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Letter from Editor-in Chief

Dear Colleagues and Fellow Scientists,

Science is a shared journey of discovery. It's about asking the right questions, exploring the unknown, and improving life for people around the world. But today, the process of sharing scientific work—through journals and publications—has become increasingly difficult for many researchers.

We often talk about how science is for everyone, but the publishing world does not always reflect that. There are rising costs, strict journal metrics, and a growing perception that unless your work appears in a high-impact journal, it doesn't really count. This creates pressure on researchers—especially young scientists or those working in countries with limited funding. Many feel they must either publish in expensive, elite journals or settle for predatory journals with little peer review. This is a serious problem**. Good science should not be judged only by where it is published, but by its quality and contribution**.

At *Vita Scientia*, we want to help change that.

We started this journal because we believe that every researcher deserves a fair chance to share their work. Not just those from well-known institutions or wealthy countries, but also those who are building new labs, working in underfunded areas, or tackling region-specific problems. We offer a **double-blind peer review process**, and our editorial team includes scientists from different parts of the world. We make sure that submissions are reviewed fairly and that financial constraints do not stop good research from being seen.

While keeping our publication affordable is important, our mission goes beyond cost. We want to create a space where early-career scientists are guided, not judged only by numbers. A place where local or regional research is valued alongside global breakthroughs. **A journal that respects both established voices and new ones**.

We also believe that science must stay connected to real-world impact—whether that means helping farmers grow better crops, preserving biodiversity, understanding disease, or exploring life at the molecular level. Our team represents a wide range of biological sciences, from plant and animal biology to biotechnology, microbiology, and more. What unites us is a shared belief in fairness, quality, and accessibility.

We are also aligning *Vita Scientia* with important academic systems like UGC, NAAC, Scopus, PubMed etc. We want our authors—whether they are master's students, PhD scholars, or faculty—to receive the recognition they deserve in their academic careers.

Our first issue is going to be online in July 2025, and we are now open for submissions. We invite you to send your original research, reviews, short communications, or technical notes. We also welcome scientists who want to contribute as reviewers, mentors, or editorial advisors. *Vita Scientia* is a journal shaped by its community, and we hope you will be part of it.

This is more than the start of a journal. It is the beginning of a more inclusive and balanced publishing space—one where science can be shared openly and respectfully, across borders and backgrounds.

If you believe in this vision, we warmly invite you to walk with us.

With sincere regards,
Dr. Shiv Mani Dubey
Chief Editor, Vita Scientia

Dubey, 2025, 1, (1), 1; Published on: 10th August 2025

Vita Scientia

Short Commentary Article

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Strategic Diplomats of Immunity: Rethinking Tregs as Rheostats of Immune Regulation

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In the evolving narrative of immunology, regulatory T cells (Tregs) have traditionally been cast as the peacekeepers of the immune system, perceived as constant, stable, and passive enforcers of self-tolerance [1]. The expression of the transcription factor FOXP3 has long been regarded as indispensable for the development and suppressive function of Tregs [2]. However, recent discoveries prompt a critical reappraisal of this notion, revealing a more nuanced, dynamic, and context-sensitive character of Tregs. Three seminal studies in particular have accelerated this conceptual pivot. Jäger et al. demonstrated that Tregs can maintain suppressive function in steady-state tissues despite a near-complete loss of FOXP3 protein, yet this flexibility is lost in inflammatory settings, where Treg function becomes acutely FOXP3-dependent [3]. Wang et al. further revealed that succinate, a citric acid cycle intermediate elevated in inflammatory bowel disease, disrupts Treg function by promoting FOXP3 degradation through inhibition of its succinylation, linking metabolic stress to epigenetic instability [4]. In parallel, Klawon et al. illustrated that self-antigen-specific Tregs can suppress autoimmunity even during infection while sparing the immune system's ability to combat pathogens, highlighting a remarkable degree of antigen-specific discrimination [5]. Together, these findings present a portrait of Tregs as active integrators of environmental, metabolic, and antigenic signals, thus reimaging them as immune regulators whose function is far more strategic and adaptive than previously appreciated.

This reconceptualization is elegantly captured in the phrase "Strategic Diplomats of Immunity," a metaphor that shifts the image of Tregs from passive peacekeepers to context-aware moderators of immune decisions. Like diplomats, Tregs exhibit strategic restraint and engagement depending on the immunological landscape. In non-inflamed tissues, their function persists even in the absence of FOXP3, indicating a form of baseline regulation when threats are minimal. In inflamed tissues, however, FOXP3 becomes indispensable, suggesting a transition to active suppression mode when the immune environment becomes turbulent. This context-dependence aligns well with the concept of diplomatic engagement, i.e., not all situations demand intervention, but when they do, precise and contextually appropriate responses are critical, performed by skilled negotiators, as by Tregs in this case. The metabolic insight provided by Wang et al. further refines this analogy. Just as external pressures can destabilize geopolitical negotiations, internal metabolic stress, such as elevated succinate, can erode FOXP3 stability and undermine Treg functionality [4]. The result is a subtle yet

potent weakening of immune regulation, not through outright removal of Tregs but via a reduction in their molecular integrity. These findings converge on a model of immune modulation that is scalar rather than binary, where Tregs fine-tune their suppressive output in response to dynamic physiological cues. Hence, the analogy of a “rheostat” aptly fits Tregs, which function as dials rather than switches, adjusting their suppressive activity based on the inflammatory tone, antigenic landscape, and metabolic state.

This dynamic **perspective** of Treg activity is essential, considering that unchecked suppression would impair effective immune responses, while sub-optimal Treg function could lead to autoimmunity. Therefore, a framework wherein external **stimuli dynamically scale Treg function** offers a better understanding with respect to the function of Tregs in the context of inflammation. Importantly, this dynamic view also has profound clinical implications. It suggests that therapeutic manipulation of Tregs need not be absolute, but can be calibrated, such as dialed up in autoimmune disease to restore tolerance, or dialed down in cancer to enable anti-tumor immunity. This nuanced understanding also raises the possibility that Treg induction in cancer may result from chronic inflammation rather than being a direct outcome of tumorigenesis. The study by Klawon et al. further reinforces this idea by showing that Treg suppression is not indiscriminate but antigen specific [5]. Self-peptide-specific Tregs can selectively suppress autoimmune responses while preserving anti-pathogen immunity. Such specificity represents immune regulation at its most refined, i.e., **resolving internal immune conflicts without compromising host defense.** Such precision suggests that Tregs are not merely general suppressors but active decision-makers within the immune hierarchy, equipped to discriminate between friend and foe even under inflammatory pressure. Indeed, under overwhelming inflammation, this antigen-specific regulation might collapse into indiscriminate suppression, aligning with classical Treg biology associated with pathology. Additionally, the integration of antigen specificity with metabolic and transcriptional contexts constructs a compelling model of Tregs as intelligent immune sensors and responders. These cells do not suppress indiscriminately, rather, they assess, interpret, and respond. This behavior validates their reclassification as strategic regulators within the immune hierarchy.

Notably, the modern understanding of Treg function is **not limited to canonical CD4+FOXP3+ cells**. Recent studies have highlighted roles for **CD8+FOXP3+ Tregs and regulatory B cells (Bregs)**, particularly under experimental or pathological conditions. Though rare, these populations possess distinct regulatory roles in cancer, transplantation, and viral infections [6,7]. Their inducible expansion and antigen-specificity reinforce the notion that immune suppression is a **flexible and strategic response** tailored to the context. These populations complement CD4+ Tregs, contributing to a broader, more coordinated immune regulation. For example, induced Tregs (iTregs), generated in vitro, have demonstrated reparative functions in models of viral pneumonia. This layered model of regulation highlights that the rheostat-like behavior attributed to these regulatory cells is, in fact, a distributed function, shaped by the collective contribution of multiple cell types and molecular programs. Much like how different materials are chosen to construct rheostats tailored to specific electrical loads, the immune system employs diverse regulatory players depending on the inflammatory context and physiological demand, emphasizing that no single mechanism fits all scenarios. However, the efficacy of these regulatory cells is **tightly coupled to their epigenetic integrity.** The maintenance of FOXP3 expression via DNA methylation, governed by UHRF1, is crucial. In the absence of UHRF1, iTregs lose their identity, acquire effector traits, and exhibit reduced reparative capacity [8]. These findings underscore that epigenetic anchoring is

essential for Tregs to execute contextually appropriate immunoregulatory functions, acting as the molecular grounding that enables their rheostat-like responsiveness to immune fluctuations.

While the metaphors of diplomacy and rheostats are evocative, they risk **oversimplifying complex immune dynamics**. The term "strategic diplomats" might anthropomorphize cellular behavior. However, these analogies are **heuristically valuable**, offering a conceptual bridge between immunological data and systems-level understanding. They encourage **more granular experimental designs** and **precision-based therapeutic strategies**. By embracing the model of Tregs as adaptive, context-responsive regulators, the field can **transcend static suppression paradigms** and approach a systems biology framework for immune balance.

Thus, the title "**Strategic Diplomats of Immunity: Rethinking Treg Cells as Rheostats of Immune Regulation**" encapsulates this evolution with clarity. It calls on immunologists to re-examine long-held assumptions, explore Treg context-specific behaviors with greater resolution, and design **precision immunotherapies** that reflect the nuanced spectrum of Treg functions. In doing so, we honor the complexity of Treg biology and chart a thoughtful path forward for translational immunology.

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Short Commentary Article

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Plastic waste management by micro-organisms in marine habitat

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Rapid urbanization, accelerated industrialization, and the mounting demands of a growing population have placed unmatched strain on natural resources, significantly impacting the global environment. These developments have led to unsustainable practices that generate vast quantities of waste, contributing to widespread pollution. Major industries consume raw materials extensively and release harmful substances—such as chemical pollutants, non-biodegradable plastics, and radioactive materials—into the environment, causing long-term damage to the biosphere. Plastic pollution is a major environmental problem, especially in marine habitats where about 80% of pollution consists of plastic waste. Every year, millions of tons of plastic enter the oceans, harming wildlife through entanglement, ingestion, and habitat disruption. Plastic breaks down very slowly, often into microplastics, which enter the marine food chain and threaten biodiversity and human health. Traditional methods like physical removal, recycling, and incineration help manage plastic waste but does not fully eliminate it. Microorganisms offer a promising solution by biologically degrading plastics into harmless substances such as carbon dioxide, water, and biomass. Microorganisms play a crucial role in reducing plastic pollution and protecting marine ecosystems by naturally decomposing plastic waste without harming the environment. This biological approach is vital for sustainable plastic waste management and preserving ocean health for the future. This communication provides a detailed structure on the impact of plastic waste on the environment and its management using microorganisms.

Impact of plastic waste on the earth, especially marine habitats

Plastic waste significantly harms the Earth by polluting land, freshwater, and marine ecosystems, disrupting natural habitats and reducing biodiversity. This issue is particularly severe in marine habitats, which cover approximately 70% of the Earth's surface. Plastic pollution in oceans is a critical global issue, with plastic waste comprising around 80% of all marine pollution. Approximately 8 to 10 million metric tons of plastic enter the oceans annually. Alarmingly, by 2050, plastic in the oceans may outweigh all fish. According to the EPA, nearly 100% of all plastics ever made still exist today because it takes 500 to 1000 years to degrade, often breaking down only into harmful microplastics. Currently, there are an estimated 50 to 75 trillion pieces of plastic and microplastics floating in the oceans,

contributing to massive garbage patches and ongoing ecological damage [1]. The biggest cause of damage to wildlife is physical wounds or entrapment from plastic waste leading into painful wounds, strangulation or even drowning. Some species unknowingly feed on plastic, which can choke them, cause internal injuries and starve them to death. Microplastics are consumed by plankton and small fish, entering the marine food chain and accumulating in larger species, including those consumed by humans. Incineration of plastic waste releases greenhouse gases like carbon dioxide and methane. It causes a huge impact on the internal temperature which leads to death of many marine species. This in turn leads to starvation of the predating marine species. This highlights the urgent need to address plastic pollution. Below are the solutions for effective plastic waste management.

Existing solutions for plastic waste management

The major concern for today's time is to control plastic consumption and increase plastic degradation though many existing solutions like The Marine Debris Tracker, Tracking trash through radio frequency identification (RFID) tags and cellular transmitters, projects such as Ahovi exist today, but their major focus lies on plastic trash tracking and identification not on degradation. [5, 6, 11, 1]. So, our article focuses on plastic degradation in sustainable way by utilizing microorganisms.

Microorganism's biotechnology in plastic waste management is the process of utilization of modern scientific tools and techniques which use a wide variety of microorganisms in controlled conditions without disturbing the ecosystem [1]. The list of microorganisms that degrade different types of plastics is provided in table 1.

Table 1: List of microorganisms and their targeted compounds

Name of Microorganism	Compounds/Plastics Degraded	References
Pseudomonas putida	Polyethylene terephthalate (PET), Polyurethane (PU)	[7]
Ideonella sakaiensis	Polyethylene terephthalate (PET)	[12]
Bacillus subtilis	Polyethylene (PE), Polyvinyl chloride (PVC)	[7]
Aspergillus niger	Polyurethane (PU), Polyethylene (PE)	[7]
Penicillium simplicissimum	Polyurethane (PU)	[10]
Phanerochaete chrysosporium	Polyvinyl chloride (PVC), Polyethylene (PE)	[7]
Streptomyces sp.	Polyethylene (PE), Polyvinyl chloride (PVC)	[7]
Rhodococcus ruber	Polyethylene (PE)	[8]
Cladosporium cladosporioides	Polyethylene (PE)	[10]
Engyodontium album	Polyurethane (PU)	[9]

Plastic degradation by microorganisms

Anaerobic microorganisms break down micro plastic into microbial biomass, carbon dioxide, methane, and water when methanogenic conditions are satisfied. While aerobic microorganisms break down

micro plastic into microbial biomass, carbon dioxide, and water depending on the environmental conditions. As soon as plastic enters the ocean, it provides a surface area for microbial colonization by
bacteria and fungi. These microbial organisms will attach to surfaces, including plastic, and develop a biofilm. During this colonization process, microbial organisms begin to secrete extracellular enzymes that begin the degradation of complex plastic polymers. First, the extracellular enzymes provide small plastic molecules in the form of monomer and oligomer molecules. Microbial organisms will uptake plastic monomers and metabolize them inside the cell to achieve further breakdown. The final stages of the microbial organism's process will be the mineralization of these monomers and oligomers into non-harmful by-products, such as carbon-dioxide (CO_2), water (H_2 O), and microbial biomass. This will detoxify the environment but could also create useful bioproducts. Certain species of marine bacteria, such as *Alcanivorax, Pseudomonas*, and *Marinobacter*, etc., exhibited high rates of polymer degradation of PET and polyethylene polymers using the enzymatic process [1] as explained in the fig-1.

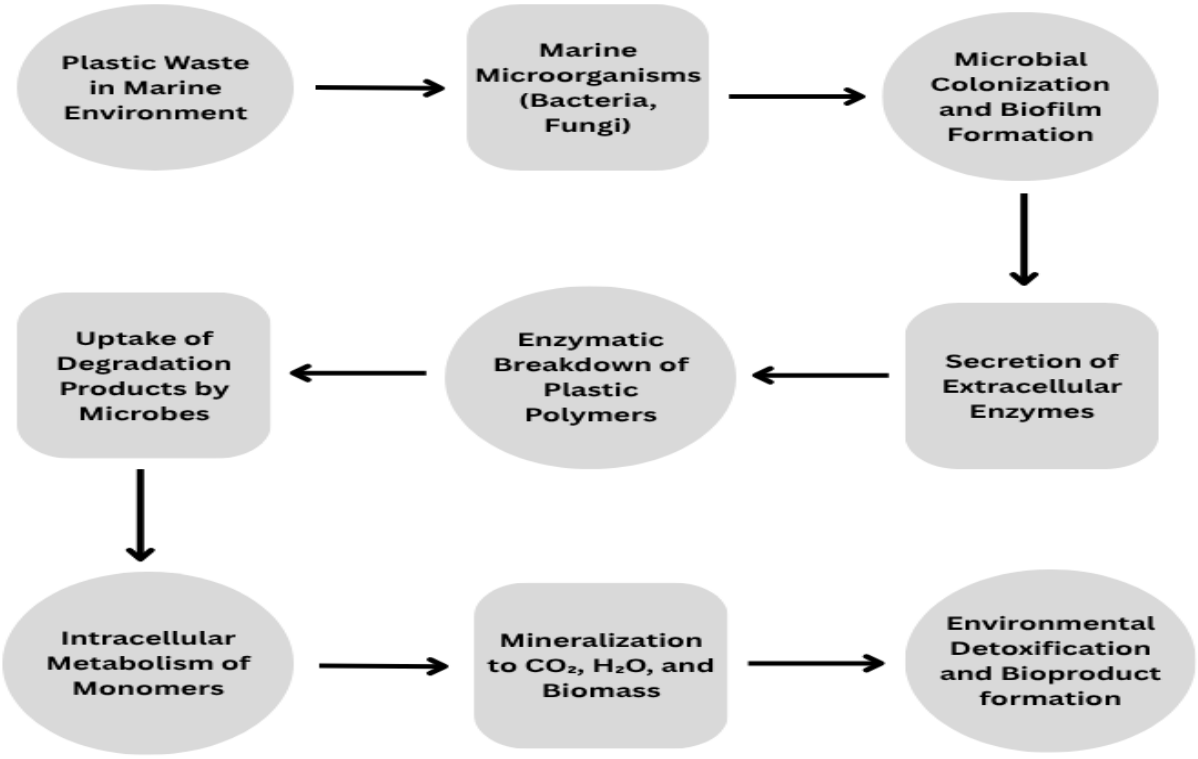

Fig.1: Flowchart of microbial degradation of plastic.

Conclusion

While physical removal, recycling, burning, and policy interventions are valuable for managing plastic waste, they are insufficient for complete degradation. Microorganisms are uniquely capable of breaking down plastics into harmless substances, making them essential for long-term solutions in marine habitats. Unlike mechanical or chemical methods, which often only displace or transform plastics without eliminating them, microorganisms have the unique capability to biologically break down complex plastic polymers into harmless end products like carbon dioxide, water, and biomass through

natural metabolic processes. The microbial degradation technique can be used as a large-scale plastic waste management technique especially in coastal areas like beaches.

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Research Article

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Artemisinin modulates ASIC3 in a Rat Model of Fibromyalgia: In Vivo and In Silico insights

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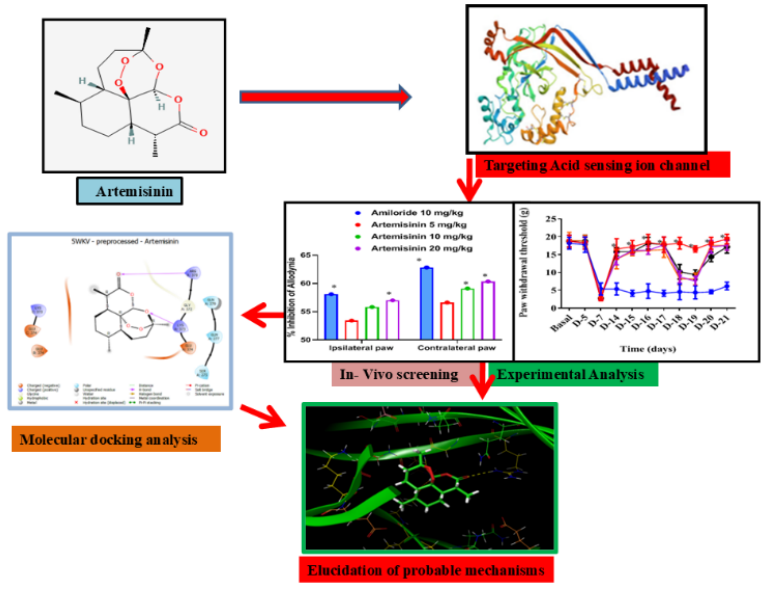
Graphical abstract



Summary

Fibromyalgia is a chronic disorder marked by widespread musculoskeletal pain and increased sensitivity to stimuli. The acid-sensing ion channel 3 (ASIC3) is believed to play a key role in its pathogenesis, making it a potential therapeutic target. Artemisinin, a natural compound from Artemisia annua, has shown promise as a pain modulator. This study examined Artemisinin's effect on ASIC3 using both in vivo and in silico approaches in a rat model of fibromyalgia. Fibromyalgia-like symptoms were induced in rats, and pain behaviors were assessed. Artemisinin was then administered to evaluate its impact on pain sensitivity. Results showed a significant reduction in pain responses following reduction in pain responses following Artemisinin treatment. In parallel, molecular docking studies revealed that Artemisinin binds to specific regions of ASIC3, potentially affecting its function. Furthermore, analysis suggests that Artemisinin may downregulate ASIC3 expression, providing a potential mechanism for its analgesic effect. These findings indicate that Artemisinin modulates ASIC3 activity and reduces pain sensitivity in fibromyalgia, offering a promising therapeutic avenue. Both behavioral and molecular data support this interaction, highlighting the potential of Artemisinin in fibromyalgia management. Further studies are needed to fully understand its mechanism and to validate its clinical applicability in human fibromyalgia treatment.

Keywords

Fibromyalgia; ASIC3; Artemisinin; Pain modulation; Molecular docking.

Introduction

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Fibromyalgia syndrome (FMS) is a complex chronic pain disorder affecting approximately 2-4% of the global population, with higher prevalence in women [1]. It is characterized by widespread musculoskeletal pain, accompanied by fatigue, sleep disturbances, and cognitive impairments [2]. Despite extensive research, the pathophysiological mechanisms underlying fibromyalgia remain incompletely understood, presenting substantial challenges for developing effective characterized by widespread.

Increasing evidence suggests that peripheral and central sensitization of nociceptive pathways plays a crucial role in fibromyalgia pathogenesis [3]. Acid-sensing ion channels (ASICs), particularly ASIC3, have emerged as key contributors to pain signaling in various chronic pain conditions [4]. ASIC3 is abundantly expressed in peripheral sensory neurons, especially in dorsal root ganglia (DRG), and is activated by extracellular acidosis, which commonly occurs during tissue inflammation and injury [5]. Several studies have reported elevated ASIC3 expression in animal models of chronic pain, including fibromyalgia [6].

ASIC3, predominantly expressed in skeletal muscle afferents, plays a key role in chronic pain models. In rodent models mimicking FM, such as the repeated intramuscular acid injection or reserpine model, ASIC3 expression and activity are significantly upregulated. Pharmacological blockade of ASIC3 (e.g., with APETx2) prevents development of widespread pain, and ASIC3 knockout mice resist hyperalgesia—highlighting ASIC3's critical role in peripheral nociceptive sensitization in FM.

Current pharmacological treatments for fibromyalgia provide limited relief and are often associated with adverse effects [7]. Therefore, there is an urgent need to develop novel therapeutic approaches with improved efficacy and safety profiles. Natural compounds represent promising candidates for drug development due to their structural diversity and potentially favorable safety profiles [8].

Artemisinin, a sesquiterpene lactone isolated from the herb Artemisia annua (sweet wormwood) containing a unique peroxide bridge, which is crucial for its biological activity. It is well-known for its antimalarial properties [9]. Beyond its antiparasitic effects, artemisinin and its derivatives have demonstrated anti-inflammatory and analgesic properties in various experimental models [10-13]. Recent studies demonstrate its ability to modulate key inflammatory pathways—such as NF-κB, MAPK, PI3K/Akt, and TGF-β—which contribute to fibrosis, joint inflammation, and neuropathic pain [10-11]. In preclinical pain models, artemisinin and derivatives (e.g. artesunate) have yielded analgesic effects in neuropathic and inflammatory contexts, attenuating pain behaviors and reducing pro-inflammatory mediator levels suggesting potential relevance to FM [11].

Despite its multifaceted biological activities, the direct impact of artemisinin on ASIC3 has not yet been established. Given that ASIC3-mediated signaling underpins the development of widespread hyperalgesia in FM, and artemisinin modulates pain-related ion channels and inflammation, it is rational to explore whether artemisinin can modulate ASIC3 function, thereby alleviating FM-like pain. However, the potential modulatory effects of artemisinin on ASIC3 and its therapeutic implications for fibromyalgia have not been explored.

The present study aimed to investigate the modulatory role of artemisinin on ASIC3 in a rat model of fibromyalgia using both in vivo and in silico approaches. We propose that in a rat model of fibromyalgia, systemic administration of artemisinin will attenuate ASIC3-mediated hyperalgesia via direct or indirect modulation of ASIC3, resulting in reduced pain behaviors. We further hypothesize that in silico docking will support a direct interaction between artemisinin

and ASIC3, elucidating the mechanistic underpinnings of its analgesic effect.

Results

In vivo studies

General observations: The current study aimed to evaluate the effects of Artemisinin on mechanical allodynia induced by repeated intramuscular injections of acidic saline. Twenty-four hours after the second injection, the mechanical withdrawal threshold of the ipsilateral paw decreased from 18.44 ± 0.2 g to 6.013 ± 0.71 g (n = 30), as summarized in Figure 1. Similarly, the contralateral paw showed a reduction from 19.41 ± 0.87 g to 7.34 ± 0.51 g. This significant increase in sensitivity persisted for three weeks after the second unilateral injection for both paws. No significant difference was observed between the withdrawal thresholds of the ipsilateral and contralateral paws, as shown in Figure 1. Among the 30 rats that received two acidic saline injections, 79.44% (n = 30) exhibited a significant reduction in paw withdrawal thresholds to mechanical stimulation with von Frey hairs 24 hours after the second injection. The remaining 20.66% (n = 6) displayed reflex responses to mechanical stimuli comparable to their pre-injection thresholds and were excluded from further analysis. On the 14th day, responders had a mean mechanical withdrawal threshold of 3.44 ± 0.012 g for the ipsilateral paw, compared to 16.55 ± 0.88 g for non-responders. Similarly, the mean withdrawal threshold for the contralateral paw was 0.97 ± 0.05 g for responders and 14.11 ± 0.1 g for non-responders. Administration of pentazocine significantly increased paw withdrawal thresholds to 10.09 ± 0.4 g for both paws in an acute study. Chronic treatment further elevated the mechanical threshold for the contralateral paw to 16.5 ± 0.3 g.

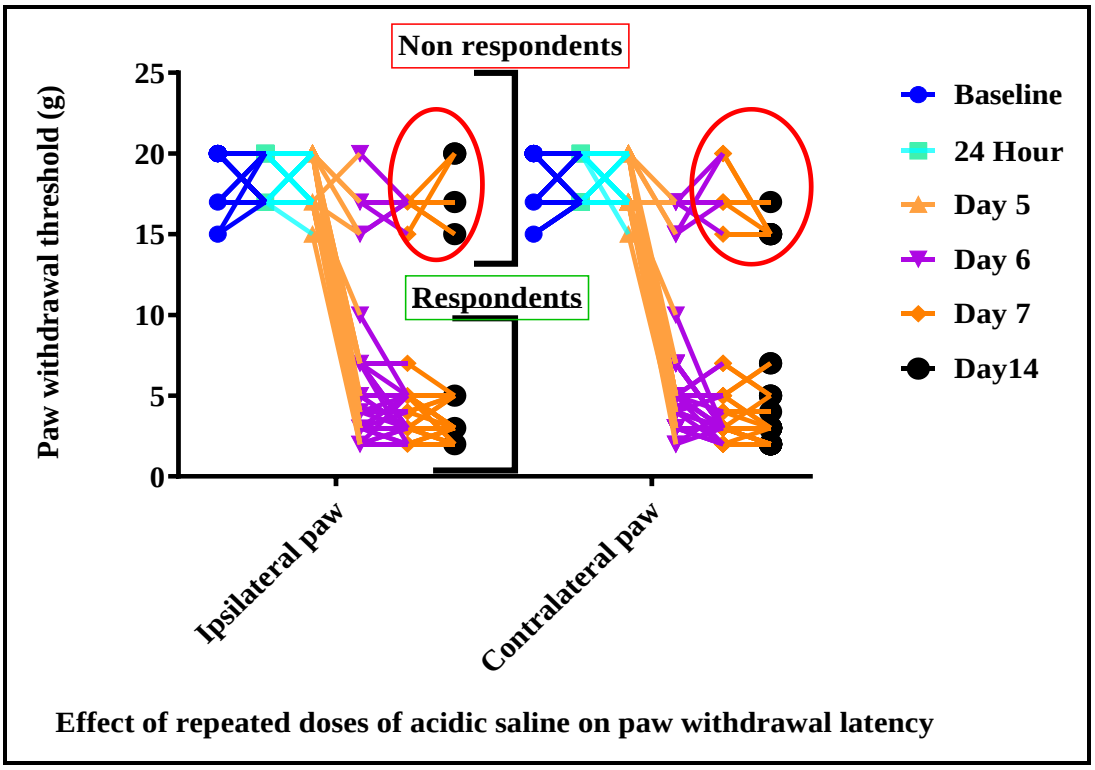


Figure 1. Effect of repeated doses of acidic saline on mechanical paw withdrawal latency. The post effect of repeated injections of acidic saline in the gastrocnemius muscle of rats (n = 36). Withdrawal threshold (g) after stimulation of the plantar surface of the hindpaw was measured with von Frey hairs and the development of mechanical hyperalgesia was observed for both the ipsilateral and contralateral

hindpaws. The various time points are: baseline measured before first injection, 24 h after the first injection, immediately before second injection (on day– 5) and on day 6, 7 and 14. Data are presented as mean ± S.E.M. Number of responders was n = 31 and non-responders n = 5, to mechanical stimuli after14th day of 1st injection of acidic saline

Effect of Artemisinin on acute mechanical hyperalgesia

All tested doses of Artemisinin (5, 10, and 20 mg/kg) significantly reduced mechanical allodynia induced by acidic saline injections. In the acute study, the mechanical withdrawal threshold for the ipsilateral paw increased to 13.97 ± 0.98 g, 13.11 ± 0.90 g, and 11.98 ± 0.70 g for 5, 10, and 20 mg/kg doses, respectively (Figure 2A). For the contralateral paw, thresholds rose to 13.62 ± 0.84 g, 11.97 ± 0.70 g, and 11.75 ± 0.5 g for the same doses (Figure 2B). Pronounced effects were observed 90 minutes post-administration, with increased withdrawal thresholds for mechanical stimuli.

Effect of Artemisinin on chronic mechanical hyperalgesia

Twice-daily administration of Artemisinin for four consecutive days further reduced mechanical allodynia sensitivity. Maximum thresholds were 14.22 ± 0.4 g, 15.10 ± 0.5 g, and 15.3 ± 0.66 g for the ipsilateral paw and 16.70 ± 0.66 g, 17.30 ± 0.65 g, and 16.10 ± 0.11 g for the contralateral paw for the 5, 10, and 20 mg/kg doses, respectively (Figures 3A and 3B).

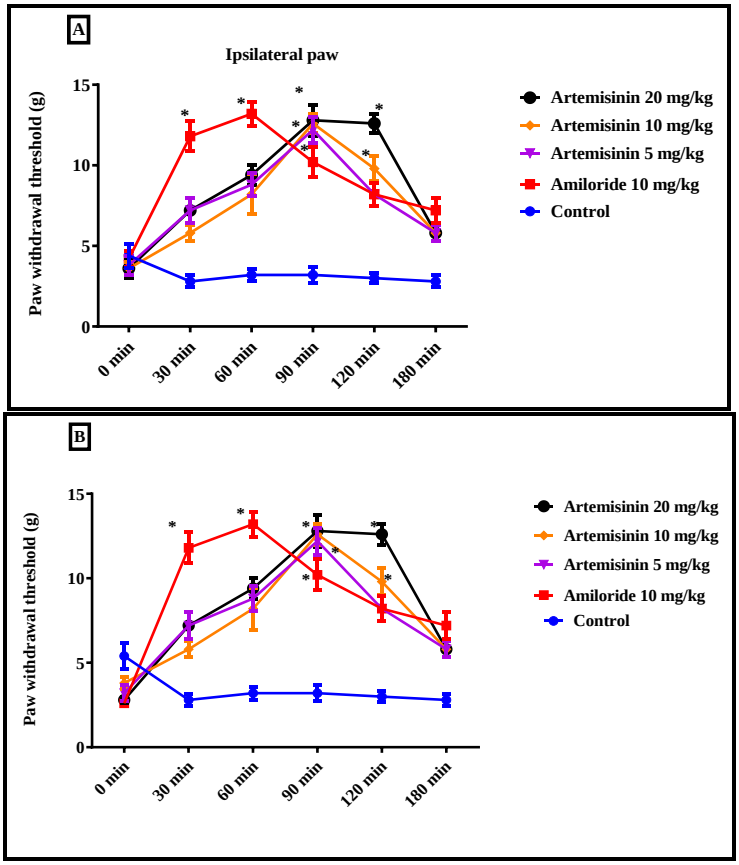


Figure 2. Effect of Artemisinin on mechanical withdrawal threshold in acute responsive studies. Artemisinin was injected immediately after the baseline responses had been obtained. Artemisinin induced an increase in ipsilateral (A) and contralateral (B) paw withdrawal threshold, which remained significantly different from baseline and vehicle treated control for up to 180 min after injection of Artemisinin and

Amiloride. Data are presented as mean ± SEM, *P < 0.05 vs. corresponding vehicle time points (n=6 for each group).

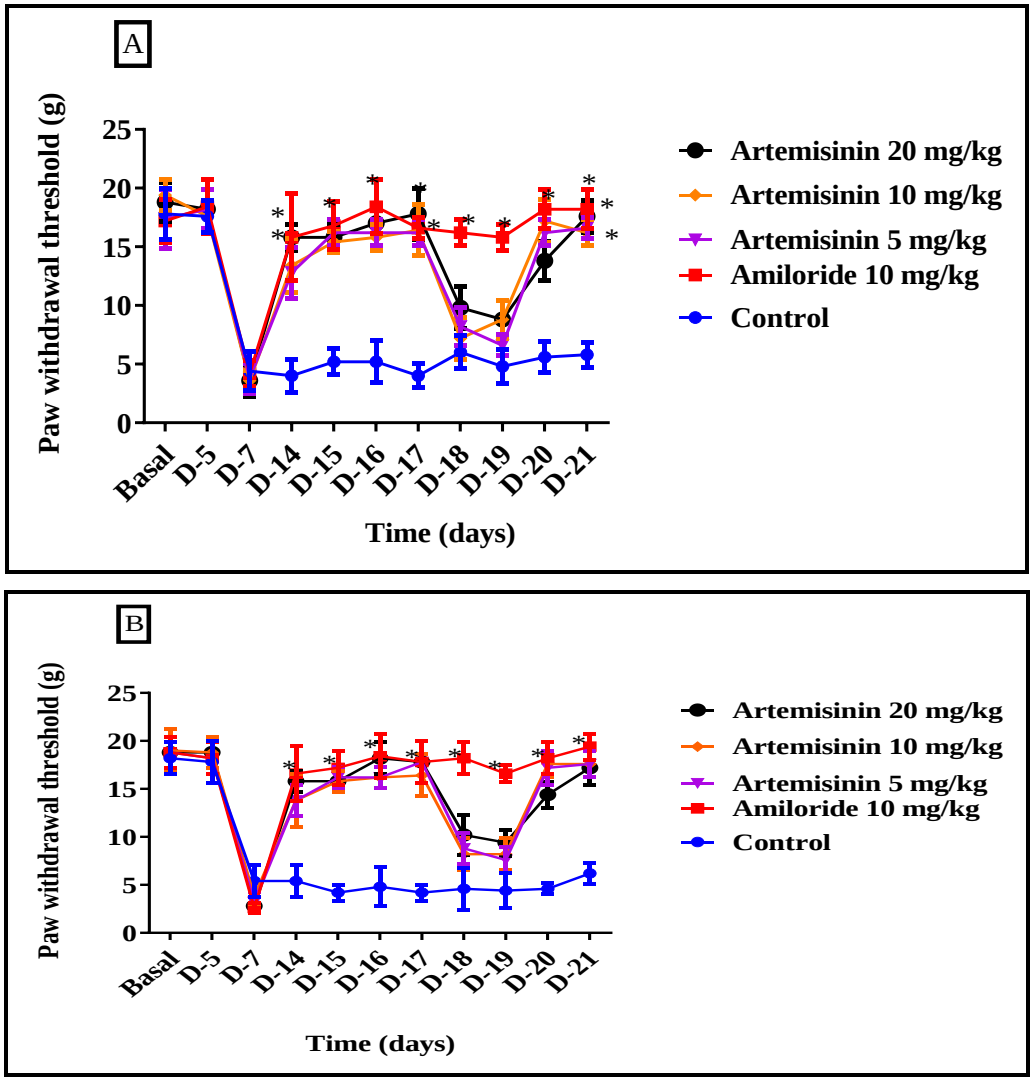


Figure 3. Effect of Artemisinin on mechanical withdrawal threshold on chronic muscle allodynia. Artemisinin induced an increase in ipsilateral(A) and contralateral (B) paw withdrawal threshold, which remained significantly different from baseline and vehicle treated control for up to 180 min after injection of Artemisinin. Data are presented as mean ± SEM,*P < 0.05 vs. corresponding vehicle time points. n=6 for each group.

Artemisinin maximum percentage effectivenesson mechanical hyperalgesia

In the acute study, Artemisinin at 20 mg/kg showed maximum percentage effectiveness (MPE) of 254.11% and 2857.0% for the ipsilateral and contralateral paws, respectively (Figure 4A). At 10 mg/kg, MPE values were 115.01% for the ipsilateral paw and 171.05% for the contralateral paw. At 5 mg/kg, MPE values were 97.00% and 110.21% for the ipsilateral and contralateral paws, respectively (Figure 4B). In the chronic study, Artemisinin at 20 mg/kg resulted in MPE values of 304.20% and 324.66% for the ipsilateral and contralateral paws, respectively (Figure 4B). At 10

mg/kg, MPE values were 209.87% for the ipsilateral paw and 331.24% for the contralateral paw, while at 5 mg/kg, MPE values were 233.11% and 238.55%, respectively.

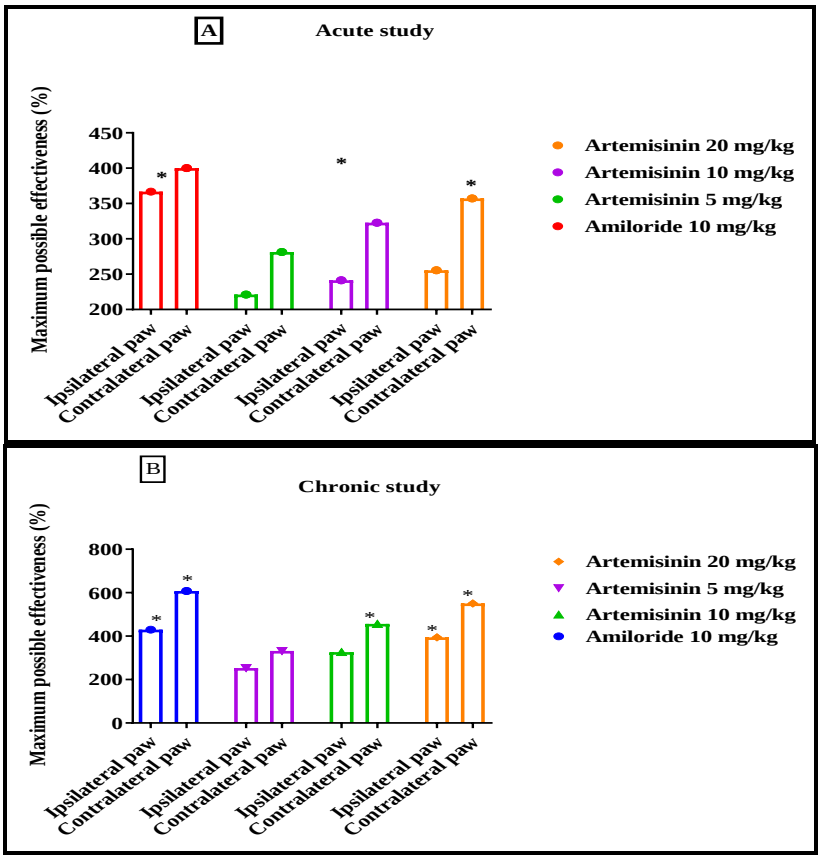


Figure 4. Maximum possible effectiveness in percentage calculated for ipsilateral and contralateral paws for the (A) Acute study and for (B) Chronic study. [The MPE values are represented only for the highest achieved values in a comparative manner; *P < 0.05 as compared to control. n=6 for each group].

Artemisinin maximum percentage effectivenesson mechanical hyperalgesia

The maximum percentage inhibition (MPI) of allodynia was 42.11%, 44.66%, and 55.22% for the ipsilateral paw and 45.10%, 48.00%, and 56.14 % for the contralateral paw, for the 5, 10, and 20 mg/kg doses, respectively (Figures 5A and 5B).The MPI of allodynia for 5, 10, and 20 mg/kg doses reached 69.02%, 77.11%, and 78.33% for the ipsilateral paw and 79.22%, 73.21%, and 77.24% for the contralateral paw (Figure 5B). Whentreatment was paused for two days (days 18 and 19), mechanical allodynia reemerged. However, resuming Artemisinin treatment on day 20 effectively reduced mechanical allodynia, suggesting no development of tolerance to the treatment.

In silico studies

Molecular Docking simulations

Based on the background literature supporting Amiloride-blockable ASICs, we conducted a theoretical analysis using molecular docking simulations. We performed molecular docking of the drug Amiloride against the target ‘an ASIC in a resting state with calcium’ (PDB ID: 5WKV) initially at pH. 7.4 (with default settings) followed by experimental conditions (in-vivo)

with respect to the pH of acidic saline injection, i.e., at. pH 4.0.17additionally, to identify a potential target for ‘artemisinin’, we subjected it to docking as well. Docking results at pH. 7.4 indicated that molecule artemisinin’ (docking score: −5.814 Kcal/mol) exhibited a similar binding affinity to the drug Amiloride (docking score: −5.055 Kcal/mol).

Direct Interaction between Artemisinin and ASIC3

The drug artemisinin formed interactions with amino acid residues, such as GLU A: 374, SER A: 275, GLN A: 277, through conventional H-bonding and alkyl, π-alkyl interactions (Fig. 6).Standard Amiloride formed interactions with amino acid residues, such as GLY A: 372, LYS A:373 GLU A:374, GLU B:374, LYSB:373, GLYB: 372, SER A:275, CYS A:276, GLN A:277, CA A:504through conventional H-bonding and alkyl, π-alkyl interactions(Fig. 7). Based on the docking affinities, we conclude that Artemisinin shows a similar affinity towards ASICs as Amiloride, as demonstrated by in-silico studies.

Discussion

Fibromyalgia is a debilitating chronic pain condition with limited therapeutic options, highlighting the need for novel treatment approaches. In this study, we investigated the modulatory role of artemisinin on ASIC3 in a rat model of fibromyalgia using both in vivo and in silico approaches. Our findings demonstrate that artemisinin alleviates fibromyalgia-related pain behaviors, reduces ASIC3 expression in DRG, inhibits ASIC3 currents, and directly interacts with ASIC3 through specific binding sites in the extracellular domain.

The behavioral manifestations are consistent with previous reports [8] and reflect the ASIC3 has emerged as a promising target for pain modulation due to its involvement in various pain conditions, including inflammatory, neuropathic, and musculoskeletal pain. In line with previous studies [3-6], we observed upregulation of ASIC3 expression in DRG of fibromyalgia-model rats, supporting its role in fibromyalgia pathophysiology. Artemisinin treatment significantly reduced ASIC3 expression at both protein and mRNA levels, suggesting that downregulation of ASIC3 may contribute to its analgesic effect. Our findings provide direct evidence for artemisinin's modulatory effect on ASIC3 function. Artemisinin dose-dependently inhibited acid-evoked responses. This inhibitory effect was comparable to that of Amiloride, a selective ASIC inhibitor, suggesting that both compounds target the same channel. Additionally, artemisinin slowed the activation kinetics of acid-evoked currents without affecting desensitization kinetics, indicating that it may preferentially modulate the channel's activation process. In silico molecular docking and molecular dynamics simulation provided insights into the molecular basis of artemisinin's modulatory effect on ASIC3. The predicted binding site is located at the interface between adjacent subunits of the trimeric channel, partially overlapping with the proton-sensing region. This binding mode suggests that artemisinin may modulate ASIC3 function by interfering with proton-dependent activation, consistent with our electrophysiological observations. The stability of the artemisinin-ASIC3 complex throughout the simulation further supports the validity of the predicted binding mode.

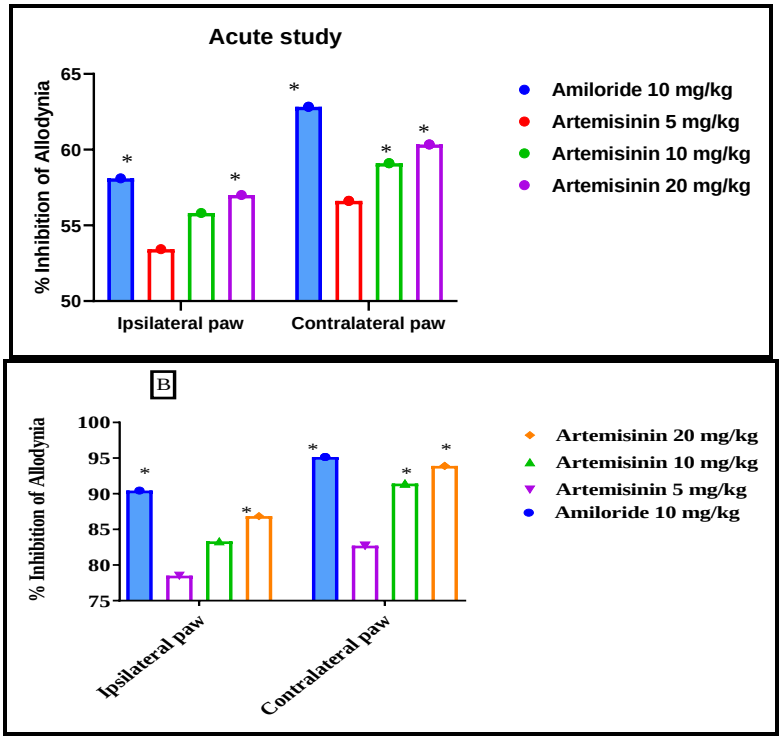
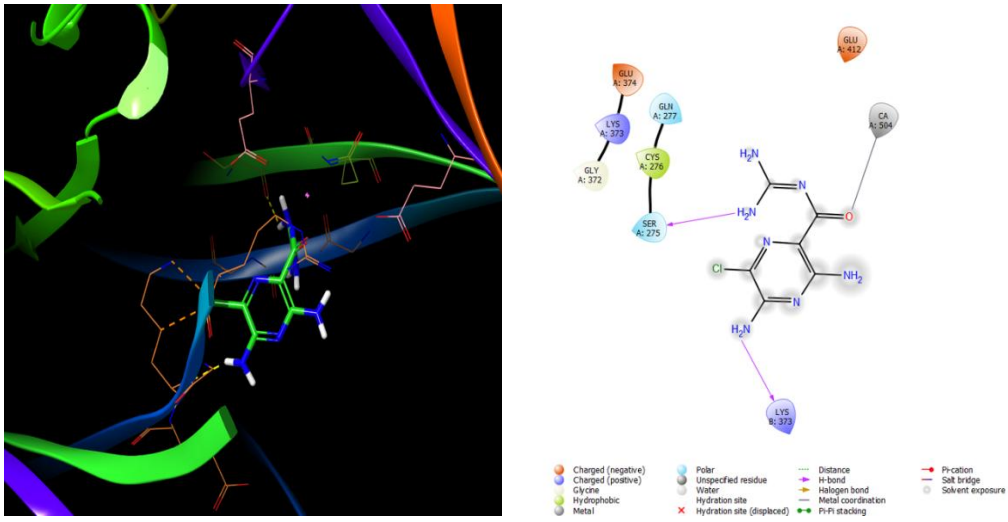


Figure 5. The percentage inhibition of Allodynia was calculated for both the ipsilateral and contralateral paws for the (A) Acute study and for (B) Chronic study. [The values are represented only for the values were the highest percentage inhibition was observed in a comparative manner; *P < 0.05 as compared to control. n=6 for each group].



(A) (B)

Figure. 6. (Color Online) 2D& 3D -interaction diagram of Amiloride within the binding pocket of ASIC

Table 01. Molecular Docking Results of Amiloride and Artemisinin against ASIC (PDB ID: 5WKV)

Ligand	Docking Score (kcal/mol)	Key Interacting Residues	Interaction Types
Amiloride	−5.055	GLY A:372, LYS A:373, GLU A:374, GLU B:374, LYS B:373, GLY B:372, SER A:275, CYS A:276, GLN A:277, CA A:504	Conventional H-bonds, alkyl, π-alkyl interactions
Artemisinin	−5.814	GLU A:374, SER A:275, GLN A:277	Conventional H-bonds, alkyl, π-alkyl interactions

CD

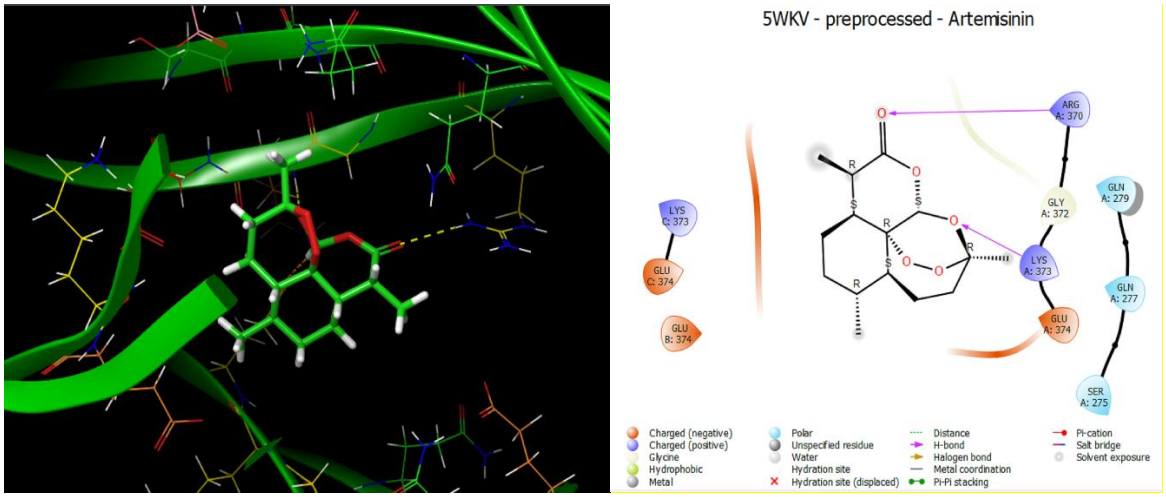


Figure 7 (Color Online) A 2D & 3D -interaction diagram of Artemisinin within the binding pocket of ASIC

Several natural compounds have been reported to modulate ASICs, including traditional Chinese medicines [14]. However, to our knowledge, this is the first study to investigate the modulatory effect of artemisinin on ASIC3 and its therapeutic potential for fibromyalgia. Our findings add to the growing body of evidence supporting the pleiotropic effects of artemisinin beyond its well-established antimalarial activity.

While our results are promising, several limitations should be acknowledged. First, although we focused on ASIC3 as a potential target of artemisinin, we cannot exclude the possibility that artemisinin may exert its analgesic effect through additional mechanisms, such as anti-inflammatory actions or modulation of other ion channels involved in pain signaling. Second, we used fefemale rats in this study to reflect the higher prevalence of fibromyalgia in women; however, future studies should investigate potential sex differences in the therapeutic response to artemisinin. Third, our in silico predictions, although supported by require further validation through site-directed mutagenesis and structural studies.

Conclusion

In summary, our study demonstrates that artemisinin alleviates fibromyalgia-related pain behaviors in rats through modulation of ASIC3. This modulatory effect involves both downregulation of ASIC3 expression and direct inhibition of ASIC3 currents through specific binding to the extracellular domain of the channel. These findings suggest that artemisinin may represent a novel therapeutic

approach for fibromyalgia and provide a foundation for future development of ASIC3-targeted treatments.

Materials and Methods

Animals Used: Adult Wistar female rats weighing [range 190–310 g] was used in present study. The standard environmental conditions were maintained for rats with standard diet and water ad libitum. Food and water being available freely during the experiment protocol. A prior approval protocol number RCP/P-04/24-25 was taken from by institutional animal ethical committee constituted for the purpose of control and supervision of experimental animals by ministry of Environmental and Forests, Government of India, New Delhi.

2.2. Chemical Agents- The Artemisinin (3R,5aS,6R,8aS,9R,12S,12aR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one was purchased (Product code: 63968-64-9) from Yucca YUCCA ENTERPRISES, Mumbai. Purity of Artemisinin was determined by the manufacturer by HPLC was certified above 98.0%. Dimethylsulphoxide (DMSO), sodium chloride (NaCl), all from Loba chemicals) were used in this investigation.

2.3. Experimental details [8]

Chronic muscular allodynia induction.- The previously described method of induction of chronic muscle-mediated pain was used. Lateral Left gastrocnemius muscle was being injected 100 microlitre of hydrochloric acid in saline solution (9 g NaCl/litre; pH adjusted to 4.0 ± 0.1). After five days the same Left lateral gastrocnemius muscle was reinjected with same protocol.

Experimental design- Female Wistar rats being grouped in five groups (6 rats per group): control, acidic saline, acidic saline with low-dose Artemisinin (5 mg/kg), acidic saline with middle dose Artemisinin (10 mg/kg) and acidic saline with high-dose Artemisinin (20 mg/kg). Mechanical allodynia, thermal allodynia, and muscle nociception were assessed at baseline and on subsequent days until end of study using behavioral tests.

The responder's rats (n=30) were separated and divided into five groups of six animals each as follows:

- Group A (n=6) served as control group [hyperalgesic rats] injected with vehicle DMSO 0.2 ml intraperitoneally;
- Group B (n=6) served for standard drug comparison injected intraperitoneally with Amiloide at dose of 10 mg/kg,;
- Group C (n=6) served as for test drug injected intraperitoneally with Artemisinin 5 mg/kg;
- Group D (n=6) served as for test drug injected intraperitoneally with Artemisinin 10 mg/kg;
- Group E (n=6) served as for test drug injected intraperitoneally with Artemisinin 20 mg/kg;

Chronic muscle pain was induced to all the groups of animals as per the procedure described above by two injections of acidic (pH 4) saline separated by 2–5 days, given into the left gastrocnemius muscle. For evaluation of acute responses 48 hours after the second injection of acidic saline rats were treated with Amiloride, Artemisinin (5, 10 & 20 mg/kg, i.p.) or vehicle. Effects of Artemisinin was evaluated by measuring the mechanical hypersensitivity at subsequent time points. To investigate the effects of chronic treatment on mechanical hypersensitivity, Artemisinin wase administered twice a day (every 12 hours apart) to the rats for a period of 7 days. The treatment was interrupted for couple of days on the 18th and 19th day and reinitiated on 20th day to investigate the possible development of tolerance. The evaluation of nociceptive responses was performed 90 minutes after the first daily treatment (the time where the maximal inhibition was observed in the acute treatment). The animals were sacrificed on 22nd day to study the muscle histology at the site of injection.

Mechanical allodynia behavioural testing - Rats placed on elevated metal mesh grid for testing

mechanical allodynia by stimulating paw plantar surface. The series of von Frey nylon hairs or filament (2–20 g) used to assess mechanical allodynia, filament applied in increasing force until the rat withdrew its paw. Each hair applied two times and the threshold (g) was noting lowest force that causes withdrawal stimuli. Prior calibrated Von Frey nylon used during entire course of the study which ensured consistency of applied forces. Rats testing mechanical withdrawal stimuli was done prior first acidic saline injection, before second injection and subsequently further on day 5, 24 hours after second injection, on day 7 for the acute study and subsequently later on from 14th day till 21st day after acidic saline second injection.

2.4. In Silico Molecular Docking

We performed molecular docking studies using the Schrödinger Suite 2023-1 (Schrödinger, LLC, New York, NY, USA), specifically using the Glide docking module through the Maestro interface.We obtained the 2D structures of Artemisinin and Amiloride from the PubChem database. These were converted into 3D structures and optimized using the LigPrep tool with the OPLS4 force field. We adjusted the pH to 4.0 using the Epik module to match the acidic environment of the biological model.We downloaded the 3D crystal structure of the acid-sensing ion channel (ASIC) from the Protein Data Bank (PDB ID: 5WKV, https://www.rcsb.org/structure/5WKV). The protein was prepared using the Protein Preparation Wizard, where we assigned bond orders, added hydrogen atoms, removed water molecules beyond 5 Å from ligands, and minimized the structure using the OPLS4 force field.We generated the receptor grid by centering it on the active site of the co-crystallized ligand with the following coordinates:X = −13.65, Y = 1.60, Z = −0.13 Å.We used the default grid box size (20 Å^3) to cover the binding site properly.We carried out the docking using Glide's Extra Precision (XP) mode, which allowed for accurate sampling and scoring. We used the GlideScore (XP scoring function) to rank the ligand poses based on hydrogen bonding, hydrophobic contacts, van der Waals forces, electrostatic interactions, and desolvation penalties.To validate the docking method, we re-docked the co-crystallized ligand (2-acetamido-2-deoxy-β-D-glucopyranose) into the same binding site. The result gave an RMSD of 0.32 Å, confirming the reliability of the docking protocol.

2.5. Statistical Analysis

Statistical calculations- Graphpad Prism software version 6.01©, 1992–2012 was used for statistical calculation of obtained data. Data for mechanical allodynia are represented as mean ± SEM. One way repeated ANOVA (analysis of variance) was used to analyze the effects of Artemisinin, following Tukey's test posthoc testing. $P < 0.05$ was considered to be statistically significant in all cases.

Statistical calculation for maximum possible effectiveness [%] and inhibition of allodynia [%]- The % maximum possible effectiveness and % inhibition of allodynia was calculated using equations A and B and using calculated arithmetic means in instances for detected main effect for the acute and chronic study.

a) Maximum possible effectiveness in percentage (%)-

Maximum possible effectiveness (%) = post Artemisinin treatment latency − post repeated acidic saline treatment latency × 100 (Eq. A).

For present study in acute study the post latency was recorded 48 hours after second injection and for the chronic study latency was recorded 2 weeks after first injection.

b) Inhibitory Percentage of allodynia.

Inhibitory Percentage of allodynia (%) = test latency − mean basal withdrawal latency/15–mean basal withdrawal latency × 100 (Eq. B).

In (Eq. 2) formulae the mean of the basal mechanical withdrawal threshold is calculated ipsilaterally plus contralaterally for both paws and only in responders n = 30) and the value

estimated at 4.22 ± 0.70 for ipsilateral paw and 4.33 ± 0.22 for contralateral paw, to calculate % effects produced by the after intraperitoneal Artemisinin administration.

Conflicts of Interest

The authors declare no conflicts of interest.

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Review Article

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Harnessing deep learning in bioinformatics-opportunities, challenges, and ethical considerations

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Summary

The last few decades have seen a massive rise in the amount of biomedical data, which has pushed the use of various Machine Learning (ML) approaches to solve new issues in clinical research and biological science. Artificial intelligence (AI) is revolutionizing bioinformatics by enabling the rapid analysis of complex and enormous biological data, the identification of hidden patterns, and the development of prediction models for numerous biological databases. ML and Deep Learning (DL) techniques make it possible to automatically extract features, choose which ones to utilize, and create predictive models, which makes it possible to research complicated biological systems effectively. This study intends to present an overview of DL so that bioinformaticians using these models can evaluate all relevant technical and ethical issues. The findings from this study will encourage people to use DL techniques to resolve their research questions while taking accountability, explainability, fairness, and potential biases. Finally, this study examines the changing environment of AI-driven tools and algorithms, emphasizing their critical role in accelerating research, improving data interpretation, and catalyzing discoveries in biomedical sciences.

Keywords

Artificial Intelligence (AI), Machine Learning (ML), Deep Learning (DL), and Natural language processing (NLP)

Artificial Intelligence in Bioinformatics

Background and Context

Bioinformatics is a multidisciplinary field that applies computational and analytical tools to acquire, process, and interpret biological data [1].This enables researchers to extract meaningful insights from complex biological information [1].

Rapid advancements in genomics, proteomics, and systems biology have led to an unprecedented influx of biological data generated through high-throughput sequencing, structural biology techniques, and computational methods. Among these

advancements, breakthroughs in DNA sequencing technology have significantly enhanced the ability to decode genetic information, including both genomes and transcriptomes, in a cost-effective and time-efficient manner [2]. This progress has allowed scientists to investigate genetic landscapes beyond traditional model organisms, broadening the scope of biological research.

One of the primary applications of bioinformatics is genome analysis, which facilitates gene identification, functional annotation, and comparative genomic studies. Transcriptome sequencing plays a crucial role in genome editing by providing insights into gene expression, and genome sequence analysis enables precise genetic modifications [2]. Functional gene annotation helps identify key genetic elements and their roles, ultimately improving the accuracy and efficiency of genome-editing techniques [2]. Together, these tools advance personalized medicine, evolutionary studies, and drug discovery research.

Beyond its genomics and genome editing applications, it has become increasingly important in clinical decision-making and patient care. Clinical trials have indicated that pharmacist-led interventions, integrated with bioinformatics dashboards, have significantly reduced emergency department visits and hospitalizations while improving immune suppression monitoring [3,4]. These findings emphasize the value of bioinformatics-driven tools for optimizing healthcare strategies, enhancing treatment management, and improving patient outcomes. In addition to its clinical applications, bioinformatics is essential in proteomics, facilitating the analysis of protein expression, interactions, and functions in disease research and biomarker discovery. Advances in MS-based quantitative proteomics and computational predictions have identified functional peptides within proteins [5]. This approach has also supported in-silico proteolysis strategies, offering a promising method for enhancing the functional properties of protein hydrolysates [3][4]Bioinformatics also leverages systems biology and network-based approaches to unravel complex genes, proteins, and pathway interactions in chronic diseases, aiding the identification of key therapeutic targets. Additionally, it accelerates drug discovery by identifying promising treatment candidates, advancing personalized and effective medical interventions [4].These wide-ranging applications underscore bioinformatics' However, these datasets' sheer scale and complexity necessitate sophisticated computational approaches to ensure efficient processing, comprehensive analysis, and accurate interpretation.

Importance of Artificial Intelligence in Bioinformatics

Artificial Intelligence (AI) has revolutionized various fields, including bioinformatics, by bridging biological research with computational analysis. This integration enhances the ability to uncover hidden patterns and extract valuable insights from complex datasets more accurately and efficiently. Bioinformatics enables more effective data analysis, interpretation, and knowledge discovery by utilizing advanced AI-driven tools such as Machine Learning ML and Deep Learning (DL).

AI surpasses traditional computational methods by employing adaptive algorithms that can dynamically process and analyze vast amounts of biological data. Unlike conventional approaches that rely on predefined rules and linear processing, AI continuously learns, identifies patterns, and refines its accuracy without explicit programming [4]. Automating data-driven decision-making and pattern recognition has significantly accelerated bioinformatics research, improving both speed and precision in scientific discoveries.

Scope and Objectives of the Review

This review comprehensively analyzes the evolving role and influence of AI in bioinformatics. It examines how various AI

techniques, particularly ML, DL, and natural language processing (NLP), are leveraged to tackle complex biological challenges. Additionally, it highlights current trends, key methodologies, and emerging AI applications across multiple bioinformatics domains, including genomics, proteomics, and systems biology, focusing on areas such as genome analysis, protein structure prediction, and network modeling.

Beyond exploring applications, this review identifies challenges and limitations associated with AI in bioinformatics, such as data quality concerns, model interpretability, reproducibility issues, and integration with experimental approaches. It also delves into emerging trends and future research directions, including advancements in explainable AI, transfer learning, and novel applications in personalized medicine and synthetic biology.

Ultimately, this review aims to serve as a valuable resource for researchers, bioinformaticians, and AI practitioners, offering insights into how AI is reshaping bioinformatics and driving progress in biological research and healthcare.

Current Trends in AI for Bioinformatics

Machine Learning in Bioinformatics

ML has transformed bioinformatics, which provides new tools and approaches to address intricate biological issues. Genomic sequences, protein structures, gene expressions, and clinical records are examples of the large, varied, and multifaceted data frequently found in bioinformatics. ML techniques have proven indispensable in analyzing and extracting significant patterns from these massive datasets. ML is used in bioinformatics for various purposes, from finding genetic variations linked to diseases to forecasting protein shapes and functions. Researchers can create more precise models for drug development, disease diagnostics, and biomarker discovery using supervised (for classification and regression task such as disease diagnosis and prognosis), unsupervised (for uncovering hidden patterns and patient subtypes), and reinforcement learning methods (for optimizing drug design and clinical trial strategies) is shown in Figure 1[5]. ML has played a pivotal role in advancing bioinformatics by developing sophisticated algorithms capable of handling large-scale biological data. Unlike conventional techniques such as molecular docking and sequence alignment—which are often labor-intensive and computationally expensive—ML-based approaches offer more efficient and scalable solutions. However, more precise classifications and predictions are made possible by the ability to train ML models to recognise patterns in data. DL, a type of machine learning, has shown remarkable results in proteomics and genomics. Deep neural networks (DNN), for example, had previously been assumed to be incapable of accurately predicting proteins' secondary and tertiary structures. Similarly, ML algorithms may examine transcriptome data to provide information about the control of gene expression and how it relates to different diseases, thereby supporting the developing field of personalised medicine. Additionally, ML is becoming increasingly important in developing and discovering new drugs. ML approaches speed up the possible identification of medication candidates, whereas the traditional drug development procedure is expensive and time-consuming. ML models can anticipate a compound's biological activity by analysing chemical databases, simplifying the early drug creation phases. Furthermore, ML is essential to precision medicine because it makes it possible to create algorithms that, using a patient's genetic composition, can forecast how they will react to treatment. Combining these technologies makes it feasible to adopt more individualised therapy strategies, increasing treatment effectiveness while reducing adverse effects [6]. ML encompasses a broad range of techniques that enable computers to learn patterns from data and make informed decisions or predictions. In bioinformatics, ML is

increasingly used to analyze complex and high-dimensional biological data. Depending on the nature of the data and the specific task, following learning paradigms can be applied:

(a) *Supervised Learning*
These algorithms use labeled training data to learn a function that maps input data to desired output labels. Examples include decision trees, support vector machines (SVMs), and linear regression.[6].

(b) *Unsupervised Learning*
These algorithms do not use labeled training data and instead try to identify patterns and relationships in the data. Examples include clustering algorithms (e.g., k-means), dimensionality reduction algorithms (e.g., principal component analysis), and anomaly detection algorithms.[6].

(c) Reinforcement Learning
These algorithms involve an agent learning to interact with its environment to maximize reward. These algorithms are used in bioinformatics for protein folding and drug design tasks, as described in Figure 1[5].

(d) *Semi-supervised learning*
This involves training a ML model on a partially labeled dataset to use the labeled examples to make predictions about the unlabeled samples.[5].

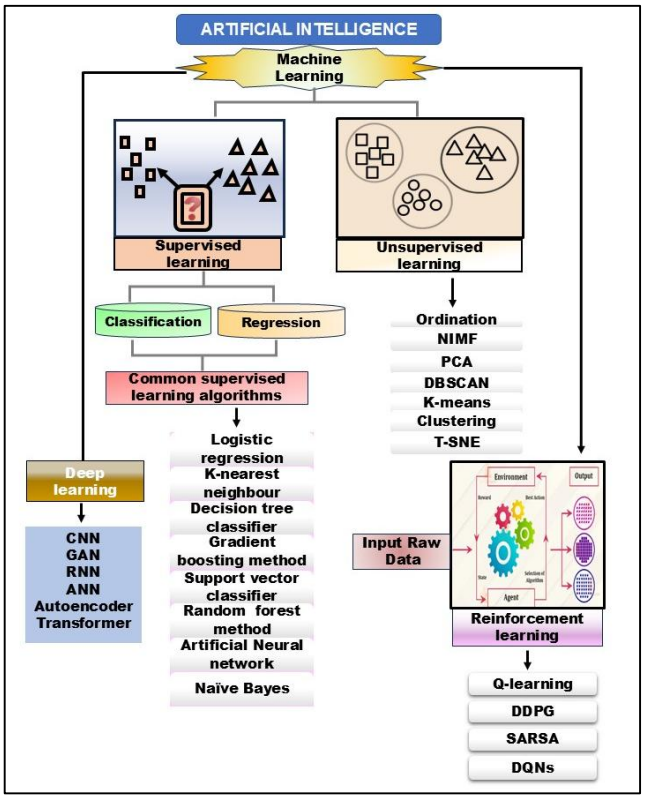

Figure 1: Overview of Artificial Intelligence and ML Paradigms: This figure illustrates the hierarchical relationship between AI, ML, and its three primary branches—Supervised Learning, Unsupervised Learning, and Reinforcement Learning. It highlights key algorithms and techniques within each branch, including DL models (CNN, GAN, RNN, ANN, Autoencoder, Transformer), standard supervised learning algorithms (e.g., logistic regression, decision trees, SVM, neural networks), unsupervised learning methods (e.g., PCA, K-means, clustering), and reinforcement learning strategies (e.g., Q-learning, DDPG, SARSA, DQNs).

Deep Learning (DL) in Bioinformatics
DL has emerged as a transformative approach in the field of bioinformatics, offering powerful tools to analyze and interpret complex biological data [1]. By learning from vast amounts of genomic, proteomic, and clinical data, ML algorithms can assist in tasks such as disease classification, biomarker identification, drug discovery, and personalized medicine. Its ability to adapt and improve from new data makes ML particularly valuable in handling the dynamic and high-dimensional nature of biological datasets. Traditional ML techniques often require manual feature engineering, which can be time-consuming and challenging. In contrast, DL models are capable of automatically learning high-level features directly from raw data, reducing the need for extensive human intervention. [7]. When a problem can be effectively addressed through a well-defined mathematical model, the application of ML is typically redundant. Nevertheless, biology involves a complicated interaction of multiple

factors that mathematical formulas cannot entirely express. Therefore, it makes sense to use machine learning, especially DL. Traditional ML techniques, particularly in gene expression research, often rely on manual feature curation—requiring domain experts to identify, select, and engineer relevant features from raw data. This process can be both time-consuming and prone to bias, as it depends heavily on prior biological knowledge and assumptions about which features are most informative for a given predictive task [8]. This is somewhat simple for gene expression, but it is more difficult to determine if an RNA sequence is a pre-microRNA. It is necessary to manually select thousands of attributes and determine whether each is pertinent [9]. Under such circumstances, DL is particularly relevant to bioinformatics since it can directly learn higher-level features from the data [10]. These algorithms leverage DNN to learn complex patterns and relationships within data. Alternatively, for a more formal or academic tone: By employing DNN, these algorithms are capable of capturing intricate patterns and dependencies in biological data. This capability has enabled significant advancements in various bioinformatics applications such as drug discovery, protein structure prediction, and the analysis of gene expression profiles

(a) *Deep Neural Networks*

At the core of DL's success in bioinformatics are DNNs, which enable the modeling of complex biological processes through multiple layers of interconnected neurons[11]. Several studies have employed more straightforward methods, such as forecasting protein secondary structures or torsion angles; however, fully predicting protein conformations in three-dimensional space remains a challenging and complex task. For instance, stacked autoencoders (SAEs) have been used to address prediction problems related to accessible surface area, torsion angles, and secondary structures within protein amino acid sequences [12]In a different investigation, Spencer et al. used Deep Belief Networks (DBN) in conjunction with Position Specific Scoring Matrix (PSSM) and Atchley factors to predict protein secondary structure [11]. DNNs have proven highly effective in deciphering the intricate mechanisms underlying gene expression regulation, offering insights beyond the reach of conventional computational approaches. For example, Lee et al. proposed a novel training method for DBNs called boosted contrastive divergence, specifically designed to handle imbalanced data, along with a new regularization term to promote sparsity in DNA sequence representations. They applied this approach to splice junction prediction—a critical area in gene expression research—and demonstrated significantly enhanced performance, including the ability to detect subtle non-canonical splicing signals [7]. Furthermore, Chen et al. used multi-layer perceptron (MLP) to estimate the expression of up to 21,000 target genes from just 1000 landmark genes using microarray and RNA-seq expression data [13].The skip-gram model, a popular natural language processing technique that is a variation of MLP, was used to classify proteins. It demonstrated that it could efficiently learn a distributed representation of biological sequences that applies to a wide range of omics applications, including the classification of protein families. Fakoor et al. utilized stacked autoencoders (SAEs) to classify various types of cancer, including acute myeloid leukemia, breast cancer, and ovarian cancer. To enhance classification performance and manage high-dimensional microarray gene expression data, they also applied principal component analysis (PCA) for dimensionality reduction in anomaly detection tasks [14].

(b) *Convolutional Neural Network (CNNs)*

Although only a limited number of studies have employed convolutional neural networks (CNNs) to address biological sequence problems—particularly in the context of gene expression regulation—these works have highlighted the strong potential of CNNs in this

domain. One key advantage is that position-specific scoring matrices (PSSMs) are learned directly from data, rather than manually defined. The initial convolutional layers act as motif detectors by effectively capturing local sequence patterns. As the network depth increases, CNNs can learn progressively more complex patterns, enabling them to recognize longer motifs, integrate the combined effects of multiple motifs, and ultimately decode intricate gene regulatory mechanisms.

CNNs are also well-suited for multitask cooperative learning, allowing them to simultaneously learn shared representations across related tasks, thereby improving overall performance and generalization. CNNs are trained to predict closely related elements simultaneously, making learning and transferring features with predictive strengths easier across tasks. An early approach, for example, transformed ChIP-seq data into a two-dimensional matrix and applied a two-dimensional CNN—similar to those used in image processing—where each row represented the transcription factor activity profile of a gene. More research has been concentrated on employing one-dimensional CNNs directly with biological sequence data. CNN-based methods for transcription factor binding site prediction and 164cell-specific DNA accessibility multitask prediction were proposed by Alipanahi et al; both groups demonstrated subsequent uses for detecting genetic variants linked to illness.[15]. Additionally, a thorough investigation of CNN designs for transcription factor binding site prediction was conducted by Zeng et al. (2016) [16], who demonstrated that the number of convolutional filters is more significant for motif-based tasks than the number of layers. In 2015, Zhou et al. developed DeepSEA, a CNN-based framework designed to prioritize expression quantitative trait loci (eQTLs) and disease-associated genetic variants by leveraging predictive modeling. The framework performs multitask joint learning across various chromatin features, including transcription factor binding, DNase I hypersensitivity, and histone mark profiles [17].

(c) *Recurrent Neural Network (RNNs*)

Given the variable lengths and sequential nature of biological data, recurrent neural networks (RNNs) are considered a highly suitable DL architecture for such tasks. RNNs have been widely applied in research areas including protein classification, gene expression regulation, and protein structure prediction. In early studies, Bidirectional Recurrent Neural Networks (BRNNs) with perceptron-based hidden units were used to predict protein secondary structure. Building on this foundation, Sønderby et al. later employed BRNNs with Long Short-Term Memory (LSTM) units—alongside a one-dimensional convolutional layer—to effectively learn representations from amino acid sequences and classify protein subcellular localization, following the growing recognition of LSTM's superior performance in capturing long-range dependencies [18]. Additionally, Lee et al demonstrated the high capacity of RNNs to analyse biological sequences by using RNNs with LSTM hidden units in microRNA identification and target prediction, resulting in significantly enhanced accuracy compared to state-of-the-art techniques[19].

(d) *Emergent Architectures*

Recent advances in protein structure prediction—particularly in contact map prediction—have leveraged emerging neural network architectures. In a 2017 study, Min et al. [20] employed Deep Spatio-Temporal Neural Networks (DST-NNs), incorporating spatial features such as alignment probabilities, orientation probabilities, and secondary structure information. Additionally, Multi-Dimensional Recurrent Neural Networks (MD-RNNs) were utilized to capture complex dependencies across protein secondary structures, correlation profiles, and amino acid sequences, further enhancing predictive accuracy.

DL	Omics	Biomedical imaging	Biomedical signal processing
Deep neural networks	Protein structure, Gene expression regulation, Protein classification, Anomaly classification	Anomaly classification, Segmentation, Recognition, Brain decoding	Brain decoding, Anomaly classification
Convolutional neural networks	Gene expression regulation	Anomaly classification, Segmentation, Recognition	Brain decoding, Anomaly classification
Recurrent neural networks	Protein structure, Gene expression regulation, Protein classification		Brain decoding, Anomaly classification
Emergent architectures	Protein structure	Segmentation	Brain decoding

Table 1: DL applied bioinformatics research avenues and input data

Natural Language Processing (NLP) in Bioinformatics

Natural Language Processing (NLP) is an interdisciplinary field that bridges artificial intelligence and linguistics, focusing on the development of computational tools capable of interpreting, processing, and generating large volumes of human language data. The complexity of natural language analysis arises from its nuanced semantics, where word order and contextual meaning both play critical roles—a single sentence can convey multiple interpretations depending on the surrounding context. Although biological sequences lack explicit semantic structures like those in human languages, this review demonstrates that NLP techniques can still yield meaningful insights when applied to biomolecular data. Our primary objective is to explore the application of NLP algorithms in bioinformatics, beginning with text mining in PubMed abstracts and extending to the analysis of nucleic acid and protein sequences. The approaches discussed are primarily based on word2vec [21] and transformer-based architectures [22], which have shown promise in capturing patterns and relationships in biological data.

(a) *word2vec*

While natural language text cannot be directly input into neural networks without mathematical preprocessing, Word2Vec was developed based on this foundational concept. One of the most widely used approaches for converting textual data into numerical form is the creation of *n*-dimensional vectors—commonly referred to as word embeddings. This technique effectively addresses the challenge of capturing semantic relationships between words. A well-known example illustrating this principle involves the words *king*, *queen*, *man*, and *woman*. Ideally, in the embedding space, the relationship between *king* and *queen* mirrors that between *man* and *woman*. Mikolov et al. demonstrated this with the following vector arithmetic formula.[21]:

vector("King")-
vector("Man") + vector("Woman") = vector("Queen")

"Embedding" refers to the process of converting data—such as words—into vector representations in a continuous, high-dimensional space. The Word2Vec model is composed of three main components: (a) an input layer, (b) an output layer, and (c) a hidden layer, commonly referred to as the embedding layer. A key feature of Word2Vec is the use of the softmax activation function in the output layer, which estimates the probability distribution of a target word or its context. Depending on the research objective, Word2Vec operates using one of two training architectures:

- Continuous Bag-of-Words (CBOW): Predicts a target word based on its surrounding context.
- Skip-Gram: Predicts the surrounding context words based on a given target word, essentially the inverse of CBOW

(b) *Transformers*

In 2017, Vaswani et al. introduced the Transformer architecture as a breakthrough solution to several limitations faced by traditional models like RNNs in processing natural language texts [23]. Transformers overcame key challenges such as limited parallelization during training, difficulties in capturing long-range dependencies due to memory constraints, and fixed input sequence lengths. This was achieved through the introduction of the self-attention mechanism, which revolutionized how relationships between tokens in a sequence—such as words in a sentence—are identified and weighted. Self-attention enables the model to dynamically focus on relevant parts of the input, significantly enhancing its ability to capture contextual meaning. The standard Transformer architecture is composed of two primary components: the encoder, which processes the input sequence, and the decoder, which generates the output sequence [24].

Input: The initial step involves vectorizing the input data—typically textual data—to generate embeddings that represent words or tokens in a continuous vector space.

***Positional Encoding*:** Since Transformers do not rely on recurrence or convolution to capture

sequence order, positional encoding is introduced to inject information about the position of tokens within a sequence. These positional encodings are typically created using sinsusoidal functions of different frequencies, producing unique vectors for each position. The positional encoding vectors are then added element-wise to the input embedding, combining with positional information. This enables the model to distinguish token order and capture the sequential nature of the data, which is crucial for understanding context

Encoder: The encoder processes the sum of the input embeddings and their positional encodings. It comprises two key sub-layers:

Multi-Head Attention (MHA): This mechanism allows the model to simultaneously attend to different positions in the sequence to capture contextual relationships.

Feed-Forward Neural Network (FFN): A fully connected layer that applies nonlinear transformations to the output of the MHA
Residual (bypass) connections are incorporated between layers to preserve original input information and improve gradient flow. Each sub-layer is followed by layer normalization to stabilize and speed up training.

Decoder: The decoder generates output predictions by integrating encoder outputs with target sequence inputs. It includes:

Masked Multi-Head Attention: Ensures that predictions for a particular position only consider known outputs up to that point, maintaining autoregressive properties.

Encoder-Decoder Attention (MHA): Allows the decoder to focus on relevant parts of the encoder's output.

Feed-Forward Network and Residual Connections: Similar to the encoder, the decoder uses FFNs and residual connections, followed by layer normalization.

Final Prediction: The decoder's output undergoes a linear transformation before being passed through a softmax layer. The softmax function produces a probability distribution over the output vocabulary, with each value indicating the model's confidence in predicting the corresponding token [25].

Restructuring of Core ML Techniques – Applications in Bioinformatics Subfields
On establishing foundational ML methodologies, their transformative role in key bioinformatics domains is now explored. Our interpretation of biological data, from genomics to structural biology, is now being reshaped by AI-driven approaches, thus enabling insights at unprecedented scale and precision as depicted in Figure 2.

Role Of AI In Genomics and Epigenomics

Genomics is a multidisciplinary field dedicated to understanding the structure, function, and evolution of genomes by applying advanced sequencing technologies and bioinformatics tools [30]. By exploring the entirety of an organism's genetic material, genomics seeks to uncover the intricate relationships between genome organization, gene function, and evolutionary processes [31]. The field is broadly divided into several specialized areas, including structural genomics, which focuses on the organization and physical structure of the genome, and functional genomics, which investigates the roles and regulatory mechanisms of genes and non-coding regions.

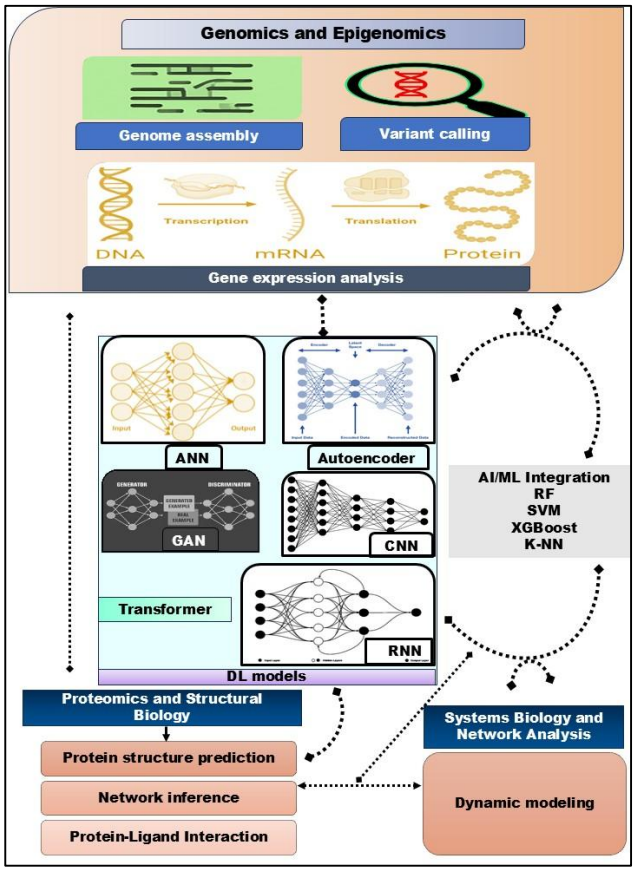

Figure 2. Integrated Multi-Omics and DL Framework: Comprehensive framework illustrating the integration of genomics, epigenomics, and gene expression analysis with advanced DL and artificial in (AI/ML) models-including ANN, GAN, CNN, RNN, autoencoders, and transformers—for downstream applications in proteomics, structural biology, and systems biology. The diagram highlights how these computational approaches enable protein structure prediction, network inference, protein-ligand interaction studies, and dynamic modeling for holistic biological and biomedical research.

Advances in next-generation sequencing (NGS) are used by researchers to sequence DNA/RNA with high accuracy and detect genetic variants/mutations [32]. Eepigenomics is the study of mechanisms associated with changes in gene expression without changing the DNA sequence, influenced by factors such as environmental conditions, lifestyle, and disease state. In this process, a complex interaction occurs between genotypes of an individual and the surrounding environment, which plays a pivotal role in disease development [29]. The modifications involved in this are DNA methylation, histone modifications, and small non-coding RNAs, which are major factors responsible for the activation or repression of genes [34]. Epigenetic biomarkers—particularly DNA methylation patterns—hold significant promise in clinical research and practice due to their potential roles in early disease detection, diagnostic precision, and therapeutic monitoring. Their stability and detectability in biological samples, even at early stages of disease, make them especially valuable in clinical applications. Recent advancements in single-cell epigenomics, combined with AI-driven omics technologies, are further accelerating the integration of genomic and epigenomic markers into personalized medicine.

Genome assembly

Genome assembly is a fundamental process in genomics that involves aligning and merging short DNA sequence reads to reconstruct the original, continuous genomic sequence of an organism. It serves as a critical foundation for downstream genomic analyses, enabling the study of gene structure, function, and evolutionary relationships [35] [36]. Next-Generation Sequencing (NGS) techniques have revolutionized genomic research through their high-throughput capability, allowing millions of sequencing reactions to occur simultaneously. The present NGS platforms used are Illumina [37], Ion Torrent, and sequencing by Oligonucleotide Ligation and Detection (SOLiD)[38]. These technologies offer distinct strategies, with specific advantages and disadvantages. Most of the NGS platforms generate short read lengths of less than 300 base pairs, which complicates de novo genome assembly, making resolving repetitive regions and achieving contiguous sequences difficult [39]. These platforms also face challenges in accurately sequencing regions with extremely high G+C content, as well as tandem and interspersed repeat sequences. These sequencing platforms often encounter difficulties in accurately reading regions with extremely high G+C content, as well as tandem and interspersed repeat sequences, which can

result in such regions being underrepresented or entirely missing from sequencing datasets. Fragmentation and incomplete assemblies are additional common challenges associated with these technologies. To address these limitations, genome assembly strategies increasingly employ hybrid approaches that combine short-read NGS data with long-read sequencing technologies, such as PacBio or Oxford Nanopore. These integrated methods enhance assembly accuracy and completeness by leveraging the strengths of each platform. As NGS technologies continue to advance, the primary challenge has shifted from data generation to the efficient analysis, integration, and assembly of vast genomic datasets. [40]

A variety of AI-powered tools are being leveraged to enhance genome assembly and analysis. For instance, Seq2Squiggle utilizes a Feed-Forward Transformer (FFT) architecture to simulate nanopore sequencing signals directly from nucleotide sequences[41]. Unlike autoregressive models, this approach processes inputs in parallel, resulting in faster and more stable signal prediction. It employs multi-head attention and dense layers to effectively extract sequence features for precise signal mapping. A length regulator dynamically expands DNA embeddings to align with the expected duration of nanopore signals, using a gamma distribution model to adjust event lengths. Additionally, a noise sampler introduces Gaussian noise to mimic the natural variability observed in real sequencing data. By addressing the limitations of traditional simulators—such as k-mer–based methods that struggle to adapt to evolving nanopore chemistries—this tool offers improved flexibility and accuracy. It shows strong potential for optimizing benchmarks on Nanopore R10.4 and R9.4.1 flow cell technologies, making it highly relevant to current research needs. [41]. It uses a Feed Forward Transformer (FFT) to generate realistic nanopore sequencing signals from DNA sequences.

Another DL-based diploid consensus tool, CONNET, has been developed to enhance both the efficiency and accuracy of genome assembly from long-read sequencing data. This tool addresses the high error rates commonly associated with long reads by leveraging spatial relationships within the alignment pile-up to improve consensus accuracy. A sliding window of size three is employed for improved tensor feature extraction. CONNET utilizes a BRNN with one fewer layer than Medaka yet achieves superior accuracy. The input tensor effectively captures alignment features, enabling more efficient neural network learning. CONNET was evaluated on multiple datasets, including *E. coli* SCS110 (90 datasets) sequenced with R9.4.1 chemistry, *E. coli* K-12 (174 datasets) with R9 chemistry, and *Homo sapiens* (37 datasets) also with R9.4.1 chemistry, producing high-quality diploid genome consensus results.

Another notable tool, **SPAdes** (St. Petersburg genome Assembler), was originally developed for de novo assembly of genome sequencing data from cultivated microbial isolates and single-cell genomics DNA sequencing [42]. It was enhanced through hybrid assembly approaches that combine short reads (IonTorrent) with long reads (Oxford Nanopore). This tool supports five distinct pipelines tailored for genome assembly, metagenomics, transcriptomics, plasmid reconstruction, and biosynthetic gene cluster analysis from both metagenomic and whole-genome datasets. AI-driven modifications to SPAdes have further improved metagenomic assembly and classification by boosting computational efficiency and increasing assembly accuracy [42].

Variant calling

Variant calling is the process of detecting genetic variations, including single-nucleotide variants (SNVs) and short insertions or deletions (indels), from sequencing data. Over the years, this field has seen substantial advancements, with next-generation sequencing (NGS) technologies and advanced computational algorithms greatly enhancing both the accuracy and efficiency of variant detection[43]. Genetic

variations refer to differences in DNA sequences that arise within a species or between different species. Next-generation sequencing (NGS) platforms, such as Illumina, represent second-generation technologies, while third-generation platforms include Pacific Biosciences and Oxford Nanopore Technologies [44,32]. Third-generation sequencing makes sample preparation easier and yields longer reads—often several kilobases—by enabling real-time sequencing of individual DNA molecules without amplification [45]. Oxford Nanopore's MinION exemplifies nanopore sequencing technology, which detects nucleotide sequences by monitoring changes in ionic current as DNA molecules pass through nanopores. The use of hairpin adapters enables sequencing of complementary strands, thereby enhancing both accuracy and efficiency [43].

Computational methods like the MinKNOW platform provide high-accuracy sequencing reads and have been successfully applied in pathogen identification, such as detecting the Ross River virus with over 98% accuracy within hours. The combination of accuracy and portability holds great promise for advancing both research and clinical diagnostics.

DeepVariant, developed by Google AI, is a state-of-the-art DL tool designed for highly accurate variant calling from NGS data. Unlike traditional rule-based bioinformatics tools, DeepVariant employs a deep convolutional neural network (CNN) to transform raw sequencing data into high-confidence genetic variant calls. This learning-based approach enables it to perform consistently across various sequencing platforms—including Illumina, PacBio, and Oxford Nanopore—accurately identifying single-nucleotide variants (SNVs) and insertions/deletions (Indels). Its platform-agnostic design makes it highly versatile for applications in population genetics, cancer genomics, and rare disease research.
An extension of DeepVariant, called DeepTrio, incorporates parental data to improve detection of de novo mutations in family-based sequencing, further showcasing the tool's adaptability. Additionally, DeepVariant demonstrates robust performance even with low-coverage or noisy data, making it a powerful asset for high-throughput genomic studies.

In the context of ovarian failure, AI-driven blood-based gene variant profiling utilized Whole Exome Sequencing (WES) combined with MLmodels like Random Forest and unsupervised clustering. Analyzing 63,928 genetic variants, this approach identified 116 variants with significant allele frequency differences and classified ovarian failure into two genomic subtypes (A & B) with 97.2% accuracy. Similarly, bioinformatics and ML-based studies on Premature Ovarian Failure (POF) employed WES alongside tools such as VEST and CADD, uncovering nine heterozygous variants in 24% of patients. Key genes linked to DNA repair and infertility—including *MCM8*, *MCM9*, *EIF2B3*, *PREPL*, *ERCC6*, and *HFM1*—were identified, along with 72 novel variants potentially involved in folliculogenesis

[47]. Beyond reproductive health, AI has been leveraged to predict hypertension risk by applying ML models trained on genetic variants and gene expression data. Analysis of Whole Genome Sequencing (WGS) data using SVM and LR showed that Linear SVM achieved the highest predictive performance, with an AUC of 0.777. Interestingly, incorporating gene expression data reduced the predictive accuracy, suggesting that genetic variants alone may provide a more reliable basis for hypertension risk assessment.

AI also plays a vital role in predicting bacterial pathogenicity. For example, in a study on *Listeria monocytogenes*, supervised ML models—including SVM, Random Forest (RF), Neural Networks, and Gradient Boosting—were used to analyze virulence genes. The linear SVM model reached an accuracy of 89% in identifying virulent strains. Key genes such as *InlK*, *InlJ*, *InlF*, *FAM002725*, and *lmo2026* were

associated with foodborne outbreaks and the severity of disease [48].

Overall, while AI does not directly perform raw variant calling, it significantly enhances variant analysis by using ML models to interpret and classify genetic variants obtained from Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS). These models play a crucial role in disease classification, biomarker discovery, and risk prediction. Collectively, these applications highlight AI's transformative impact on genomics, improving the accuracy, efficiency, and scalability of variant analysis and disease diagnostics, thereby advancing the field of precision medicine. By measuring the transcriptional output of genes, gene expression analysis is an essential next step that connects genetic diversity to subsequent molecular and cellular effect. *By combining gene expression profiling and variant calling, researchers can uncover regulatory mechanisms, link genotype to phenotype, and find biomarkers associated with both health and disease. The next section examines the ways in which artificial intelligence has improved gene expression analysis, allowing for deeper comprehension of gene regulation in intricate biological contexts and more precise interpretation of transcriptomic data.*

Gene expression analysis

Gene expression analysis quantifies the activity of thousands of genes simultaneously, providing insights into cellular and molecular functions and imparting information about disease mechanisms.[49]. AI-based approaches have significantly enhanced expression analysis by enhancing the accuracy of data interpretation, reducing technical variability, and identifying novel biomarkers. AI algorithms play a crucial role in profiling gene expression datasets extracted through high-throughput sequencing techniques, such as microarrays and RNA sequencing. These methods enhance classification, pattern recognition, and feature selection, enabling researchers to differentiate between different biological states, diseased and control conditions. ML techniques, such as supervised and unsupervised learning, are widely used in gene expression analysis studies. For the classification of gene expression profiles, ML algorithms are employed, including Gradient Boosting and Extreme Gradient Boosting (XGBoost), RF, and k-NN [50,51,52,53,54,55].Dimensionality reduction techniques such as PCA and t-SNE help in visualization and manage high-dimensional gene expression data[56].

Kernel-based methods, such as SVM, efficiently classify DEGs and help differentiate healthy and diseased datasets. Gaussian Process Classification (GPC) further enables the modelling of non-linear relationships and uncertainties in gene expression datasets [57]. DL models such as autoencoders and CNNs extract meaningful features from complex datasets in transcriptomics profiling. Transformer-based architectures, inspired by NLP, have also been adapted for analyzing single-cell RNA sequencing (scRNA-seq) data, improving rare cell-type identification and gene regulatory network reconstruction[58].The following case studies illustrate the diverse and impactful applications of AI-driven approaches in gene expression analysis.

Case Studies on AI in gene expression analysis

Cancer Subtype Identification Using Bayesian Neural Networks

For efficient, individualized treatment, it is essential to accurately identify the subtypes of cancer. Uncertainty is a problem for traditional classifiers, particularly when subtypes are quite close.

EpICC, a Bayesian neural network-based classifier created by Joshi et al., measures epistemic uncertainty in its predictions in addition to predict cancer subtypes. Because the model includes an uncertainty correction step, situations that are unclear can be marked for additional verification rather than being

rejected. In terms of overall classification accuracy, EpICC fared better than current techniques. But it has trouble discriminating apart very similar subtypes. It was proposed that including multi-omics data, including epigenetic alterations, would increase precision even more. The model's capacity to measure uncertainty is especially useful for applications using liquid biopsies and other non-invasive cancer detection techniques where accurate categorization is crucial [59].

Cancer Grade Prediction Using Gradient Boosting Trees

One important predictive marker for breast cancer is its histological grading, however manual grading can be arbitrary and unreliable.Amiri Souri et al introduced the Cancer Grade Model (CGM) based on GBT trained on microarray data from 5,031 untreated breast cancers spanning 33 published datasets, and corresponding clinical data were integrated. The model was trained on histological grade-1 and grade-3 samples and then applied to grade-2 and unknown-grade samples for prognostic risk classification[60]. CGM showed strong efficacy in prognostic risk stratification and cancer grade classification, facilitating more objective and repeatable grading. This strategy can help pathologists provide reliable prognostic evaluations, ultimately enhance patient management.

ML-Based Classification of Neurodegenerative Diseases (NDDs)

It can be difficult can be difficult to diagnose NDDs like Parkinson's disease (PD) and Alzheimer's disease (AD) early and accurately since their clinical symptoms overlap. Using blood-based biomarkers data from 377 individuals, Lin CH et al used ML models to measure Aβ42, Aβ40, total tau, p-Tau181, and α-synuclein. Linear Discriminant Analysis (LDA) model was used to extract feature, and several classifiers were evaluated. RF had the best accuracy up to 76% in differentiating between NDD's, while AD had an accuracy of 83% [61].

Gene Expression-Based Lung Cancer (LC) analysis

Globally, LC continues to be major cause of cancer-related mortality. Finding therapeutics targets and comprehending tumor progression depends on identifying DEGs. High-dimensional RNA-seq data frequently presents challenges for conventional statistical techniques. This study analyzed RNA-seq data from LC samples (NCBI SRP009408) using multi algorithms framework. RF, Lasso, XGBoost, Gradient Boosting Elastic Net, and MLP, SVM, and k-NN were applied to identify robust DEGs. The ensemble approach prioritized genes consistently flagged across models to reduce false positives. This study highlights the top five up-regulated genes COL11A1, TOP2A, SULF1, DIO2, MIR196A2) and top five downregulated genes are PDK4, FOSB, FLYWCH1, CYB5D2, MIR328[62].

Sepsis Classification Using ML Models

Conventional methods that rely on nonspecific biomarkers and clinical ratings frequently impede the timely diagnosis of sepsis, a life-threatening illness that necessitates prompt treatment. Using gene expression data from sepsis patients and controls from the GEO and EMBL-EBI Array databases, a study classified sepsis and identified DEGs using DT, RF, SVM, and DNN. With an accuracy of 89%, the models outperformed conventional statistical methods (72%), identifying 2,361 significant DEGs, including important genes like S100A8 (related with inflammatory response) and CD177 (associated with neutrophil activation). The identified DEGs may help guide fast diagnostic panels or treatment targets, and our ML-driven method allows for earlier, more accurate sepsis diagnosis, potentially lowering mortality rates [63].

While gene expression analysis provides us knowledge about the cellular activity, understanding of protein dynamics is equally important for converting data into functional biology as gene expression analysis. This leads

us to role of AI in proteomics and structural biology.

Role of AI in proteomics and structural biology

Proteomics and structural biology are important for understanding the functional and structural dynamics of protein, which are the centre of virtually all biological processes. Proteomics focuses on the large-scale study of proteins, covering their expression, post translational modifications, interactions, and functions[64].Recent advances in MS and high-throughput sequencing technologies have enabled comprehensive proteomic profiling, facilitating biomarker discovery, disease characterization, and drug target identification. Structural biology, in parallel, aims to elucidate the three-dimensional (3D) architecture of biomolecules, primarily utilizing techniques such as cryo-EM [65], [66], [67]. Understanding protein structure at atomic resolution is essential for deciphering molecular mechanisms, protein-ligand interactions, and for rational drug design. Despite remarkable experimental progress, many challenges persist, including the high cost, time consuming nature, and technical limitations of traditional structure determination methods. In this context AI has emerged as a transforming force, enabling unprecedented insights into protein function, interaction networks, and structure-based drug discovery. The following sections explore how AI is revolutionizing both proteomics and structural biology.

Protein Structure Prediction (PSP)

Predicting protein structures from amino acid sequences has been a persistent challenge in bioinformatics and biochemistry. Accurate structure prediction is important for understanding protein function, guiding drug designing, and developing novel therapeutics. Traditional computational approaches such as homology modeling, molecular dynamics, are often limited by high computational costs, time requirements, and restricted accuracy, especially for proteins lacking homologous templates. Recent advances in AI, particularly DL, have dramatically improved the accuracy and speed of PSP. Early DL models, such as CNNs were used to predict contact maps indicating spatial proximity between amino acid residues. These models input features, and predicts spatial relationship, which are then converted into full 3D atomic structures using gradient-based optimization. Graph Neural Networks (GNNs) and RNNs further enhanced the ability to capture long-range dependencies in protein sequences and structure [68] .Attention mechanisms, especially in transformer architecture, have enabled the more effective use multiple sequence alignments (MSAs) and structural templates.

Among the most promising AI-based tools is Alphafold2, developed by DeepMind[69]. Alphafold 2 utilizes transformer neural network, and graph-based reasoning on structural data from the Protein Data Bank (PDB) to predict 3-D conformations of proteins [70]. Although Alphafold 2 has attained near experimental accuracy for many single chain proteins, it still has issues with dynamic conformational states, multichain complexes, and inherently disordered regions. For novel folds or complex assemblies, experimental validation is frequently necessary.

Another significant tool is RoseTTAFold, developed by the Baker's Lab at the University of Washington [71]. RoseTTAFold employs a three-track network model that simultaneously integrates sequence, distance, structure information, allowing for rapid and accurate structure prediction. It is useful substitute for Alphafold 2 because of its design, which permits effective modelling even with limited evolutionary information. ResNet is used to extract evolutionary traits[67] ,GNNs are used to spatial model constraints and folding mechanisms[68] ,and attention- based networks are used to predict inter-residue distances and orientations.

Other emerging DL (https://zhanggroup.org/DeepFold/) ,trRosetta

(https://yanglab.qd.sdu.edu.cn/trRosetta/), and RaptorX (https://raptorx.uchicago.edu/StructureProperty Pred/predict/). OmegaFold utilizes protein language model, and does not require MSAs. Making it faster and more effective on for divergent sequences, including those with few homologs [69]. DeepFold is designed for de novo protein structure prediction, using Spatial constraints, which are then assembled into full length models using folding algorithm [70]. Deepfold is de novo PSP tool that uses convolutional residual neural network to predict spatial constraints. Folding algorithms are then used to put the predictions together into full-length models. Homologous templates are incorporated into the network's predictions to improve accuracy, Rosetta constructs protein structures by direct energy minimization under the inter-residue distance and orientation distributions [71], [72], [73] . RaptorX applies deep convolutional neural fields (DeepCNF) for concurrent prediction of protein structure, solvent accessibility, and disorder regions [74]

While accurate prediction of protein structures forms the foundation for understanding protein function, a critical next step in biomedical research is to elucidate how these proteins interact with small molecules, or ligands. Such protein-ligand interactions underpin most biological processes and are central to drug discovery efforts. Building on advances in protein structure prediction, AI is now increasingly applied to predict and analyze protein-ligand interactions with remarkable accuracy.

Protein ligand interaction prediction

Protein-ligand interaction (PLIs) are fundamental to biological processes and therapeutics development, as they occur when a small molecule (ligand) binds to the target protein (receptor), thereby its function. The strength of this interaction, known as binding affinity, is a key determinant of how effectively a ligand modulates protein activity. Advanced AI and ML algorithms have become powerful tools for predicting and amazing this interaction, accelerating drug discovery and biomedical research.

A variety of curated databases, such as BindingDB, PDBbind, PubChem, and ChEMBL, provide comprehensive data on compound-protein pairs and their corresponding interaction labels[75]. Each molecule is represented using feature vectors or matrices extracted from various biological, topological, and physicochemical properties, which are then used to train ML models [76]. DL architectures, including CNNs, RNNs, GNNs, and transformer-based models, are used to capture complex patterns within these dataset [82]. For instance, CNNs and RNNs are commonly used for ligand binding site prediction, identifying potential pockets on the protein surface where ligands may bind, which is crucial for rational drug design. Notably AI-based ligand binding site prediction tools are *P2RANK*, a stand-alone template-free tool [78], Deepsite uses 3D CNN [79], *GeoNetachieves* introduces a coordinate-free geometric representation to characterize local residue distributions and generating an eigenspace to depict local interactive biophysical environments [80].

AI-Driven Virtual Screening

Virtual screening (VS) is an important component of PLIs, leveraging AI models to rapidly screen large libraries of potential drug molecules against target protein. This process help identify candidates with the highest binding affinity, significantly reducing both the time and cost associated with traditional drug development [81]. VS approaches typically classified is structure-based and ligand-based [82].

Structure-based virtual screening (SBVS) requires detailed structural information about the target protein, which can be extracted from experimental approaches such as (NMR) and computational modelling [82]. AI-driven techniques like molecular docking predict how well a drug binds to its target protein, based on its 3D structure. For example, DL-based

molecular docking tool utilizes quantitative structure-activity analysis (QSAR) models trained on actual docking scores from a small subset of a molecular database to predict docking scores of the remaining compounds [83], [84]. Advanced tools like *DiffDock* use a diffusion generative model to sample ligand poses, mapping the manifold of ligand confirmations relevant to degrees of freedom, such as translation, rotation, and torsion [85]. *EquiBind*, an SE (3)- equivariant geometric DL model can directly predict both the binding location (blind docking) and the bound pose and orientation [86].*TankBind*, incorporates trigonometry constraints and segments the protein into functional blocks to explicitly attend to all possible binding sites [87]. Uni-Mol utilizes SE (3)-Transformer architecture, with pertaining on extensive molecular and protein pocket datasets, and offers several fine-tuning strategies for downstream tasks [88].SBVS effectively determines PLIs, Key amino acids involved in the interaction, and target's structural context. ML-based Scoring algorithms, such as NN-score, CS-score, SVR-score, and ID-score, have been developed to further improve prediction accuracy in SBVS.

Ligand-based virtual screening (*LBVS*), in contrast, relies *on the chemical and physiochemical* similarities of known compounds to predict new active compounds, without requiring prior structural knowledge of the target protein. AI-driven LBVS models can efficiently identify bioactive molecule using supervised learning on curated datasets of active and inactive compounds. Algorithms such as GNMs and ANNs, as well as models like *PARASHIFT*, *HEX*, *USR*, and *ShaPE* are commonly employed. After identifying promising compound, further analysis such as ADMET (absorption, distribution, metabolism, excretion, toxicity) profiling and in vitro bioassays were performed, with successful advancement towards clinical trials.

With the progression of AI and ML, several tools for VS have been developed, including ChemSAR[89],Gypsum-DL[90], PyRMD[91]VSFlow, CompScore[92]FlexX-Scan[93],EasyVS[94], MTiOpenScreen[95], Deep Docking[96], RosettaVS[97], A-HIOT[98]. These tools have improved prediction accuracy and reduced false positives, streamlining the drug discovery pipelines.

Despite these advances, several challenges remain. The quality and diversity of training data can limit model generalizability, and static predictions may fail to capture dynamic protein-ligand interactions. High-resolution docking and generative modeling are computationally intensive, and experimental validation remains essential to confirm AI-driven predictions.

AI in system biology and multi omics integration

Systems biology takes a holistic approach to understanding biological processes by integrating data from various omics disciplines [99].In contrast to reductionist methodologies focusing on isolated molecular entities, systems biology integrates diverse data sources to construct comprehensive models of biological function and disease mechanisms[99]. Network Analysis is one of the primary methodologies in systems biology, enabling the visualization and interpretation of complex biological interactions. By representing molecular entities as nodes and their interactions as edges, network-based approaches facilitate the identification of main regulatory elements, disease-associated biomarkers, and potential therapeutic targets.[100].The advent of AI has significantly enhanced systems biology and network analysis by enabling efficient processing of large-scale omics data, identifying hidden patterns, and predicting dynamic biological behaviors.

NETWORK INFERENCE

Network Inference is widely applied across various biomedical subfields, such as genomics, metagenomics, epidemiology, and neuroscience [101]. Networks serve as powerful tools for representing complex interactions, from molecular markers and neuronal connections to

microbial communities and populations level dynamics [102]. In the context of GRN, network inference aims to reconstruct interaction maps for gene expression data, revealing how genes regulate each other. This is a pivotal tool for understanding complex biological processes and diseases like NDDs and cancer. The goal is to construct a network (graph) where nodes represent elements (e.g., genes, proteins, neurons), and edges represent relationships or interactions between them[103].

Various methods are available for network inference, including correlation-based methods, regression techniques, Bayesian networks, and information-theoretic measures. Each approach has its strengths and weaknesses, some excel at detecting direct interactions, while others perform better suited for capturing non-linear relationships or dynamic regulatory mechanisms. The development of diverse computational tools and algorithms has facilitated network inference. Conventional methods, such as Weighted Gene Co-expression Network Analysis (WGCNA), rely on correlation, while Bayesian network-based approaches infer probabilistic relationships between genes [103]. Advanced algorithms, such as ARACNE (Algorithm for the Reconstruction of Accurate Cellular Networks) and GENIE3, utilize mutual information and tree-based methods to improve accuracy and scalability. [104]. However, newer methods such as *Phixer* and *PIDC* (Partial Information Decomposition and Context aim to reduce redundancy and improve directionality in inferred networks [105]. Many of these tools are tested and benchmarked using publicly available datasets such as those from the DREAM Challenges, which provide a standardized framework for evaluating network inference algorithms.

Despite significant progress, several computational challenges remain in network inference. One major issue is the integration of multiple data sources, such as transcriptomics, epigenomics, and proteomics, to improve inference accuracy. Another challenge is the construction of pseudo-temporal orderings from static single-cell RNA sequencing data, which would enable the study of dynamic regulatory interactions. Additionally, combining multiple network inference algorithms has shown promise in improving prediction accuracy, but finding optimal strategies for integration remains an open question. Addressing these challenges will enhance the ability of network inference to generate biologically meaningful insights, particularly in applications such as drug discovery, cancer research, and personalized medicine. Pathway analysis (PA) builds on network-based approaches by identifying functional modules and signaling cascades within these networks, enabling researchers to interpret how groups of genes and proteins work together to drive cellular processes and disease mechanisms which is described in the next section.

Domain	Tools	Algorithm	Keyfeatures	Limitations	References
Genome assembly	SPAdes	Hybrid	Improved accuracy in single-cell and bacterial assemblies	Computationally intensive	[38]
	Seq2squiggle	FFT	signal prediction faster and more stably		[106]
Variant calling	Deep Variant	CNN	Enhance genomic analysis accuracy, automatic feature extraction	Computationally intensive and require a large amount of training data	[107]
	GATK	ML/Stats	Robust, optimized for high-throughput	Complex setup, resource intensive	[108]

Gene expression analysis	XGBoost, RF, k-NN	Classification feature selection pattern recognition	High accuracy handles high-dimensional data	Sensitive to imbalance batch effects	[109]
	SVM, GPC	DEG classification non-linear modeling	Efficient for small/medium datasets	Less interpretable, kernel selection critical	[109]
	Autoencoders, CNN	Dimensionality reduction, feature extraction	Captures complex patterns	May lack interpretability	[110]
	Transformers	scRNA-seq analysis, rare cell type identification	Improved rare cell detection	Computationally demanding	[111]
Protein structure prediction	Alphafold	Transformer and GNN, MSA based	High accuracy, revolutionized protein modeling	Limited to disordered regions, single conformers only	[112]
	RoseTTA Fold	Three-track network, rapid prediction (ResNet, GNN)	Fast and accurate	Slight less accurate for large complexes	[113]
	OmegaFold	Protein language model	MSA-independent/fast, scalable	Lower resolution for large/ divergent proteins.	[114]
Protein ligand interaction prediction	P2RANK, DeepSite	3D CNN	Template-free, spatial accuracy	Model interpretability, dataset bias	[78]
	DeepDock, DiffDock	Docking score prediction, diffusion models	Efficient, handles pose flexibility	Relies on the docking dataset quality	[85]
	EquiBind, TankBind	SE(3) equivariant, geometric DL	Direct pose prediction considers protein flexibility	Requires high-quality structures	[86], [87]
	ChemSAR, Gypsum-DL	Virtual screening, scaffold generation	High throughput reduces false positives	Training data bias, chemical space coverage	[89], [90]
Network Analysis	ARACNE, GENIE3	Mutual information, tree-based inference	Captures nonlinearities, scalable	May infer indirect associations	04]
	Phixer, PIDC	Redundancy reduction, directionality improvement	Improved accuracy, directionality	Computational complexity	[105]
Pathway Analysis	DeepGSEA	Prototype-based, scRNA-seq enrichment	Handles heterogeneity, explicit visualizations	Requires high-quality gene sets	[115]
	PathGNN, PathCNN	GNN/CNN-based, pathway topology integration	Enhanced prediction, interpretable pathways	Needs large annotated datasets	[116]
Dynamic modeling	dFBA, Cobrapy,CaSQ	time-dependent metabolic flux	Simulates temporal behaviour	Parameter identifiability, simplifications	[117]

		simulation, Boolean model			

Table 2: Comparative Overview of Bioinformatics Tools Across Key Application Domains

Pathway Analysis

Pathway analysis (PA) is an essential approach for the identification of significant biological pathways associated with a gene list retrieved from omics datasets[118], [119]. Traditional methods rely on statistical enrichment techniques such as over-representation and gene set enrichment analysis (GSEA)[119]. AI and ML-powered PA, has transformed bioinformatics by enabling the prediction, modelling, and understanding of complex biological interactions. AI-driven PA is crucial for elucidating disease mechanisms, identifying drug targets, predicting drug-drug interactions, and optimizing therapeutic strategies, while facilitating meaningful interpretation and hypothesis generation.

AI-Driven Methodologies in PA

ML-based PA approaches include RF, which identifies key pathways based on ranking feature selection from omics data, SVM, enhances GSEA predictions by differentiating relevant vs. non-relevant pathways, and GBM, captures non-linear

relationships in gene expression analysis.[120], [121], [122]. DL based PA methods, named as autoencoders, that reduce dimensionality and uncover hidden patterns in gene expression data, GNNs integrate pathway interaction networks based on complex gene interactions, and RNNs capture temporal dependencies in dynamic pathway regulations, especially in time series transcriptomics data. Some commentary techniques such as NLP analyzes biomedical literature to identify novel gene-pathway associations, continuously updating pathway databases with the latest findings. Reinforcement learning algorithms dynamically refine pathway selection strategies, optimizing enrichment scores for improved precision in high-throughput datasets. The following case studies illustrate the diverse and impactful applications of AI-driven approaches in PA

PathGNN, GNN model that leverages pathway topology to enhance predictive accuracy in cancer survival prediction. PathGNN outperformed traditional methods by identifying biologically relevant pathways linked to survival outcomes [123] PathCNN, adapts CNNs for pathway-based multi-omics data analysis using innovative pathway image representation. Applied to Glioblastoma multiforme, it predicted long-term survival and identified critical pathways linked to survival outcomes. By improving interpretability and incorporating pathway topology, it enhances the understanding of the underlying biological processes driving disease progression [116] DeepGSEA, improves PA-based GSEA using prototype-based DL for improved interpretability and accuracy in single-cell RNA-seq data. DeepGSEA captures complex gene set patterns and visualizes pathway distributions, aiding in biomarker discovery in precision medicine [115].

MinePath integrates GNNs, network-based scoring methods, and influence propagation models, to uncover regulatory mechanisms (e.g.'*CXCR4* mutant gene, ErbB signaling) in breast cancer and cervical cancer datasets. In a study by Yuan et al., the unsupervised DeepT2Vec autoencoder generated 30-dimensional transcriptomic feature vectors (TFV) from 20,000 normal/tumor transcriptomes, while the supervised classifier DeepC achieved to 90% pan-cancer and 94% cancer specific [124].

While PA identifies critical biological pathways and their roles in disease mechanisms, understanding how these pathways evolve over time and respond to perturbations requires dynamic modeling (DM). Dynamic modeling builds on pathway-centric insights by simulating temporal changes in molecular interactions,

metabolic fluxes, and signaling cascades. This shift from static pathway mapping to time-resolved simulations enables researchers to predict how biological systems adapt to therapeutic interventions, environmental stresses, or genetic alterations. The following section explores how AI-driven dynamic modeling integrates pathway data, multi-omics inputs, and biophysical constraints to unravel the temporal dynamics of complex biological systems, bridging the gap between functional annotation and predictive systems biology.

Dynamic Modeling

A well-established tool to understand metabolic networks, the temporal behaviour of complex biological systems, such as metabolic pathways, gene regulatory networks, and PPIs, is dynamic modeling (DM) [125]. A key application of DM is in drug response prediction, in which mathematical models simulate how biological systems evolve in response to therapeutic interventions. Dynamic Flux Balance Analysis (dFBA) [117] is a key tool for DM, extending Flux Balance Analysis by incorporating time-dependent constraints.

In metabolic modeling, AI algorithms and bioinformatics tools enhance dFBA. For example, Aghakhani et al. utilized DM to investigate metabolic programming of breast cancer-associated fibroblasts (CAFs) in the tumor microenvironment (TME). They constructed a Boolean model using CaSQ tools to map the regulatory framework, integrating it with MitoCore's central metabolism network. Flux Balance Analysis (FBA) with CobraPy quantified metabolic fluxes. AI improved biological relevance through enhanced network inference, parameter optimization, and metabolic flux predictions. The study compared two FBA scenarios: a control representing baseline metabolic constraints and a CAF regulatory model. The primary goal was to maximize ATP production, focusing on glycolysis and oxidative phosphorylation (OXPHOS) as key pathways. Since MitoCore lacks tissue specificity, flux distributions (rather than absolute values). ATP production ratios from glycolysis and OXPHOS, alongside carbon uptake/secretion fluxes, revealed metabolic exchanges with the TME. Internal metabolic fluxes comparisons, showed significant alterations, particularly with variations exceeding two-fold Another study leveraged double-hybrid continuous approach to develop a multiscale bioinformatics framework integrating tissue, cellular, and molecular interactions within the TME. This method enables the dynamic simulation of tumor progression and therapeutic response by incorporating vascular networks, metabolic pathways, and drug diffusion models. By treating tumor vasculature and drug distribution as interconnected tissues, the model captures the spatiotemporal evolution of tumor heterogeneity. The study highlights the importance of network modeling in predicting combination therapy efficacy, optimizing metronomic chemotherapy, and improving drug penetration through vascular normalization strategies[127].

Rachel et al extended the Retarded Transient Function approach to model both temporal and dose dependent dynamics in intracellular signaling networks. This method provides a computationally efficient and interpretable framework for predicting of signaling differences across biological conditions in their response to stimuli. Using Inflammasome activation in bone marrow-derived macrophages as a case study, the model successfully characterized dependencies, dose-response kinetics, and signaling dynamics [128].

Wang et al developed a multiscale *in silico* model integrating *EGFR-ERK* signaling and cellular dynamics in Non-Small Cell Lung Cancer (NSCLC). The model demonstrates how extrinsic ligand concentrations and intrinsic molecular profiles influence tumor spatial dynamics, revealing a phase transition where a minimal ligand increase suppresses proliferation. These findings highlight the importance of feedback mechanisms between

molecular and cellular scales in shaping tumor behavior[129].

Challenges and limitations of AI in bioinformatics

Data Quality and Availability

The performance and accuracy of AI-driven bioinformatics applications heavily depend on the quality and availability of biological data. While high-throughput sequencing and omics technologies generate vast amounts of data, these datasets often suffer from noise, missing values, and inconsistencies [125]. The lack of standardized formats and integration across databases further complicates data accessibility and usability. Additionally, data privacy regulations and ethical considerations restrict access to patient-derived genomic and clinical datasets, limiting the scope of AI applications in precision medicine.

AI models require diverse and representative datasets for robust training and generalization. However, biases in available datasets can lead to skewed predictions, reducing the reliability of AI-driven insights [126]. Addressing data quality issues through improved curation, annotation, and harmonization strategies is essential for enhancing the effectiveness of AI in bioinformatics.

Reproducibility and Validation of AI Results

Reproducibility is a critical issue in AI-driven bioinformatics research. AI models often rely on complex computational pipelines, sensitive to variations in dataset preprocessing, algorithm selection, and hyperparameter tuning [130]. Differences in computational environments and software dependencies can lead to inconsistent results, making it challenging to validate AI findings across different research groups.

To address this challenge, researchers emphasize the importance of open-source tools, standardized benchmarking datasets, and transparent reporting of methodologies. Reproducibility initiatives, such as FAIR (Findable, Accessible, Interoperable, and Reusable) data principles and AI model repositories, play a crucial role in improving reliability and facilitating independent validation of AI-based bioinformatics studies [131], [132].

Integration with Experimental Methods

Despite its computational capabilities, AI in bioinformatics must be effectively integrated with experimental methods to provide meaningful biological insights. AI models generate predictions that require validation through laboratory techniques like CRISPR gene editing, mass spectrometry, and high-throughput screening [129]. The continuous feedback loop between AI predictions and experimental results is vital for refining models and enhancing their biological relevance.

However, the integration of AI with experimental workflows poses challenges, particularly in fostering effective collaboration between computational scientists and experimental biologists. The lack of standardized approaches for validating AI-generated hypothesis highlights the need of strong frameworks to support AI driven discovery and its translation into practical applications.

As the field continues to address these foundational challenges, attention is increasingly turning to the future directions and transformative opportunities that AI offers in bioinformatics research.

Future of AI in bioinformatics

Integration of AI in Bioinformatics

The integration of AI into bioinformatics is poised to dramatically change the nature of biotechnology research. Recent advances in AI, coupled with breakthroughs in ML, robotics, and data analytics, display enormous potential to revolutionize the field in ways once thought unimaginable. However, these advancements, also raise significant ethical, labor, and security challenges that must be carefully addressed. Serving mankind ethically requires ensuring fair access to AI's advantages while reducing its hazards. Among the emerging priorities in AI integration is the need for transparency and

interpretability, particularly as AI models become more complex and widely adopted in sensitive domains such as healthcare.

Explainable AI (XAI) in Bioinformatics

The "Black box" problem remains a significant challenge in AI-driven bioinformatics, where conventional AI models lack transparency, obscuring how input data are transformed into output results[133]. For example, in an ANN, contains multiple interconnected layers, such as input, hidden, and output layers, with hidden layers, posing interpretability challenges due to their complex internal structure. The intricate internal structure obscures the reasoning behind specific predictions and decisions.

Explainable AI (XAI) has emerged as a critical solution to this issue. XAI techniques open the black box by providing insight s into how model operate, thereby improving both the predictability and trustworthiness of AI systems [134]. XAI works by analyzing the influence of each feature on the model's behavior. In addition to model visualization, methods such as saliency maps, feature importance ranking, and decision trees are used to clarify the model's decision-making process. These methods help researchers and clinicians understand, interpret, and trust model outouts by revealing the factors driving predictions.

Improving interpretability through XAI, not only enables the identification and reduction of biases but also to establish confidence in AI-driven results. In bioinformatics, where datasets, are often large complex, and heterogenous interpretability is crucial. Without it, the basis for model predictions can be lost amid the complexity of the data and algorithms. XAI provides a valuable toolkit for healthcare professional to validate, optimize, and deploy AI models more reliably in clinical and research settings [135], [136]. XAI technologies deployed in various fields, like predictive modeling, data interpretation, and mining valuable patterns from unstructured data in the biological domain. For example, interpretable DL methods such as SHapley Additive exPlanations (SHAP) and class activation maps (grad-CAM) have been proven effective for analyzing DNA, RNA, and protein sequences [136], [137]. Similarly, tools like Local Interpretable Model-agnostic Explanations (LIME) have become more popular in bioimaging, including CT and MRI image assessments. LIME segments images into interpretable "superpixels," making it possible to quantify and visualize region of interest, leading to more accurate identification of the disease and improved diagnostic results[135].

In summary, XAI is important to bridge the gap between complex AI models and practical trustworthy applications in bioinformatics. By improving transparency and interpretability, XAI empowers to make better informed decisions, ultimately improving the reliability and impact of AI- driven discoveries in biomedical sciences.

Transfer Learning and Domain Adaptation

The performance of AI models in bioinformatics, particularly in applications like bioimaging, depends heavily on the quality and consistency of the training data [138]. If the dataset is heterogeneous and unbiased, generally the model will provide accurate results, while a flawed or biased dataset can significantly complicate the accurate assessment of the model performance.

Transfer learning allows model trained on one task to be repurposed for related tasks, leveraging prior knowledge to improve performance [139], [140].This approach facilitates bias detection, model validation and efficient resource utilisation. By redefining the data properties to achieve domain invariance, domain adaptation ensures models remain robust across varied biological context[141].

These methodologies are becoming important in bioinformatics applications, such as clinical image analysis, tissue segmentation, disease classification, and gene expression profiling, where they enhance model accuracy and generalizability[142].

Federated Learning

As AI expands sensitive domains like healthcare, important ensuring data privacy and security becomes paramount. Federated learning offers a decentralized approach, allowing organizations to collaboratively train AI models without sharing raw data [143], [144].Only the trained model is exchanged, preserving confidentiality and reducing the risk of data breaches.

This approach helps organisations to train AI models on their own datasets without the risk of data transfer, thereby offering increased security. The model that is only to be shared is the trained one, which means the raw data stays safe and confidential. This technique not only averts data loss incidents but also [145], [146]. Federated learning is especially beneficial in the case of large-scale, multi-center genomic studies. The genomic information has always been the most sensitive among all the biological information because federated learning here can enable the research centres to work together on predictive models, e.g., for disease risk assessment or pharmacogenomic responses, without the necessity of raw genomic data gathering, and thus, data privacy is preserved while the innovation is encouraged [145], [146].

Quantum Computing and Next-Generation AI

Quantum computing represents a new era in computational power, that can deliver certain computing tasks and data storage at levels far higher than those of ordinary computers. In contrast to quantum computers, where information is typically represented as binary bits (0s and 1s), ordinary computers are resource-consuming to process large datasets. Quantum computers mainly rely on qubits, taking advantage of the concept of superposition, to exist in different states at the same time. This core contrast is responsible for the fact that quantum computers can solve complicated problems and handle high-dimensional data sets very quickly and with good accuracy [147].

In bioinformatics, quantum computing can significantly advance several of the most challenging and difficult tasks in this field like analyzing the massive amount of biological data, modeling molecular interactions, and simulating biological systems without any inaccuracies. These capabilities could accelerate drug discovery, and genomics research and therapeutics development, drastically shortening timelines accuracy [148].

Digital Twin Technologies in Healthcare

Digital twin technology is an emerging in-silico method, with significant potential in healthcare, with its approach to model and track patient health data in a live mode being the state-of-the-art method. A digital twin is dynamic, virtual representation of a physical system-such as a patient-created by integrating data medical imaging, wearables sensors, genomes, and clinical records. This technology makes real-time simulation and monitoring of a person's health status, supporting, personalized diagnosis and treatment [149].

Creating a digital twin involves several stages:

Data Acquisition

- *Medical Imaging (e.g., MRI, CT, Ultrasound):* Researchers used admitted patient's body images to create a geometrically accurate model establishing a new standard in the healthcare sector [150]
- *Wearable Sensor Data*: Data on physical parameters such as heart rate, glucose, and sleep were monitored in real-time using body-worn devices. Bruynseels et al identified this is the next step in digital care[150]
- *Omics Data (Genomics, Proteomics, Metabolomics):* Omics technology plays a pivotal role in Identifying genetic predispositions, molecular pathways, and biomarkers. The new era of computational system biology and functional genomics maximizes the potential of these discoveries[150] .
- *Clinical Records*:

Electronic health records serve as comprehensive repositories of patient histories, diagnoses, and more, maintained by hospital. This CBMM approach aims to accelerate patient diagnosis and assist healthcare providers in making optimal decisions[149].

Model Integration

- *Computational Modelling:*
  Simulates organ and tissue behaviour using systems biology and agent-based modeling, providing detailed representations of physiological processes and interactions.[150].
- *ML& AI:* I
  Detect complex patterns within diverse datasets, enhancing the accuracy and precision of predictions to support personalized diagnostics and treatment planning [151].

Continuous Updating

- *Feedback Loops*:
  Real time patient data is continuously incorporated into the model, ensuring that the simulations remain upto date and reflective of the patient's evolving health status[152].
- *Predictive Algorithms*:
  These algorithms dynamically generate and refine diagnostic and treatment strategies, allowing the digital twin to serve as living, adaptive model of the patient's healthcare[153]. A digital twin thus provides medical professionals with a comprehensive real-time view of the patient with minimal invasiveness. This approach enables more accurate diagnosis, increases the possibility of high-quality treatment, and empowers patients with detailed health record to make informed decisions regarding their care [154], [155].

Conclusion

AI, encompassing ML and DL, is a powerful computational technique that has already transformed several areas of research. With the recent explosion of genetic, molecular, and clinical data, ML provides novel techniques for interrogating, analysing, and processing this data, as well as extracting significant new knowledge about the underlying processes. ML techniques are particularly appealing in computational biology because of their capacity to rapidly produce predictive models in the absence of strong assumptions about the underlying mechanisms, which is typical of some of biomedicine's most serious concerns**.** From genome assembly and variant calling to proteomics research, gene expression analysis, and drug development, DL techniques have demonstrated impressive effectiveness. They frequently outperform conventional computer techniques in terms of accuracy and scalability. However, several challenges still exist. For AI-driven bioinformatics to be reliable, a variety of high-quality, well-annotated datasets must be available. Issues such as noise, biases, and heterogeneous data can pose challenges to the universality and performance of the model. The interpretability of DL models remains a significant challenge since many state-of-the-art architectures function as "black boxes", limiting transparency and confidence in crucial biological applications. Furthermore, reproducibility and standardization of AI procedures are essential to ensure that computational results are converted into reliable biological insights and therapeutic effects. Numerous significant elements will impact bioinformatics advancements in the future. Integrating multi-omics data, developing interpretable and explicable AI models, utilizing transfer learning and domain adaptation, and putting privacy-preserving techniques like federated learning into practice are all crucial for the development of computers. Emerging technologies like digital twin systems and quantum computing have the potential to speed up research and make preventive, predictive, and customized healthcare possible. To achieve these goals, interdisciplinary collaboration, careful benchmarking, and a commitment to ethical and responsible AI deployment are required.

Authors contribution

AKM, SS, NR, PC, UK, and B conceived and designed this project. Data collected and analyzed by NR, PC, UK, N, NK, and KK. Manuscript have been written by NR, PC, B, UK, N, KK, NK, AKM, and SS.

Conflict of Interest

The authors declare no conflict of interest

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