DOI: https://doi.org/10.36094/sc.v91.2025.Decoding_Bioactive_Compounds_from_the_River_Ganga.Gadnayak.425

ARTICLE

Sci. and Cult. 91 (7–8) : 425-430 (2025)

DECODING BIOACTIVE COMPOUNDS FROM THE RIVER GANGA BY TARGETING HUMAN CXCR4 FOR ADVANCES IN REGENERATIVE MEDICINE

AYUSHMAN GADNAYAK, HIRAK JYOTI CHAKRABORTY, VIKASH KUMAR AND BASANTA KUMAR DAS*

The human C-X-C chemokine receptor 4 (CXCR4), indicating its potential as a CXCR4-targeting drug in regenerative therapy, calcium alginate, hyaluronic acid, chitin, and a collagen prolyl hydroxylase inhibitor toward the human CXCR4 receptor. The research employed molecular docking, active site prediction, and molecular dynamics simulations to evaluate how strongly four bioactive compounds interact with the target. Although the other compounds showed diminished binding capacity, they are known to facilitate regeneration through extracellular matrix remodeling and biochemical signaling. The findings highlight the effectiveness of bioinformatics techniques in evaluating natural substances for medicinal purposes and suggest further experimental investigations.

Background

Bioactive compounds, such as peptides, polysaccharides, polyphenols, and fatty acids, are crucial in regenerative medicine, stimulating cell proliferation, differentiation, and repair, and are particularly useful in tissue engineering, wound healing, neuroregeneration, and managing degenerative diseases[1,2,3]. The growing global interest in regenerative medicine is driven by the aging population and the limitations of current therapies in restoring damaged tissues and organs[4,5]. Marine environments are abundant in bioactive compounds due to their diverse biodiversity and unique ecological conditions, where organisms have evolved to produce potent biological activity[6]. Freshwater ecosystems, despite their diverse and beneficial organisms, are underexplored. However, scientists are slowly uncovering bioactive molecules from lakes, rivers, and wetlands, which have promising biomedical applications[7,8]. The Ganga, or Ganges River, is a vital freshwater system in the Indian subcontinent, holding immense ecological, cultural, spiritual, and historical importance, supporting millions and sustaining a complex ecosystem across 2,500 kilometers and five Indian states[9,10]. The water supports a diverse aquatic ecosystem with over 140 fish species, algae, plankton, fungi, and microbial communities[11]. These organisms are adapted to unique environmental pressures, potentially giving rise to novel bioactive compounds. Furthermore, the River Ganga has historically been a key element of Ayurvedic and traditional Indian medicine, with ancient texts and practices detailing the therapeutic properties of plants and aquatic materials from the Ganga basin, which modern science can now investigate using advanced biochemical and molecular tools[12].

The area of regenerative medicine is advancing as researchers pursue innovative bioactive chemicals that facilitate tissue regeneration, stem cell migration, and immunological regulation. The C-X-C chemokine receptor type 4 (CXCR4) is a crucial molecular target in stem cell homing, tissue regeneration, and angiogenesis[13]. Due to its function in several physiological and pathological

ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata, West Bengal

* Corresponding author: basantakumard@gmail.com

processes, CXCR4 has become a prospective target for therapeutic intervention. This work used an *in silico* method to investigate the regeneration potential of bioactive chemicals sourced from microbial and natural resources of the River Ganga, specifically targeting the human CXCR4 receptor. We used bioinformatics methods to evaluate the binding affinity of potential compounds to CXCR4[14,15,16].

The amalgamation of traditional ecological knowledge with computational biology not only enables economical and fast screening of natural substances but also corroborates old beliefs with scientific data[17]. This work signifies a synthesis of drug discovery and regenerative science, presenting a novel viewpoint on how revered ecosystems like as the River Ganga may inform the development of future medicinal medicines.

Materials and Methods

Retrieval of Ligands: Four bioactive compounds, Calcium Alginate, Hyaluronic Acid, Chitin, and a Collagen Prolyl Hydroxylase Inhibitor, were selected for their roles in tissue repair, regeneration, cellular migration, and chemokine signaling pathways. The selected compounds, obtained in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov), were chosen based on their potential roles in influencing cell migration, remodeling of the extracellular matrix, and regulation of chemokine signaling pathways[15].

Target Protein Preparation: The 3D structure of the human Chemokine Receptor CXCR4 (UniProt ID: P61073) was retrieved from the UniProt database (https://www.uniprot.org) and cross-referenced with the the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/) using the UniProt ID: P61073, corresponding to AlphaFold model AF-P61073-F1.

Active Site Prediction: The hypothesized binding pockets of CXCR4 were determined using the PrankWeb server (https://prankweb.cz/), a machine learning-based technology for predicting ligand-binding sites. The AF-P61073-F1 structure was uploaded, and the highest-ranked binding cavities were documented according to probability ratings[15].

Molecular Docking: Molecular docking offers a comprehensive understanding of the interaction between target proteins and potential therapeutic ligands. The coordinates of the highest-confidence predicted site were used to delineate the docking grid box in AutoDock Vina, so assuring precise targeting of probable interaction zones[16]. AutoDock Vina was used to conduct docking study between each ligand and the anticipated binding site of the CXCR4 receptor.

Molecular Dynamics (MD) Simulation: Molecular dynamics (MD) simulations were carried out using the Desmond module within the Schrödinger suite (D. E. Shaw Research: Resources) to evaluate the conformational stability and dynamic interactions of the protein-ligand complex. The complex was immersed in a simple point charge (SPC) water model within a 10 Å orthorhombic box. To maintain charge neutrality, counterions were added, and a physiological salt concentration of 0.15 M NaCl was introduced. The simulation was run under NPT ensemble conditions at 300 K and 1.63 bar for a duration of 50 nanoseconds. Energy data were recorded every 1.2 picoseconds, while trajectory snapshots were saved at 50 picosecond intervals. The OPLS-4 force field was applied for all calculations. Graphical outputs of molecular interactions were produced using the Desmond simulation interaction diagram tool.

Results and Discussion

Active Site Prediction: Active site residues were identified using the PrankWeb server, which pinpointed several high-probability binding pockets on the AlphaFold-predicted structure of CXCR4. A total of six binding pockets are formed, shown in Fig.1, out of six binding pockets we confirmed that calcium alginate snugly fit into pocket 1 of the predicted pockets for docking, establishing direct contacts with multiple high-confidence residues, lending additional validity to both the docking accuracy and the selected binding site.

Molecular Docking Analysis

Molecular docking was conducted to evaluate the binding affinity of four bioactive compounds that is Calcium Alginate, Hyaluronic Acid, Chitin, and a Collagen Prolyl Hydroxylase Inhibitor toward the CXCR4 receptor (Table 1). The findings identified Calcium Alginate as the most advantageous ligand, demonstrating the maximum binding affinity with a docking score of –10.076 kcal/mol, markedly surpassing the other molecules in Fig. 2A and B. The interaction between calcium alginate and CXCR4 was reinforced by a robust network of hydrogen bonds, carbon hydrogen bond, and van der waals interactions, especially involving critical amino acid residues in a 2D interaction such as ARG30, TYR45, ASP97, CYS186, ASP187,

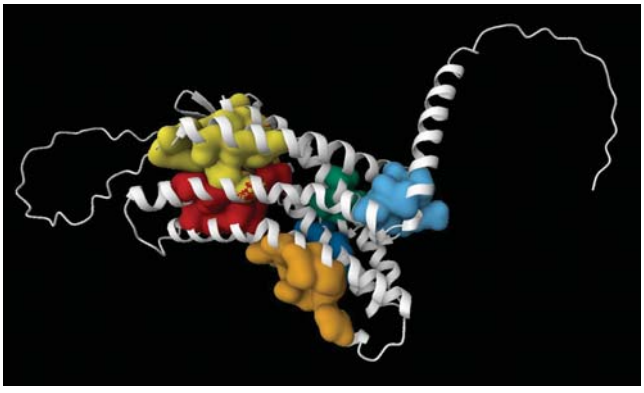

Fig. 1: Predicted binding pockets on the CXCR4 receptor using PrankWeb.

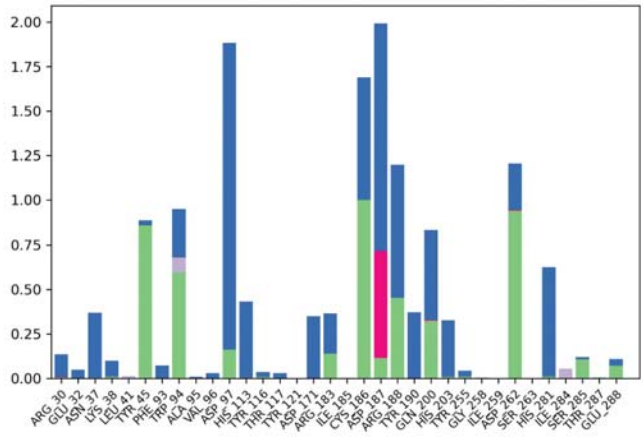

Fig. 4: Protein–ligand contact histogram for the CXCR4–calcium alginate complex

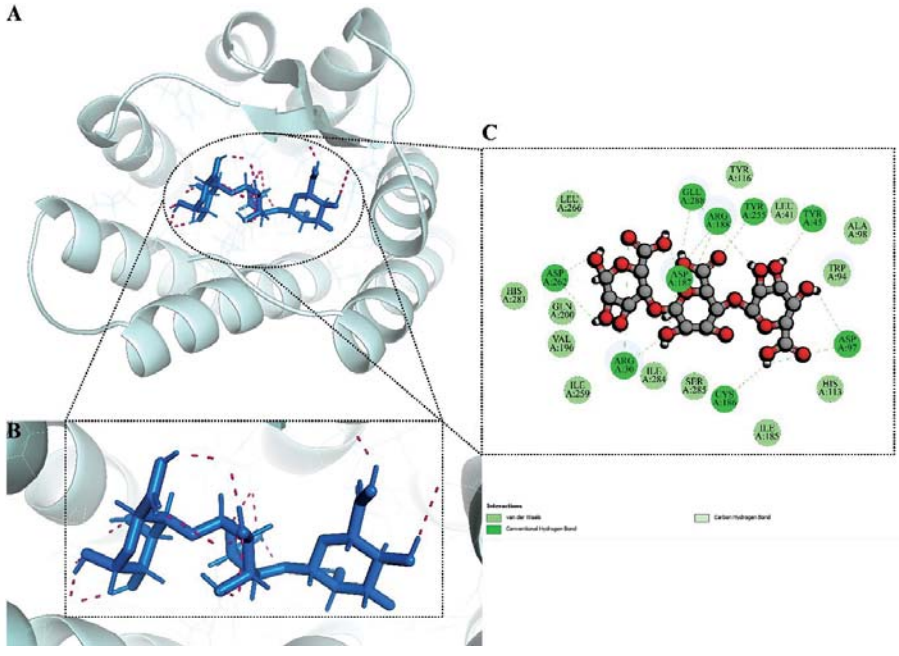

Fig. 2: Molecular docking of bioactive compounds with the CXCR4 receptor.

ARG188,TYR255, ASP262, and GLU288 for hydrogen bond interaction similarly LEU41, TRP94, ALA98, HIS113, TYR116, ILE185, VAL196, GLN200, ILE259, LEU266, HIS281, ILE284, and SER285 are carbon hydrogen bond and van der waals interactions which are recognized for their roles in ligand recognition and signal regulation shown in Fig. 2C. Conversely, the remaining ligands exhibited diminished interactions and less advantageous binding energies, indicating a reduced probability of stable or specific binding to the active site.

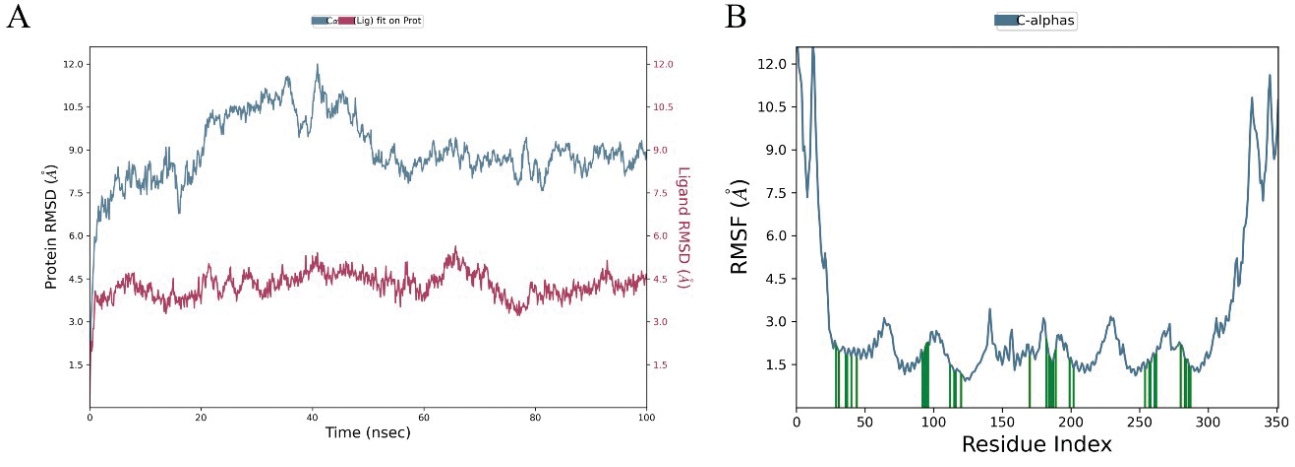

Fig. 3: Molecular dynamics (MD) simulation of the CXCR4–calcium alginate complex. (A) RMSD plot of the CXCR4 backbone and calcium alginate ligand over a 100 ns MD simulation, (B) RMSF plot showing residue-wise flexibility throughout the simulation.

Table 1:

Compound	Docking Score (kcal/mol)
Calcium Alginate	–10.076
Hyaluronic Acid	–6.583
Chitin	–5.514
Collagen Prolyl Hydroxylase Inhibitor	–3.442

Molecular Dynamics Simulation

The molecular dynamics simulation provides valuable insights into the stability and interactions of the protein-ligand complex. The protein-ligand contact histogram reveals several residues that frequently interact with the ligand throughout the simulation. The root mean square deviation (RMSD) plot further supports the complex's structural stability over time. The protein backbone (Cá atoms) shows a gradual increase in RMSD, stabilizing within the 8–12 Å range after an initial rise. This indicates moderate conformational changes while maintaining overall structural integrity. The ligand RMSD, calculated after fitting to the protein, remains within the 3.5–5 Å range, demonstrating that the ligand maintains a relatively stable position in the binding site without significant displacement. This behavior reflects a consistent binding mode and reasonably strong affinity, which is shown in Fig. 3A.

Furthermore, the root mean square fluctuation (RMSF) plot illustrates the flexibility of individual protein residues. As expected, the N- and C-terminal regions exhibit higher fluctuations (above 10 Å), while most core residues show low RMSF values (1–3 Å), indicating a stable and rigid structure. Occasional spikes in RMSF suggest flexible loop regions. The green bars in the plot likely denote active or binding site residues, and their correspondence with regions of low fluctuation reinforces their structural importance in ligand binding (Fig. 3B).

Notably, in protein ligand contacts histogram, residues such as TYR45, TRP94, ASP97, ARG183, CYS186, ASP187, ARG188, GLN200, ASP262, SER285, and GLU288 are involved in hydrogen bond interactions. Additionally, residues including ARG30, GLU32, ASN37, LYS38, TYR45, PHE93, TRP94, VAL96, ASP97, HIS113, TYR116, THR117, ASP171, ARG183, CYS186, ASP187, ARG188, TYR190, GLN200, HIS203, TYR255, ASP262, HIS281, and GLU288 participate in water bridge interactions. Hydrophobic interactions are observed with residues LEU41, TRP94, and ILE284, while ASP187 forms an ionic interaction with the ligand. These residues show strong and persistent contacts, suggesting their critical roles in stabilizing the ligand within the binding pocket. The variety of interactions, hydrogen bonds (green), hydrophobic contacts (violet), ionic interactions (magenta), and water-mediated bridges (blue) highlight the complex and robust nature of ligand binding, as depicted by the multi-colored bars in the histogram, depicted in fig. 4.

Overall, the simulation results indicate that the protein maintains a stable conformation, and the ligand remains consistently associated with the binding pocket through diverse and persistent interactions. These findings are essential for understanding the molecular basis of binding and could inform future efforts in mutagenesis studies or rational drug design.

Discussion

The interaction between Calcium Alginate and the human CXCR4 receptor observed in this study demonstrates significant potential for therapeutic application in regenerative medicine. The high docking affinity (–10.076 kcal/mol) suggests a strong and specific binding interaction, which was further validated by stable behavior throughout a 100 ns molecular dynamics (MD) simulation. These results are particularly notable because CXCR4 is a crucial chemokine receptor involved in stem cell migration, angiogenesis, and tissue repair[18]. Calcium Alginate, a biopolymer widely used in wound healing and drug delivery, has been previously reported to enhance cell proliferation and extracellular matrix formation, largely due to its ability to create a moist and bioactive environment[19]. Its interaction with CXCR4, as shown here, may suggest a novel mechanism beyond structural support—possibly influencing cell signaling pathways related to regeneration.

The Root Mean Square Deviation (RMSD) plot for the protein's backbone (Cá atoms) indicates a gradual increase in deviation during the initial phase, followed by stabilization within the range of 8–12 Å. While this deviation suggests some conformational flexibility, particularly in loop regions or at termini, the overall structural framework remains preserved—consistent with a system that has reached equilibrium[15]. The ligand RMSD, which remains confined between 3.5–5 Å, suggests that the ligand maintains a stable orientation and interaction profile with the protein throughout the simulation, indicating good binding stability[16,20]. The Root Mean Square Fluctuation (RMSF) analysis reveals the degree of

flexibility of individual amino acid residues. As expected, the N- and C-terminal residues display higher fluctuations (>10 Å), typical of terminal regions that are not tightly constrained[15]. Conversely, most residues within the core of the protein exhibit low fluctuations (1–3 Å), suggesting that the ligand-binding region is structurally stable. Notably, residues involved in binding interactions generally display low RMSF values, implying reduced flexibility and supporting their role in ligand stabilization. The alignment of green bars likely denoting binding site residues with low RMSF regions further supports this observation[16]. The protein-ligand contact histogram reveals a diverse range of interactions maintained throughout the simulation, indicating stable and specific binding[21]. Hydrogen bond interactions were predominantly observed with residues TYR45, TRP94, ASP97, ARG183, CYS186, ASP187, ARG188, GLN200, ASP262, SER285, and GLU288. These hydrogen bonds likely play a pivotal role in anchoring the ligand within the binding pocket, as such interactions are often essential for high-affinity binding and specificity[22]. Water bridge interactions mediated by water molecules that form hydrogen bonds with both protein residues and the ligand, were seen with a larger set of residues, including ARG30, GLU32, ASN37, LYS38, PHE93, VAL96, HIS113, TYR116, THR117, ASP171, TYR190, HIS203, TYR255, HIS281, among others. Water bridges are known to enhance binding stability and facilitate transient interactions in aqueous environments[23]. Hydrophobic interactions, observed with LEU41, TRP94, and ILE284, contribute to the entropic component of binding, helping to exclude water molecules from the binding interface and reinforcing the non-polar character of the pocket[24]. Additionally, the residue ASP187 formed a notable ionic interaction, which further stabilizes the ligand, especially when charged moieties are involved[25].

Conclusion

This *in silico* investigation found Calcium Alginate as a strong binder to the CXCR4 receptor, exhibiting the highest docking score and stable molecular dynamics, hence highlighting its potential use in regenerative medicine. Although Hyaluronic Acid, Chitin, and the Collagen Prolyl Hydroxylase Inhibitor exhibited decreased binding affinity, their established biological roles substantiate their significance in tissue healing. These results underscore the efficacy of bioinformatics methods in identifying natural substances with medicinal promise and provide a basis for further experimental confirmation.

Acknowledgement

The authors sincerely acknowledge the National Mission for Clean Ganga for offering a platform to conduct experiments and pursue research activities. We also thank the Director, ICAR–Central Inland Fisheries Research Institute for their extensive support and for providing the necessary research infrastructure. Special thanks to BioCompin for their valuable assistance and collaboration in this research. ❐

References

1. Angeline Duval, Thierry Sayd, Sylvie Bourillon, Laurent Aubry, Vinciane Mosconi, Vincenza Ferraro and Véronique Santé-Lhoutellier, *Food & Function*, 2078-2089 (2024).
2. C.G. Awuchi, C.N. Chukwu, A.O. Iyiola, S. Noreen, S. Morya, A.O. Adeleye, H. Twinomuhwezi, K. Leicht, N.B. Mitaki, C.O. Okpala, *Biomed Res. Int.* 1, 3661866 (2022).
3. A. Kumar, R. Naraian, In *New and Future Developments in Microbial Biotechnology and Bioengineering Elsevier*, pp.205-221 (2019).
4. M.C. Mantovani, I.B. Silva, F.H. Lojudice, P.M. Kossugue, M.C. Sogayar, In *Resident Stem Cells and Regenerative Therapy, Academic Press*, pp.75-103 (2024).
5. L. Xiao, Q. Wang, H. Ni, T. Xu, X. Cai, T. Dai, L. Wang, C. Song, Y. Li, F. Li, T. Meng, *Heliyon*, 10 (2024).
6. A. Karthikeyan, N. Gopinath, & B.G. Nair, *Microbial Pathogenesis*, **194** 106836 (2024).
7. D.P. Cook, *Understanding airway smooth muscle molecular physiology using the cystic fibrosis pig* (2018).
8. Saloni Sachdeva and P. Indira, Sarethy. *International Journal of Environmental Health Research*, ***35***(2) 282-300 (2025).
9. Luna Bharati, R. Sharma Bharat and Smakhtin Vladimir, *Ganges River Basin*. Taylor & Francis, (2016).
10. B.K. Das, A. Gadnayak, H.J. Chakraborty, S.P. Pradhan, S.S. Raut, and S.K. Das, *Scientific Reports*, **15**(1), p.4784 (2025).
11. Q.Y. Zhang, F. Ke, L. Gui, and Z. Zhao, *Water Biology and Security*, **1**(4), p.100062 (2022).
12. M.K. Shukla, A. Prakash, A. Shukla, N. Tiwari, and B.K. Gupta, *International Journal of Plant and Environment*, ***9***(04), pp.301-311 (2023).
13. U.M. Domanska, R.C. Kruizinga, W.B. Nagengast, H. Timmer-Bosscha, G. Huls, E.G. de Vries, and A.M. Walenkamp, *European Journal of Cancer*, **49**(1), pp.219-230 (2013).
14. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, and A.J. Olson, *Journal of Computational Chemistry*, **30**(16), pp.2785-2791 (2009).
15. A. Gadnayak, B. Dehury, A. Nayak, S. Jena, A. Sahoo, P.C. Panda, A. Ray, and S. Nayak, *PLoS One*, **1***7*(7), p.e0271956 (2022).

16. A. Gadnayak, A. Nayak, S. Jena, A. Sahoo, P.C. Panda, A. Ray and S. Nayak, *Plant Science Today*, **10** (2024).

17. B. Patwardhan, *EPMA Journal*, **5,** pp.1-7 (2014).

18. B.A. Teicher, and S.P. Fricker, *Clinical Cancer Research*, **16**(11), pp.2927-2931 (2010).

19. K.Y. Lee, and D.J. Mooney, *Progress in Polymer Science*, **37**(1), pp.106-126 (2012).

20. S.P. Pradhan, A. Gadnayak, S.K. Pradhan, V. Epari, *Cureus*, **16**(12) (2024).

21. A. Gadnayak, and B. Dehury, *Recent Frontiers of Phytochemicals*, pp.85-93 (2023).

22. Caterina Bissantz, B. Kuhn, and M. Stahl. *Journal of Medicinal Chemistry*, **53**.14, 5061-5084 (2010).

23. Yaakov Levy and Onuchic N. José, *Annu. Rev. Biophys. Biomol. Struct.*, **35**.1, 389-415 (2006).

24. K.A. Dill, *Biochemistry*, **29.31**, 7133-7155 (1990).

25. Barry Honig and Nicholls Anthony, *Science*, **268**. 5214, 1144-1149 (1995).