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Review

Chromatin mechanics and regulatory protein function: insights from single-molecule force spectroscopy

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Single-molecule force spectroscopy probes chromatin mechanics by resolving force-induced conformational transitions across multiple length scales. Recent studies extend these measurements beyond canonical nucleosomes to chromatin assembled on native genomic DNA, incorporating histone variants, post-translational modifications, and specialized chromatin architectures. These approaches further scale to chromatosomes, folded fibers, and intact chromosomes, demonstrating that chromatin composition and organization modulate mechanical properties across multiple length scales. Single-molecule measurements further demonstrate that regulatory proteins, including transcription factors, architectural proteins, and chromatin-associated cofactors, actively sense and reshape this mechanical landscape. Together, these observations support a unified view of chromatin as a mechanically encoded medium that can be both written and read.

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Introduction: from force to function — mechanical views of chromatin

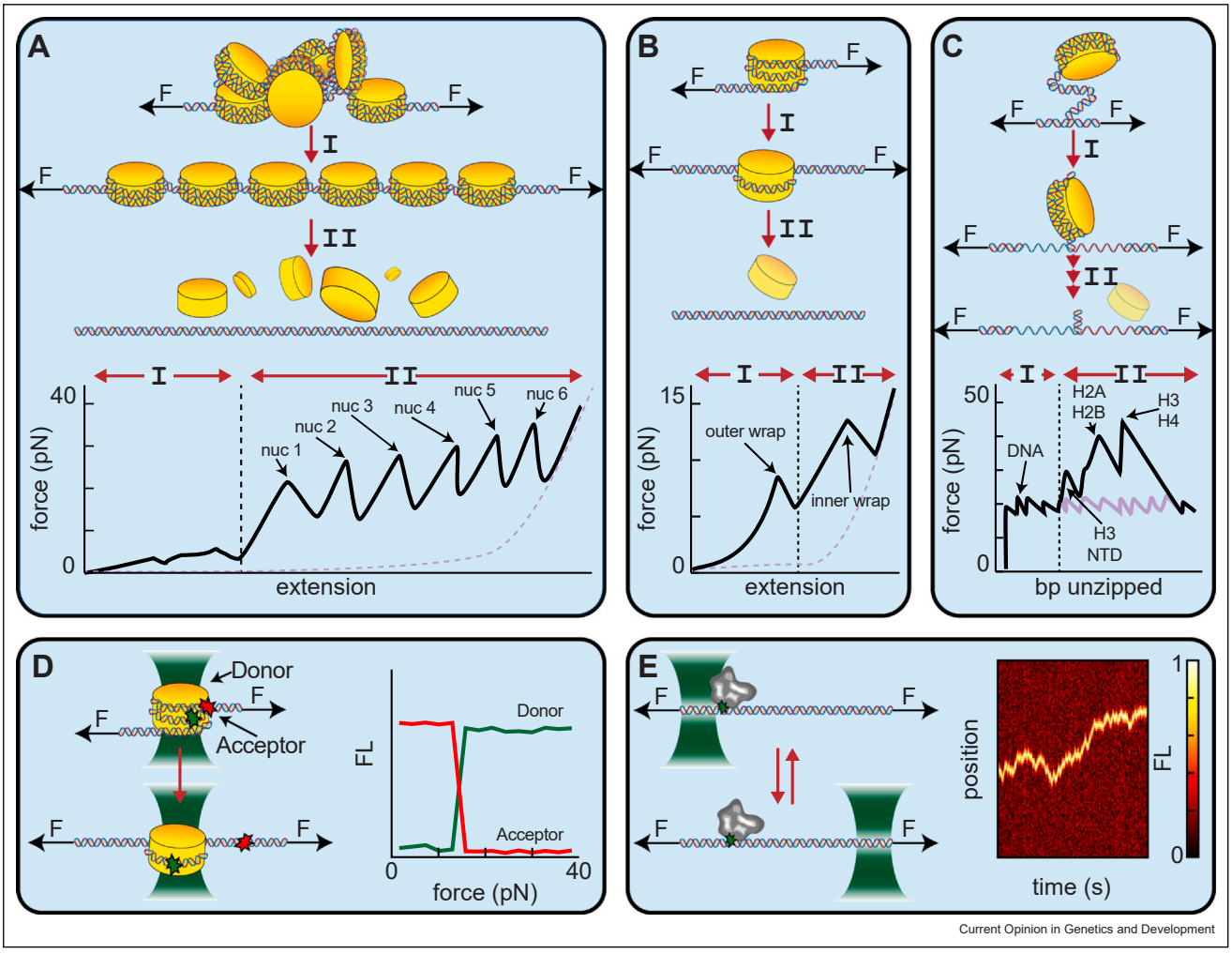
Gene expression begins with access. To bind their motifs and initiate transcription, transcription factors (TFs) must engage DNA packaged into chromatin, where each nucleosome presents a physical barrier and higher-order folding adds additional constraints. Accessibility in this setting is not a simple open-or-closed state but reflects the probability and timescale with which DNA segments become transiently exposed. This dynamic view was formalized by Widom's equilibrium site-exposure model, which described nucleosomal DNA as a thermally fluctuating polymer that transiently unwraps to reveal transcription-factor binding sites [1]. Single-molecule force spectroscopy [2] transformed this idea into quantitative energy landscapes, contributing to a biophysical perspective of chromatin dynamics [3].

Optical and magnetic tweezers enabled real-time interrogation of chromatin, primarily through two experimental geometries: pulling to probe unfolding pathways (Figure 1a,b) and unzipping to map histone–DNA contacts (Figure 1c). Initial pulling experiments [4] stretched chromatin fibers with optical tweezers and revealed weak inter-nucleosomal attractions that govern higher-order chromatin structure. Subsequent stretching of reconstituted fibers revealed stepwise nucleosome loss and distinct unfolding pathways [5–7]. Magnetic-tweezers measurements later established that chromatin arrays behave as compliant helices whose stiffness depends on nucleosome repeat length, ionic environment, and linker-histone content [8,9]. For individual nucleosomes, a two-stage unwrapping pathway was identified, in which the outer DNA turn releases at low force while the inner turn requires substantially higher ones [10], consistent with the underlying histone–DNA contact organization [11]. In a major conceptual advance, combined force–fluorescence measurements (Figure 1d) further showed that nucleosome unwrapping is asymmetric and dictated by sequence-encoded DNA flexibility [12]. Subsequent work assigned structural intermediates to these force-defined transitions, linking mechanical signatures to specific nucleosomal disassembly states [13].

Unzipping provided a complementary view by peeling nucleosomal DNA apart one base pair at a time. Early unzipping experiments resolved individual histone–DNA contacts as discrete force peaks and showed how chromatin remodelers alter these interactions [14]. Subsequent high-resolution mapping provided near–base-pair structural assignments, and established the periodic contact clusters that underlie the nucleosome's mechanical barrier [15].

In parallel, single-molecule manipulation methods were further integrated with fluorescence readouts. In some implementations, fluorescence served as an additional

Figure 1



Single-molecule force-spectroscopy approaches to chromatin mechanics. **(a)** Pulling experiments on chromatin fibers using optical or magnetic tweezers probe higher-order folding, internucleosome interactions, and fiber elasticity. **(b)** Pulling experiments on individual nucleosomes resolve stepwise unwrapping pathways and nucleosome stability under tension. **(c)** Unzipping experiments peel nucleosomal DNA base pair by base pair to map histone–DNA interactions and nucleosome energy landscapes. **(d,e)** Hybrid force–fluorescence assays combine mechanical manipulation with fluorescence readouts to correlate chromatin conformational changes measured by FRET **(d)** or protein positioning and dynamics **(e)**.

probe during force spectroscopy, allowing mechanical transitions to be correlated with protein position or structural state (Figure 1d) [12]. In other implementations, low tension is applied only to extend or immobilize the DNA while fluorescence tracks proteins in real time (Figure 1e; reviewed in [16]). These hybrid assays complement force spectroscopy by revealing dynamical changes that cannot be resolved from mechanical signals alone.

Together, these experimental geometries established the foundations for modern single-molecule force-spectroscopy studies of chromatin. In this review, we focus on insights from force-manipulation experiments into nucleosome structure, chromatin mechanics, and the mechanical interplay between chromatin and regulatory proteins. Our scope is limited to approaches in which force actively probes chromatin or is used to extend or immobilize substrates for fluorescence-based detection. We do not cover torque-dependent chromatin behaviors, ATP-dependent chromatin remodelers, or RNA polymerase acting on nucleosomes, each of which constitutes a substantial field beyond the scope of this review.

Nucleosome composition shapes chromatin mechanics

With the foundational mechanical behaviors of nucleosomes established, more recent force-spectroscopy studies have begun to reveal how histone composition, including variants, post-translational modifications

(PTMs), and specialized assemblies, modulates these properties. These advances have shifted the focus from a single 'canonical' nucleosome toward a heterogeneous mechanical landscape in which each compositional feature specifies distinct physical behaviors.

Histone variants illustrate how compositional changes can substantially affect nucleosome mechanics. One example is H2A.Z, which is enriched at active promoters and regulatory elements. Unzipping measurements showed that H2A.Z-containing nucleosomes unwrap more readily than canonical particles [17] and exhibit increased spontaneous mobility, enhancing exposure of transcription-factor binding sites [18]. High-resolution mapping further refined the identity and position of variant-dependent mechanical contacts [19]. Germline-specific chromatin provides another example in which histone variants tune nucleosome mechanics toward structural plasticity. Optical-tweezers studies of nucleosomes containing the testis-specific variants H2B.W1 and H2B.W2 revealed weakened histone–DNA contacts, slower rewrapping kinetics, and reduced higher-order compaction, features thought to support the extensive chromatin remodeling required during spermatogenesis [20,21]. By contrast, variants such as CENP-A and macroH2A1.2 show little intrinsic mechanical destabilization, indicating that their regulatory roles arise primarily through context-dependent interactions [22,23].

PTMs modulate nucleosome mechanics through region-specific mechanisms. Early optical-tweezers experiments showed that selective tail clipping and acetylation globally weaken histone–DNA contacts [24]. Later studies using chemically defined histones showed that tail acetylation preferentially promotes entry–exit unwrapping, whereas histone-fold modifications destabilize inner DNA turns [25]. More recent work on defined trinucleosome arrays showed that H3K36me3 and multisite H4 acetylation both promote open conformations, but through distinct mechanisms. H3K36me3 loosens outer DNA turns, while H4 acetylation reduces inter-nucleosome stacking interactions [26]. Ubiquitination adds a further layer of mechanical regulation. H2A monoubiquitination stabilizes nucleosomes and introduces pronounced asymmetry into the unwrapping pathway [27], and both H2A and H2B ubiquitination reprogram the nucleosome barrier for transcription by locally modifying histone–DNA contact stability [19].

DNA sequence has long been known to influence nucleosome stability and dynamics through encoded DNA mechanical properties [28,29], and the first force-based characterization of nucleosome mechanical stability came from single-molecule pulling experiments [6]. More recent force-spectroscopy studies using native genomic DNA further showed that sequence context modulates not only nucleosome mechanical stability but also nucleosome mobility [18]. Local perturbations reinforce this view: single-base mismatches increase local DNA flexibility and enhance nucleosome mechanical stability under force [30], demonstrating that the mechanical role of DNA is highly context dependent.

Regulatory proteins as mechanical agents

The mechanical properties encoded in chromatin shape how regulatory proteins engage their substrates. TFs and chromatin-binding proteins therefore operate within a mechanically heterogeneous landscape. Single-molecule force spectroscopy has revealed that these proteins are not passive readers of DNA sequence but actively probe, deform, and in some cases reshape this mechanical landscape.

Unzipping experiments on the zinc-finger TF Egr-1 bound to bare DNA showed that bases flanking the canonical motif alter binding forces and affinity, demonstrating that the genomic environment shapes the energetic landscape of recognition [31]. Epigenetic modifications add a further layer of regulation: CpG methylation alters dissociation kinetics even when positioned outside the core motif [32]. Beyond sensing local DNA mechanics, TFs can also reshape nucleosome behavior. Optical-tweezers studies tracking nucleosome motion during Egr-1 binding showed that H2A.Z increases the frequency with which internal Egr-1 sites become transiently exposed, enhancing binding probability, while Egr-1 binding in turn biases nucleosome mobility [18]. Mechanical interplay becomes even more pronounced in the context of pioneer TFs. Sox2–DNA co-condensates generate several piconewtons of tension on naked DNA, yet nucleosomes dissipate this stress and mechanically sequester Sox2, limiting its ability to restructure wrapped DNA [33]. In contrast, Klf4 undergoes a sequence-dependent surface-condensation transition that reorganizes the local chromatin environment without generating substantial forces [34]. Together, these examples show how TFs can locally modify chromatin microenvironments.

Chromatin-binding cofactors also display pronounced mechanosensitivity, exemplified by FACT. In force-manipulation assays of nucleosomes, FACT can destabilize histone–DNA contacts, yet in partially unwrapped states it stabilizes nucleosome integrity in a pathway-dependent manner [35]. FACT selectively disassembles macroH2A1.2 nucleosomes and accelerates transcription initiation [22], indicating that variant-dependent nucleosome stability shapes FACT activity. Complementary single-molecule force studies further showed that human FACT subunits act cooperatively to catalyze both nucleosome disassembly and reassembly, stabilizing partially disrupted intermediates [36].

Architectural chromatin-binding proteins provide additional examples of regulatory factors that modulate

chromatin mechanics through diverse, non-canonical binding modes. MeCP2 displays distinct one-dimensional diffusion kinetics on unmethylated vs. methylated DNA and preferentially localizes to nucleosomes, where it stabilizes them [37]. High-mobility group B proteins such as Nhp6A and Hmo1 similarly bind preferentially to nucleosomes, destabilizing specific histone–DNA contacts and facilitating partial unwrapping, with Hmo1 even perturbing inner-turn interactions [38]. Recent optical-tweezers measurements showed that CTCF rapidly samples multiple zinc-finger conformations when bound to DNA to modulate cohesin barrier function and loop-extrusion boundaries [39].

Beyond static binding interactions, chromatin's mechanical landscape also serves as a substrate for target search. Ultrafast force-clamp tweezers showed that LacI alternates between sliding and tightly bound states as it navigates naked DNA, with sequence-dependent barriers modulating speed and dwell times [40]. Recent experiments extended these ideas to chromatinized templates, showing that the GAGA zinc-finger TF employs a 1D–3D facilitated diffusion strategy to efficiently locate its targets [41]. Combined optical tweezers and fluorescence measurements further revealed that the linker histone H1.0 undergoes force-dependent one-dimensional diffusion on DNA and loads onto nucleosomes through multiple pathways regulated by histone chaperones [42]. A further layer of regulation arises from intrinsically disordered regions. In the yeast TF Msn2, these regions promote nonspecific interactions and one-dimensional scanning of naked DNA, enhancing both the speed and specificity of promoter search [43].

Together, these studies show that transcription emerges from reciprocal mechanical interactions between regulatory proteins and chromatin. TFs, architectural proteins, and chromatin-associated cofactors perturb nucleosome wrapping, mobility, and condensation, while the mechanical properties of DNA and chromatin govern protein access and residence times, shaping regulatory outcomes such as motif exposure, boundary formation, and target-search efficiency.

Higher-order chromatin and chromosome mechanics

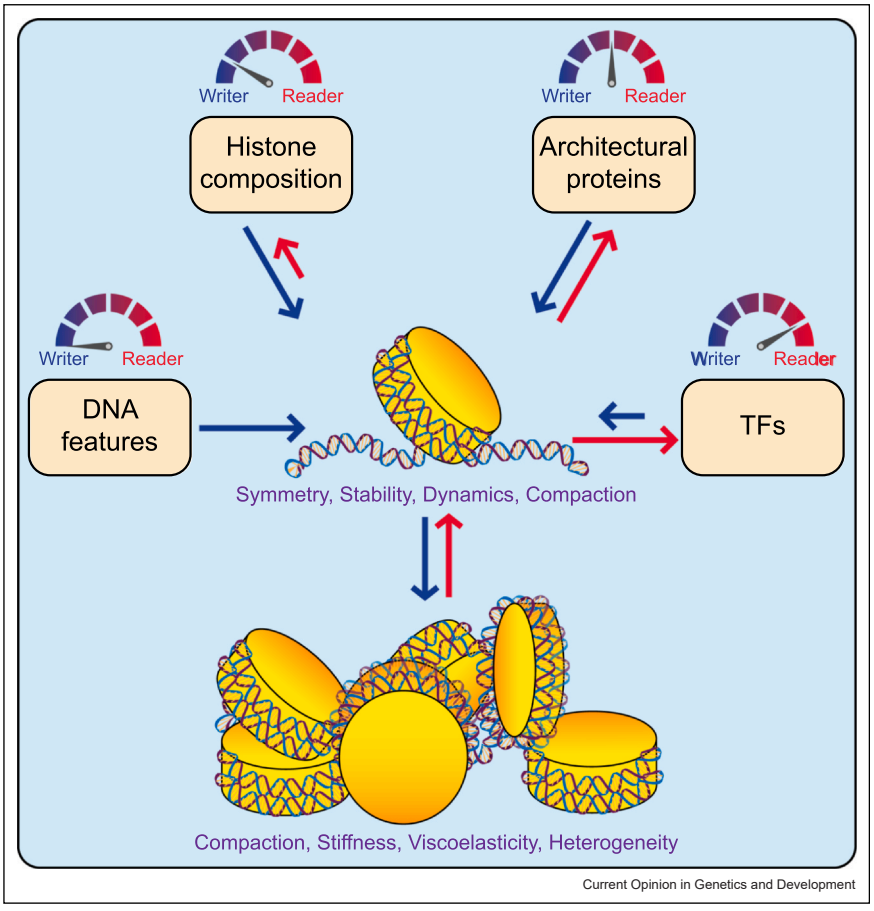
The mechanical behavior of chromatin emerges hierarchically, beginning with nucleosome-level mechanics and scaling upward into chromatosomes, folded arrays, specialized chromatin domains, and finally entire chromosomes. Single-molecule studies have revealed that each organizational tier introduces new mechanical constraints while inheriting features from the level below. These observations are consistent with polymer-physics and multiscale modeling descriptions of chromatin in which local nucleosome mechanics propagate into mesoscale fiber and nuclear properties, reflecting how chromatin's material behavior contributes to nuclear shape and function [44–46].

The first step toward higher-order structure occurs with the addition of the linker histone H1, which forms the chromatosome by bridging the DNA entry–exit region. Early magnetic-tweezers experiments showed that H1 can compact naked DNA under sub-piconewton forces and accelerate nucleosome assembly in extract-based systems, suggesting that it stabilizes nucleosome geometry and biases wrapping equilibria [47]. High-resolution unzipping later revealed that H1 tunes chromatosome compaction, making extended, dynamic and tunable contacts with DNA [48]. Beyond individual chromatosomes, H1 also contributes to mesoscale condensation. It binds single-stranded nucleic acids and undergoes liquid–liquid phase separation into droplets with tunable viscoelastic properties that stiffen or relax under force [49]. Complementary measurements using a newly developed DNA origami nanocaliper approach reconstructed the compaction free-energy landscape of tetranucleosomes and showed that H1 biases a broad, magnesium-stabilized ensemble toward more compact states [50].

Single-molecule approaches have now been extended to specialized chromatin architectures. Force-spectroscopy measurements of telomeric nucleosome arrays revealed reduced fiber stiffness and lower unstacking free energy compared with canonical arrays, consistent with weaker nucleosome stacking and histone–DNA interactions [51]. These results demonstrate that specialized sequence contexts can support compact chromatin organization while remaining mechanically compliant under load. Similarly, archaeal hypernucleosomes display Mg^{2+}-dependent compaction and mechanical stability, underscoring the general role of ionic context in chromatin-like fiber mechanics [52].

At the largest scale, single-molecule manipulation of intact chromosomes has revealed that mitotic chromosomes behave as nonlinear viscoelastic materials. Stretching of human metaphase chromosomes uncovered force-induced softening transitions attributed to rupture of condensin-mediated crosslinks, demonstrating that chromosome mechanics emerge from long-range crosslinking, chromatin tension, and topological constraints [53]. Subsequent work has resolved how distinct structural layers contribute to chromosome stiffness and heterogeneity. The chromosome periphery functions as a mechanically active shell modulating global elastic response [54], while ionic conditions tune chromosome condensation and stiffness by modulating chromatin polymer behavior and loop entanglement [55]. Atomic Force Microscopy-based force spectroscopy and microrheology revealed spatial heterogeneity within individual chromosomes, resolving distinct internal and

Figure 2



Chromatin as a mechanical integration hub. DNA features, histone composition, transcription factors, and architectural proteins act as mechanical writers, readers, or both, depending on context. The nucleosome integrates these inputs into force-dependent mechanical states that propagate to higher-order chromatin organization, ultimately influencing gene regulatory outcomes.

interfacial mechanical responses [56]. Extending force-based approaches to the nuclear scale, measurements on isolated nuclei demonstrated heterogeneous chromatin mechanics across nuclear regions, linking chromosome material properties to nuclear organization [57].

Together, these studies show that chromatin's mechanical grammar persists across orders of magnitude in length. Principles established at the nucleosome scale propagate into the stiffness of folded fibers, the accessibility of specialized domains, and the heterogeneous, environment-sensitive rheology of entire chromosomes. Higher-order chromatin thus acts as a multiscale mechanical continuum, translating forces applied at one genomic locus into structural responses that shape genome accessibility.

Conclusions and outlook

A major trend in recent single-molecule force spectroscopy work is the move toward studying chromatin in increasingly realistic mechanical contexts. Instead of focusing exclusively on canonical nucleosomes assembled on high-affinity positioning sequences, these studies now probe nucleosomes formed on native genomic DNA and incorporate histone variants, PTMs, and defined chromatin architectures. These approaches further extend to chromatosomes, folded fibers, specialized domains, and even intact chromosomes, revealing how local nucleosome mechanics scale into emergent material properties at higher levels of organization. Together, these advances establish how chromatin composition and DNA properties dictate the mechanical properties of chromatin, which in turn influence downstream processes, motivating the concept of a 'mechanical code'. Importantly, while we use this term as an organizing framework, we do not imply a deterministic or one-to-one mapping, but rather a probabilistic model in which mechanical properties bias regulatory outcomes in a context-dependent manner.

By analogy to classical epigenetic regulation, where post-translational modifications and DNA methylation act as

molecular 'marks' written by specific enzymes and interpreted by reader proteins, we consider chromatin mechanics itself as the functional mark. In this view, features such as histone variants, post-translational modifications, and DNA sequence act as 'writers' in a functional sense, as they establish the mechanical properties (such as stability, flexibility, and accessibility) that influence downstream regulatory processes.

Complementing this mechanical encoding, regulatory proteins emerge as mechanosensitive 'readers' of this code. Single-molecule measurements show that TFs, architectural proteins, and chromatin-binding cofactors adjust their binding modes, search strategies, and residence times in response to DNA flexibility, nucleosome dynamics, and higher-order chromatin organization. Importantly, many of these proteins also actively reshape chromatin mechanics by stabilizing alternative states, inducing partial unwrapping, or reorganizing local structure, thereby feeding back into and rewriting the mechanical landscape they sense. In this view, mechanical writing and reading represent context-dependent functional roles along a continuum rather than fixed molecular identities (Figure 2).

Together, these insights support a view in which chromatin functions as an active mechanical medium rather than a passive scaffold. Histone composition, DNA sequence, and chromatin architecture collectively define mechanical states that govern factor engagement, positioning the nucleosome as an integration hub where biochemical and mechanical signals converge to regulate transcription. These mechanically encoded states propagate to higher-order chromatin structures, linking local molecular interactions to global genome organization. Understanding gene regulation, therefore, requires not only identifying molecular regulators, but also characterizing how their interactions are mediated by, and feed back into, the mechanical properties of chromatin itself. Single-molecule force spectroscopy is central to this effort.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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