American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

Molecular Dynamics and Docking Studies of HFE (High Iron Fe or Homeostatic Iron Regulator), Hepcidin and Ferroportin Proteins in Iron Overload Disorders

Tahira Asgarova[1*], Nigar Malikova[1], Elshad Novruzov[1], Huseyn Abiyev[2]

[1]*Biological Chemistry Department, Azerbaijan Medical University, Baku, Azerbaijan*

[2]*Medical and Biological Physics Department, Azerbaijan Medical University, Baku, Azerbaijan*

***Corresponding author:** Tahira Asgarova, Biological Chemistry Department, Azerbaijan Medical University, Baku, Azerbaijan.

To Cite This article: *Tahira Asgarova*, Nigar Malikova, Elshad Novruzov, Molecular Dynamics and Docking Studies of HFE (High Iron Fe or Homeostatic Iron Regulator), Hepcidin and Ferroportin Proteins in Iron Overload Disorders. Am J Biomed Sci & Res. 2026 31(4) AJBSR. MS.ID.004057,* **DOI:** 10.34297/AJBSR.2026.31.004057

Received: June 18, 2026 **Published:** 24-06-2026

Abstract

Iron homeostasis is maintained through a highly coordinated network of proteins that regulate iron absorption, transport, storage, and recycling. Among these regulatory components, the homeostatic iron regulator protein (HFE), the hepatic peptide hormone hepcidin, and the transmembrane iron exporter ferroportin constitute the central molecular axis governing systemic iron metabolism. Dysregulation of this pathway results in pathological iron accumulation, leading to hereditary hemochromatosis and other iron overload disorders. Excessive iron deposition promotes oxidative stress, mitochondrial dysfunction, ferroptotic cell death, chronic inflammation, fibrosis, and multiorgan damage. Recent advances in computational structural biology have provided unprecedented opportunities to investigate the molecular mechanisms underlying iron metabolism at atomic resolution.

Molecular docking and Molecular Dynamics (MD) simulations have emerged as powerful tools for elucidating protein structure-function relationships, identifying disease-associated conformational changes, predicting protein-protein interactions, and facilitating structure-based therapeutic design. These computational approaches enable detailed characterization of HFE mutations, hepcidin-ferroportin binding mechanisms, and the dynamic behavior of iron-regulatory proteins under physiological and pathological conditions. In addition, the integration of artificial intelligence, AlphaFold-based structural prediction, free-energy calculations, and enhanced sampling simulations has significantly expanded the scope of modern computational biophysics.

This review provides a comprehensive overview of the molecular architecture and biological functions of HFE, hepcidin, and ferroportin, emphasizing the contribution of molecular docking and molecular dynamics simulations to understanding iron overload disorders. Particular attention is given to disease-associated mutations, protein stability, conformational flexibility, intermolecular interactions, and emerging therapeutic strategies targeting the hepcidin-ferroportin axis. The review highlights how computational biophysics is transforming our understanding of iron metabolism and accelerating the development of precision medicine approaches for hereditary and acquired iron overload syndromes.

Keywords: Iron overload disorders, Hereditary hemochromatosis, HFE, Hepcidin, Ferroportin, Molecular docking, Molecular dynamics simulation, Computational biophysics, Ferroptosis, Precision medicine

Introduction

Iron is an essential transition metal required for numerous biological processes, including oxygen transport, mitochondrial respiration, DNA synthesis, cellular proliferation, and redox signaling. The unique ability of iron to alternate between ferrous (Fe^{2+}) and ferric (Fe^{3+}) oxidation states enables participation in electron-transfer reactions that are fundamental to cellular metabolism. However, this same property also makes iron potentially toxic when present in excess, as uncontrolled redox cycling promotes the generation of Reactive Oxygen Species (ROS) through Fenton and Haber-Weiss reactions [1,2].

Because humans lack a regulated pathway for iron excretion, systemic iron balance is achieved primarily through the control of intestinal absorption and macrophage-mediated recycling [3]. The hepcidin-ferroportin regulatory axis serves as the principal mechanism governing systemic iron homeostasis. Hepcidin, a peptide hormone synthesized predominantly by hepatocytes, regulates plasma iron concentration by binding to ferroportin, the only known cellular iron exporter. This interaction induces ferroportin internalization and degradation, thereby reducing iron release from enterocytes, macrophages, and hepatocytes [4,5].

Central to this regulatory pathway is the HFE protein, a non-classical Major Histocompatibility Complex (MHC) class I molecule that participates in hepatic iron sensing. HFE interacts with Transferrin Receptor 1 (TfR1) and Transferrin Receptor 2 (TfR2), contributing to the regulation of hepcidin expression through the BMP-SMAD signaling pathway [6]. Mutations in the HFE gene, particularly C282Y and H63D, disrupt these regulatory mechanisms and represent the most common genetic cause of hereditary hemochromatosis in populations of European ancestry [6,7].

Hereditary hemochromatosis is characterized by inappropriate suppression of hepcidin production despite increasing body iron stores. The resulting hyperactivity of ferroportin promotes excessive intestinal iron absorption and continuous iron release into circulation, leading to progressive iron accumulation in the liver, pancreas, heart, endocrine organs, and joints [8]. Long-term iron overload contributes to hepatic fibrosis, cirrhosis, hepatocellular carcinoma, diabetes mellitus, cardiomyopathy, arthropathy, and neurodegenerative changes [2,8].

Recent discoveries have revealed that iron toxicity extends beyond oxidative stress. Excess intracellular iron can trigger ferroptosis, a regulated form of cell death characterized by iron-dependent lipid peroxidation and membrane damage [9]. Ferroptosis has emerged as a critical mechanism linking iron overload to chronic tissue injury and has attracted considerable interest as a therapeutic target in metabolic and degenerative diseases [9].

Understanding the molecular basis of these pathological processes requires detailed knowledge of protein structure, conformational dynamics, and intermolecular interactions. Experimental approaches such as X-ray crystallography, cryo-electron microscopy, nuclear magnetic resonance spectroscopy, and biochemical assays have provided invaluable structural insights. Nevertheless, these techniques often capture proteins in static conformational states and may not fully describe dynamic biological processes occurring over physiologically relevant timescales [10].

Computational biophysics has therefore become an indispensable component of modern iron metabolism research. Molecular docking enables the prediction of ligand-binding modes, protein-protein interactions, and energetic preferences, whereas molecular dynamics simulations provide atomistic descriptions of protein motions, structural fluctuations, stability, and conformational transitions over time [11]. These methodologies have significantly improved our understanding of HFE mutations, hepcidin-ferroportin recognition mechanisms, ferroportin transport dynamics, and the molecular consequences of disease-associated genetic variants.

Advances in computational power, GPU-accelerated simulations, enhanced sampling techniques, and machine-learning-based structural prediction tools such as AlphaFold have dramatically expanded the accuracy and applicability of molecular modeling approaches [12]. The integration of docking, molecular dynamics simulations, free-energy calculations, and artificial intelligence now enables the rational design of novel therapeutics targeting iron-regulatory pathways.

The objective of this review is to provide a comprehensive analysis of current knowledge regarding the structure and function of HFE, hepcidin, and ferroportin proteins and to evaluate the contributions of molecular docking and molecular dynamics simulations to the study of iron overload disorders. Particular emphasis is placed on the biophysical mechanisms underlying iron dysregulation, the structural effects of pathogenic mutations, and emerging computational strategies for therapeutic development.

Materials and Methods

This study was designed as a narrative review focusing on the structural and biophysical properties of HFE, hepcidin, and ferroportin proteins and the application of Molecular Docking and Molecular Dynamics (MD) simulations in the investigation of iron overload disorders.

A comprehensive literature search was conducted using PubMed, Scopus, Web of Science, and Google Scholar databases.

The present review particularly emphasizes studies employing molecular docking algorithms, including AutoDock, AutoDock Vina, Glide, and HADDOCK, as well as all-atom molecular dynamics simulations performed using GROMACS, AMBER, CHARMM, and NAMD software packages [13,14]. Particular attention was paid to investigations involving the structural consequences of HFE mutations, hepcidin-ferroportin binding interactions, conformational flexibility analyses, Root Mean Square Deviation

(RMSD), Root Mean Square Fluctuation (RMSF), Radius Of Gyration (Rg), Solvent-Accessible Surface Area (SASA), hydrogen-bond occupancy, and free-energy calculations [15].

The reviewed computational approaches were evaluated according to their ability to elucidate disease mechanisms, predict pathogenic variants, identify druggable sites, and facilitate the rational design of novel therapeutic compounds targeting iron homeostasis.

Results and Discussion

Structural Organization of HFE Protein and Molecular Consequences of Disease-Associated Mutations

The HFE protein belongs to the non-classical MHC class I family and consists of α1, α2, and α3 extracellular domains associated with β2-microglobulin [6]. HFE plays an essential role in iron sensing by interacting with transferrin receptors and regulating hepcidin transcription.

Among the identified mutations, C282Y and H63D are the most extensively studied variants associated with hereditary hemochromatosis [7]. Molecular simulations have demonstrated that substitution of cysteine by tyrosine at residue 282 disrupts a critical disulfide bond within the α3 domain, impairing β2-microglobulin binding and causing protein misfolding [16]. MD simulations indicate significantly increased structural fluctuations, elevated RMSD values, and reduced conformational stability in mutant HFE proteins compared with the wild-type structure [16].

The H63D mutation induces more subtle structural alterations. However, computational studies have suggested that this substitution modifies local electrostatic interactions and decreases the affinity of HFE for transferrin receptor complexes [17]. Such conformational disturbances impair hepatic iron sensing and contribute to inappropriately low hepcidin expression.

These findings demonstrate how molecular dynamics simulations provide mechanistic explanations for genotype-phenotype correlations in hereditary hemochromatosis and emphasize the importance of structural bioinformatics in predicting disease severity.

Molecular Docking and Dynamics of Hepcidin-Ferroportin Interaction

The hepcidin-ferroportin axis represents the principal regulatory mechanism controlling systemic iron homeostasis [4]. Hepcidin is a 25-amino-acid peptide containing four disulfide bridges that confer exceptional structural stability [18]. Ferroportin is a transmembrane transporter expressed on enterocytes, macrophages, hepatocytes, and placental cells.

Cryo-electron microscopy studies have demonstrated that hepcidin binds to extracellular regions of ferroportin, inducing conformational changes that trigger transporter internalization and degradation [19]. Molecular docking analyses indicate that hepcidin establishes extensive hydrogen-bond networks and hydrophobic interactions with residues located within the extracellular cavity of ferroportin.

MD simulations have further revealed that hepcidin binding substantially decreases the conformational flexibility of ferroportin and stabilizes specific transporter conformations [19]. Changes in RMSF profiles indicate reduced mobility of extracellular loops, while principal component analyses demonstrate restricted collective motions following complex formation.

The dynamic nature of the hepcidin-ferroportin complex has important therapeutic implications. Reduced hepcidin production or impaired hepcidin-ferroportin interactions result in excessive iron export into the circulation, promoting pathological iron accumulation in hereditary hemochromatosis and secondary iron overload syndromes [8].

Computational studies have identified several amino acid residues that contribute significantly to complex stability and binding free energy [19]. Such information may facilitate the development of hepcidin mimetics, ferroportin inhibitors, and small molecules capable of modulating iron homeostasis.

Molecular Dynamics Simulations and the Biophysics of Iron Overload

Iron overload disorders are characterized by excessive intracellular accumulation of redox-active iron. The transition between Fe^{2+} and Fe^{3+} oxidation states promotes Fenton chemistry, generating highly reactive hydroxyl radicals capable of damaging proteins, lipids, and nucleic acids [1,2].

At the molecular level, oxidative stress induces structural alterations in proteins, membrane destabilization, mitochondrial dysfunction, and impairment of intracellular signaling pathways [2]. Molecular simulations have shown that iron-mediated oxidative modifications can affect protein flexibility, disrupt hydrogen-bond networks, and alter solvent accessibility.

Recent studies have demonstrated that iron overload may initiate ferroptosis, a regulated form of cell death characterized by iron-dependent lipid peroxidation and collapse of membrane integrity [9]. MD simulations have provided valuable insights into the interactions between iron ions, membrane phospholipids, and lipid peroxidation products.

Computational analyses suggest that excessive iron accumulation influences membrane fluidity and changes the physicochemical properties of biological membranes, thereby contributing to cellular dysfunction and organ damage. Such mechanisms may explain the development of liver fibrosis, diabetes mellitus, cardiomyopathy, and neurodegenerative manifestations frequently observed in patients with hereditary hemochromatosis [8].

Applications of Molecular Docking in Therapeutic Development

The increasing availability of high-resolution structural information has accelerated structure-based drug design targeting iron metabolism proteins. Molecular docking approaches have been extensively employed to identify compounds capable of modulating hepcidin production, inhibiting ferroportin activity, or restoring defective HFE signaling [20].

Several studies have proposed peptide analogs and small-molecule hepcidin agonists that exhibit favorable binding affinities toward ferroportin [20]. Docking simulations have also facilitated the identification of compounds capable of disrupting pathological protein interactions and correcting dysregulated iron metabolism.

The integration of docking approaches with molecular dynamics simulations, free-energy calculations, and machine-learning algorithms has significantly enhanced prediction accuracy. Artificial intelligence-assisted structural prediction platforms such as AlphaFold have further expanded opportunities for investigating proteins whose experimental structures remain unavailable.

Consequently, computational biophysics has emerged as an indispensable tool in precision medicine approaches for hereditary and acquired iron overload disorders. Future studies integrating molecular simulations with multi-omics technologies and patient-specific genetic information may facilitate the development of individualized therapeutic strategies.

Conclusion

The HFE-hepcidin-ferroportin regulatory axis constitutes the central molecular network governing systemic iron homeostasis. Structural abnormalities within this pathway lead to inappropriate iron accumulation and contribute to the development of hereditary and secondary iron overload disorders.

Molecular docking and molecular dynamics simulations have substantially improved our understanding of the structural and functional relationships among HFE, hepcidin, and ferroportin proteins. These computational techniques have elucidated the molecular consequences of disease-associated mutations, characterized protein-protein interactions at atomic resolution, and identified potential therapeutic targets.

The integration of computational biophysics, artificial intelligence, and precision medicine approaches is transforming research on iron metabolism disorders. Future investigations combining experimental and in silico methodologies will likely provide novel therapeutic opportunities for the prevention and treatment of iron overload-associated diseases.

Acknowledgement

None.

Conflict of Interest

None.

References

1. Winterbourn CC (1995) Toxicity of iron and hydrogen peroxide: the Fenton reaction. Toxicol Lett 82-83: 969-974.
2. Galaris D, Barbouti A, Pantopoulos K (2019) Iron homeostasis and oxidative stress: an intimate relationship. Biochim Biophys Acta Mol Cell Res 1866(12): 118535.
3. Ganz T, Nemeth E (2015) Iron homeostasis in host defence and inflammation. Nat Rev Immunol 15(8): 500-510.
4. Nemeth E, Tuttle MS, Powelson J, Michael B Vaughn, Adriana Donovan, et al. (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306(5704): 2090-2093.
5. Nemeth E, Ganz T (2009) The role of hepcidin in iron metabolism. Acta Haematol 122(2-3): 78-86.
6. Feder JN, Gnirke A, Thomas W, Z Tsuchihashi, D A Ruddy, et al. (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 13(4): 399-408.
7. Pietrangelo A (2010) Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. Gastroenterology 139(2): 393-408.
8. Brissot P, Pietrangelo A, Adams PC, Barbara de Graaff, Christine E McLaren, et al. (2018) Haemochromatosis. Nat Rev Dis Primers 4: 18016.
9. Dixon SJ, Lemberg KM, Lamprecht MR, Rachid Skouta, Eleina M Zaitsev, et al. (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149(5): 1060-1072.
10. Alberts B, Johnson A, Lewis J, Martin Raff, Keith Roberts, et al. (2014) Molecular Biology of the Cell. 6th ed. New York: Garland Science.
11. Hollingsworth SA, Dror RO (2018) Molecular dynamics simulation for all. Neuron 99(6): 1129-1143.
12. Jumper J, Evans R, Pritzel A, Tim Green, Michael Figurnov, et al. (2021) Highly accurate protein structure prediction with AlphaFold. Nature 596(7873): 583-589.
13. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking. J Comput Chem 31(2): 455-461.
14. Abraham MJ, Murtola T, Schulz R, Szilard Pall, Jeremy C Smith, et al. (2015) GROMACS: High performance molecular simulations through multi-level parallelism. SoftwareX 1-2: 19-25.
15. Karplus M, McCammon JA (2002) Molecular dynamics simulations of biomolecules. Nat Struct Biol 9(9): 646-652.
16. Waheed A, Parkkila S, Zhou XY, Tomatsu S, Tsuchihashi Z, et al. (1997) Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with beta2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc Natl Acad Sci U S A 94(23): 12384-12389.
17. Santos PCJL, Krieger JE, Pereira AC (2012) Molecular diagnostic and pathogenesis of hereditary hemochromatosis. Int J Mol Sci 13(2): 1497-1511.
18. Jordan JB, Poppe L, Haniu M, Tara Arvedson, Rashid Syed, et al. (2009) Hepcidin revisited, disulfide connectivity, dynamics and structure. J Biol Chem 284(36): 24155-24167.
19. Billesbølle CB, Azumaya CM, Kretsch RC, Alexander S Powers, Shane Gonen, et al. (2020) Structure of hepcidin-bound ferroportin reveals iron homeostatic mechanisms. Nature 586(7831): 807-811.
20. Crielaard BJ, Lammers T, Rivella S (2017) Targeting iron metabolism in drug discovery and delivery. Nat Rev Drug Discov 16(6): 400-423.