**Journal of Advanced Biomedical and Pharmaceutical Sciences**

Journal Homepage: http://jabps.journals.ekb.eg

# Binding of curcumin near the GBT440 binding site at the alpha cleft in the sickle cell hemoglobin model [Pdb ID: 1NEJ]

**Roohallah Yousefi***

Department of Biochemistry, Faculty of biology of Tarbiat Modares University, Tehran, Iran.

Received: November 8, 2023; revised: December 30, 2023; accepted: January 6, 2024

**Abstract**

**Introduction:** In sickle cell anemia, deoxygenated HbS molecules polymerize into long fibers in deep tissues during hypoxia, leading to erythrocyte sickling and capillary and vessel blockage. We are searching for new ligands, such as gbt440, that can inhibit HbS polymerization.
**Methods and Materials:** We obtained the molecular model of hemoglobin [PDB ID: 1NEJ] from the Protein Data Bank and the molecular models of GBT440 and curcumin from the PubChem database. The physicochemical properties were investigated using SwissADME software, followed by docking to identify protein-ligand binding sites and determine their affinity. Finally, molecular dynamics simulations were conducted to assess the stability of the protein-ligand complex in the alpha-cleft of the hemoglobin tetramer model [PDB ID: 1NEJ].
**Results and Discussion:** The docking results revealed that curcumin exhibited a higher binding affinity than GBT440 to the nearby binding site of gbt440 in the alpha-cleft of the hemoglobin model. The hydrogen bonding and electrostatic interactions of the 1NEJ-GBT440 complex differed from those of the 1NEJ-curcumin complex. The results of the molecular dynamics simulation showed no significant difference in the flexibility of hemoglobin among the different ligand-hemoglobin complexes.
**Conclusion:** The binding of curcumin to hemoglobin in the alpha cleft may inhibit the transition of hemoglobin to the tense state and stabilize the hemoglobin tetramer model [PDB ID: 1NEJ] in the R2 state. However, further investigation in the laboratory is necessary to confirm this.

**Keywords**
HbS, Gbt440, molecular docking, curcumin.

## Introduction

Sickle cell disease is a type of beta-hemoglobinopathy in which valine replaces glutamic acid at the sixth amino acid position in the beta globin chain of hemoglobin. Under conditions of tissue hypoxia, these hemoglobin tetramers change their conformation to a tense state, resulting in the polymerization of hemoglobin tetramers through hydrophobic interactions. This polymerization causes the collapse of red blood cells, leading to the rupture of the cell membrane and activation of the endothelial cells membrane. The sedimentation of hemoglobin further blocks blood flow in tissue vessels, resulting in tissue ischemia and painful crises (1-3). Gbt440, an FDA-approved drug for sickle cell anemia, inhibits the conformational change to the tense state of hemoglobin, preventing the formation of hemoglobin polymers (4).
On the other hand, curcumin (PubChem CID number: 969516), a phenolic compound with a yellow color, belongs to the ginger family and is extracted from Curcuma Longa. It is known for its antioxidant, anti-inflammatory, and anticancer properties. Curcumin inhibits certain reductase and dehydrogenase enzymes involved in electron and proton transfer reactions. These enzymes include aldehyde reductase, shikimate dehydrogenase, IMP dehydrogenase, thioredoxin reductase, and NAD (P) H-dehydrogenase (5-7). In this study, we investigated the physicochemical properties of curcumin, its docking with hemoglobin modal [Pdb ID: 1NEJ] compared to Gbt440, and performed molecular dynamic simulations of the docking results.

## Methods and Materials

*Model preparation:* The molecular model of sickle cell Hemoglobin [Pdb ID: 1NEJ] was obtained from the RCSB PDB database. A carbon monoxide hemoglobin crystal, which shows the R2 quaternary state at neutral pH in the presence of polyethylene glycol with a resolution of 2.1 angstroms, was used. Models of GBT440 and Curcumin were obtained from the PubChem database (5).
*Molecular docking:* Molecular docking was performed using AutoDock Vina software. Docking was done on all hemoglobin tetramers, but only the binding sites in the alpha cleft of hemoglobin were selected for molecular dynamic simulations. Ligands with higher docking scores for the alpha cleft (between the two alpha globin chains in the hemoglobin tetramer) were studied (8).
*Molecular dynamics simulation and analysis:* Molecular dynamics simulations were performed using CABS-flex, a near native dynamic simulation obtained from 10-nanosecond MD simulations. CABS Flex predictions of protein fluctuations correlate well with fluctuations observed in the NMR ensemble. RMSF and contact frequency were obtained from CABS-flex online software (9).

* ***Correspondence:*** *Roohallah Yousefi*
***Tel.:*** **+09168741235**
***Email Address:*** *r.yosefi@modares.ac.ir*

*The process of conducting the in silico study:* The physicochemical properties of each ligand were calculated based on its structure using the online SwissADME software. The ligand-binding site and its affinity were calculated using AutoDock Vina software. The molecular docking results of the best binding sites at the alpha cleft of the hemoglobin tetramer were used for molecular dynamics simulations using CABS-flex online software (9-10).

## Results

Curcumin is a hydrophobic herbal compound with higher solubility in fats, making it better absorbed by the digestive system. However, its low solubility in aqueous solutions limits its use in medicine. Additionally, its higher weight limits its passage through the blood-brain barrier. Curcumin has a more polar surface than gbt440, leading to more electrostatic interactions. It has six hydrogen bond acceptors, two hydrogen bond donors, and eight rotatable bonds. It is flexible and a good hydrogen bond acceptor. The binding affinity was calculated using AutoDock Vina software, and the results were consistent with the molecular dynamics simulation results (11).

**Table 1.** Physicochemical Properties of Curcumin and GBT440.

| Compounds | Molecular weight | Heavy Atoms (number) | Water Solubility (Log S) | Lipophilicity (Log Po/w) | Polar Surface Area(Å²) | GI absorption | BBB permeability |
|---|---|---|---|---|---|---|---|
| **Gbt440** | 337.37 g/mol | 25 | -3.73 | 2.68 | 77.24 Å² | High | Yes |
| **Curcumin** | 368.38 g/mol | 27 | -3.94 | 3.27 | 93.06 Å² | High | No |

In our study, curcumin was found to bind to hemoglobin with higher affinity than gbt440. Curcumin binds to the alpha cleft, located between two alpha chains, with a binding affinity of -7.2 (kcal/mol), while Gbt440 binds to a nearby region with a binding affinity of -6.4 (kcal/mol). The specific binding sites for curcumin in hemoglobin include valine 1, leucine 2, lysine 7, valine 73, aspartic acid 74, aspartic acid 75, methionine 76, proline 77, serine 131, and threonine 134 of the alpha globin chains.

**Table 2.** The results of molecular docking conducted using AutoDock Vina software, which involved curcumin and GBT440 docking with the molecular model of hemoglobin (Pdb ID: 1NEJ).

| Mode | Gbt440 | | Curcumin | |
|---|---|---|---|---|
| | affinity (kcal/mol) | RMSD ligand binding site. (distance from best mode) | affinity (kcal/mol) | RMSD ligand binding site. (distance from best mode) |
| **pos1** | -6.8 | 0.000 | -7.8 | 0.000 |
| **pos2** | -6.7 | 3.525 | -7.8 | 4.956 |
| **pos3** | -6.7 | 2.718 | -7.3 | 18.653 |
| **pos4** | -6.4 | 25.113 | -7.3 | 4.663 |
| **pos5** | -6.3 | 3.510 | -7.2 | 5.568 |
| **pos6** | -6.3 | 27.388 | -6.8 | 8.523 |
| **pos7** | -6.1 | 26.094 | -6.7 | 19.469 |
| **pos8** | -6.0 | 2.970 | -6.6 | 19.329 |
| **pos9** | -6.0 | 3.376 | -6.5 | 18.883 |

In the binding site (Pos 3), curcumin forms six hydrogen bonds with hemoglobin. These bonds involve the nitrogen of the amide group of valine 1 (α1-chain), the nitrogen of the amide group of methionine 76 (α1), the ketone group of valine 73 (α1), the ketone group of valine 73 (α2), and the oxygen of the hydroxyl group of the side chain of threonine 134 (α2) (Figure 1).

The binding sites for GBT440 in hemoglobin include valine 1, leucine 2, valine 73, aspartic acid 74, aspartic acid 75, methionine 76, proline 77, serine 131, threonine 134, and valine 135 of the alpha globin chains. In the binding site of GBT440 (pos. 4), hydrogen bonds are formed between GBT440 and the oxygen of the hydroxyl group of the C-terminal alpha chain of threonine 134 (α1, α2), as well as the nitrogen of the amino group of valine 1 (α1) (Figure 1).

In our study, the molecular dynamics simulations are based on the results of molecular docking between proteins and ligands. For these simulations, we utilize the CABS Flex-2 software, which is capable of predicting protein fluctuations. The results of this software align well with the fluctuations observed in the NMR ensemble. CABS-flex utilizes the coarse-grained protein model CABS as an efficient simulation engine. The protein fluctuations in aqueous solution are derived from molecular dynamics (MD) simulations, which involve all atoms of globular proteins and last for 10 nanoseconds. These simulations are conducted with different force fields (9).

In molecular dynamics simulation studies, specific attention is often given to amino acids that display increased or decreased RMSF compared to the ground state. This analysis takes into account factors such as temperature, solution content, and the type of ligand. Molecular dynamics simulations are frequently employed to identify amino acids that exhibit altered RMSF (14, 16-18).

The RMSF scale, calculated for the dynamic simulation of the alpha globin chain sequence with or without a ligand, demonstrates a distinct and similar pattern.

The type of ligand and its binding affinity have a noticeable impact on the RMSF of each amino acid during the dynamic simulation, suggesting that ligand binding stabilizes the protein. Specifically, the molecular dynamics simulation for the curcumin-1NEJ binding site (Pos 3) reveals that the targeted

amino acids exhibit minimal fluctuation, indicating a stabilization of the ligand-protein complex. Conversely, when hemoglobin is not bound to a ligand, a higher RMSF value is observed. The binding of a ligand is associated with a decrease in the RMSF index of the target amino acids (14, 16-18). Finally, curcumin forms more electrostatic and hydrogen bonds with two alpha-globin chains compared to gbt440, resulting in a more stable hemoglobin structure.

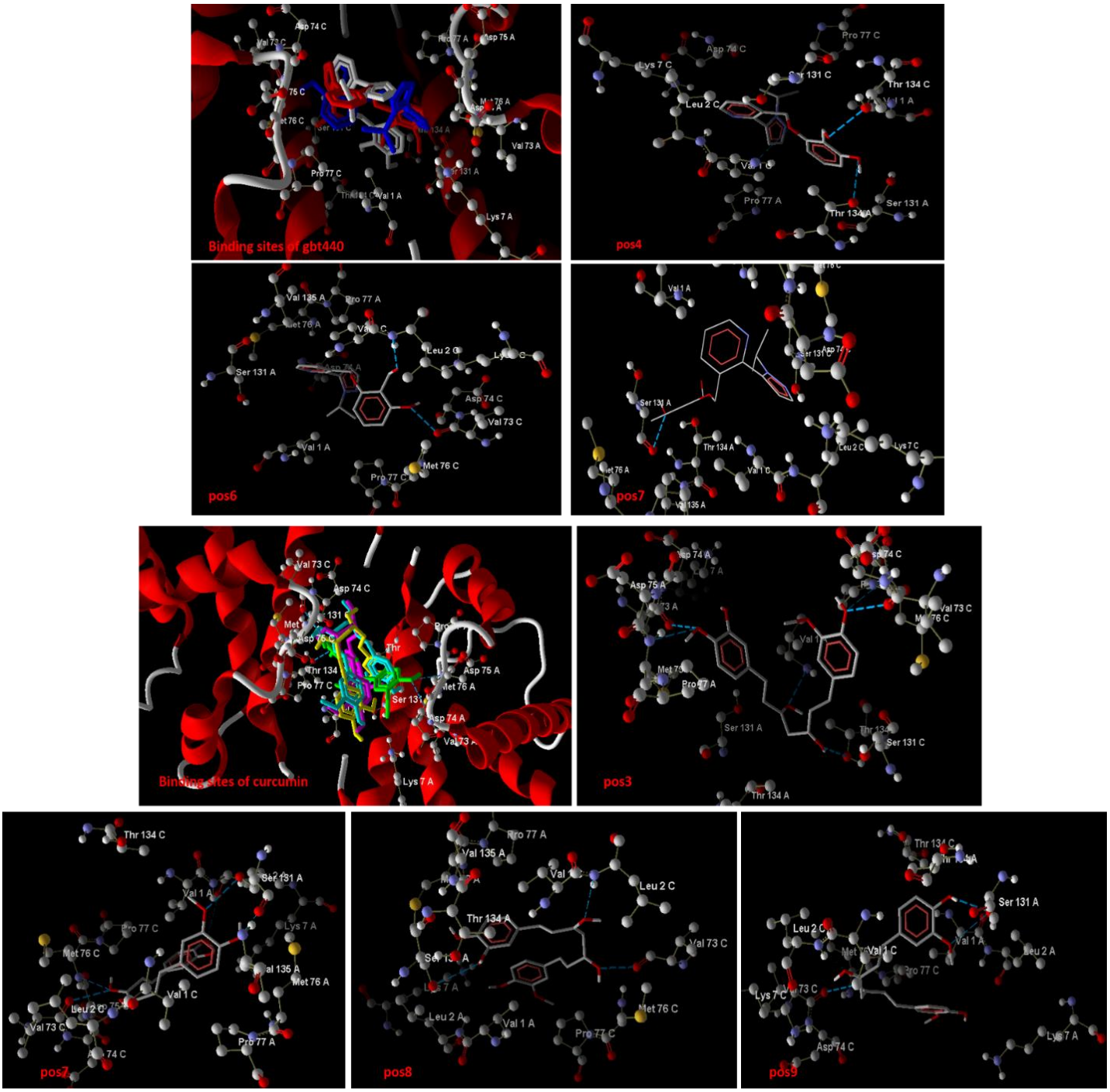


**Figure 1**. shows the binding sites of ligands to hemoglobin, with ligands represented in wireframe and alpha-globin represented in ball and stick. Hydrogen bonds are depicted as blue dotted lines.

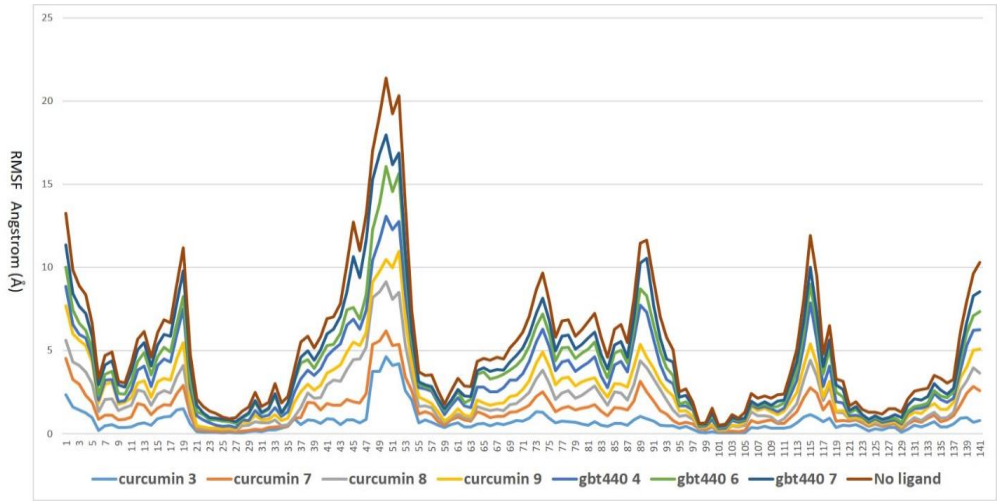


**Figure 2.** Stacked line chart comparing the Root Mean Square Fluctuation (RMSF) of amino acid sequences in the alpha globin chain during the molecular dynamics simulation of binding sites (pos) for curcumin and GBT440 at the alpha cleft, located between alpha globin chains. The simulation was performed using CABS-flex online software, which can be accessed at the following address: https://biocomp.chem.uw.edu.pl/CABSflex2/queue.

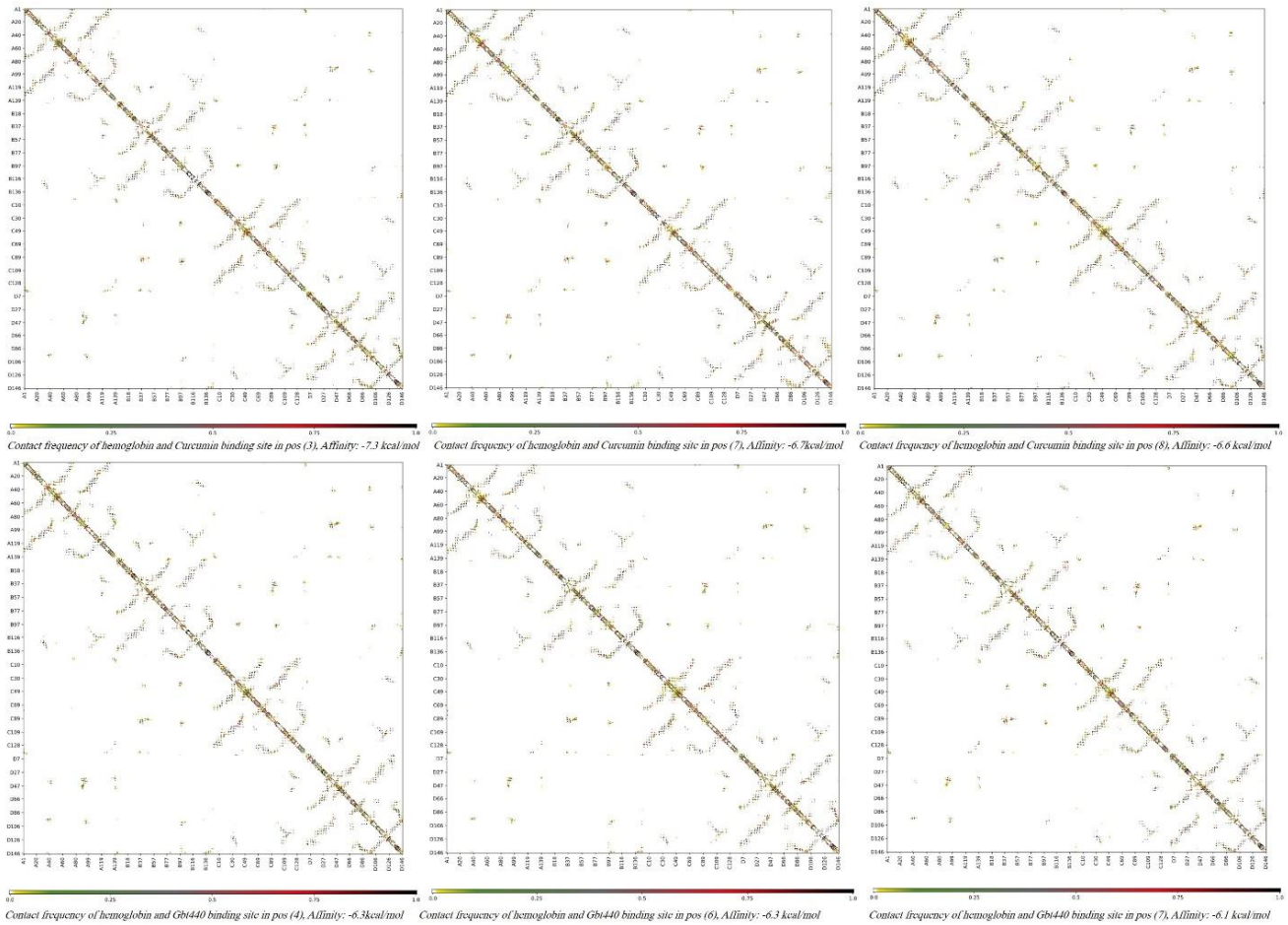


**Figure 3**. Contact frequency diagram of molecular dynamics simulation results for hemoglobin-ligand docking at the binding site in the alpha cleft. The simulation was performed using CABS-flex online software, which can be accessed at the following address: https://biocomp.chem.uw.edu.pl/CABSflex2/queue.

## Discussion

The calculation of protein ligand binding affinity is based on docking results using models that consider the structural potentials of ligand, protein, water, and ion molecules. Compounds designed to inhibit the formation of a tense state of hemoglobin should prevent hydrophobic and electrostatic interactions that promote the tense state, while maintaining interactions that promote the relaxed state. The formation of a salt bridge leads to the convergence of the two alpha or beta globin chains in the hemoglobin tetramer, promoting the tense state. During the conversion from the tense state to the relaxed state, certain interactions are disrupted, such as arginine 141 with

valine 1 between the two alpha globin chains and aspartic acid 126 with arginine 141 within the alpha chain.

Interactions between lysine 40 of the alpha globin chain and histidine 146 of the beta globin chain in α1β2 and α2β1 dimers are also broken, as well as the interaction between histidine 146 and aspartic acid 94 within each beta chain.

In the relaxed state, arginine 141 interacts with tyrosine 140 within each alpha chain. Lysine 40 is released in each alpha chain, while valine 1 is released with a positive charge of nitrogen and aspartic acid 126 is released with a negative charge. Amino acids such as valine 1 and arginine 141 from the two alpha chains play a role in the conversion from the tense state to the relaxed state, and are involved in the development of a suitable ligand for sickle hemoglobin to maintain the relaxed state. Previous studies have shown that gbt440 forms electrostatic interactions and hydrogen bonds with valine 1 and serine 131 in the alpha globin chains of hemoglobin, resulting in the formation of a large space between the two alpha globin chains and facilitating the stabilization of the relaxed state.

In our study, we identified the binding sites of curcumin on hemoglobin, which include several amino acids in the alpha globin chains. The binding site of curcumin between two alpha globin chains is very close and similar to the binding site of gbt440, suggesting that curcumin may have a similar effect as gbt440. Molecular dynamics simulations showed that both ligands stabilize the sequence of alpha globin chains. However, it cannot be concluded with certainty that curcumin stabilizes the R2 state of hemoglobin.

## Conclusion

This study investigated the physicochemical properties of curcumin and gbt440, and their binding affinity for hemoglobin based on molecular docking at the binding sites between two alpha chains. The results showed that curcumin has a higher binding affinity than gbt440 for the binding site between two alpha globin chains, resulting in decreased fluctuation of amino acids during molecular dynamics simulations. This suggests that curcumin stabilizes hemoglobin in its ground state.

## Declaration of Interests

The author declares no conflict of interest for the current study.

## Acknowledgments

We would like to thank the Department of Biology at Tarbit Modares University of Tehran for their support. This study was approved by the Ethics Committee of the Faculty of Medicine at Tarbit Modares University, in accordance with the Code of Ethics IR.MODARES. REC.1401.149.

## References

[1] Rees, D.C., Williams, T.N., and Gladwin, M.T. Sickle-cell disease. Lancet, 2017; 376: 2018–2031.

[2] Azar, S., and Wong, T.E. Sickle-cell disease: a brief update. Med. Clin. North Am, 2017; 101: 375–393.

[3] Hebbel, R.P. Ischemia-reperfusion injury in sickle-cell anemia: relationship to acute chest syndrome, endothelial dysfunction, arterial vasculopathy, and inflammatory pain. Hematol. Oncol. Clin. North Am, 2014; 28: 181–198.

[4] Oksenberg, D., Dufu, K., Patel, M.P., Chuang, C.H., Li, Z., Xu, Q., Silva-Garcia, A., Zhou, C.J., Hutchaleelaha, A., Patskovska, L., Patskovsky, Y., Almo, S.C., Sinha, U., Metcalf, B.S.W., Archer, D.R. GBT440 increases hemoglobin oxygen affinity, reduces sickling, and prolongs RBC half-life in a murine model of sickle cell disease. Br. J. Haematol, 2016; 175: 141−153.

[5] Patskovska, L.N., Patskovsky, Y.V., Almo, S.C., Hirsch, R.E. COHbC and COHbS crystallize in the R2 quaternary state at neutral pH in the presence of PEG 4000. Acta Crystallographica Section D: Biological Crystallography, 2005 May 1; 61(5): 566-573.

1. [6]APA American Psychological Association. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 969516, Curcumin. Retrieved September 30, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/Curcumin.
2. [7] Urošević, M., Nikolić, L., Gajić, I., Nikolić, V., Dinić, A., Miljković, V. Curcumin: Biological activities and modern pharmaceutical forms. Antibiotics, 2022 Jan 20; 11(2): 135.
3. [8] Trott, O., Olson, A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry, 2010; 31: 455-461.
4. [9] Jamroz, M., Kolinski, A., Kmiecik, S. (2013), CABS-flex: Server for fast simulation of protein structure fluctuations. Nucleic acids research, 41, W427-431.
5. [10] Daina, A., Michielin, O., Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug likeness, and medicinal chemistry friendliness of small molecules. Scientific Reports, 7: 42717.
6. [11] Nguyen, N.T., Nguyen, T.H., Pham, T.N.H., Huy, N.T., Bay, M.V., Pham, M.Q., Nam, P.C., Vu, V.V., Ngo, S.T. Autodock Vina Adopts More Accurate Binding Poses but Autodock4 Forms Better Binding Affinity. J Chem Inf Model, 2020 Jan 27; 60(1): 204-211. doi: 10.1021/acs.jcim.9b00778. Epub 2020 Jan 7. PMID: 31887035.
7. [12] Mihailescu, M.R., Russu, I.M. A signature of the T ---> R transition in human hemoglobin. Proc Natl Acad Sci U S A, 2001 Mar 27; 98(7): 3773-3777. doi: 10.1073/pnas.071493598. Epub 2001 Mar 20. PMID: 11259676; PMCID: PMC31128.
8. [13] Storz, J.F. Hemoglobin structure and allosteric mechanism. Hemoglobin: Insights into Protein Structure, Function, and Evolution, Jay F. Storz, Oxford University Press (2019).
9. [14] Yao, L., Yan, H., Cukier, R.I. A Molecular Dynamics Study of the Ligand Release Path in Yeast Cytosine Deaminase. Biophysical Journal, 2006, 92(7), 2301–2310.
10. [15] Barazorda-Ccahuana, H.L., Ernesto Valencia, D., Aguilar-Pineda, J.A., Gomez, B. Art v 4 Protein Structure as a Representative Template for Allergen Profilins: Homology Modeling and Molecular Dynamics. ACS Omega, 2018, 3, 17254−17260.
11. [16] Singh, W., Karabencheva-Christova, T.G., Black, G.W., Sparagano, O., Christov, C.Z. Conformational flexibility influences structure–function relationships in tyrosyl protein sulfotransferase-2. RSC Advances, 2016, 6, 11344.
12. [17] Mihailescu, M.R., Russu, I.M. A signature of the T3R transition in human hemoglobin. PNAS, 2001, vol (98): 7; 3777.
13. [18]Taghizadeh, M., Goliaei, B., Madadkar-Sobhani, A. Variability of the Cyclin-Dependent Kinase 2 Flexibility Without Significant Change in the Initial Conformation of the Protein or Its Environment; a Computational Study. Iran J Biotechnol, 2016 Jun; 14(2): 1-12. doi: 10.15171/ijb.1419. PMID: 28959320; PMCID: PMC5435026.