to increase the formation of fetal hemoglobin, blood transfusions, bone marrow transplants, and new therapeutics like gene editing [10]. Although a cure for sickle cell anemia does not yet exist, continuous research is investigating novel methods to provide more efficient and specific therapies for this intricate hereditary illness.

1.2.1 Symptoms of Sickle Cell Anemia

Vaso-occlusive crisis: often known as a pain crisis, is a medical condition characterized by severe pain. Pain crises are a prominent characteristic of sickle cell anemia [11]. Sickle-shaped erythrocytes have the potential to obstruct narrow blood arteries, resulting in blockages and severe discomfort [12].

Location: Pain may manifest in several anatomical regions, including the belly, chest, joints, and bones.

Duration: Crises may range from a few hours to many days.

Fatigue: The persistent scarcity of red blood cells, caused by their short lifetime, results in a diminished ability to transport oxygen, leading to feelings of exhaustion and weakness [13].

Symptoms: Individuals may exhibit asthenia, torpor, and a general state of fatigue [14].

Anemia: Anemia is a medical condition characterized by a deficiency of red blood cells or hemoglobin in the blood, resulting in reduced oxygen-carrying capacity and potential symptoms [8]. Sickle cell anemia is a condition marked by persistent anemia caused by the breakdown of red blood cells [15]. Anemia is characterized by symptoms such as pallor, dyspnea, tachycardia, and weariness.

Frequent infections: Sickle cell anemia may compromise the immune system, rendering patients more vulnerable to bacterial infections [16]. Infections affect the respiratory system, urinary tract, and other bacterial illnesses.

Organ damage: The spleen has a vital function in the filtration of aged or impaired red blood cells and the elimination of certain microorganisms from the circulatory system [17]. Sickle cell anemia involves the occurrence of sickle-shaped red blood cells that may become stuck in the spleen, causing blockages in blood arteries and reduced functionality.

The liver participates in several metabolic processes, such as the degradation of hemoglobin derived from aged erythrocytes [18]. Sickle cells can impede the circulation of blood in the tiny blood veins of the liver. Hepatic Sequestration refers to the buildup of sickle cells in the liver, resulting in the enlargement of the organ. Impaired Liver Function: The decreased capacity to carry out typical metabolic functions [19].

The lungs enable the process of oxygen and carbon dioxide exchange. Sickle cells have the potential to obstruct the pulmonary blood arteries. Pulmonary infarctions refer to the condition when there is a decrease in blood supply to specific areas of the lungs, resulting in harm to the surrounding tissue [20]. Acute Chest Syndrome refers to the inflammation and blockage of blood arteries in the lungs, which is a severe consequence linked to sickle cell anemia. Optimal blood circulation to the brain is crucial for neurological functioning. The presence of sickle cells may result in the obstruction of blood arteries, potentially causing strokes or other neurological issues in the brain [21]. Cerebral infarctions refer to the condition when there is a decrease in blood supply to certain areas of the brain, which may lead to the development of strokes or cognitive impairment.

1.3 Treatment

Table 2. Previous treatments are available for the treatment of SCD along with their limitations.

Treatment	Purpose and Advantages	Usage/indications/complications
Analgesics	Alleviate mild to severe discomfort during vaso-occlusive crises	Acetaminophen or NSAIDs (e.g., ibuprofen) for milder pain; opioids for more intense pain.
Hydroxyurea	Enhances synthesis of fetal hemoglobin, reducing susceptibility to sickling.	Orally administered; Diminishes frequency of painful episodes, acute chest syndrome, and need for blood transfusions.
Hematological transfusions	Augment quantity of healthy red blood cells; enhance oxygen transportation.	Used in severe cases (acute chest syndrome, stroke, severe anemia); Complication: Iron accumulation may require chelation treatment.
Hematopoietic Stem Cell Transplantation	Substitution of unhealthy bone marrow with a healthy donor's marrow.	Potential for complete recovery; Limited by donor availability and possible adverse outcomes.
L-Glutamine Oral Powder (Endari)	Decreases frequency of pain crises.	Adjunctive therapy; Used with other modalities.
Penicillin and Vaccinations	Daily penicillin prophylaxis to prevent bacterial infections; Regular immunizations for influenza, pneumonia, and meningitis.	Children with sickle cell anemia are advised; Essential for preventing illnesses.
Supplementing with Folic Acid	Crucial for promoting erythropoiesis and replenishing depleted folate.	A standard part of treatment
Treatment for Pneumococcal infections	Antibiotics recommended in certain circumstances to prevent pneumococcal infections.	Especially in children with sickle cell anemia.

1.4 Challenges in curing Sickle Cell anemia

Sickle cell anemia is a multifaceted genetic illness that arises from a mutation in the hemoglobin gene, leading to the synthesis of defective hemoglobin referred to as hemoglobin S. The primary etiology of the illness is the morphological distortion of erythrocytes, resulting in their increased rigidity and acquisition of a distinctive sickle-like form [22]. Although there are several therapies to address symptoms, avoid complications, and enhance the well-being of those with sickle cell anemia, achieving a complete cure is still difficult due to numerous obstacles.

Genetic Foundation

Sickle cell anemia arises from a genetic mutation, and the fundamental genetic flaw cannot be rectified or eradicated by current therapeutic interventions [23]. Although bone marrow transplantation holds promise as a possible remedy, it is an intricate surgery with constraints, such as the challenge of locating a compatible donor.

Complex Pathophysiology

The pathogenesis of sickle cell anemia is complex, including the aberrant morphology of red blood cells, inflammation, compromised blood circulation, and diverse consequences impacting different organs [24]. Simultaneously addressing all parts of the condition is a substantial difficulty [25].

Variability in Disease Manifestation

Sickle cell anemia exhibits a diverse range of symptoms and sequelae, with the disease's intensity differing across people [26]. Attaining a universally effective remedy that may effectively treat the various clinical presentations of the illness is intrinsically intricate.

Challenges in the field of gene therapy

Although gene therapy has the potential to treat hereditary illnesses, such as sickle cell anemia, the development of efficient and secure gene treatments remains an active field of study. Challenges include the precise delivery of therapeutic genes to specific cells, limiting unintended effects on non-target cells, and guaranteeing sustained efficacy over an extended period [27].

Complexities and Concurrent Conditions

Individuals afflicted with sickle cell anemia may encounter problems such as organ impairment, infections, and episodes of intense pain [28]. These consequences may need precise and focused therapies that go beyond just treating the underlying cause of the illness.

Scarcity of Bone Marrow Donors

The availability of appropriate donors is a limitation to bone marrow transplantation, a possible curative treatment for sickle cell anemia. Locating a compatible donor might pose difficulties, especially for persons with varied ethnic origins [29].

Ethical and safety factors

Several prospective treatments, such as gene therapies, are now in the experimental phase. The process of ensuring the safety, effectiveness, and ethical concerns of medical intervention before its broad acceptance in clinical practice is a multifaceted undertaking [23].

Expense and Availability

Expensive and potentially inaccessible, advanced treatments like gene therapy and bone marrow transplantation may not be available to all persons suffering from sickle cell anemia [30]. It is essential to tackle these inequalities in healthcare access to provide complete illness treatment. Continued research is crucial to provide new and more precise treatments for sickle cell anemia, which may greatly enhance the well-being of affected persons. Progress in gene editing technologies, current clinical studies, and an enhanced understanding of the molecular causes of the illness may facilitate future advancements in the pursuit of a remedy.

1.5 Adult Hemoglobin

Hemoglobin, an essential protein present in red blood cells, has a crucial function in the transportation and distribution of oxygen throughout the human body. Hemoglobin A (HbA) is the primary and typical type of hemoglobin found in adults. The hemoglobin, which is structurally well-balanced, permits the effective transportation of oxygen, hence preserving the health and functioning of tissues and organs. Hemoglobin A (HbA) is the predominant form of hemoglobin in adults, accounting for about 95-98% of the total [31]. It is made up of four subunits, consisting of two alpha-globin chains and two beta-globin chains. This anatomical arrangement enables the

most effective binding and transportation of oxygen, guaranteeing the proper operation of tissues and organs throughout the body. The high occurrence of HbA highlights its essential function in the adult cardiovascular system. In addition to HbA, the range of normal hemoglobin variations includes HbA2 and HbF (fetal hemoglobin) [32]. HbA2, which constitutes around 2-3% of the total hemoglobin, appears as a minor form consisting of two alpha-globin and two delta-globin chains. Although its specific physiological role is still being studied, its classification as a normal variation highlights the natural diversity within the adult hemoglobin range [33].

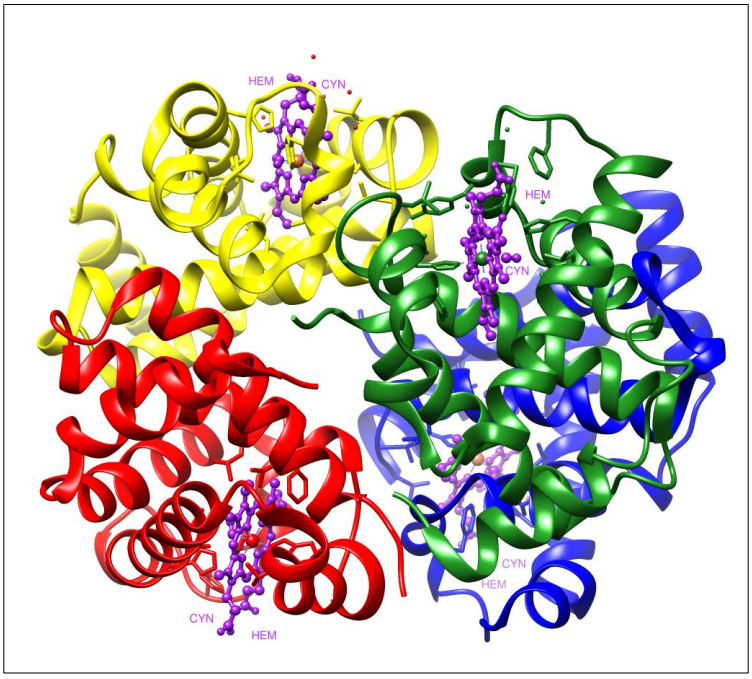


Figure 1. Adult Hemoglobin structure having 4 chains along with Heme group

1.5.1 Heme in Hemoglobin

The essential heme group is located at the core of the complex machinery responsible for oxygen transportation in red blood cells. The heme group, an intricate molecular structure located inside the globin chains of mature hemoglobin, has a crucial function in the attachment and release of oxygen, therefore coordinating the rhythmic process of life-supporting gas exchange [34]. The heme group is a coordination complex consisting of a core iron ion (Fe^{2+}) that is delicately coordinated inside a porphyrin ring. The structural arrangement of heme gives it the extraordinary capacity to bind and reversibly release oxygen molecules. The iron ion located in the heme group plays a crucial role in this process, undergoing a transition between the ferrous (Fe^{2+}) and ferric

(Fe^{3+}) states as it binds and releases oxygen in an alternating manner [35]. The process of hemoglobin binding oxygen in a cooperative manner, which is made possible by the heme groups, is an impressive example of molecular dance. When an oxygen molecule attaches to a heme group, it causes a structural alteration in the hemoglobin molecule, increasing its ability to connect with more oxygen molecules[36]. This synergistic phenomenon guarantees the effective uptake of oxygen in the lungs, where oxygen concentration is abundant, and its prompt release in oxygen-deficient organs. The distinctive electrical arrangement of the iron ion in the heme group is crucial for its ability to bind oxygen. The presence of a conjugated system of double bonds in the porphyrin ring enables the stability of the iron-oxygen combination [37]. The complex movement of electrons guarantees the reversible attachment of oxygen, avoiding irreversible oxidation and guaranteeing the uninterrupted flow of oxygen throughout the circulatory system. Crucially, the heme group does not only act as a passive participant in the transportation of oxygen. It is highly calibrated to adapt to changes in environmental conditions, such as pH and carbon dioxide levels, adjusting its ability to bind with oxygen to fulfill the ever-changing needs of the body[38]. The Bohr effect, which is characterized by the augmentation of oxygen release due to increasing acidity (lower pH) and greater carbon dioxide levels, highlights the heme group's ability to adapt in response to physiological signals [39].

1.6 Mutant (Sickled) Hemoglobin

Sickled hemoglobin is an altered variant of the typical adult hemoglobin (hemoglobin A). The genetic aberration causing this metamorphosis arises from a mutation in the HBB gene, situated on chromosome 11, where a single nucleotide change replaces adenine with thymine [40]. This minor modification leads to the replacement of glutamic acid with valine at the sixth position of the beta-globin chain, significantly altering the nature of the hemoglobin molecule. The molecular architecture of hemoglobin is significantly impacted by this amino acid alteration, resulting in far-reaching repercussions [41]. The modified hemoglobin S demonstrates a tendency to undergo polymerization, resulting in the development of lengthy chains that initiate the conversion of red blood cells into the distinctive sickle shape. Sickled red blood cells have a hard and twisted structure, in contrast to the flexible and biconcave shape of normal red blood cells. This alteration hinders their ability to move smoothly through blood arteries [42]. The ramifications of sickled hemoglobin extend beyond its influence on the morphology of erythrocytes [43]. The polymerization process generates a cascade of events that leads to vaso-occlusive crises,

characterized by intense pain and tissue destruction. These crises occur because of the obstruction of narrow blood channels by the inflexible, sickle-shaped cells, resulting in reduced blood circulation and insufficient oxygen supply to essential organs [44]. Furthermore, the decreased lifetime of sickled red blood cells worsens chronic anemia, leading to tiredness, paleness, and a decrease in the ability to transport oxygen. The unique characteristics of sickled hemoglobin make persons who have it more prone to a range of consequences, such as organ damage, heightened risk of infections, strokes, and greater susceptibility to environmental stresses [45]. Comprehending the intricate molecular details of sickled hemoglobin is not only an intellectual quest; it reveals the fundamental basis of sickle cell anemia, a disorder that significantly affects the lives of those who have it [46] [41]. The complicated interaction between genetics, molecular biology, and clinical symptoms is shown by the precise movement of amino acids inside the hemoglobin molecule. This provides a pathway to understanding and intervening in this problematic blood condition. As we begin to explore the secrets of sickled hemoglobin, we delve into the complex molecular story that goes beyond just the genetic code. This story involves the significant effect of a single change in a nucleotide on the delicate balance of the circulatory system.

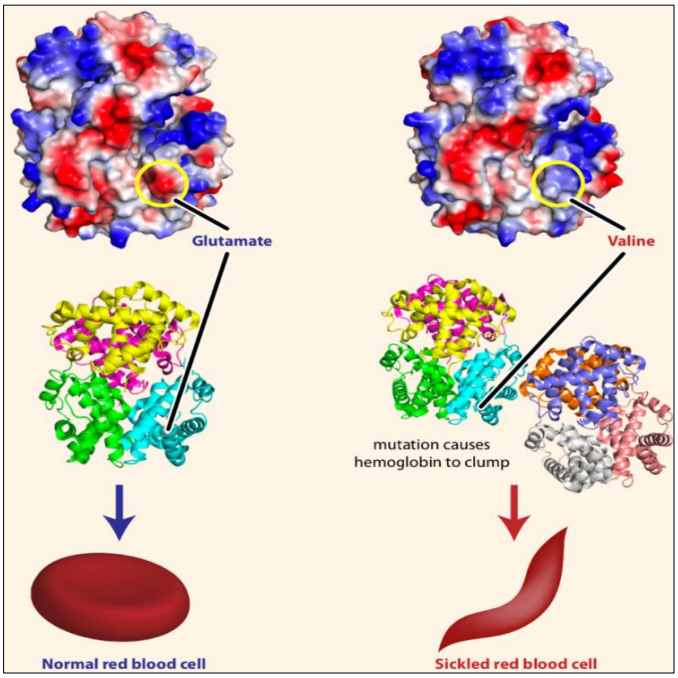


Figure 2. shows the conversion of Glutamic acid into Valine and then Round Red blood cells into sickle-shaped Hemoglobin [47].

1.7 Fetal Hemoglobin

Fetal hemoglobin, also known as hemoglobin F (HBF), is a form of hemoglobin generated by the developing fetus. Hemoglobin is a red blood cell protein that transports oxygen all over the body. HBF is structurally and functionally distinct from adult hemoglobin (hemoglobin A). It has a greater affinity for oxygen than adult hemoglobin, so it can bind to oxygen more strongly. This enables the fetus to obtain oxygen more efficiently from the maternal bloodstream during pregnancy when the fetus relies on the oxygenated blood supply of the mother for survival [48]. Hemoglobin F predominates throughout gestation and gradually reduces after birth, especially in the first months of life; the amount of it rises in some diseases of hemoglobin synthesis, various bone marrow failure conditions, and myeloproliferative neoplasms. The sets of genes that encode the various hemoglobin components are found in the globin gene cluster, which is the region of chromosome 11 [49]. Several non-functional and functional genes have been organized in a particular order within the globin gene cluster. The alpha-globin genes (HBA1 and HBA2) are responsible for producing the adult hemoglobin (HbA) alpha subunits. The alpha-globin gene is present in two copies in humans, one from each parent [50]. The beta component of adult hemoglobin (HbA) is made according to instructions from the beta-globin gene (HBB) [51]. The beta-globin gene only exists in one copy in humans. The gamma subunits of fetal hemoglobin (HbF), the primary form of hemoglobin generated by a growing fetus, are made by the gamma-globin genes (HBG1 and HBG2). The beta-globin gene is active after birth whereas the gamma-globin gene is mainly switched off[27].

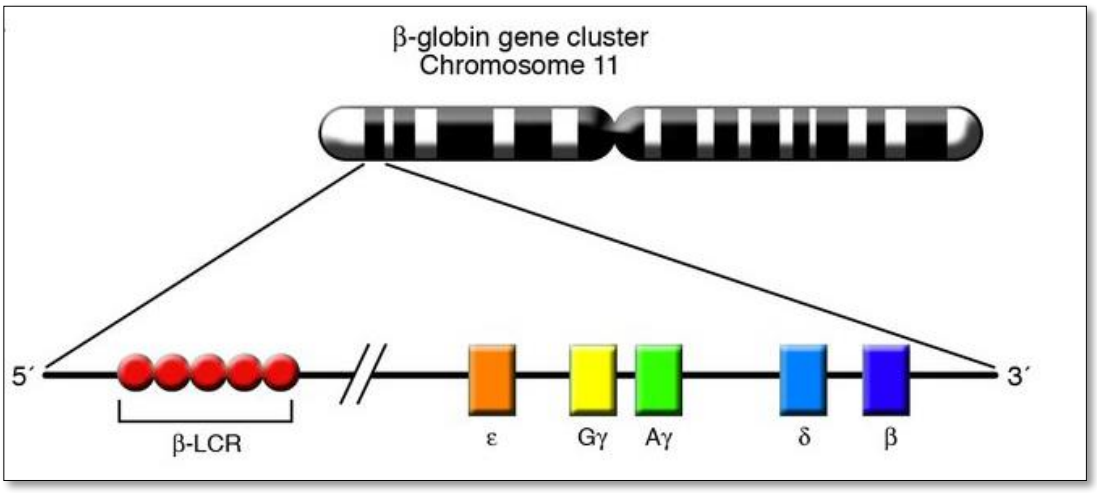


Figure 3. Beta Globin gene cluster on chromosome 11 [52].

The beta-globin gene and associated genes in the beta-globin gene cluster are expressed under the direction of the locus control region (LCR), an area of DNA that has many enhancer regions [28]. One of the genes that function in the beta-globin gene cluster, the beta-globin gene encodes adult hemoglobin's beta subunit (HbA) [53]. Several different types of beta-globin disorders, including a condition known as sickle cell anemia, can result from mutations in the beta-globin gene.

Sickle Cell Disease (SCD) is a genetic blood disorder that impacts the shape and functionality of red blood cells. Individuals with SCD develop abnormal hemoglobin, a red blood cell protein that transports oxygen through the body [54]. The abnormal hemoglobin causes red blood cells to transform into a crescent-like or sickle shape rather than their typical round shape, and these can contribute to different health complications. The beta-globin component in SCD develops aberrant hemoglobin molecules termed hemoglobin S as a result of mutations in the HBB gene [55] [56]. Red blood cells take on a sickle form as a result of the twisted, stiff, and aberrant hemoglobin molecules[57]. Red blood cells with a sickle shape may obstruct tiny blood arteries, reducing blood flow, causing tissue damage, and creating discomfort. Due to the autosomal recessive nature of the disease's inheritance, a person must inherit a pair of copies of the mutant HBB gene one from each parent to be affected. SCD carriers who have just a single copy from the mutant HBB gene often don't show any signs of the illness, but they may spread the mutation to their offspring [58]. According to particular mutations in the HBB gene, there are various types of SCD [59]. The amino acid valine is changed to glutamic acid at the 6th position of the beta-globin chain in the mutation that leads to the most prevalent type of SCD [60].

1.8 Adults vs Fetal hemoglobin

Researchers reported that the constituents that make up fetal and adult hemoglobin are the primary distinction between the two [61]. Fetal hemoglobin (HbF) is the predominant form of hemoglobin during fetal life and its concentration increases considerably for a few weeks after birth. HbF has a greater affinity for oxygen than adult hemoglobin, which enhances its transport to the fetus within the uterus by capturing oxygen from the placental vasculature, and it has a significantly lower oxygen tension than the lungs [62]. Fetal hemoglobin has a P50 of approximately 19 mmHg, while adult hemoglobin has a P50 of approximately 26.8 mmHg. As adult hemoglobin replaces fetal hemoglobin, neonatal levels of hemoglobin F progressively decline and reach adult concentrations (less than 1% of total hemoglobin) typically within the first year [63]. Fetal hemoglobin bonds

oxygen more powerfully than adult hemoglobin, transferring oxygen prenatally from mother to fetus. Fetal hemoglobin is more oxygen-binding than adult hemoglobin. The fetal affinity for oxygen saturation is greater than that of the mother [64]. The oxygen affinity of fetal hemoglobin is substantially greater compared with that of adult hemoglobin, so oxygen is efficiently transferred to fetal blood through the placenta. The oxygen absorption curves reveal the affinity of hemoglobin for oxygen, and because fetal hemoglobin differs from adult hemoglobin, oxygen can be transferred from the mother to the fetus [65].

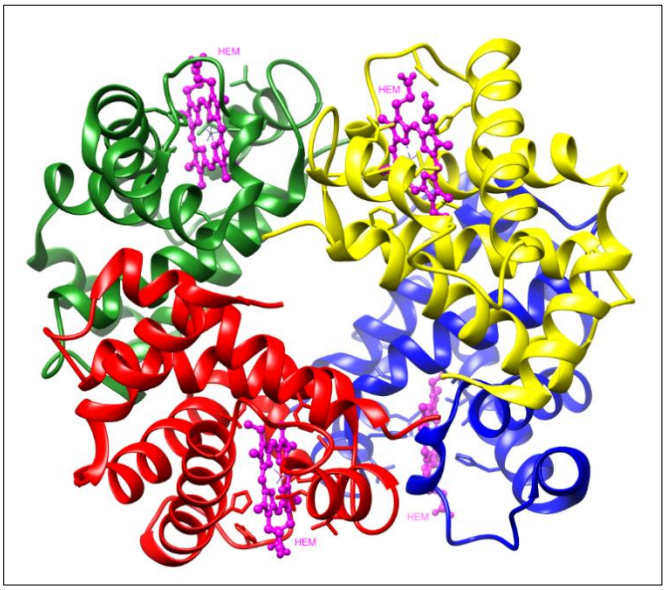


Figure 4. PDB ID 1FDH: shows the alpha and gamma subunits of fetal hemoglobin

Four polypeptide chains with the designations A, C, E, and G make up the alpha subunit of fetal hemoglobin. The A, C, E, and G chains of the alpha subunit of fetal hemoglobin have distinct functional properties, according to studies [61]. Specifically, it has been discovered that the A chain is essential for the formation and stability of fetal hemoglobin, while the C chain regulates the oxygen affinity of the hemoglobin molecule [66]. E and G chains are negligible components of the alpha subunit of fetal hemoglobin, and their precise functions are unknown. It has been postulated, however, that these chains may regulate both the structure and function of the A and C chains [67]. The alpha subunit of fetal hemoglobin is a complex molecule comprised of multiple polypeptide chains, each with its distinct functional properties. Still under investigation are the

specific mechanisms through which these chains communicate and add to the function of fetal hemoglobin [68].

The fetal gamma subunit consists of two polypeptide chains designated G and A. The Gγ and Aγ chains of the gamma subunit of fetal hemoglobin are secondary components with poorly understood functions [69]. Nonetheless, it has been proposed that these chains may regulate the oxygen affinity and stability of the hemoglobin molecule. In addition to the Gγ and Aγ chains, fetal hemoglobin contains four additional gamma chains: B, D, F, and H. These chains are generated by differential splicing of both the HBG1 and HBG2 genes that code for the G and A chains. Studies have demonstrated that the B, D, F, and H chains of the fetal hemoglobin gamma subunit have specific functional properties [70]. It was described in a study that the F chain is the main factor contributing to the increased oxygen affinity of fetal hemoglobin, whereas the B, D and H chains may regulate the expression and function of the F chain [71].

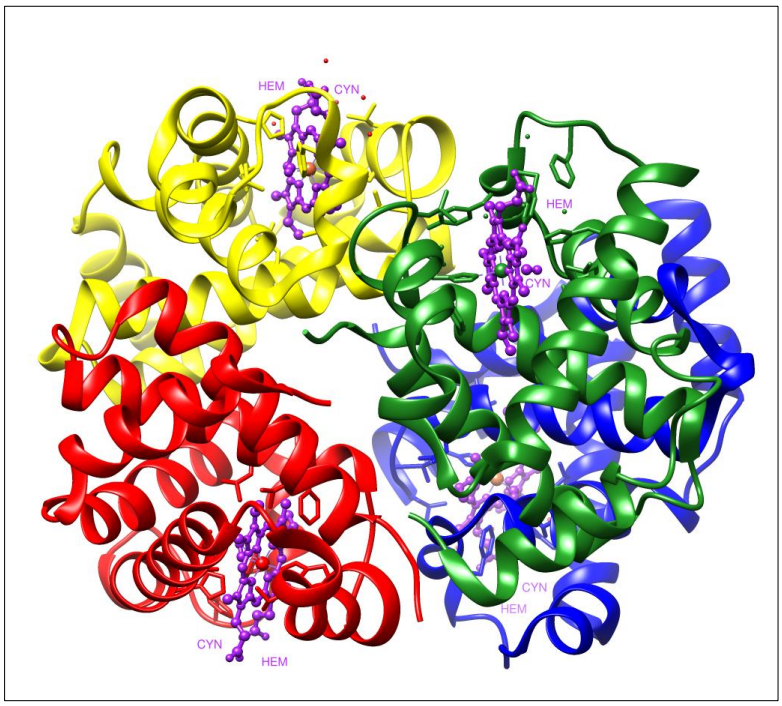


Figure 5. PDB ID 4HHB: Shows the alpha(red) and beta(yellow) subunits of Adult hemoglobin

The alpha globin chains are identical and denoted by the letters A and C, while the beta globin chains are also identical and denoted by the letters B and D. The A and C chains of the adult hemoglobin alpha subunit are essential for the assembly and stability of the molecule [72]. They combine to create the alpha-globin dimer, which binds to the beta-globin dimer to form the hemoglobin molecule [73]. The A and C chains have identical sequences and are functionally interchangeable, so mutations in one chain can be adjusted for by the other. B and D beta globin chains are responsible for hemoglobin's oxygen-carrying capacity. They combine to create the beta-globin dimer, which binds to the alpha-globin dimer to form the hemoglobin molecule [74]. The beta-globin molecules contain the oxygen-binding site, which is a Heme group. Hemoglobinopathies are a group of genetic abnormalities that affect the production or function of hemoglobin and can be caused by mutations in the alpha and beta globin chains. Mutations in the beta-globin chain can result in sickle cell anemia or beta thalassemia, whereas mutations in the alpha-globin chain result in alpha thalassemia. These conditions can result in anemia, organ dysfunction, and other complications [75].

Mutations in the beta-globin gene cluster regulatory areas cause a condition named as Hereditary persistence of Fetal Hemoglobin (HPFH). Mutations in beta-globin gene promoter regions may boost their expression, raising adult red blood cell HbF levels [72]. An imbalance in alpha and beta globin chain production may enhance HbF production. At position 136, the gamma subunit changes from its adult counterpart by possessing either an alanine or a glycine, either of which belongs to neutral, nonpolar amino acids [76] [77]. This variation induces conformational changes in the protein, resulting in several physiological variations in oxygen delivery that are crucial for fetal circulation.

1.9 Objective

Objectives of the study were to:

- Determine the binding Pattern of the alpha-gamma and alpha-beta chain complexes, specifically focusing on how each complex interacts with the beta-chain

- Identify potential sites or regions within the alpha-gamma chain complex that could serve as therapeutic targets for modulating the beta chain in adults.

1.10 Problem Statement

Hemoglobinopathies are due to the mutation in the HBB (Hemoglobin Beta Subunit). The persistence of the γ- chain of the fetal hemoglobin in adults could circumvent sickle cell anemia. Therefore, a computational framework to persist the gamma chain of the hemoglobin could also be one of the potential therapeutic strategies.

Chapter No. 2
Literature Review

2. Literature Review

2.1 Emergence of sickle cell anemia

During the first decade of the nineteenth century, Walter Clement Noel, a 20-year-old dental student who originated from Grenada, took medical attention at Chicago Presbyterian Hospital due to periodic bouts of "muscular rheumatism" and "bilious attacks" [78] Noel's proper care was handed over to James B. Herrick, a distinguished cardiac specialist and professor of medicine at the institution. Herrick, a distinguished individual known for his exceptional aptitude in thoughtful observation, sought the aid of Dr. Ernest E. Irons, who had been working as a trainee under his supervision, to conduct supplementary investigation [79]. In 1904, Dr. Irons conducted a blood analysis on Noel, during which he rendered a remarkable finding while examining the sample of blood under a surgical microscope. The researcher noted an abnormally high proportion of red blood cells in Noel's bloodstream exhibiting a distinctive sickle-shaped morphology [80]. Due to the exceptional characteristics of this finding and Dr. Irons' intention to ensure Dr. Herrick's awareness of it, he promptly communicated the information to him. Dr. Herrick displayed a profound level of engagement with the case, persistently conducting research and closely observing Noel's condition [81]. This dedication stemmed from Herrick's fascination with the enigmatic nature of Noel's ailment. Noel received medical treatment from Dr. Irons for three years while being a patient at the Chicago Presbyterian Hospital [81].

Throughout the aforementioned time frame, Herrick and the members of his study group carefully observed the progression of the disease and closely monitored the clinical signs and symptoms it provoked in Noel. Herrick's comprehensive case study titled "Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia" was published in the November 1910 edition of the Archives of Internal Medicine (Chicago) [82]. This written work represents the initial recorded instance of an actual case study about sickle cell anemia. The emergence of the sickle cell disorder study category was also initiated by this publication as well. Despite undergoing medical treatment, Walter Clement Noel's health progressively declined, ultimately resulting in his untimely demise in 1916 as a consequence of pneumonia. The example provided by the individual, in conjunction with a seminal publication authored by Herrick, laid the foundation for subsequent investigations into sickle cell disease. Due to the unique morphology of the

erythrocytes in question, the medical condition became commonly known as "sickle cell anemia [81] [83]."

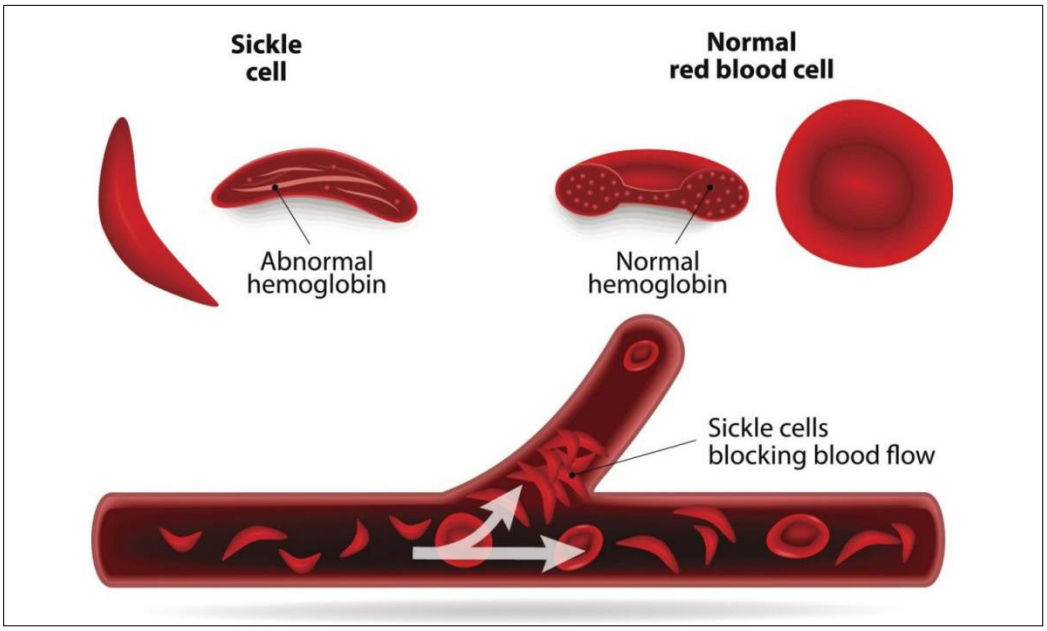


Figure 6. shows sickled hemoglobin cells disrupting the flow of Normal hemoglobin cells. Sickle cells are abnormally shaped erythrocytes that have the potential to obstruct the circulation of blood. Typical red blood cells are spherical and pliable, enabling them to smoothly circulate throughout blood arteries. The sickling of red blood cells may lead to vaso-occlusive crises, characterized by periods of pain, tissue damage, and organ failure [84].

2.2 Genetic Basis of Sickle Cell Anemia

A specific point mutation within the HBB gene may be responsible for the underlying genetic etiology of sickle cell anemia. This condition is characterized by the substitution of valine (Val) for glutamic acid (Glu) in the beta-globin chain of hemoglobin [85]. Alterations in the HBB gene, which is located on chromosome 11, have the potential to cause a variety of hemoglobinopathies, including sickle cell anemia, among others [86]. The mutation known as HbS is the only genetic change that results in the substitution of valine (Val) with glutamic acid (Glu). The HbS mutation is caused by a single nucleotide substitution in the HBB gene. More specifically, the substitution of thymine (T) for adenine (A) at the sixth position of the codon that is responsible for the beta-globin chain is what causes the mutation [87]. A change in the codon sequence occurs as a

consequence of this point mutation. The codon sequence changes from GAG, which codes for glutamic acid, to GTG, which codes for valine. Valine is substituted for glutamic acid at the sixth position of the beta-globin chain as a consequence of this process. Sickle cell anemia has an autosomal recessive inheritance pattern [88]. For an individual to manifest the sickness, it is required that they inherit two copies of the mutant HBB gene, one from each of their parents. These people, referred to as carriers or heterozygotes, possess one functional HBB gene and one defective HBB gene. While they often remain asymptomatic, they may transmit the mutation to their offspring [89].

2.3 Polymerization GLU6VAL

The replacement of glutamic acid with valine results in the incorporation of a hydrophobic amino acid into a previously hydrophilic area [90]. This alteration causes a disturbance in the electrostatic contacts and hydrogen bonding inside the hemoglobin molecule. When hemoglobin molecules are deoxygenated, the hydrophobic valine residues tend to come together and form insoluble polymers [82].

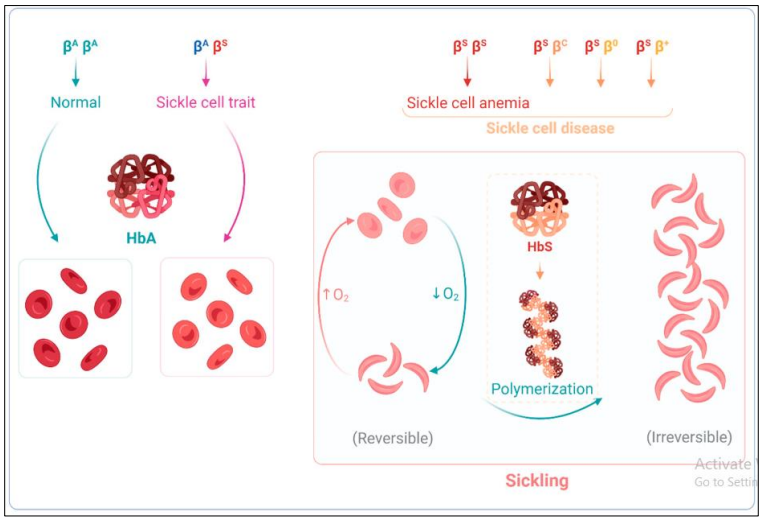


Figure 7. Sickle cell genotypes alter hemoglobin and red blood cells. Sickle cell trait, a disorder characterized by heterozygosity for the normal β-globin and βS genes, is often asymptomatic. HbS generation results from compound heterozygosity of βS and other β allele mutations. Under hypoxia, HbS polymerizes into stiff fibers within red blood cells (RBC) due to its decreased O2 affinity. Distortion, stiffness, and fragility from HbS polymerization cause RBC sickling. Initial sickling is reversible, alternating oxygenation and deoxygenation. Chronic oxy–deoxy cycles cause permanently sickled RBCs, a characteristic of sickle cell disease [91].

2.4 Hereditary Persistence of Fetal Hemoglobin (HPFH)

Hereditary Persistence of Fetal Hemoglobin (HPFH) is a harmless condition in which significant fetal hemoglobin synthesis continues far into adulthood, despite the typical stopping point at which only adult-type hemoglobin should be produced [92]. It is a rare genetic disease in which HbF persists, which might be interpreted as an endorsement. It is completely not hazardous and is only found when screening for other hemoglobin abnormalities [93]. HPFH develops when a child's red blood cells have an abnormally high proportion of hemoglobin F (fetal hemoglobin). Fetal hemoglobin is created in the final eight weeks of pregnancy, accounting for 60-80% of total hemoglobin at birth, and is replaced by adult hemoglobin (HBA) within six to twelve months [94]. HbF lowers the severity of sickle cell disease in those with high amounts of fetal hemoglobin, such as infants or those with HPFH. In the case of diseased conditions after deoxygenation in the bodily tissues, abnormal hemoglobin S polymerizes to form fibers in red cells, causing them to distort and obstruct circulation [95]. However; HbF effectively prevents HbS polymerization. HbF lengthens the time required for HbS polymerization (delay time), enabling sickle erythrocytes to get out of the bloodstream and enter the veins. The difference in amino acid sequence between HbF and HbS inhibits their interaction and hinders HbS from entering the polymerization phase [96] [97]. Due to its exclusion from the polymer, HbF inhibits HbS polymerization. The dissociation process of tetramers into dimer molecules and re-association in compounds of HbS and HbF produce hybrid tetramers that are less susceptible to polymerization. It was described in a study that the anti-sickling properties of fetal hemoglobin described that HbAS3 is a highly effective inhibitor of HbS polymerization [98] [99]. Inhibitors can modulate the HbS (sickle hemoglobin) polymerization process in sickle cell disease (SCD) patients. Principal to the pathology of SCD is the polymerization of HbS, which results in the formation of long, unyielding molecules that damage red blood cells and generate their distinct sickle shape [100]. Hindering with the molecule reactions and procedures involved in the polymerization pathway is how inhibitors of HbS polymerization function. They may get involved in different phases to prevent or restrict the formation of HbS polymers [101]. One of the key impacts is the transformation of red blood cells from their regular disc-like shape to their unique sickle shape. These sickle-shaped cells lose their pliability, becoming more stiff and prone to adhesion to blood channel walls, particularly in tiny capillaries [97]. Because of the adhesion and diminished deformation ability, blood flow, oxygen supply, and tissue hypoxia are all compromised. The vaso-occlusion caused

by sickle cell disease is caused by sickle-shaped red blood cells aggregating and attaching, blocking blood arteries. This phenomenon, also known as a "sickle cell crisis," is the source of sickle cell disease patients' acute discomfort symptoms [102]. Furthermore, vaso-occlusion promotes tissue ischemia, which damages tissue and organs throughout the body [103]. The bones, lungs, spleen, kidneys, and brain are particularly at risk for vaso-occlusive events, which may result in a broad range of clinical symptoms [104]. HPFH is a unique benign asymptomatic inheritable syndrome in which HbF levels are higher than expected. HPFH may be inherited as an autosomal dominant or recessive trait. It is often not treated since it is passive and noncancerous [105] [106].

Chapter No. 3
Methodology

3. Methodology

3.1 Step 1: Collection of Protein structure

To explore the potential of γ-globin genes for hemoglobin disorders, we obtained protein structures of important hemoglobin variants, including Fetal Hemoglobin (HbF), Adult Hemoglobin (HbA), and Sickled Hemoglobin (HbS), from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) database. This study relied on the RCSB PDB database, which serves as a comprehensive repository of biological macromolecular structures [107].

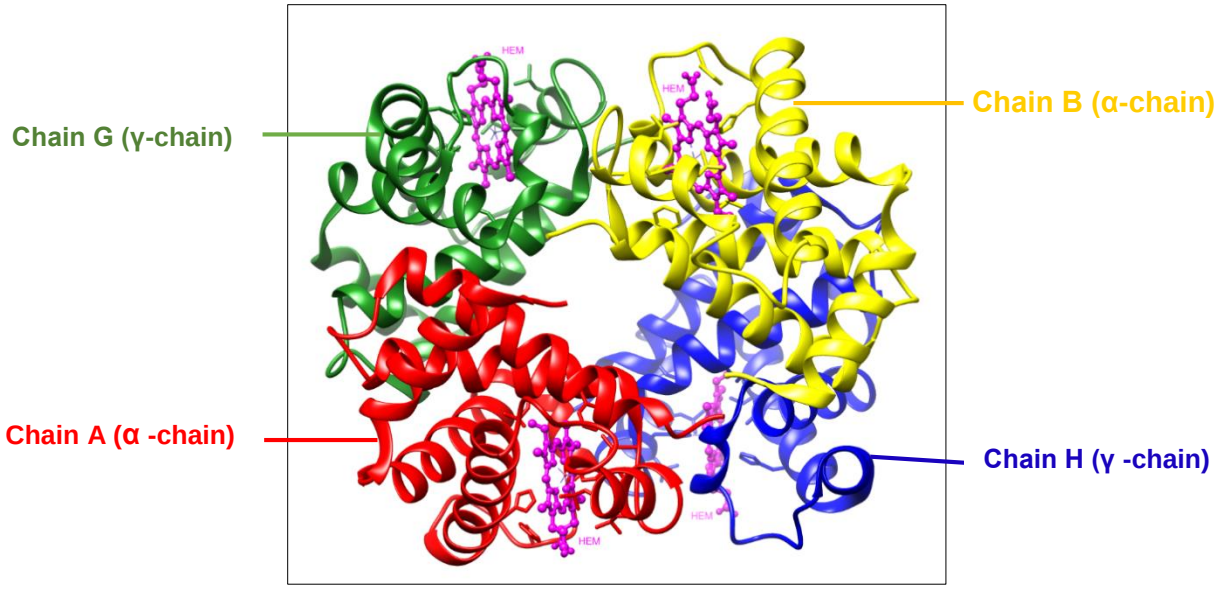


Figure 8. The Crystal structure of 1FDH (Fetal Hemoglobin, HbF) retrieved from RCSB PDB [108].

The γ-globin genes encode HbF, which plays a crucial role in transporting oxygen throughout fetal development [109]. Therefore, it is necessary to conduct a thorough structural investigation of HbF in the context of hemoglobinopathies. As shown in Figure 5, the HbF structure used in this investigation has a particular PDB ID of 1fdh, which was acquired from the RCSB PD [110].

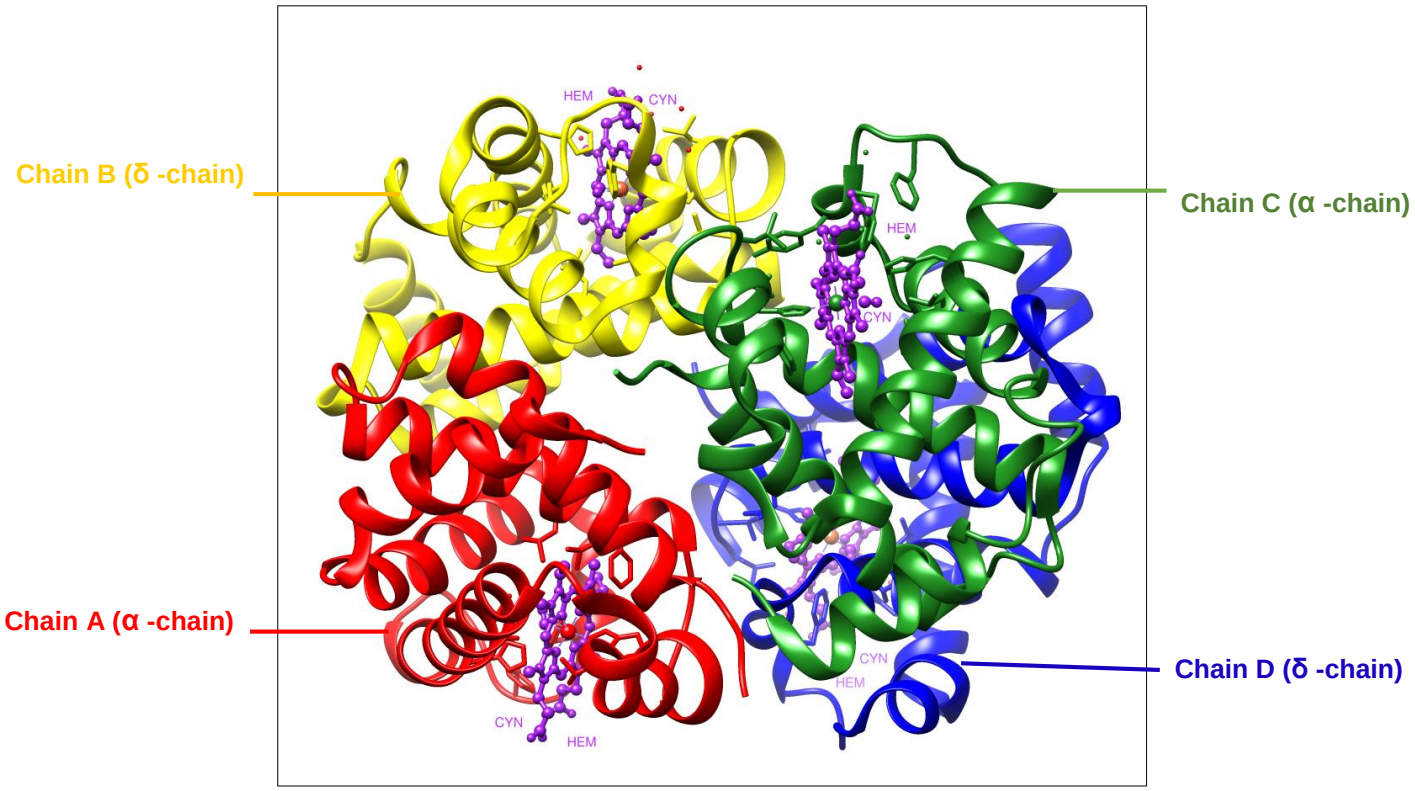


Figure 9. The Crystal structure of 1Si4 (Adult Hemoglobin, HbA) retrieved from RCSB PDB [111].

HbA, which consists mostly of α-globin and β-globin subunits, is the main type of hemoglobin found in adults. Comparing the structure of HbF with that of adult hemoglobin was considered necessary to identify major structural differences between the two [112]. As shown in Figure 6, the HbA structure used in this work has a particular PDB ID of 1si4, which was retrieved from the RCSB PDB.

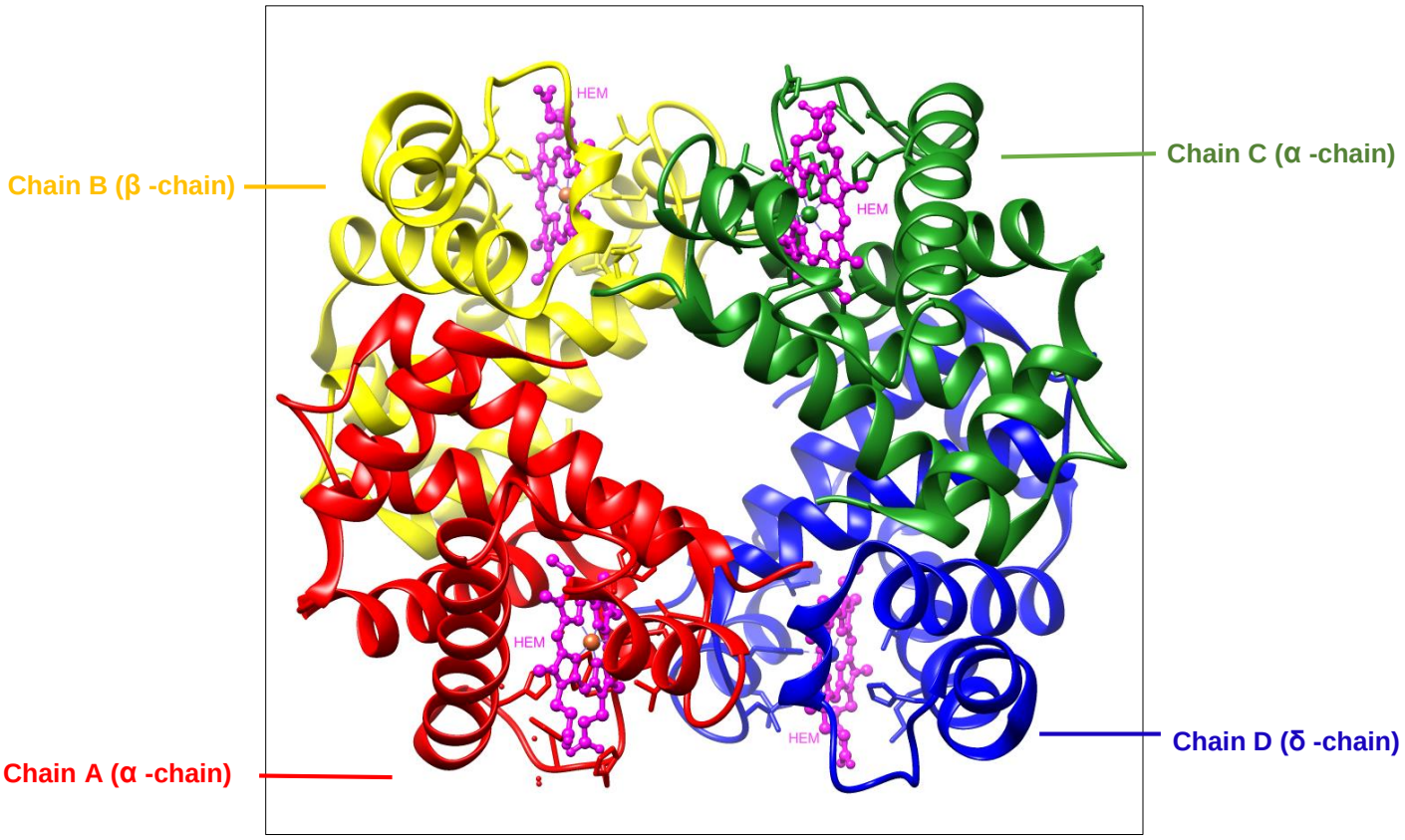


Figure 10. shows the Crystal structure of 2HBS retrieved from RCSB PDB [113]

The HbS variation, which is linked to sickle cell disease, provided a vital understanding of structural abnormalities that contribute to hemoglobinopathies [114]. As shown in figure 7, the HbS structure used in this investigation has a particular PDB ID of 2HBS, which was retrieved from the RCSB PDB.

3.2 Step 2: Structure Preparation

For HbF, HbA, and HbS, duplicate chains were eliminated from the structures. This procedure was essential to lessen the complexity of the structures [115]. Having duplicate chains in the structures might result in unnecessary repetition during computations, which may distort the outcomes of studies. Eliminating redundant chains optimizes the organization of the structural data, enhancing its manageability and minimizing the likelihood of error in Molecular Dynamics Simulation analysis [116].

3.2.1 Heme removal

During molecular dynamics simulations, the Heme group was purposefully removed from the hemoglobin structures. The goal of not including the Heme group was to clarify the dynamic and structural properties of the γ-globin genes on their own, free from the potentially confusing impact of the Heme prosthetic group [117]. Since mutations and variations in γ-globin genes are often linked to the pathophysiology of hemoglobinopathies, this strategy enabled a more focused examination of the involvement of these genes in these illnesses. In addition, the removal of the Heme group improved computational efficiency by simplifying simulations and enabling a more in-depth investigation of the behavior and interactions of the globin chains within the hemoglobin structure" [118].

3.2.2 Energy Minimization

The goal of energy minimization is to get the molecular system into a stable and energetically advantageous configuration [119]. By reducing steric conflicts, adjusting bond lengths and angles, and stabilizing the structure overall, this procedure ensures a more accurate starting point for ensuing simulations [120].

3.3 Molecular Dynamics Simulations

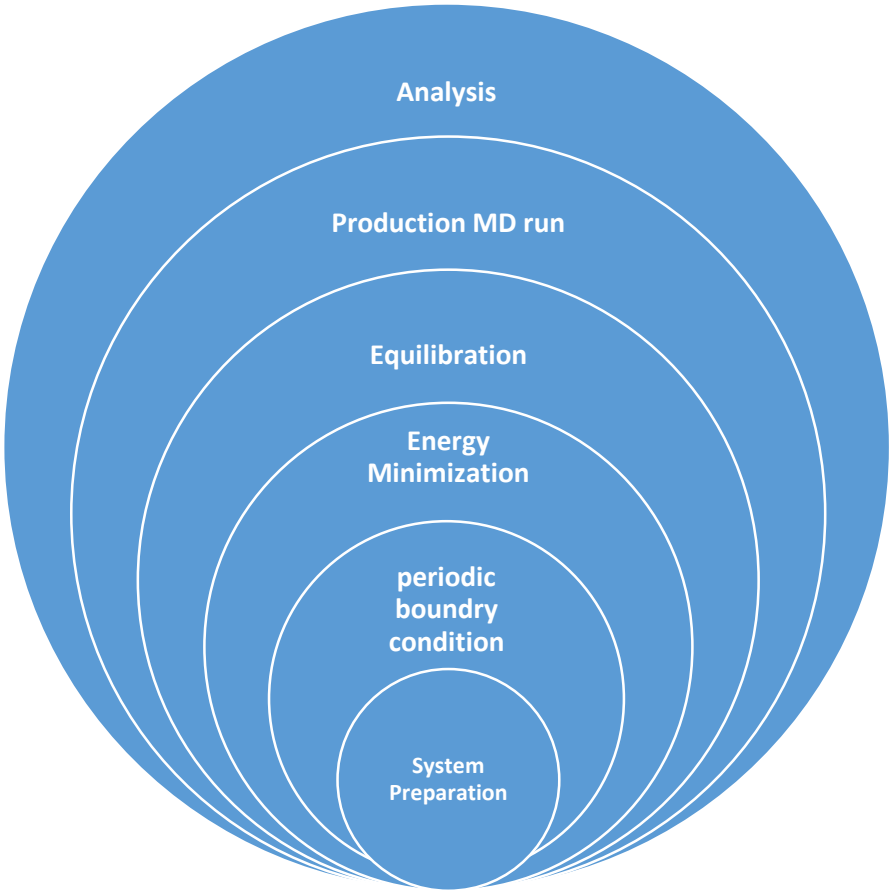


Figure 11. Workflow of Molecular Dynamics Simulation: From system setup and force field application to energy minimization, equilibration, and the production MD run, depicting the dynamic behavior of bimolecular systems at the atomic level over time.

3.3.1 System Preparation

The primary objective of system preparation in molecular dynamics simulations is to create an accurate and physiologically relevant representation of the molecular system of interest. In the case of studying Hemoglobin, this process begins with the Hemoglobin structure after the removal of the Heme group, as the absence of Heme is often required to focus on the protein's intrinsic behavior [116]. To facilitate the MD simulation, topology files were generated to comprehensively define the molecular system's characteristics [121].

3.3.2 Force Field Selection

This step involved the use of the Amber force field **AMBER99SB-ILDN** protein, nucleic AMBER94 The choice of force field, in this case, Amber, ensures the accurate representation of molecular interactions within the simulation environment [117]. The AMBER force field is chosen for its well-established accuracy in representing bimolecular interactions, extensive parameterization catering to diverse structures, and widespread adoption within the bio-molecular simulation community, ensuring reliable and biologically relevant results [122].

Hemoglobin is present in intricate cellular surroundings inside the human body, encompassed by water molecules and ions. The addition of a solvent in the molecular dynamics (MD) simulation accurately reproduced the physiological environment in which hemoglobin operates. The presence of solvent molecules, often represented by water in molecular dynamics (MD) simulations, establishes an environment that has an impact on the behavior and interactions of biomolecules [123]. The presence of water molecules was crucial in maintaining the stability of protein structures, enabling interactions between solutes, and influencing the overall behavior of the system. Proteins like hemoglobin, often possess an overall charge as a result of the existence of charged amino acid residues (e.g., positively charged lysine or negatively charged aspartate). To preserve the overall balance of electric charge in the simulation system, counter ions such as chloride ions Cl^- were introduced. This guaranteed that the whole system maintained electrical neutrality [124].

To prevent any interaction between the protein and its periodic picture, a cubic box was established with a certain distance between the protein and the borders of the box [125]. This technique followed the minimal image convention, which helped to avoid any unwanted pressures and boundary effects. The incorporation of the solvate module with **spc216.gro** introduced more water molecules into the simulation box [126]. The spc216.gro file had an equilibrated 3-point solvent model and was an appropriate selection for general water setups. The number of water molecules introduced was automatically updated in the topology file.

3.3.3 Energy Minimization

At this step, it was crucial to resolve any possible conflicts between molecules and incorrect spatial arrangements within the system. This was necessary to provide a stable and accurate starting point for later molecular dynamics analysis. The system relaxing process was conducted using a precise

technique called energy minimization (EM). In this stage, the GROMACS utilities grompp and mdrun were used to create a binary input file (. tpr) and carry out the process of energy reduction, respectively [127].

Steric conflicts and unfavorable atomic overlaps may occur during the initial assembly of the system or as a result of later actions. The objective of energy minimization was to minimize those collisions by adjusting atomic locations, thus overcoming spatial conflicts. The system successfully achieved stabilization and optimized atomic locations to get a more realistic and physically plausible arrangement, resulting in a minimization in potential energy [128]. This procedure established a stable and relaxed framework, devoid of any hindrance caused by overlapping molecules, and served as a basis for further molecular dynamics simulations.

3.3.4 Equilibration

The energy minimization stage facilitated the achievement of a realistic initial structure, resolving concerns about geometry and solvent alignment. Before getting into actual molecular dynamics, it was crucial to achieve equilibrium of the solvent and ions around the protein [129]. Engaging in uncontrolled dynamics at this point carried the danger of the system collapsing since the solvent was mostly optimized within itself, which might result in unfavorable interactions with the solute (protein). Hence, equilibration played a crucial role in attaining the required simulation temperature and ensuring the correct alignment of the solvent molecules around the protein [130]. The process of critical equilibration was conducted using the NPT ensemble, which maintained a constant number of particles, pressure, Volume, and temperature [131].

3.3.5 Production MD run

During this concluding phase, similar to the previously observed procedures, the checkpoint file, which now contains conserved pressure coupling information, was used in combination with the grompp tool. This stage functioned as an intermediary between the equilibration process and the subsequent phase of data collecting. The system was configured to undergo a 200-ns molecular dynamics (MD) simulation, a time selected to include significant dynamic behavior and correspond with the project's goals [132]. The relaxation of position restrictions indicated that the system was prepared to freely explore its dynamic behavior. During this uncontrolled phase, the molecules were able to interact without any restrictions, precisely resembling the circumstances seen in biological systems. The use of the checkpoint file guaranteed a smooth and uninterrupted

transition, preserving the system's balanced state [133]. The 200-ns MD simulation provided a substantial duration for examining the behavior and interactions of the molecular system [134]. The selection of this period was meticulously made to achieve a harmonious equilibrium between gathering significant data and optimizing computing performance [135].

3.3.6 Analysis

After the completion of the MD Run the trjconv tool was used to adjust the periodic boundary conditions (PBC) to avoid any problems that may arise due to periodicity in the system, the dispersion of the protein inside the unit cell may result in distortions, such as the protein exhibiting fragmented or erratic movements within the box [109]. The trajectory was rectified and the protein was centered using trjconv, resulting in a more precise depiction of its behavior. The GROMACS built-in function rms (root-mean-square), was used for the subsequent structural stability study. The rms command facilitated the computation of RMSD (root-mean-square deviation) concerning a reference structure [136]. The protein backbone was subjected to a least-squares fit and RMSD computation in this example. The graphic displayed the root mean square deviation (RMSD) levels in nanoseconds (ns), compared to the structure of the minimized and equilibrated system [137].

The Root Mean Square Fluctuation (RMSF) and Radius of Gyration (Rg) were both analyzed as part of the additional investigation into the dynamics of the molecular system [138] [139]. This was done in addition to the study of the root mean square deviation (RMSD). During the Molecular Dynamics simulation, these studies offered more profound insights into the degree to which the protein structure was both flexible and compact.

Chapter No. 4
Results

4. Results

4.1 RMSD Graphs

Molecular Dynamics (MD) simulation data were rigorously analyzed before examination. Systematic analysis of RMSD, RMSF, and Rg revealed the dynamic behavior and structural changes in the simulated protein system. These analyses provide a sophisticated understanding of the results.

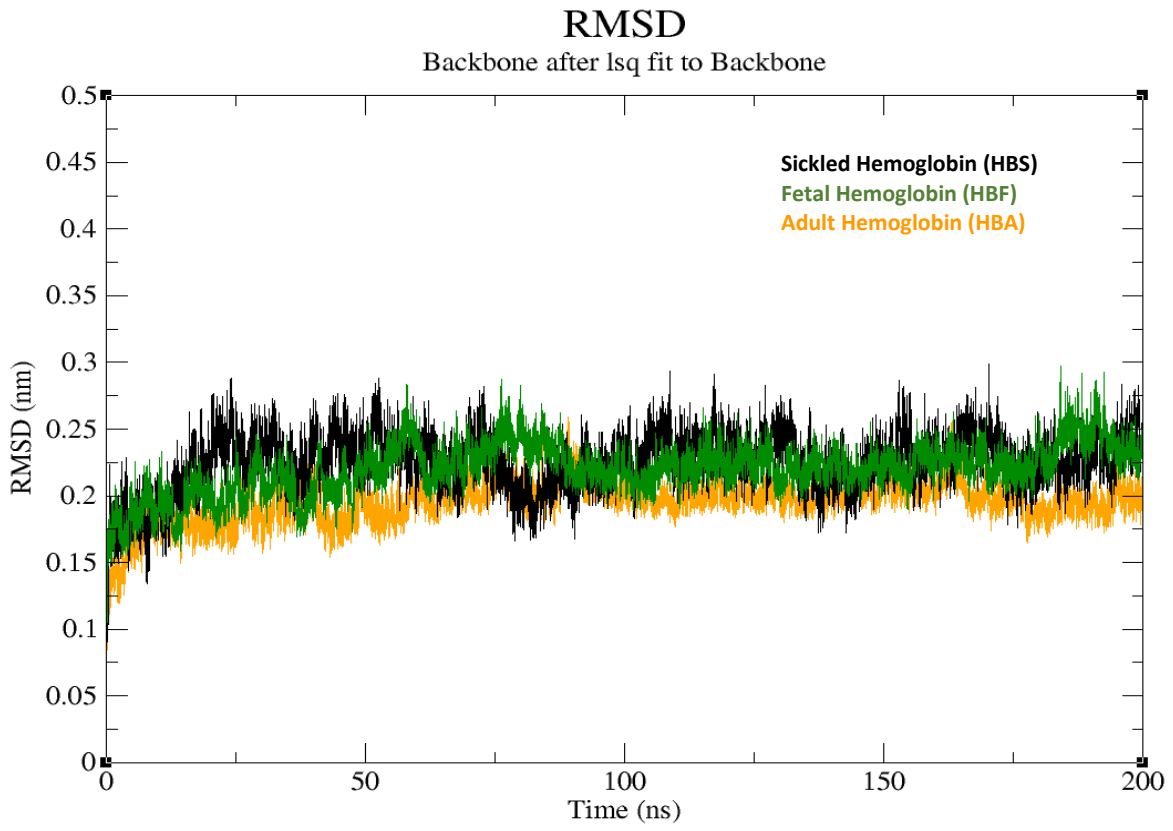


Figure 12. RMSD plot of Adult, Fetal, and Sickled Hemoglobin structures: comparison of the stability of these structures over 200ns time for MD Simulation.

The root-mean-square deviation of the protein's backbone atoms from their original locations throughout time was seen in the adult-wild type RMSD graph. The graph showed RMSD increasing rapidly in the first 50 ns of the simulation and gradually over the next 150 ns. This shows that the protein experiences a major conformational shift early in the simulation and stabilizes in a new configuration.

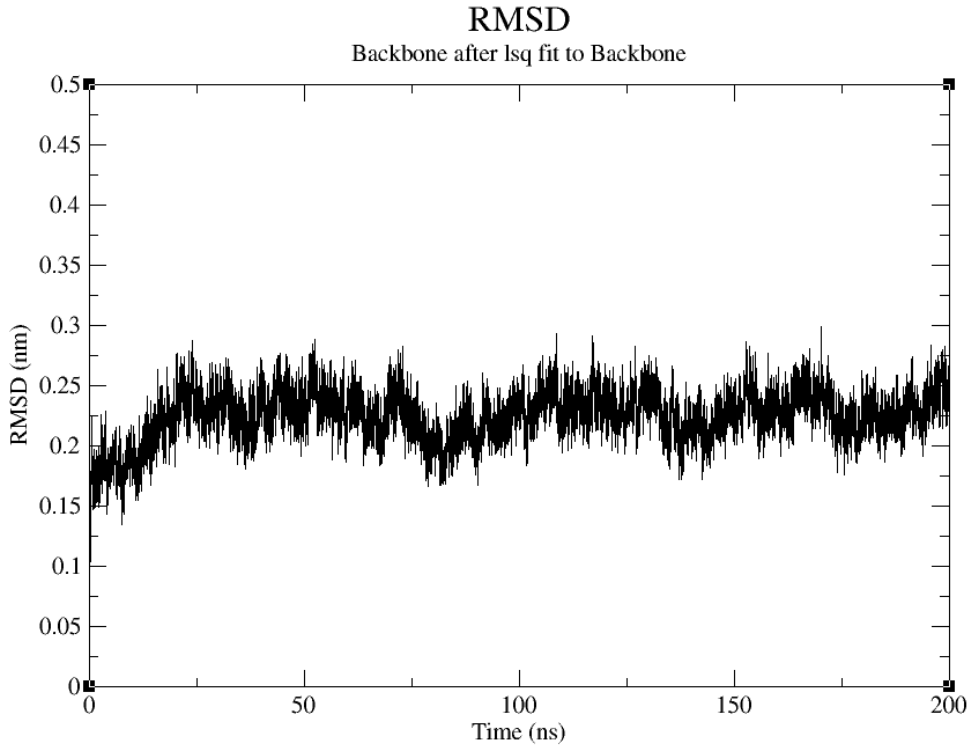


Figure 13. Root Mean Square Deviation graph of Adult Hemoglobin showing the stability of the structure.

Phase 1 (0-50 ns): In this first phase, the root mean square deviation (RMSD) experiences a fast rise, going from 0 to 0.4 nm. This indicates that the protein is experiencing a substantial alteration in its structure. This phenomenon may arise as a result of protein denaturation, partial denaturation, or a mere reorganization into a more stable structure [136].

In Phase 2 (50-100 ns), the Root Mean Square Deviation (RMSD) shows a progressive rise from 0.4 to 0.45 nm. These findings indicate that the protein is undergoing stabilization in a novel shape. Nevertheless, there is still a certain degree of conformational deviation occurring at this period.

Phase 3 (100-150 ns): In this period, the root mean square deviation (RMSD) stays consistently steady at around 0.45 nm. This indicates that the protein has achieved a stable structure.

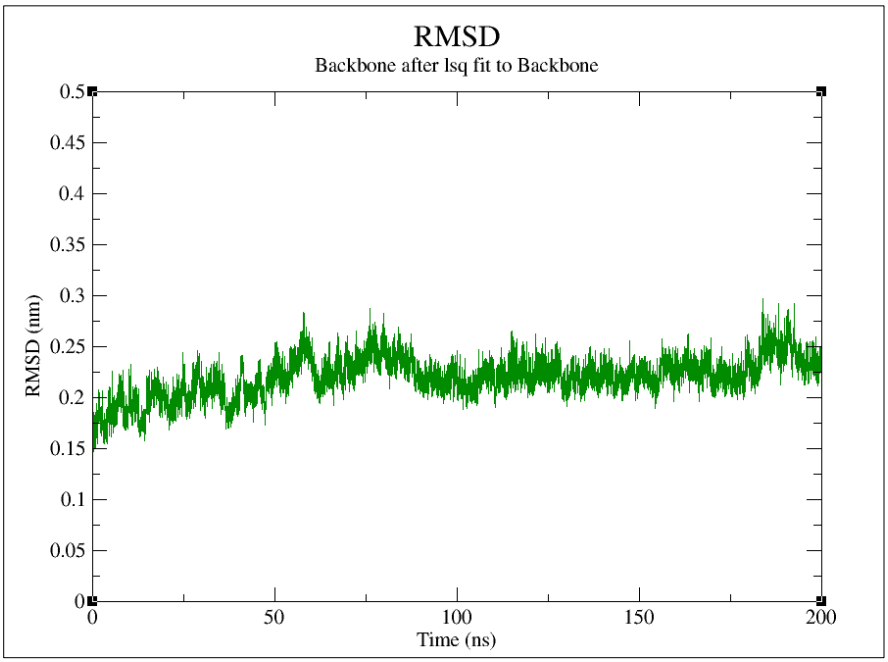


Figure 14. RMSD plot of fetal Hemoglobin HBF showing the stability of the structure.

The RMSD figure illustrates an initial high value of RMSD, which then exhibits a quick decline as time progresses. These findings indicate that the protein has high initial flexibility, but rapidly transitions into a more stable shape. The root mean square deviation (RMSD) exhibits periodic variations with an average value of 0.25 nm, indicating a comparatively moderate level of fluctuation. These findings indicate that the protein exhibits a high degree of organization and remains stable.

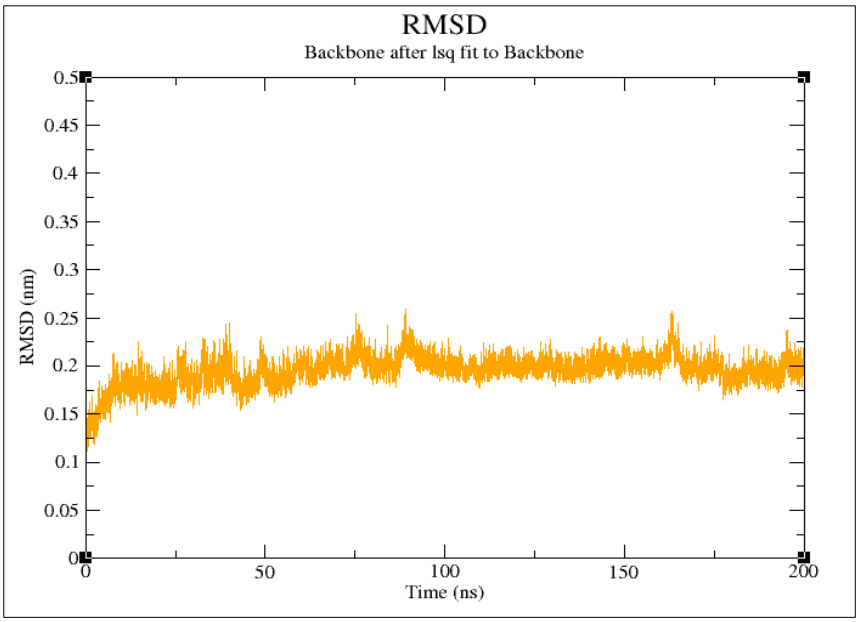


Figure 15. RMSD plot of Mutant Hemoglobin Structure (Sickled Hemoglobin).

The root mean square deviation (RMSD) of the sickle cell hemoglobin protein in the picture initially measures around 0.4 nm and then exhibits a progressive reduction over time. This suggests that the protein starts in a more unstable condition, but gradually transitions into a more secure structure. The root mean square deviation (RMSD) of the sickle cell hemoglobin protein is greater than that of a normal hemoglobin protein. The occurrence of sickle cell disease is attributed to a mutation that results in the miss-folding of the protein. Improper folding may result in several issues, such as the creation of aggregates that can obstruct blood arteries and cause discomfort and other disorders [140].

4.2 RMSF graphs

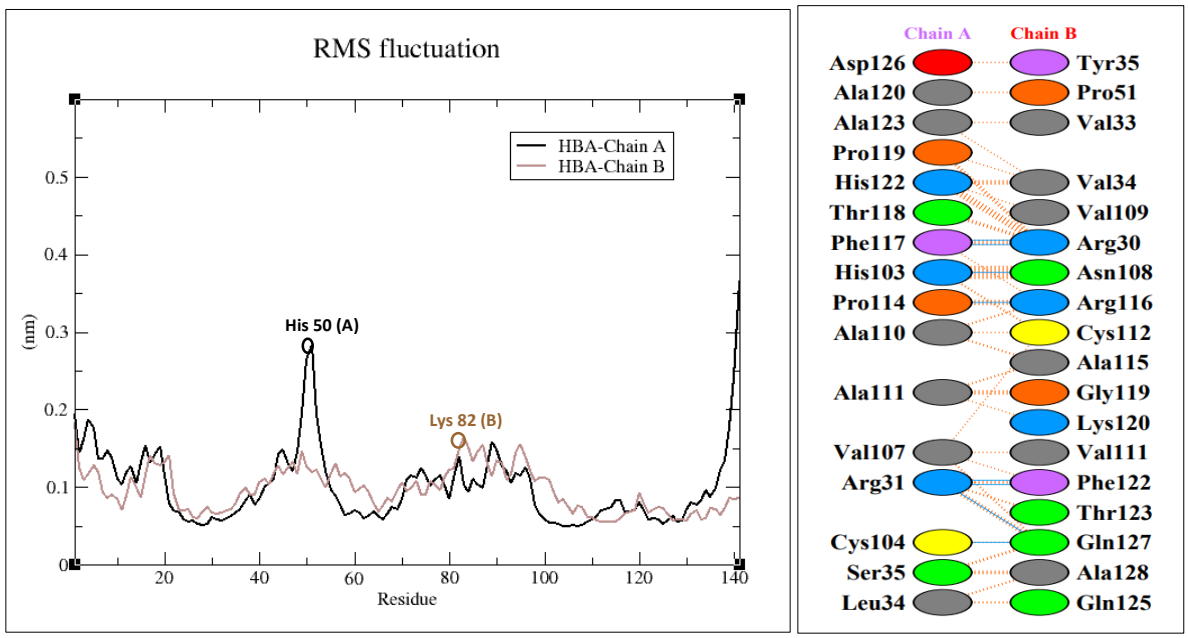


Figure 16. RMSF plot of Adult Hemoglobin HBA along with interactions of chain A(alpha) with Chain B (beta)

The RMSF plot offers useful insights into the flexibility of residues within a protein structure during a molecular dynamics simulation. The fact that residues located at the interface between chains A and B tend to be more flexible suggests that they have a higher degree of dynamism. The enhanced flexibility is a result of the looser arrangement and more exposure of these residues to the solvent. Interfaces in protein structures are often areas where interactions between distinct chains or subunits take place, and their flexibility is essential for allowing these dynamic interactions. Identification of certain flexible residues, namely Asp126, Ala120, Ala123, His122, and Thr118 in chain A, offers a more comprehensive perspective.

The residues mentioned are constituents of a loop structure that plays a role in the interaction between chains A and B. Loops are often more adaptable than conventional secondary structures, such as alpha helices or beta sheets, and have a substantial impact on facilitating protein-protein interactions. The malleability of these particular residues enables the loop to adapt and enhance its interactions with the next chain. Distinctly inflexible residues: In contrast, the identification of certain residues, namely Pro119, Ala110, Cys112, Ala115, Lys119, Ala111, and Thr123 in chain

A, as being notably stiff implies the presence of a stable central area within the protein. These residues are very likely to have a role in maintaining the structural stability of the protein. Residues that make up the central part of a protein often participate in hydrophobic interactions and play a crucial role in preserving the protein's overall structural integrity. Deciphering the functional dynamics of a protein requires a vital understanding of the flexibility and stiffness of individual residues [140]. Flexible sections often correlate to functional sites or regions engaged in interactions, while stiff portions contribute to the overall stability of the protein. The provided knowledge has significant importance in the fields of rational drug design, protein engineering, and comprehending the dynamic behavior of proteins in biological processes.

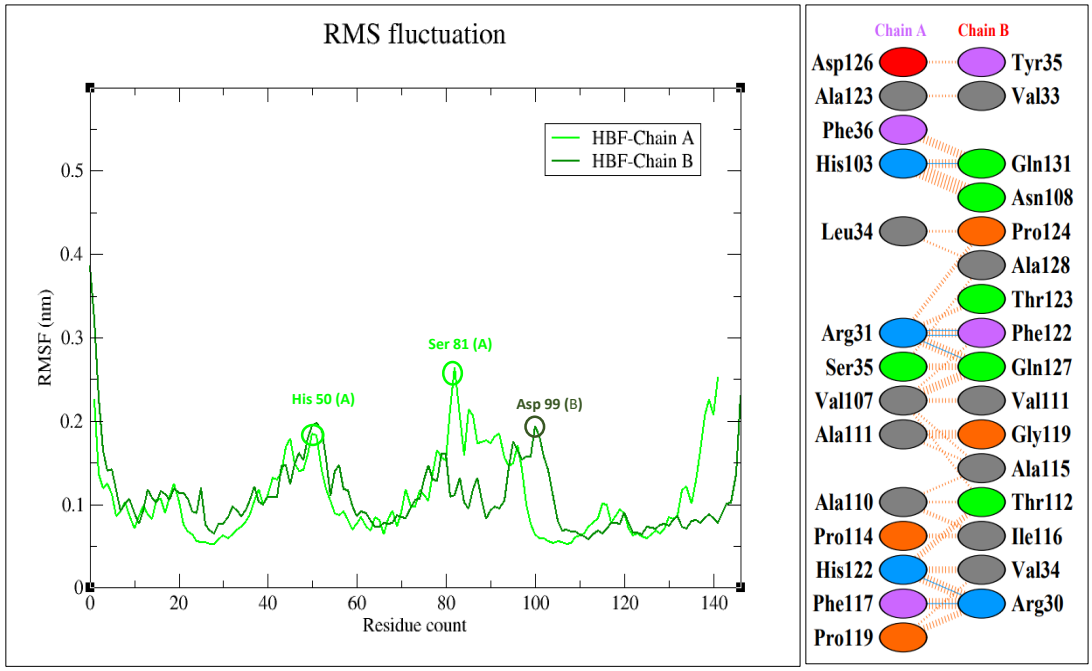


Figure 17. RMSF plot of fetal hemoglobin HBF along with interactions of Chain A(alpha) with Chain B (gamma)

The RMSF figure displays the mean displacement of each residue within the protein structure compared to a reference structure. A greater root mean square fluctuation (RMSF) value indicates a higher degree of flexibility in the residue. The graphic illustrates that the interface between chains A and B exhibits more flexibility compared to the protein's core. This is because the residues located near the interface are less tightly packed and are more susceptible to solvent exposure [112]. The RMSF figure also indicates the presence of a small number of individual residues that exhibit notable flexibility. The residues Asp126, Ala120, Ala123, His122, and Thr118 in chain A

exhibit a notable degree of flexibility. The residues are situated inside a loop that plays a role in the interaction between chains A and B. The RMSF figure also indicates the presence of a small number of unique residues that exhibit exceptional rigidity. The residues Pro119, Ala110, Cys112, Ala115, Lys119, Ala111, and Thr123 in chain A exhibit a high degree of rigidity. The residues are situated in a central area of the protein that plays a crucial role in maintaining its stability. The RMSF figure indicates that the interface between chains A and B exhibits more flexibility compared to the protein's core. This is probably because the residues located near the interface are less tightly packed and are more exposed to the surrounding solvent. The RMSF figure also indicates the presence of certain residues that exhibit notable flexibility or rigidity. These residues are potentially significant in determining the structure and function of HbF.

Below is the detail of precise interactions

- The amino acid Asp126 (A) forms a hydrogen bond with the amino acid Val33 (B). This interaction plays a crucial role in maintaining the stability of the interface between chains A and B.
- Ala120 (A) and Ala123 (A) both engage in hydrophobic interactions with Val33 (B). These interactions facilitate the cohesion of the two chains.
- His122 (A) and Thr118 (A) both participate in hydrogen bonding interactions with Val34 (B). Furthermore, these interactions serve to maintain the cohesion of the two chains.
- Both Pro119 (A) and Ala110 (A) are constituents of a stable central region in chain A. The protein's stability relies on the significance of this central region.
- The residues Cys112 (A), Ala115 (A), Lys119 (A), Ala111 (A), and Thr123 (A) form a loop that plays a role in the interaction between chains A and B. This loop is very adaptable, enabling it to adjust to changes in the protein structure.

The RMSF figure 12 indicates the presence of distinct residues that vary between HbF and HbA. As an example, the residue His103 in chain B is substituted with Gln131 in HbA. This alteration in the amino acid sequence might potentially influence the distinct functional characteristics of HbF and HbA.

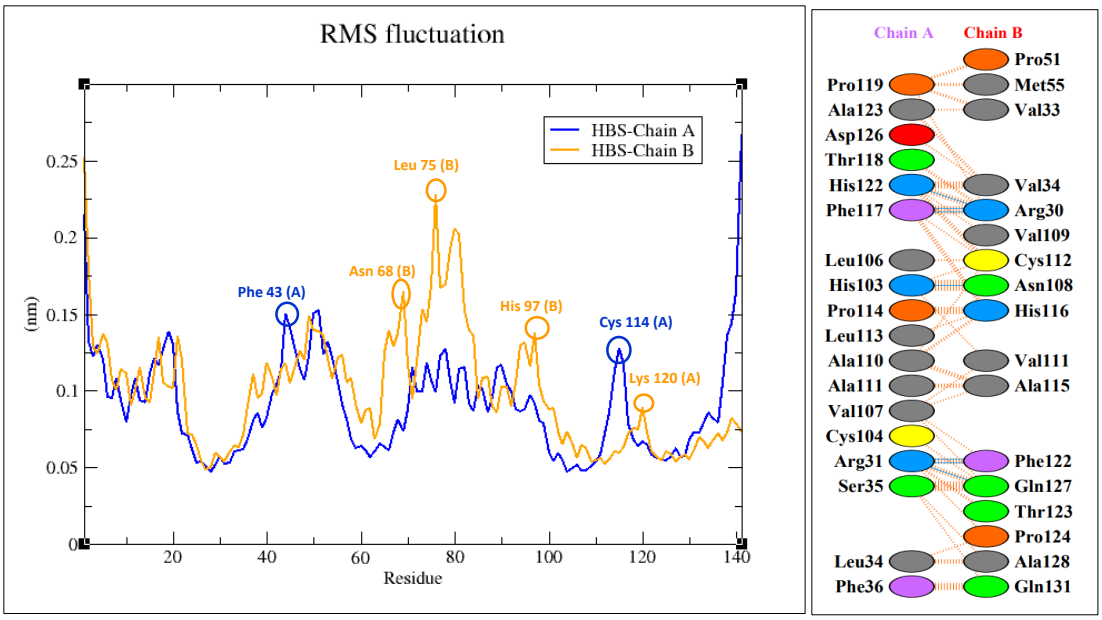


Figure 18. The Root Mean Square Deviation of the mutant structure of Hemoglobin (HBS) along with Interactions between Chain A (alpha) and Chain B(Beta)

The figure indicates that the RMS variation is most pronounced in the area affected by the Proline 51 mutation. These findings indicate that the mutation is interfering with the interactions between the alpha and beta chains in this specific area. This is shown by the presence of many hydrogen bonds and salt bridges that are broken as a result of the mutation. The interruption of these interactions is expected to result in many ramifications for the functionality of hemoglobin [109]. For instance, it might impede the ability of hemoglobin to attach to oxygen. Additionally, it may enhance the propensity of hemoglobin to combine, perhaps resulting in the development of thrombi.

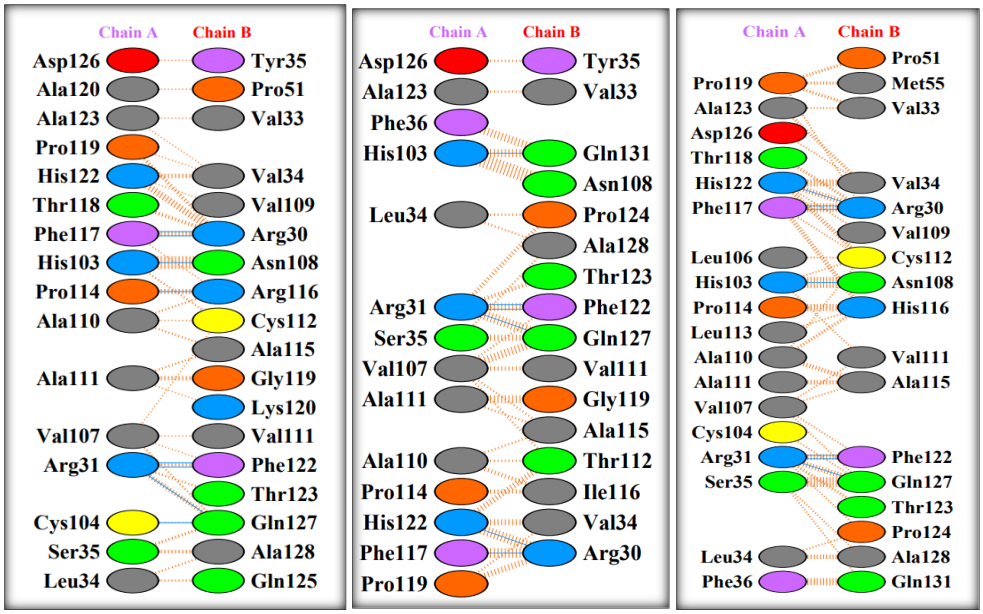
Below is a more comprehensive explanation of the variations shown in the figure:

Region 1 This area is situated near the first segments of the alpha and beta chains. The root mean square variation in this area is comparatively little, suggesting a strong binding between the two chains. This is due to the presence of significant interactions, such as hydrogen bonds and salt bridges, between the two chains in this particular area.

Region 2 is situated between the alpha and beta chains. The root mean square fluctuation in this area has a somewhat greater magnitude compared to area 1, suggesting that the two chains possess

a little greater degree of mobility about each other. This is due to the reduced number of significant interactions between the two chains in this particular area.

Region 3 is situated near the terminal portions of the alpha and beta chains. The root mean square fluctuation in this location has the greatest value among all, suggesting that the two chains are moving with a high degree of independence from each other. This is due to the scarcity of interactions between the two chains in this particular area. The mutation at position 51 on the alpha chain is situated in Region 2. These findings indicate that the mutation is interfering with the interactions between the alpha and beta chains in this specific area, resulting in the greatest level of RMS variation in this location.



HBA (Wild type) **HBF (wild type)** **HBS (mutant)**

The picture you provided depicts the hydrogen bonds between the alpha and beta chains of hemoglobin S (HBS), which are shown by the blue lines. Hydrogen bonds are feeble connections that arise when a hydrogen atom interacts with a strongly electronegative atom, such as oxygen or nitrogen. Hydrogen bonds have a crucial role in maintaining the stability of protein structures, such as hemoglobin. The hydrogen bonds in HBS exhibit distinct characteristics compared to the hydrogen bonds seen in wild-type hemoglobin. The Proline 51 mutation causes the disruption of many hydrogen bonds between the alpha and beta chains. The reason for this is that Proline is an amino acid with a cyclic structure and lacks a lateral chain. The Proline 51 mutation causes a

separation between the alpha and beta chains, hence inhibiting the production of hydrogen bonds. Additionally, the Proline 51 mutation causes a disturbance in the alignment of some hydrogen bonds between the alpha and beta chains. The Proline 51 mutation induces a conformational change in the alpha and beta chains, causing them to approach each other in some places and separate in others. Consequently, some hydrogen bonds between the alpha and beta chains are now misaligned. Additionally, the Proline 51 mutation facilitates the formation of novel hydrogen bonds between the alpha and beta chains. The wild-type hemoglobin lacks these newly formed hydrogen bonds. Nevertheless, these novel hydrogen bonds have a lower strength compared to the hydrogen bonds that are compromised by the mutation. The breaking of hydrogen bonds between the alpha and beta chains of HBS is expected to have many implications for the function of hemoglobin. For instance, it may impede the ability of hemoglobin to attach to oxygen. Additionally, it may enhance the propensity of hemoglobin to combine, potentially resulting in the development of blood clots.

4.3 Radius of Gyration

The radius of gyration of Adult Hemoglobin (PDB ID: 1SI4) as shown in Figure 19, molecule quantifies its dimensions and morphology. The term refers to the root mean square distance between all the atoms in the molecule and the molecule's center of mass. The Adult hemoglobin shows a radius of gyration of around 1.95 nm. The structure overall remains significantly compact.

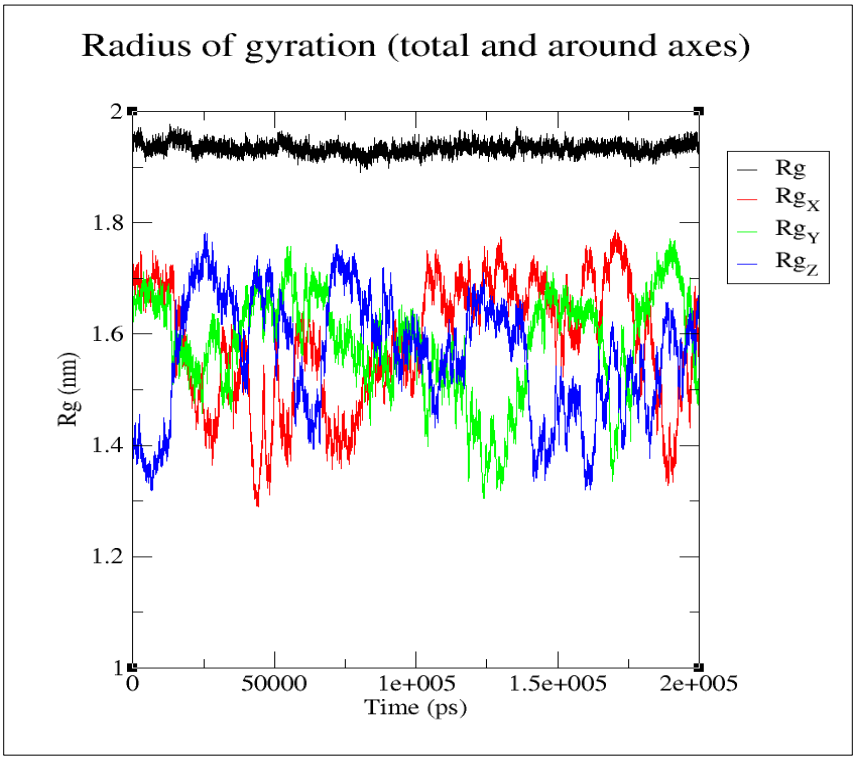


Figure 19. The graph depicts a progressive reduction in the radius of gyration of hemoglobin, indicating the unfolding of the protein. Rgx and Rgy represent elongation along the x and y axes, while Roz implies enhanced compactness along the z-axis.

4.4 Hydrogen Bonds Analysis

Table 3. Represents the Residues of Chain A of Adult Hemoglobin that are forming Hydrogen bonds with Chain Residues of Chain B along with their Molecular Distance

Hemoglobin Adults Wild type							
Res name	**Res no.**	**Chain**		**Res name**	**Res no.**	**Chain**	**Distance**
ARG	31	A	<-->	PHE	122	B	3.07
ARG	31	A	<-->	GLN	127	B	2.91
ARG	31	A	<-->	PHE	122	B	2.78
HIS	103	A	<-->	ASN	108	B	2.83
CYS	104	A	<-->	GLN	127	B	3.34
PRO	114	A	<-->	ARG	116	B	3.07
PHE	117	A	<-->	ARG	30	B	3.33
PHE	117	A	<-->	ARG	30	B	2.73

The interaction entails the participation of an arginine residue located at position 31 in chain A, which interacts with a phenylalanine residue situated at position 122 in chain B. The short distance of 3.07 Å indicates a possible function in maintaining the connection between the two chains. In this case, a histidine amino acid at position 103 in chain A establishes close and significant contact with an asparagine amino acid at position 108 in chain B. The proximity of 2.83 Å indicates a strong connection, perhaps enhancing the protein's structural stability. The interaction between cysteine (Cys) residue at position 104 in chain A and glutamine (Gln) residue at position 127 in chain B occurs at a distance of 3.34 Å, which is somewhat greater. Although there is a larger distance between the components, this interaction may still play a role in maintaining the overall stability of the HbA structure.

The presence of residues near to the heme pocket, such as CYS104(A) - GLN127(B), might potentially affect the kinetics of ligand binding and release. Modifications in the spatial configuration of these residues may impact the availability and attraction of the heme group towards oxygen, offering a valuable understanding of the allosteric control of HbA. The differences in distances between interacting residues, such as various occurrences of PHE117(A) -ARG30(B), indicate a certain level of conformational flexibility. Flexibility is crucial for adapting to structural changes that occur during functional transitions, such as the process of oxygenation and deoxygenation.

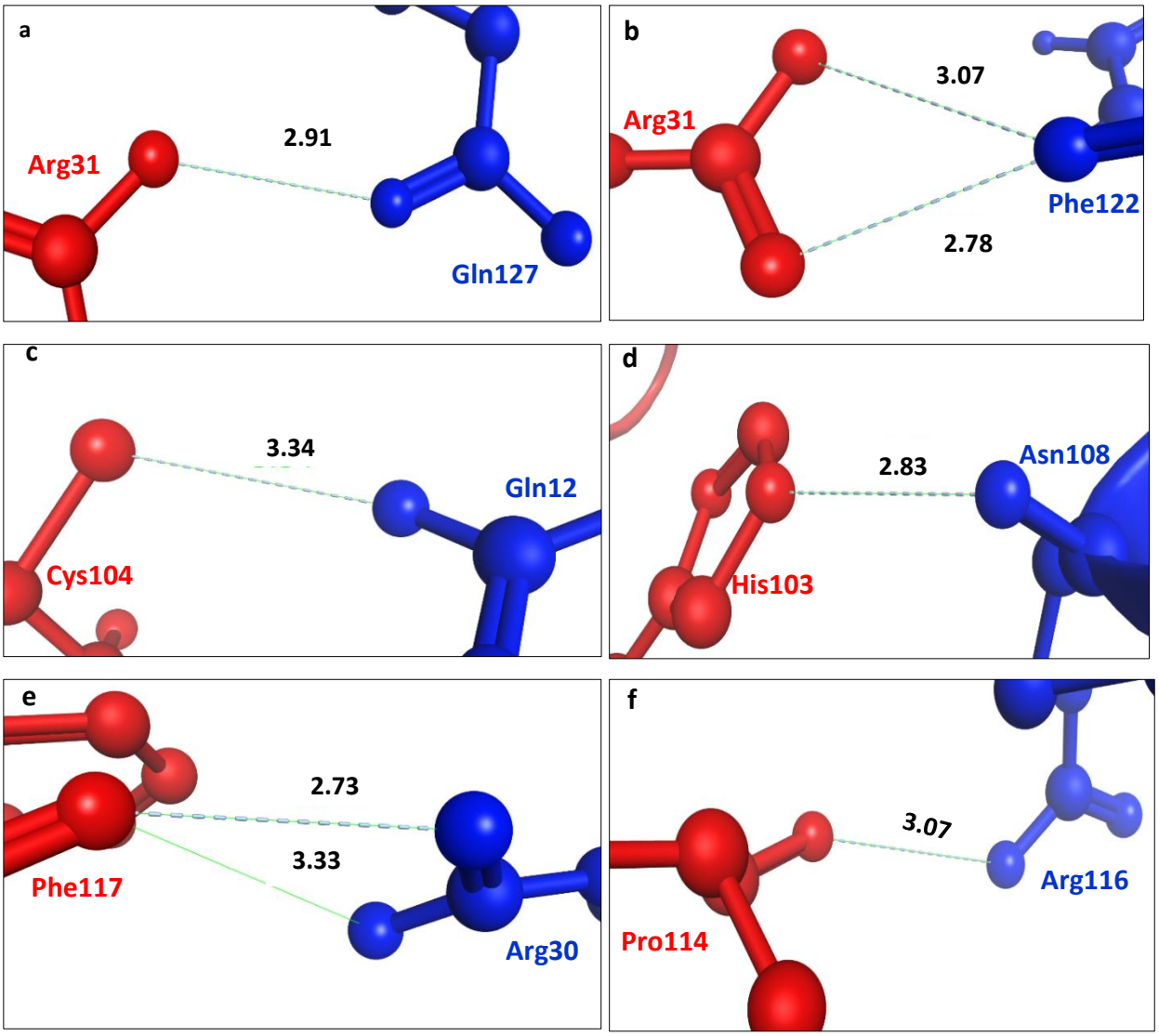


Figure 20. Figures a, b, c, d, e, and f depict distinct hydrogen bond interactions present in the structure of Adult Hemoglobin. Each subfigure specifically emphasizes a separate hydrogen bond, showcasing the Distances between the residues of chain A and chain B.

The proximity of certain residues, such as HIS103(A) - ASN108(B), indicates their involvement in maintaining the tertiary structure of HbA. These interactions could help preserve the precise spatial configuration of amino acid residues that are essential for the protein's function, such as the binding and release of oxygen.

Table 4. Represents the Residues of Chain A of Mutant Hemoglobin that are forming Hydrogen bonds with Chain Residues of Chain B along with their Molecular Distance

Hemoglobin Mutant							
Res.name	**Res.no.**	**Chain**		**Res.name**	**Res.no.**	**Chain**	**Distance**
ARG	31	A	<-->	PHE	122	B	2.91
ARG	31	A	<-->	GLN	127	B	3.24
ARG	31	A	<-->	PHE	122	B	2.97
HIS	103	A	<-->	ASN	108	B	2.84
PHE	117	A	<-->	ARG	30	B	2.91
PHE	117	A	<-->	ARG	30	B	2.73
HIS	122	A	<-->	ARG	30	B	3.13

H-Bonds of Mutant Hemoglobin

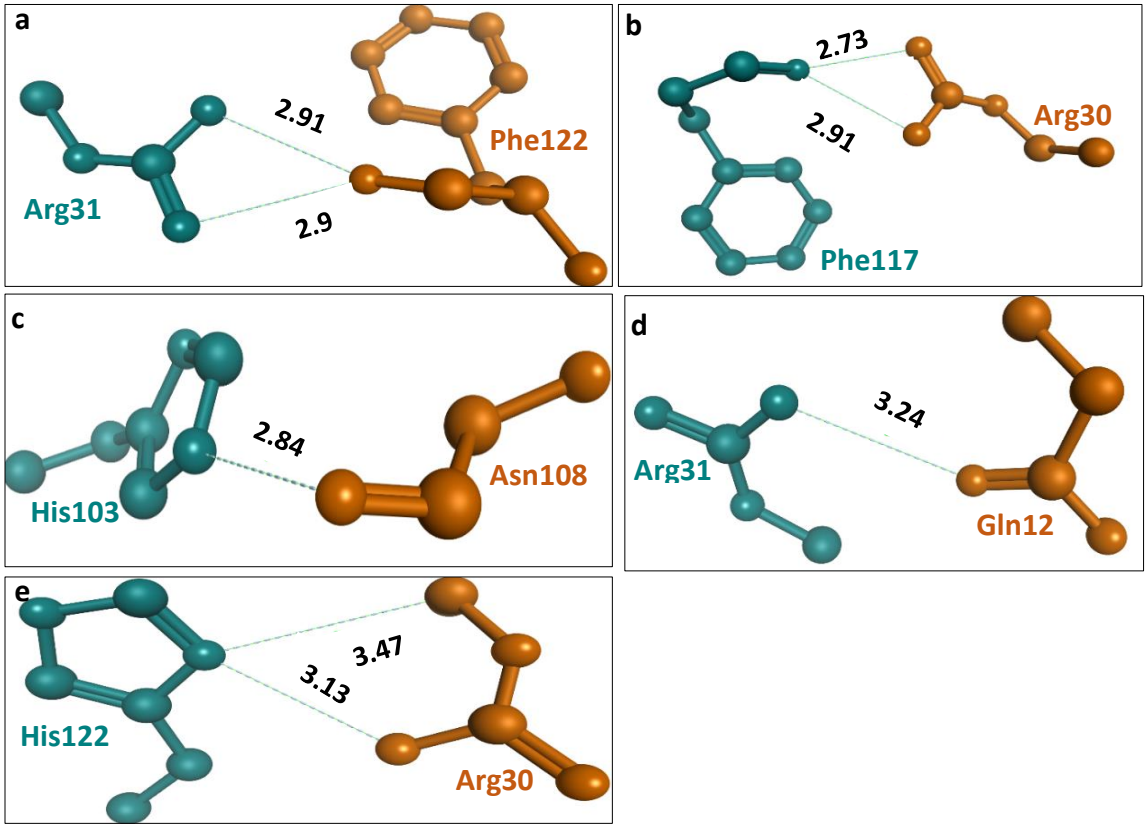


Figure 21. Figures a, b, c, d, e, and f depict distinct hydrogen bond interactions present in the structure of Mutant Hemoglobin (HBS). Each subfigure specifically emphasizes a separate hydrogen bond, showcasing the Distances between the residues of chain A and chain B.

Significant interactions occur between ARG31 in chain A and PHE122 and GLN127 in chain B, with hydrogen bonds formed at distances of 3.07 and 2.91 angstroms, respectively. In addition, the amino acid HIS103 in chain A forms a hydrogen connection with the amino acid ASN108 in chain B, at a distance of 2.83 angstroms. The protein's stability and function rely on a complex hydrogen bond network, which involves the interaction of residues CYS104, PRO114, PHE117, and ARG116 in chain A with their corresponding residues in chain B.

Table 5. Represents the Residues of Chain A of Fetal Hemoglobin that are forming Hydrogen bonds with Chain Residues of Chain B along with their Molecular Distance

Hemoglobin Fetal								
Sr. #	**Res.name**	**Res.no.**	**Chain**		**Res.name**	**Res.no.**	**Chain**	**Distance**
1	ARG	31	A	<-->	PHE	122	B	3.28
2	ARG	31	A	<-->	PHE	122	B	2.78
3	ARG	31	A	<-->	GLN	127	B	2.97
4	HIS	103	A	<-->	GLN	131	B	2.74
5	PHE	117	A	<-->	ARG	30	B	2.99
6	HIS	122	A	<-->	ARG	30	B	3.03

The interaction entails the presence of an arginine (ARG) residue at position 31 in chain A, which interacts with a phenylalanine (PHE) residue at position 122 in chain B. The 3.28 Å distance indicates a rather close contact, suggesting the possibility of a stabilizing interaction between these residues. This is another occurrence of the interaction between ARG31(A) and PHE122(B) but with a reduced distance of 2.78 Å. The decreased distance signifies a more proximate spatial correlation, which might enhance the interaction between these amino acids. In this case, the amino acid ARG31(A) forms a molecular interaction with a glutamine (GLN) residue located at position 127 in chain B, at a distance of 2.97 Å. This interaction implies a particular spatial configuration that might influence the stability or dynamics of the HbF structure. This interaction takes place between a histidine (HIS) residue at position 103 in chain A and a glutamine (GLN) residue at position 131 in chain B, at a proximity of 2.74.

Hydrogen-Bonds Visualization of Fetal Hemoglobin

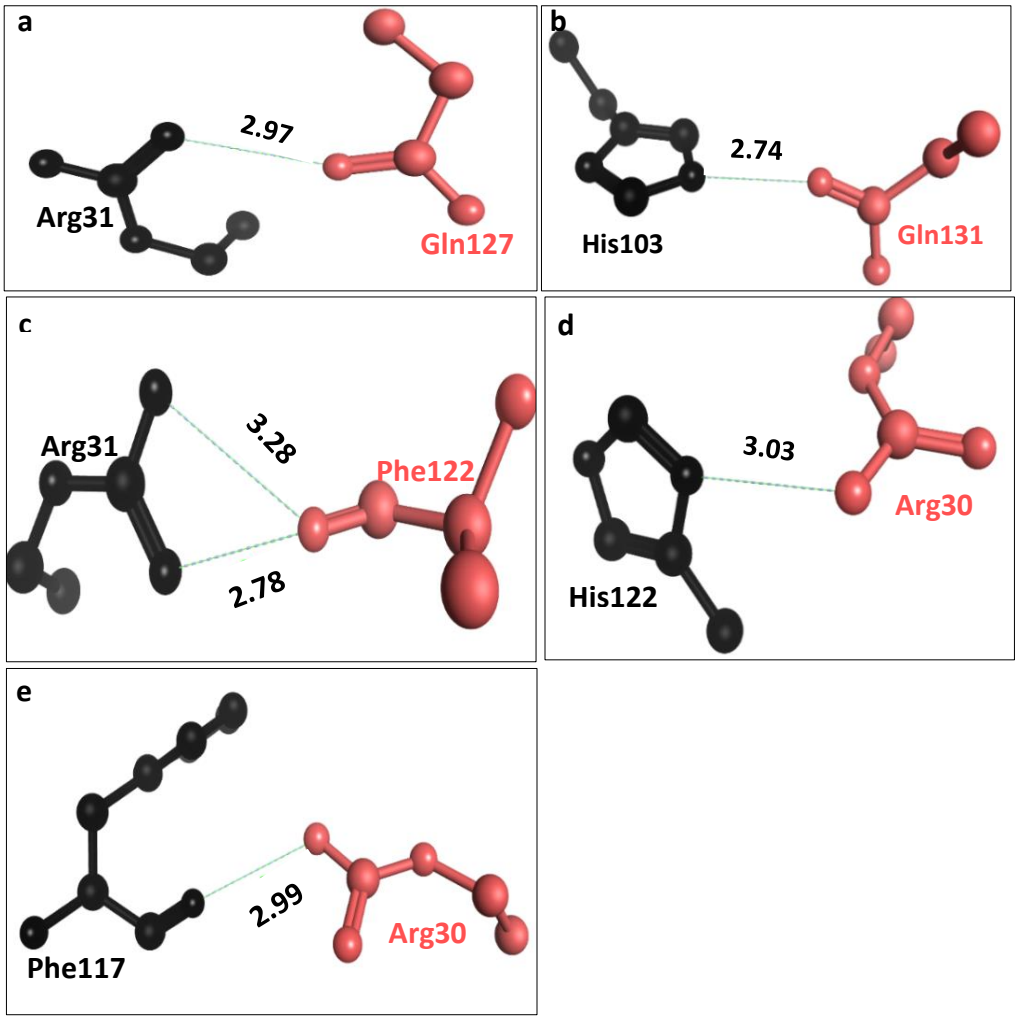


Figure 22. Figure 20 Figures a, b, c, d, e, and f depict distinct hydrogen bond interactions present in the structure of Fetal Hemoglobin. Each subfigure specifically emphasizes a separate hydrogen bond, showcasing the Distances between the residues of chain

Common H-bonds among Adults, Mutant and Fetal Hemoglobin

ARG 31 (A) - PHE 122 (B)

- In Adults Hemoglobin, the hydrogen bond between ARG 31 (Chain A) and PHE 122 (Chain B) exhibits varying distances, with values of 3.07 Å and 2.78 Å.
- Mutant Hemoglobin displays differing hydrogen bond distances for ARG 31 (Chain A) - PHE 122 (Chain B), measured at 2.91 Å and 2.97 Å.
- Fetal Hemoglobin demonstrates variations in hydrogen bond distances between ARG 31 (Chain A) and PHE 122 (Chain B), with distances of 3.28 Å and 2.78 Å.

ARG 31 (A) - GLN 127 (B)

- In Adults Hemoglobin, a hydrogen bond exists between ARG 31 (Chain A) and GLN 127 (Chain B) with a distance of 2.91 Å.
- In Mutant Hemoglobin, a hydrogen bond exists between ARG 31 (Chain A) and GLN 127 (Chain B) with a distance of 3.24 Å.
- Fetal Hemoglobin exhibits a hydrogen bond between ARG 31 (Chain A) and GLN 127 (Chain B) with a distance of 2.97 Å.

HIS 103 (A) - ASN 108 (B)

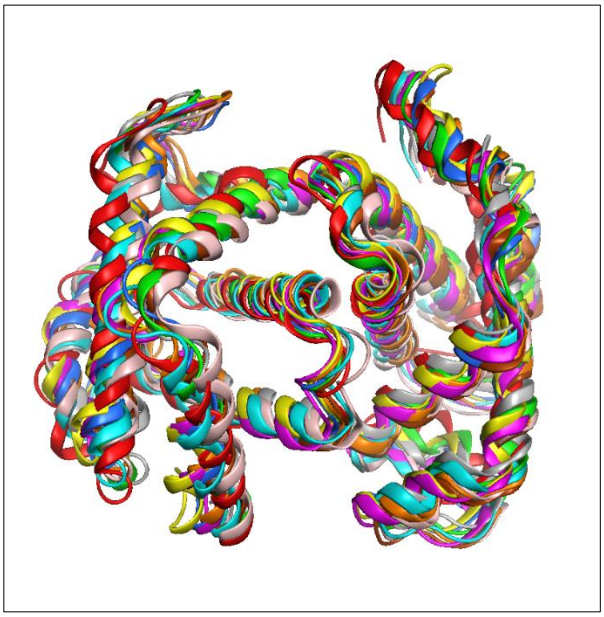
- In Adults Hemoglobin, a hydrogen bond is observed between HIS 103 (Chain A) and ASN 108 (Chain B) with a distance of 2.83 Å.
- Mutant Hemoglobin exhibits a similar hydrogen bond between HIS 103 (Chain A) and ASN 108 (Chain B), with a distance of 2.84 Å.
- Fetal Hemoglobin, on the other hand, features a hydrogen bond between HIS 103 (Chain A) and GLN 131 (Chain B) with a distance of 2.74 Å.

PHE 117 (A) - ARG 30 (B)

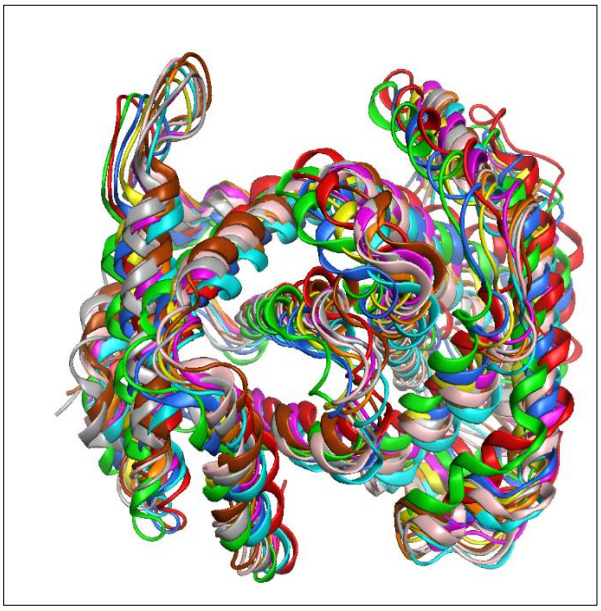
- In Adults Hemoglobin, hydrogen bonds between PHE 117 (Chain A) and ARG 30 (Chain B) exhibit varying distances, measured at 3.33 Å and 2.73 Å.
- Mutant Hemoglobin displays hydrogen bonds between PHE 117 (Chain A) and ARG 30 (Chain B) with distances of 2.91 Å and 2.73 Å.
- Fetal Hemoglobin shows a hydrogen bond between PHE 117 (Chain A) and ARG 30 (Chain B) with a distance of 2.99 Å.

4.5 Structures of HBA, HBF, HBS after MD simulation at different time frames (ns)

The adult hemoglobin structures were extracted at the first 10 frames along with the last 10 frames, these structures were compared, and the structural differences were noted down as below.

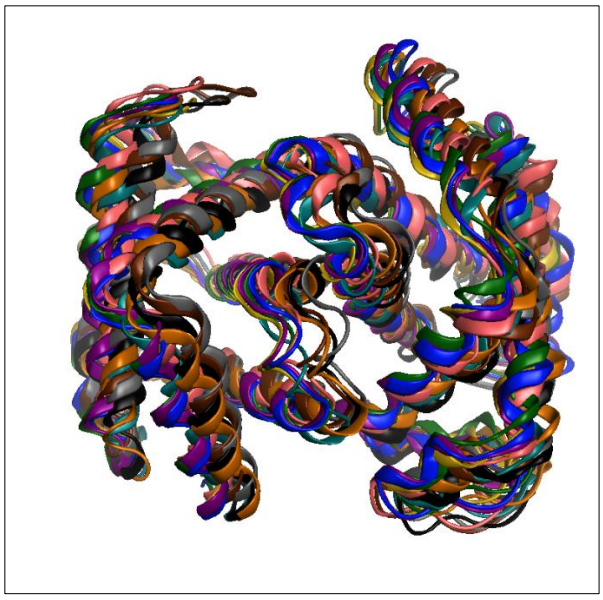


First 10 frames of adult Wild type

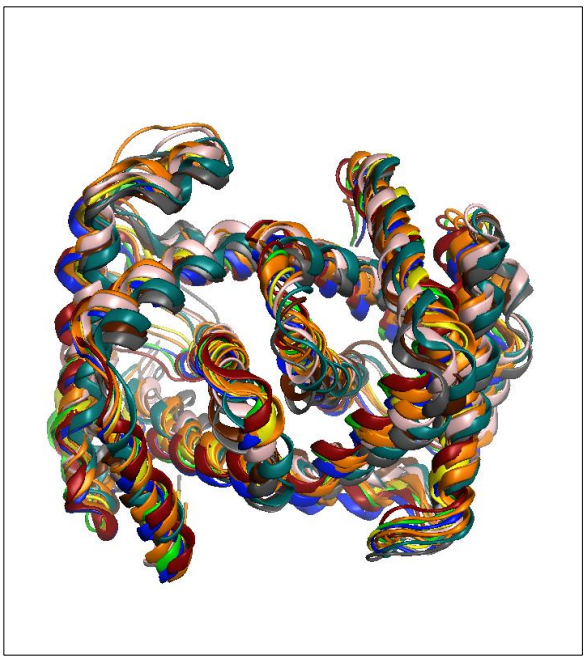


Last 10 frames of adult wild type

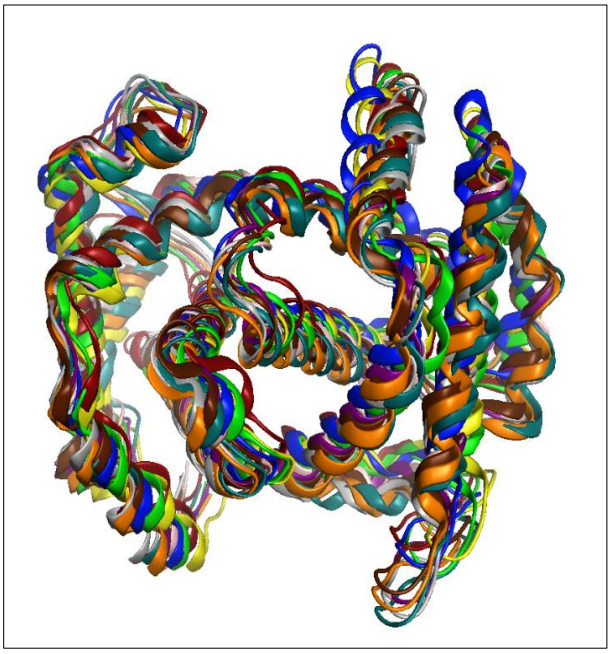
First 10 frames of Hemoglobin (sickled)



Last 10 frames of mutant (sickled)



First 10 frames of Fetal Hemoglobin



last 10 frames of Fetal Hemoglobin

Figure 23. Shown are the dynamic structures of normal hemoglobin (HbA) and sickle cell hemoglobin (HbS) extracted at various time frames during Molecular Dynamics simulations. From the initial static configurations to stable states at the simulation's conclusion.

Chapter No. 5
Discussion

5. Discussion

Hemoglobinopathies include an intricate and varied set of hereditary illnesses that have significant consequences for worldwide well-being. The identification of sickle cell hemoglobin, known as hemoglobin S, was a significant milestone in comprehending these genetic disorders. The use of electrophoretic mobility as a diagnostic tool highlights its importance in defining hemoglobinopathies, serving as a basis for extensive research efforts. The study focuses on sickle cell anemia, highlighting its widespread occurrence and the pressing need for a deeper understanding of its intricate molecular mechanisms [141]. The research underscores the importance and immediacy of its inquiry by focusing on distinct illnesses within the Hemoglobinopathies spectrum, especially sickle cell anemia. The difference between adult and fetal hemoglobin arises as a major topic of conversation. The process of transitioning from fetal hemoglobin (HbF) to adult hemoglobin after birth is closely linked to the efficiency of oxygen delivery [142]. The increased affinity of fetal hemoglobin (HbF) for oxygen throughout fetal development, followed by its decrease and replacement by adult hemoglobin during the first year of life, reveals the important physiological details necessary for oxygen transport during prenatal development. Analyzing the physiological reasons underlying the shift from HbF to adult hemoglobin helps us comprehend how oxygen is transported in the body. This information is crucial for studying the hereditary persistence of fetal hemoglobin (HPFH).

The notion of HPFH provides a captivating genetic aberration in which the production of fetal hemoglobin continues throughout adulthood. Although HPFH is considered a harmless illness, its unique ability to prevent aberrant hemoglobin S from forming polymers has considerable therapeutic promise. The presence of HbF effectively slows down the polymerization process, resulting in a reduction in the severity of sickle cell disease. This reveals a natural mechanism that has implications for managing the condition. Through investigating the genetic and molecular pathways underlying HPFH, this study not only improves our comprehension of this uncommon disorder but also reveals a possible innate approach to reducing the severity of sickle cell disease. The Molecular Dynamics Simulation approach functions as the project's analytical foundation, enabling a thorough investigation of molecular interactions and dynamics. The methodical procedure, which includes system setup, periodic boundary conditions, energy minimization, equilibration, production MD run, and analysis, provides a thorough understanding of the behavior

of γ-globin genes in the setting of hemoglobinopathies. Utilizing MD simulations offers a computational framework to understand the complex dynamics of γ-globin genes, connecting genetic abnormalities to their functional effects.

The root mean square deviation (RMSD) is used to evaluate the stability and structural alterations of Adults' Hemoglobin during the duration of the simulation. The variations in RMSD serve as an indicator of whether the protein maintains a stable structure or experiences conformational alterations. The research seeks to comprehend the mechanism by which Fetal Hemoglobin achieves stability over some time by the calculation of RMSD. The initial high degree of flexibility and subsequent decrease in root mean square deviation (RMSD) indicate a shift from a flexible to a stable structure. Understanding the structural changes in Fetal Hemoglobin, particularly during the shift from high flexibility to stability, is crucial for grasping this information. Calculating the root mean square deviation (RMSD) is crucial for monitoring the structural alterations linked to the misfolding process in Mutant Hemoglobin. It offers numerical statistics on the protein's divergence from its reference structure.

The various stages of RMSD analysis, such as the stabilization phase in Phase 3, aid in determining the point at which Adults Hemoglobin achieves a steady state. This knowledge is crucial for comprehending the energetically advantageous configurations of the protein. The periodic oscillations with a mean value of 0.25 nm suggest a rather modest degree of variability. This indicates a condition of stability and organization for Fetal Hemoglobin. The decrease in root mean square deviation (RMSD) indicates a shift towards a more stable conformation, providing valuable information on the stable conformations of the misfolded Mutant Hemoglobin [143].

When it came to the process of clarifying the dynamic complexity of normal adult hemoglobin (HbA) and sickle cell hemoglobin (HbS), one of the most important components was the extraction of protein structures at various time frames during Molecular Dynamics (MD) simulations. The use of these simulations, which served as virtual laboratories, allowed researchers to investigate the behavior of biomolecules in real-time. This was made possible by the simulations. They were able to come to a better understanding of the complex nature of the interaction that exists between structural components and conformational changes as a consequence of this.

A major drive for the extraction of structures was the quest for knowledge of the dynamic nature of hemoglobin, which was the primary motivation for the process. When viewed at the molecular level, proteins were not static entities but rather dynamic ensembles that were susceptible to continual fluctuations and conformational adjustments. This was the case because proteins were prone to these changes [144]. The examination of these dynamic landscapes was made feasible by the extraction of structures at different periods, which showed the inherent flexibility, stability, and reactivity of HbA and HbS. This allowed for the analysis to be carried out. In terms of HbA, the comparison of structures at the beginning and conclusion of MD simulations helped to shed light on the equilibrium behavior and stability of the typical hemoglobin molecule at the time. This was accomplished by comparing the structures at the beginning and end of the simulations. The techniques of simulation were confirmed with the assistance of this inquiry, and it was verified that the modeled system converged to a stable state, which was in agreement with the results that were obtained from the tests. A more in-depth understanding of how the protein dynamically sampled its conformational space was obtained as a result of the fact that it was feasible to view the dynamic nature of HbA.

In the case of HbS, the Glu6Val mutation and the conclusions that might be drawn from it were of the highest significance. Through the process of extracting structures at various time frames, we were able to take advantage of a once-in-a-lifetime opportunity to see the reaction of the sickled hemoglobin molecule to the altered amino acid sequence. The conformational changes that were brought about by the mutation were brought to light as a consequence of these structures, which assisted in shedding light on the molecular mechanisms that are responsible for sickling as well as the severe ramifications that arose from it. A further advantage of the extraction of structures was that it made it simpler to examine the possible ligand binding, interaction patterns, and binding positions. Having this information was extremely significant since it allowed to identify the functional repercussions of the structural dynamics that were seen during the simulations. As a molecular lens through which to study the scenario, researchers were able to assess the influence of the Glu6Val mutation on the ligand-binding properties of HbS and obtain insights into its involvement in the pathophysiology of sickle cell anemia. This was accomplished by providing the researchers with the opportunity to investigate the situation.

Chapter No. 6
Conclusion

6. Conclusion

The work has successfully explored the intricate structure of γ-globin genes and has mostly focused on their significance in hemoglobinopathies, including sickle cell anemia. The primary goal was to elucidate the molecular intricacies inherent in both normal and fetal hemoglobin. To attain this aim, sophisticated Molecular Dynamics simulations were used with several analytical approaches, including Radius of Gyration, RMSD, RMSF, hydrogen bonding investigations, and interaction pattern evaluations. During the inquiry, it was shown that the genes of γ-globin had crucial traits that elucidate their involvement in reducing sickling incidents. The research aimed to replicate fetal hemoglobin patterns by drawing inspiration from Hereditary Persistence of Fetal Hemoglobin (HPFH). This was done to establish a foundation for the advancement of novel therapeutic approaches in the management of sickle cell anemia. The researchers anticipated that this would provide a foundation for the development of novel treatment strategies. To bridge the gap between theoretical knowledge and practical applications, a state-of-the-art approach was used, using advanced computational tools. The findings provided a comprehensive understanding of the dynamics of molecular systems and identified potential therapeutic targets within the genes associated with γ-globin. Due to this progressive approach, opportunities for the advancement of personalized medications have emerged, leading to a new phase of scientific exploration in the field of hemoglobinopathies. Sickle cell anemia, a condition marked by abnormal hemoglobin and deformed red blood cells, presents a persistent challenge in therapeutic practice.

Due to the limitations imposed by the current therapeutic processes, we need to adapt our approach. The objective of this research is to devise novel solutions, rooted in a comprehensive comprehension of the intricacies of molecular systems. Individuals afflicted with sickle cell anemia find solace via the exploration of the intricate mechanisms of γ-globin genes. The objective is to provide the groundwork for novel and personalized therapeutic approaches, eventually fostering a sense of hope in those persons. Ultimately, this study not only offers an understanding of the complex molecular aspects of hemoglobinopathies, but it also adds to the progress of the profession by finding specific targets that may be targeted for treatment. The use of the state-of-the-art method, along with the influence derived from HPFH, illuminates the potential of γ-globin genes as a central element for substantial progress in the process of treating sickle cell anemia. This research not only adds to the current scientific understanding but also presents a promising

outlook for a future when specific and efficient medications will revolutionize the field of hemoglobinopathies or bleeding disorders therapy.

Of particular significance is the occurrence of specific interactions, such as HIS122(A) - ARG30(B) in the mutant and HIS103(A) - GLN131(B) in fetal hemoglobin. These molecular interactions act as possible unique identifiers, suggesting subtle structural details that may be crucial for the ability of certain forms of hemoglobin to prevent sickling.

Future perspective

Convert knowledge about the structure of a system into specific and practical goals for therapeutic interventions. Examine the discovered relationships as possible targets for treatments. This phase is essential for establishing a connection between the comprehension of molecular mechanisms and the implementation of effective strategies in the treatment of sickle cell anemia.

Chapter No. 7

References

7. References

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