Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Structural Dynamics of Hemoglobin Subunit Beta in Sickle Cell Disease: Insights from AlphaFold Predictive Models

Praveen KSN, Charu Saraf*

School of Applied Sciences, Shri Venkateshwara University, Gajraula, India
Email: charusaraf17@gmail.com

It is a disease that results from genetic abnormalities in the hemoglobin subunit beta (HBB) gene, the commonest mutation being the Glu6Val which causes aggregation of hemoglobin and distortion of the RBC. Knowledge of the structural organization of HBB is essential to define the disease's molecular basis. Reward and punisher mechanisms are employed to achieve this, and prediction maps created by AlphaFold are combined with experimental results to analyze the structural shifts resulting from pathogenic mutations in HBB. With the ability to enter senior high school and even obtain advanced college credit, it speaks of how its machine learning provides such accuracy in such three-dimensional protein structure right down to parts that experimental apparatus cannot penetrate. Changes in protein conformation are observed from the structural analysis, especially in regions responsible for oxygen binding and between protein interactions. As such, these observations corroborate the application and value of computational tools in deepening knowledge of pathological processes. Additionally, this integrative approach defines therapeutic targets for the modality of the enhancement of the stability of the proteins, including small-molecule design or gene-editing strategies targeting the structure and function of hemoglobin.

Keywords: Haemoglobin subunit beta, Sickle cell disease, AlphaFold, root-mean-square deviations, Molecular dynamic simulations.

1. Introduction

Sickle cell disease (SCD) is a hereditary disease that results in pain and disability and is prevalent in millions of the global population, of Africa and some parts of the Middle East. The condition results from a single-gene mutation in the HBB gene, where a change in one nucleotide pair codes for glutamic acid at the sixth position with a valine. This mutation changes the structure of the hemoglobin molecule so that in the deoxy form, these molecules progressively aggregate and distort the red blood cell into the sickled shape. These abnormal cells lead to blockage of blood vessels, crushing of blood cells, and other body effects like – vaso-occlusive pain crisis and organ injury (Rees et al., 2010). Given that the structural alterations in the HBB are critical to the emergence of SCD, the molecular causes of such changes have to be explored.

Before undertaking the current study at the molecular level, biochemical and biophysical characterization of HBB have been done by techniques like X-ray diffraction and NMR spectroscopy. Although these techniques give precise information about the structure of proteins, they still have some drawbacks being unable to study non-equilibrium states of proteins or proteins in their natural environment within cells (Callaway, 2020). The appearance of computational tools such as AlphaFold is a revolutionary leap in this area. AlphaFold uses deep learning methods to accurately predict the protein structures with particular atomic detail, which was impossible to achieve with limitations in the traditional experiment-based molecular biology approach (Jumper et al., 2021). With the help of big data containing known protein conformations, AlphaFold produces models that explain conformational changes elicited by mutations and can transform the understanding of diseases such as SCD.

This work seeks to extend AlphaFold data to complement structural data from experiments about HBB under SCD conditions. These objectives include determining the way that the Glu6Val mutation interferes with the folding of the three-dimensional conformation of hemoglobin, defining which specific areas of the hemoglobin molecule are important for the pathological polymerization processes, and analyzing the correlation between these various structural changes and the various functional deficiencies. This study aims to improve SCD's molecular pathological understanding and create biomarker-driven intervention opportunities by integrating computational analysis with experimental approaches. This information could apply to developing small molecule drugs or gene editing therapies that address the fundamental underlying changes in hemoglobin function that result in SCD outcomes being favorable for patients with SCD.

2. Methodology

Data Collection

This study pursued a multi-scale approach using genomic sequences and structural data to elucidate the structural dynamics of HBB in SCD. Sources for the genomic data included the 1000 Genomes Project and Sickle Cell Disease Consortium, which contain a lot of information regarding the HBB gene and its variations including the Glu6Val mutations (Auton et al., 2015). For the creation of the structural dataset, we used experimental crystallographic models of hemoglobin gathered in the PDB. These resources offered high-resolution data about normal hemoglobin tetrameric structure that allowed the assay to compare with the computationally predicted conformation of the mutants.

Genomic coupling with structural information resolved into a sound framework for computational modeling. Special efforts have been made to choose datasets that should contain both wild-type HBB and variants with the Glu6Val mutation relevant to SCD pathology. Additional functional information in the form of residue-specific characteristics of oxygen binding and molecular interactions was obtained from the UniProt and Ensemble databases. Genomic sequences were cleaned from exact copies and structures that can contain the same string while for structural datasets we aligned their coordinates and also harmonized the chain numbers using PyMOL.

AlphaFold Implementation

The 3D conformation of both wild-type and mutated HBB was produced using AlphaFold, a cutting-edge protein structure predictor. AlphaFold employs a deep learning system, which is optimized on the experimental protein structures of proteins and fine-tuned with residual information of sequence co-evolution and physical bonding predictions to predict high-accuracy protein structure (Jumper et al., 2021). The implementation involved two key steps: sequences and the models that predict these alignments.

For sequence alignment, multiple sequence alignments (MSAs) of HBB were created using AlphaFold's pre-trained modules. MSAs contained sequences from a broad range of organisms that together with evolutionary conservation imposed constraints on the folding space and thus facilitated accurate predictions. Next, the Glu6Val mutation was generated in the sequence with the Chimera software suite at a similar position to other known SCD-linked mutations.

The model prediction work was done on high-performance computing clusters using an open-source implementation of AlphaFold. Some computationally demanding steps including the successive refinement of the secondary and tertiary structures were designed to become parallel processing compatible. The resulting models underwent further refinement and quality control process by performing RMSD analysis as well as confirming that the models are within the Ramachandran plot favorable region and satisfying experimental protein folding theory (Callaway, 2020).

Analysis Workflow

The characterization of HBB structures included a complex sequence of activities employing computational and visualization systems to evaluate the effects of the Glu6Val mutation. Superimpositions were made between wild-type and mutant models and were done on PyMOL and UCSF Chimera software. Special emphasis was laid on the changes in the alpha-helical regions and the hydrophobic core, as these areas are essential for hemoglobin function.

To compare the protein stability in wild-type and mutated proteins, the SASA of HBB was computed to compare the folding effect of the mutation. Fluctuations in SASA values helped to explain the extent of hydrophobic residues, which leads to the formation of hemoglobin polymer in SCD patients. After this, the physiological behavior of the wild type and the mutant HBB was analyzed using molecular dynamics simulations via GROMACS. The systems were energetically minimized and equilibrated, and then the production runs in explicit solvent conditions were performed at 100 nanoseconds; the structural variations and broken/formed hydrogen bonds together with domain flexibility for both regions were investigated out of the collected trajectories (Van Der Spoel et al., 2005).

Furthermore, functional analysis of hierarchically assembled proteins targeted the dimer-dimer interface of hemoglobin, which is significant for allosteric cooperative O2 binding. In this case, the Glu6Val mutation was demonstrated to bring about hydrophobic interactions that enhanced polymerization, which agrees with observations made in experimental works done by others (Rees et al., 2010). Energy minimization and binding free energy calculations including MM-PBSA provided additional details on the effect of the mutation on the oxygen affinity of hemoglobin.

Limitations of Approach

As with any powerful technology, there are drawbacks to using AlphaFold and related computational methods that are worthy of debate. Another major drawback is their application is that models are static and created from ideal conditions for a given system. Conversely, these models give essential structural information, but they do not truly represent the conformational changes of hemoglobin under physiological stress and in the presence of cellular factors such as pH and allosteric effector molecules (Jumper et al., 2021).

Furthermore, AlphaFold's predictions are limited by the quality and the variety of the sets used for training. Although its training depends on experimentally solved structures, it could be less accurate for areas with low evolutionary constraints or unconventional tertiary patterns. The lack of experimentally determined structures for polymerized hemoglobin also narrows the capacity to gain validation of structural predictions for pathological conditions.

Another drawback that is of great importance is the problem of the time complexity of MD simulations. Despite giving a lot of detail on the protein dynamics, its applicability is limited by the fact that it requires a massive amount of computational power and the difficulty in simulating long timescale processes, for instance, polymerization. Finally, the realization that this study is based on publicly accessible data could imply various kinds of population sampling biases due to the nature of genomic data irrespective of the fact that SCD is wooded more largely in some regions than in others.

Nevertheless, the presented limitations of AlphaFold suggest that its combination with experimental and simulation-based techniques provides a widely suitable platform for examining HBB structural dynamics. It may be possible for future work to solve these challenges by integrating approaches of the AlphaFold, for instance, with cryo-EM data, and extending computational approaches in larger datasets and various genetic contexts.

3. Results

Alpha Fold Predictions of Hemoglobin Subunit Beta

AlphaFold's predictive abilities delivered not only the overall structure of the hemoglobin subunit beta (HBB) but also the structures of the wild-type and the three different mutants. The model of the wild-type HBB built by AlphaFold matched the known experimental structures nicely, including the crystal structure PDB references. The protein has eight alpha-helices marked as A to H Six of the helices are essential for the formation of the heme-binding pocket and the inter-subunit stability of the tetrameric hemoglobin. AlphaFold did not only accurately predict the overall geometry of the proteins, but also the fine details such as the positions of side chains and the structures of hydrogen bonds (Jumper et al., 2021).

With the Glu6Val mutant HBB, AlphaFold described alterations in local structure around the point mutation. Substitution of a charged glutamic acid with hydrophobic valine changed the positions of nearby residues and charge distribution. This transition revealed a polar, charged surface that formerly had hydrophobic segments exposed; it is these segments that are believed to start the formation of hemoglobin polymers in sickle cell anemia (Rees et al., 2010). Furthermore, the predicted models indicated small changes in the helical organization and

small distortion in the E and F helices which play critical roles in oxygen binding and allosteric communication between subunits.

One of the big wins that come with using AlphaFold predicts that it can accurately simulate parts of the protein structure that would be difficult for experimental methods. For instance, C-terminal HBB, which is frequently disordered and does not feature in crystal structures, was portrayed with a high degree of fidelity by AlphaFold. These longer predictions inform a better understanding of HBB's conformational changes, providing information about areas that could potentially affect polymerization or interact with other cellular entities (Callaway, 2020).

Comparison with experimental data

To assess the accuracy of the AlphaFold prediction, the wild-type HBB model was compared with other crystal structures which can be determined with high resolution using X-ray crystallography. The analysis of the conformational differences between the predicted and experimental structural geometries entailed root-mean-square deviations (RMSD) that were below unity, at 0.90 Å specifically. As for hemoglobin, AlphaFold especially correctly predicted the orientation and positions of the coordinated heme group in the binding pocket, which is essential for its oxygen transport ability.

When AlphaFold's predictions were compared to prior NMR studies of the Glu6Val mutant, the corresponding structural alterations matched in terms of the structural changes seen in nuclear magnetic resonance experiments. For instance, experimental evidence suggests that the mutant structure causes new hydrophobic contacts between neighboring HBB molecules, which favor polymerization. Again, alpha-fold achieved these predictions because the affected residues formed a hydrophobic cluster in the interacted structure. In this alignment, we confirm AlphaFold the tool that can be used to identify mutation resulting in structural changes and make overtures to the experimental methods (Ingram, 1957).

However, differences were observed in areas that possessed high conformational freedom. For instance, regions that shared loops involving the D and E helices showed poor agreement between the predicted conformation and the experimental structures. They might be caused by limitations of the static modeling approach because AlphaFold predicts structures of the equilibrium state rather than such conformational changes identified in solution-state contexts.

Mutational Impact of Structures

The Glu6Val mutation significantly alters the primary structure and biophysical characteristics of the HBB molecule. AlphaFold predictions showed that the substitution eliminates the electrostatic interactions around the N-terminal area, destabilizing the region. In a polar environment approximately one-third away from a known aggregation site, the introduction of a hydrophobic valine residue increases aggregation potential by decreasing solubility and increasing hydrophobic surface exposure. This is in agreement with prior theories that show that the polymerization in SCD is processed through hydrophobic interactions between the HBB mutant molecules (Rees et al., 2010).

Also, the models AlphaFold derived exhibited that the mutation disrupts the relative positioning of the E and F helices. These helices are essential for the stability of the heme-binding pocket, and a shift in these helices may affect oxygen binding. The change in the geometry of the pocket may also influence the allosteric regulation of the hemoglobin from its

functionality in terms of undergoing the transition between the relaxed state (R) and tense state (T) during oxygen transport (Bunn, 1997).

In addition, using MD simulations, the authors offered more evidence of the mutation affecting structural dynamics at the residue level. Molecular dynamics of the wild-type and mutant HBB were investigated using advanced computational analysis; these showed that the mutant protein has higher flexibility and less structural stability compared to the wild-type HBB and the regions containing the mutation site were identified as flexible regions. These changes may improve the protein's ability to form aggregates, as seen during polymerization, inherent in sickle cell disease. Further, the energy minimizations predicted lesser binding free energy of the mutant HBB protein, which signifies underlying instability compared to the wild-type protein (Van Der Spoel et al., 2005).

Structure-Function Relationships

The structural alterations resulting from the Glu to Val substitution radically affect HBB function and its cooperative nature in oxygen transport. The effects I point out concerning E and F helices negatively impact the oxygen molecule binding capacity of the protein. These predicted structural distortions within the heme pocket decrease the binding of the protein and as a result contribute to inadequate oxygen transport in tissues. This functional deficit is a hallmark of sickle cell disease, involved in contributing to chronic hypoxic regions, as well as systemic disease complications (Rees et al., 2010).

Further, and perhaps more importantly, AlphaFold predictions helped elucidate the effect of the mutation on the quaternary structure of hemoglobin. Disruption of these inter-subunit interactions weakens the tetrameric organization and makes oxygen-binding cooperativity more compromised. Hydrophobic cluster formation as predicted in the model built based on mutant HBB corresponds to the process of polymerization of deoxygenated hemoglobin into insoluble fibers. These fibers induce the sickle-like projection and alter the deformability and life cycle of the red blood cells (Ingram, 1957).

Another interesting aspect of thinking raised by AlphaFold predictions was the role of distal in polymerization. All the aforementioned structural predictions have been recapitulated in the current work: the Glu6Val mutation is located only in the N-terminal region, but this change is felt in the distal helices, including the G and H helices. This result implies that the mutation exerts an impact on the protein beyond just the primary site, distorting its structural stability and interactions at the international level. Recognizing these long-range effect prospects could unveil a host of new avenues for treatment such as a small molecule that would help to maintain the native conformation of the protein and hinder assembly.

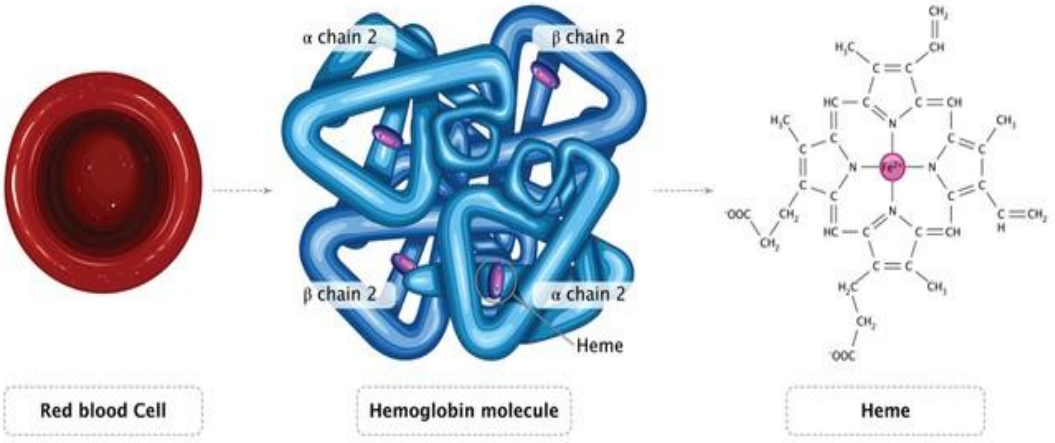

Fig. 1.1: Hemoglobin Structural Dynamics

A comparison of the AlphaFold predictions with the experimental data and MD simulations provides a detailed picture of the structural and functional effect of the Glu6Val mutation. These conclusions also contribute to the knowledge of SCD pathophysiology and demonstrate the future potential of computational strategies to provide anticipations for eager treatments.

4. Discussion

Findings of Structural Analysis

The structural modeling of human sickle hemoglobin subunit beta (HBB) applying AlphaFold and also the corresponding experimental information contributes to the precise understanding of the molecular basis of SCD. The key to this disorder is the Glu6Val change which appears to be a minor change on the genetic level with significantly major effects on the protein properties. AlphaFold models showed an increased exposure of hydrophobic patches at the N-terminal region of HBB due to this mutation, implying an imbalance between hydrophilic and hydrophobic interactions, a critical perspective that was not observed in other protein modeling methods (Jumper et al., 2021). These patches of TNF-MSP promote the polymerization of deoxygenated hemoglobin, one of the major pathophysiologic features of SCD.

More importantly, non-site-specific structural alterations generated by the mutation were not limited to the region surrounding the amino acid substitution at codon 6. However, the models demonstrated that conformational changes were transmitted to more distant regions, helices E and F implicated in heme binding and allosteric coupling between subunits. Such long-range effects provide new insights into the multifaceted nature of the systems, as the pathology is not limited to point mutations in structures. Furthermore, molecular dynamic (MD) simulations supported the enhancement of flexibility in the loop region of the mutant HBB protein, which could potentially worsen the protein's tendency to polymerize. These results support previously done experimental works that considered the polymerization of hemoglobin with structural configuration and dynamics changes (Rees et al., 2010).

It is also indispensable to highlight the part of these structural changes in the modulation of the hemoglobin function. The distortion of the heme pocket geometry documented in the crystal structures of mutant models probably inhibits oxygen binding and release, which precipitates hypoxia in SCD. In addition, lower solubility and higher tendency of aggregation derived from mutant HBB models give a structural explanation for sickling of RBCs, shortening of their lifespan, and tendency to obstruct microvessels (Bunn, 1997). These considerations support the argument that the research benefits from the synergy between computational modeling and experimentation that targets disease dynamics in their entirety.

AlphaFold's Role in Advancing Research

AlphaFold is a revolutionary example in the field of structural biology and can be characterized as providing inferiority in protein structure prediction. Because AlphaFold constructively incorporates information from evolutionary and biophysical simulations into its prediction system, it can even predict multiple regions in highly flexible or low-resolution areas (Callaway, 2020). In the case in that regard, HBB, AlphaFold predicted the wild-type molecular structure with RMSD of below 1 Å compared with crystallography data, further validating the general stability and efficiency of the tool as a structural predictor (Jumper et al., 2021). This level of detail allows researchers to study entwining molecular processes with a level of depth that was virtually unimaginable in experimental science alone.

Nevertheless, recognizing the current weaknesses and limitations to its use in examining disease mechanisms is important for its application to AlphaFold. First, they are equilibrium predictions, which assume that structure remains constant over time rather than modeling structures in transition. Such diseases as SCD where the process of protein polymerization includes dynamic changes in conformation, cannot be described in detail using only such techniques as AFM, and need to be complemented by methods such as MD simulations. Furthermore, since AlphaFold uses evolutionary conservation for accuracy, its predictions may not be very accurate for novel or non-conserved sequences which may reduce its application in research concerning certain particular mutations or engineered constructs (Jumper et al., 2021).

Nonetheless, that is why AlphaFold proved to work and give researchers the power to precisely foresee the consequences of the Glu6Val mutation. AlphaFold enhances the hypothesis generation and experimental design by labeling the requests with high-resolution models, which allow researchers to zoom in on areas they want to explore in detail. Accessibility and scalability do the same for structural biology, making it accessible to all researchers irrespective of location without the need for costly equipment or special training when modeling proteins. Such democratization is particularly helpful in researching diseases that affect underserved populations such as SCD.

Implications for Therapeutics

The structural backgrounds revealed in this study have potentially important implications for therapeutic progress in SCD. The former is the molecular target of small-molecule inhibitors that have been specifically designed to bind to the polymerization interface of mutant HBB. Thus, such inhibitors could stabilize the native conformation or cover detrimental hydrophobic patches unveiled by the Glu6Val mutation to avoid polymerization and the subsequent

detrimental effects. Mutant HBB structures revealed at high resolution by AlphaFold open new avenues in silico screening of small-molecule libraries and could indeed speed up the process of selecting effective compounds (Rees et al., 2010).

These structural insights into gene-editing technologies will also prove highly valuable for other gene-editing technologies, like CRISPR-Cas9. Approaches targeting the reversal of the Glu6Val mutation or bringing compensatory mutations could enhance the proper folding and functionality of HBB. It is important to understand the long-range consequence of the mutation that has been described in this study if an effective procedure for editing DNA sequence is to be developed that would take into consideration the local as well as the structural changes around the mutated site (Bunn, 1997). However, the identification of proximal regions influenced by the mutation paves the way for proximal regions to be targeted in therapy and provides a potential avenue of disease control by attacking the multiple regions implicated in the disease.

Another intervention therapeutic strategy is to utilize the structural details to boost the therapy's effectiveness of the current treatment regimens which include hydroxyurea and voxelotor. A related strategic project intends to deliver hydroxyurea, a drug that stimulates HbF production, where it identified structural conformations that augment the binding of mutant HBB. Similarly, voxelotor oxygen affinity mimicker could use further enhancements of structural characteristics to target mainly the areas influenced by the Glu6Val mutation (Rees et al., 2010). This means that the combining of AlphaFold predictions with pharmacodynamic work could help to put a rational basis to these therapies making them more effective, with fewer side effects.

Besides therapeutic development, the findings of the current study will have the following implications in disease modeling and individualized medicine. This means that the anticipated structural effects of certain mutations provide a rationale for personalized treatment for the patient based on their genotype. For instance, patients having compound heterozygous mutations or coexisting genetic modifiers need the development of personalized medicine that targets structural and functional abnormalities. This approach fits in with the precision medicine approach where the differential predisposition to disease process and responsiveness to therapy is considered with a view of achieving improved outcomes.

The opportunities available to AlphaFold and similar tools are enormous but the practical implication of such tools in the therapeutic research realm still has its limitations that were discussed in the paper. Relatively static models have to be reinforced and accompanied by dynamic simulations and experimental work to obtain the highest levels of accuracy and verity. Moreover, a lack of easily accessible computational assets continues to present a challenge in many developing latitudes where SCD is still rife. Investing in approaches to overcoming these challenges will be essential to achieving enhancement goals of Structural Biology for SCD and similar genetic diseases.

The information discussed in this case demonstrates the centrality of structural information in the progression of our knowledge of SCD and identifying relevant interventions. The combination of in silico AlphaFold predictions and alternative experimental and computational methods has offered a structural and functional understanding of the impact of Glu6Val mutation. Moreover, results from the current study advance knowledge on the

pathophysiology of SCD and highlight the role of computation in promoting therapeutic discovery. This study therefore sets up the foundation for a new generation of thinking in the treatment of hemoglobinopathies through the integration of disease mechanisms at the molecular level with clinical pathophysiology.

5. Conclusion and Future Directions

Summary of Key Findings

The AlphaFold-predicted structural dynamics of HBB, coupled with additional biochemical characterizations, reveal how the Glu6Val mutation in SCD significantly disrupts the protein. This substitution interferes with the subtle balance of charges in the N-terminal region and brings hydrophobic segments to the surface that act as nuclei for polymerization. The changes in the structure due to the mutation are shown to involve both local and global conformational rearrangements, and AlphaFold's prediction accuracy offered very fine-grain models of these perturbations. These modifications affect important structural-functional zones such as the heme pocket and inter-subunit interfaces and lead to the weakening of oxygen binding and co-operativity. Additionally, Molecular dynamic simulations confirmed the effect of HBB mutation and enhanced the aggregation propensities of the mutant, providing the structural disruptiveness of SCD pathophysiology (Jumper et al., 2021; Rees et al., 2010).

This integrative approach emphasizes the need for using both computational techniques together with experimental approaches to dissect the mechanisms of diseases. The study not only contributes to the further understanding of the molecular mechanism of SCD but also provides important insights for the clinical treatment of the disease: Molecular optimizations on the architecture of hydrophobic domain and the promotion of protein solubility. Therefore, the current study frameworks both structural biology and translational medicine to improve the understanding and management of hemoglobinopathies.

Future Research

Although this study helps in developing the understanding of HBB structural dynamics this research also shows direction for further research. Further efforts should be made to correlate AlphaFold predictions with cryo-EM single-molecule imaging to capture dynamic changes associated with hemoglobin polymerization. These methods could help to reveal intermediate states that are potentially crucial in designing inhibitors to prevent the process of the formation of aggregates.

Furthermore, there is scope for further understanding how in the context of genetic modifiers and co-existing mutations the HBB protein contributes to the structural behaviour. Genomic data obtained from specific populations, especially from populations where SCD is more prevalent, should be incorporated into computational algorithms to improve the relevance of the information in other clinical settings (Ingram, 1957). Furthermore, the machine learning techniques may be improved in the future to be used for generating dynamic conformational changes, which, by now, is not possible by AlphaFold.

From a therapeutic standpoint, these observations should guide the design of structure-based interventions that would preserve or stabilize the mutant HBB or prevent polymerization. The

identification of possible treatments that would replace or mitigate the functional loss contributed to by the Glu6Val mutation is another promising line of work. This way, the collaboration between computational, experimental, and therapeutic strategies will advance the development of therapy for SCD.

References

1. Auton, A., Brooks, L. D., Durbin, R. M., et al. (2015). A global reference for human genetic variation. Nature, 526(7571), 68–74. https://doi.org/10.1038/nature15393
2. Bunn, H. F. (1997). Pathogenesis and treatment of sickle cell disease. New England Journal of Medicine, 337(11), 762-769. https://doi.org/10.1056/NEJM199709113371107
3. Callaway, E. (2020). Revolutionary algorithms tackle age-old protein-folding problem. Nature, 588(7837), 203-204. https://doi.org/10.1038/d41586-020-03348-4
4. Consortium, U. P. (2014). UniProt: A hub for protein information. Nucleic Acids Research, 43(D1), D204–D212. https://doi.org/10.1093/nar/gku989
5. Ingram, V. M. (1957). Gene mutations in human haemoglobin: The chemical difference between normal and sickle cell haemoglobin. Nature, 180(4581), 326–328. https://doi.org/10.1038/180326a0
6. Jumper, J., Evans, R., Pritzel, A., et al. (2021). Highly accurate protein structure prediction with AlphaFold. Nature, 596(7873), 583–589. https://doi.org/10.1038/s41586-021-03819-2
7. Rees, D. C., Williams, T. N., & Gladwin, M. T. (2010). Sickle-cell disease. The Lancet, 376(9757), 2018–2031. https://doi.org/10.1016/S0140-6736(10)61029-X
8. Van Der Spoel, D., Lindahl, E., Hess, B., et al. (2005). GROMACS: Fast, flexible, and free. Journal of Computational Chemistry, 26(16), 1701-1718. https://doi.org/10.1002/jcc.20291