

DNA as a Model in Polymer Mechanochemistry

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Date

Signature

*You can fail at what you don't want,
so you might as well take a chance on doing what you love.*
—Jim Carrey

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Summary

Mechanical forces shape the behavior of materials across all scales, from geological processes to molecular reactions. In polymers, such forces can activate specific chemical bonds, which forms the principle of the field of polymer mechanochemistry. This discipline seeks to harness mechanical energy to drive selective reactions along polymer backbones. Yet, despite major progress, the field remains limited by the synthetic complexity of mechanophores and by analytical tools that cannot resolve bond scission with molecular precision.

DNA provides an exceptional alternative to conventional polymers. Its monodispersity, sequence control, and structural programmability make it a uniquely tunable material for studying force–reactivity relationships. Furthermore, next-generation sequencing (NGS) enables single-nucleotide resolution of fragmentation events, which transforms DNA into a quantitative probe for mechanochemical reactions.

This thesis investigates where mechanochemical activation occurs within macromolecules and how specific structural features influence the localization of force-induced reactivity. Here we show that DNA can be engineered as a programmable mechanochemical platform in which structural motifs, such as nicks (single-strand breaks in the backbone) and hairpins, act as intrinsic mechanophore analogs that focus and direct mechanical stress. Under ultrasound and freeze–thaw activation, these motifs guide bond rupture to predetermined sites, as verified by NGS and supported by molecular dynamics simulations.

Our findings reveal that DNA’s stiff, highly programmable structure can be exploited to tune the precision and spatial localization of mechanochemical scission far beyond what is achievable with synthetic polymers. A single nick provides a weak point for site-selective activation, while hairpins enable enhanced control through geometric force focusing. Extending the concept to freezing-induced stress uncovers a new mechanochemical regime relevant to cryopreservation, where ice formation exerts sufficient tension to cleave DNA covalent bonds.

Together, these results establish DNA as a model system for decoding how molecular structure governs mechanical reactivity, laying the foundation for the rational design of responsive materials and molecular devices that translate mechanical energy into precise chemical transformations.

Zusammenfassung

Mechanische Kräfte bestimmen das Verhalten von Materialien über alle Größenskalen, von geologischen Prozessen bis hin zu molekularen Reaktionen. In Polymeren können solche Kräfte gezielt chemische Bindungen aktivieren, was die Grundlage des Forschungsfeldes der Polymermechanochemie bildet. Dieses Gebiet untersucht, wie mechanische Energie genutzt werden kann, um selektive Reaktionen entlang des Polymerrückgrats auszulösen. Trotz großer Fortschritte wird das Feld jedoch weiterhin durch die aufwendige Synthese kovalenter Mechanophore und durch begrenzte analytische Auflösung bei der Identifizierung einzelner Bruchstellen eingeschränkt.

DNA stellt eine außergewöhnliche Alternative zu konventionellen Polymeren dar. Aufgrund ihrer Monodispersität, ihrer präzisen Sequenzkontrolle und ihrer strukturellen Programmierbarkeit eignet sie sich ideal zur Untersuchung von Kraft-Reaktivitäts-Beziehungen. Darüber hinaus ermöglicht Next-Generation-Sequencing (NGS) die Analyse von Fragmentierungen mit Auflösung einzelner Nukleotide, was DNA zu einer quantitativen Sonde für mechanochemische Prozesse macht.

Diese Arbeit untersucht, wo innerhalb von Makromolekülen mechanochemische Aktivierung auftritt und wie strukturelle Merkmale die Lokalisierung kraftinduzierter Reaktivität beeinflussen. Insbesondere wird erforscht, wie sich dieser Prozess gezielt durch molekulare Architektur steuern lässt.

Hier wird gezeigt, dass DNA als programmierbare mechanochemische Plattform dienen kann, in der strukturelle Motive, wie Einzelstrangunterbrechungen (Nicks) oder Haarnadelstrukturen, als intrinsische Mechanophor-Analoga wirken, die mechanische Spannung gezielt lenken. Unter Ultraschall und Gefrierzyklen führen diese Strukturen zu Bindungsbrüchen an definierten Positionen, was durch NGS-Analysen und molekulardynamische Simulationen bestätigt wurde.

Die Ergebnisse zeigen, dass die steife, hochprogrammierbare Struktur der DNA genutzt werden kann, um die Präzision und Lokalisierung mechanochemischer Spaltungen weit über das hinaus zu steuern, was mit synthetischen Polymeren möglich ist. Ein einzelner Nick fungiert als Schwachpunkt für regiospezifische Aktivierung, während Haarnadelstrukturen eine noch höhere Kontrolle durch geometrische Kraftfokussierung ermöglichen. Die Übertragung des Konzepts auf das Einfrieren von Wasser offenbart zudem ein neues mechanochemisches Regime, das für die Kryokonservierung relevant ist, da hier Eiskristallbildung ausreichende Spannungen erzeugt, um kovalente Bindungen zu brechen.

Insgesamt etabliert diese Arbeit DNA als Modellsystem zur Entschlüsselung des Zusammenhangs zwischen molekularer Struktur und mechanischer Reaktivität und legt damit die Grundlage für das rationale Design responsiver Materialien und molekularer Systeme, die mechanische Energie in präzise chemische Reaktionen umwandeln können.

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Aim and motivation

Polymer mechanochemistry explores how externally applied forces can be transduced into selective chemical transformations along a polymer backbone. The field opened up applications ranging from optical stress sensing, on-demand catalysis to stimuli-immolative materials. Yet, despite impressive progress, the domain encounters three persistent bottlenecks: (i) the reliance on covalently embedded mechanophores with demanding synthetic efforts and sometimes ambiguous activation pathways; (ii) limited spatial resolution in locating where bonds actually break along the backbone; and (iii) incomplete mechanistic understanding of how force is focused by polymer architecture and environmental conditions. A modular platform that decouples synthetic complexity from analytical power, while enabling precise, quantitative mapping of scission, would address these limitations and sharpen mechanochemical design rules.

DNA could offer a distinctive, elegant answer. As a perfectly sequence-defined, fully monodisperse polymer with intrinsically programmable secondary structure, DNA can be produced with exact length and composition. Further it can be analyzed with molecular tools unparalleled in synthetic polymer science. In particular, DNA can be interrogated with next-generation sequencing (NGS) which enables the conversion of *post-mortem* fragments into monomer-resolved "maps" of scission positions.

In this thesis, I aim to establish and exploit DNA as a sequence-defined, analyzable, and architecturally programmable platform for polymer mechanochemistry.

Concretely, the goals are to:

1. demonstrate mechanophore-free, near-nucleotide precision scission in nicked double-stranded DNA under ultrasound, and quantify scission distributions by NGS while rationalizing force focusing by MD;
2. introduce DNA secondary structures as 'biomechanophores' that guide and sharpen site-specific scission, mapping how loop size, stem length, and complementary mismatches shape the mechanochemical outcome; and
3. extend the platform to cryomechanochemistry by probing freeze-thaw-induced scission in nicked and non-nicked DNA across different contour lengths and architectures in order to elucidate kinetics, length scaling, and interfacial effects.

Methodologically, replacing covalent mechanophores with DNA features (nicks, hairpins) removes synthetic barriers and allows high-throughput, quantitative readouts by NGS with single-monomer resolution. Scientifically, coupling experiments to all-atom MD links observed scission distributions to local force distributions, base-pair stability, and fraying dynamics. This type of mechanistic information is difficult to access in conventional polymers.

Practically, the insights enable rational placement of "weak links" in DNA-based materials (e.g., nanostructures and devices), and suggest general strategies to focus mechanical stress in soft matter without bespoke mechanophore chemistry. The freeze–thaw results speak to sample handling, storage, and formulation in biotechnology, while also offering a gentle mechanochemical stimulus for short-contour-length systems that are typically inactive under ultrasound (US).

In **Chapter 1**, the state of the art, foundational background, recent advances and current limitations in the relevant fields are concisely reviewed to contextualize the aims of this thesis.

In **Chapter 2**, we establish nicked double-stranded DNA (dsDNA) as a mechanophore-free model for selective scission under 20 kHz ultrasound and analyze products by NGS. Our results show a sharp, centered scission distribution opposite the nick with near-nucleotide precision, tunable by sequence context and chain length. Constant-force MD attributes the finite distribution width to force-induced fraying that exposes a short single-stranded DNA (ssDNA) domain under high local tension. Thereby, we are capable of explaining both the high precision and its modulation with GC content. The chapter also demonstrates strategic placement of multiple or off-center nicks to program hierarchical fragmentation, thus decoupling mid-chain bias from genuine force distributions and offering a blueprint for multi-site activation.

In **Chapter 3**, we introduce DNA hairpins as biomechanophores, *i.e.*, secondary-structure motifs that focus tensile force and guide site-specific scission without any chemical mechanophore. Using a ligation-based assembly to embed a central hairpin motif into ~750 bp dsDNA, we map how loop size, stem length, and unpaired nucleotides opposite the junction govern the position, precision, and apparent two-step character of scission. Agarose gels reveal narrowly defined fragment sizes indicative of localized cleavage, while MD shows elevated force on nucleotides opposite the hairpin junction. NGS resolves strand-specific distributions as sharp, well-defined patterns on the strand opposite the hairpin and a broader or bimodal profile on the complementary strand. Our results support a mechanistic picture in which the hairpin geometry defines the force distribution along the polymer and thus the regioselectivity of cleavage. Notably, the best hairpin designs improve precision beyond earlier nick-based systems, despite requiring two coordinated backbone scissions. All in all, this highlights the power of secondary-structure engineering for mechanochemical control. In **Chapter 4**, we extend the platform from ultrasound to cryomechanochemistry to interrogate nicked and non-nicked DNA across different contour lengths and architectures (10–30 bp hairpins; 400, 800, 1500 bp duplexes). After repeated freeze–thaw cycles, nicked DNA shows a similarly regioselective scission pattern as we observed under US. Non-nicked DNA shows more uniform scission lacking mid-chain bias. Kinetic analyses reveal a near-linear growth of conversion with the number of cycles and a positive correlation with molecular weight. The Young’s modulus of the substrate (glass vs. stainless steel) modulates scission, supporting a universal role of microcrack-induced stretching during ice formation. Remarkably, even short hairpins (*e.g.*, 20 bp) show substantial conversion with freezing force. This reveals a distinctly different confined activation mechanism under freeze–thaw-induced stress compared to shear-flow-induced polymer scission.

Chapter 1

Introduction

1.1 Polymer Mechanochemistry

Polymer mechanochemistry is the study of chemical transformations initiated directly by mechanical force transmitted through polymer chains. This sets it apart from reactions driven by traditional stimuli such as heat, light, or chemical reagents. In this context, mechanical force acts as a selective and directional input, traveling along the polymer backbone to activate incorporated mechanophores – molecular motifs specifically designed to undergo bond scission, rearrangement, or other chemical changes in response to mechanical stress.^[1]

This approach reframes polymers as dynamic systems where mechanical input can control chemical reactivity and functionality. Research in this area began with the observation of mechanical degradation, where the application of mechanical force led to chain scission. Pioneering work by Staudinger and Melville in the mid-20th century provided early evidence that mechanical force could induce polymer chain cleavage, a process initially regarded as purely destructive.^[2,3]

A paradigm shift occurred with the realization that force could be used constructively to trigger specific chemical transformations at defined locations along the polymer chain.

This concept led to the development of mechanophores, enabling site-specific and predictable reactivity under force (Fig. 1.1). Early mechanophores included benzocyclobutene^[4] and gem-dichlorocyclopropane,^[5] with the latter undergoing highly efficient ring opening under mechanical load. These examples revealed that mechanochemical reactivity depends strongly on the mechanophore's structure, placement within the chain, and its molecular environment. Collectively, they established core design principles for tailoring mechanochemical responses in polymers.

Building on these foundations, the field has branched into covalent and supramolecular (non-covalent) mechanochemistry. Covalent systems are often characterized by transformations that are treated as irreversible, such as bond cleavage or certain isomerizations, though

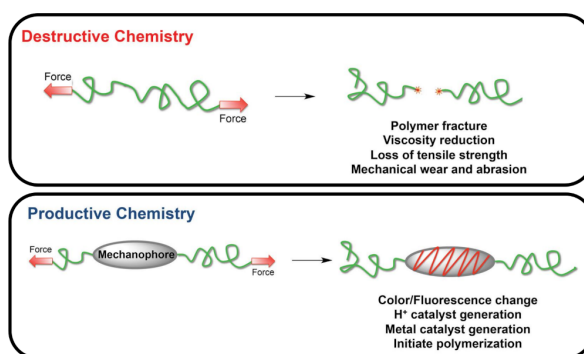


Figure 1.1: Transition of polymer mechanochemistry from destructive to productive (adapted with permission from Ref. [2], Copyright 2015, American Chemical Society).

some covalent mechanochemical reactions can in fact be reversible under appropriate conditions. Supramolecular systems, by contrast, predominantly feature reversible structural changes involving non-covalently bound units such as hydrogen bonds, host–guest complexes, or metal–ligand interactions.^[6,7] Although research has largely focused on covalent mechanisms, recent advances have begun to quantify non-covalent force responses with single-molecule precision.^[7]

Polymer mechanochemistry offers a unique perspective on how stress propagates from the macroscopic to molecular scale and therefore challenges traditional models of reaction kinetics. It also enables the creation of materials with advanced functions, including self-healing,^[8,9] stress sensing,^[10–13] force-activated catalysis,^[14–16] and mechanically triggered release.^[17–20]

1.1.1 Mechanistic Foundations and Experimental Milestones

To contextualize these advancements, it is necessary to examine the mechanistic principles that govern force-induced reactivity and the experimental milestones that established them. Mechanistic understanding was first formalized through the Bell model,^[21] which describes how an applied force lowers the activation energy barrier by an amount proportional to the product of force and the distance to the transition state. This yields a force-dependent rate constant:

$$k(F) = k_0 \exp\left(\frac{F\Delta x^\ddagger}{k_B T}\right) \quad (1.1)$$

where k_0 is the zero-force rate constant, $\Delta x^\ddagger$ the distance to the transition state, k_B the Boltzmann constant, and T the temperature. This framework was later extended in the Bell–Evans model, which introduced time-dependent force application and rupture statistics to enable quantitative interpretation of single-molecule force spectroscopy data.^[22]

At the molecular level, applied force first induces geometric deformations such as bond angle bending, torsion, and stretching. Once a certain threshold is reached, this leads to homolytic bond rupture (Fig. 1.2a). This process is commonly modeled using a Morse potential $V_0(r)$. When an external load is applied, it is replaced by the force-modified potential $V_F(r)$, which lowers the effective dissociation energy from D to D' (Fig. 1.2b).^[24]

Empirical validation of these models has been achieved *via* single-molecule force spectroscopy, which showed that force can accelerate or redirect reactivity and even suppress it under certain conditions ("catch bonds").^[25] Advanced models now account for barrier shapes (Dudko–Hummer–Szabo),^[26] multidimensional paths, and polymer-specific mechanics.^[27] Experimental studies using poly(methyl acrylate) chains demonstrated how substitution patterns, chain symmetry, and mechanophore position affect activation thresholds.^[28,29] For example, Moore and col-

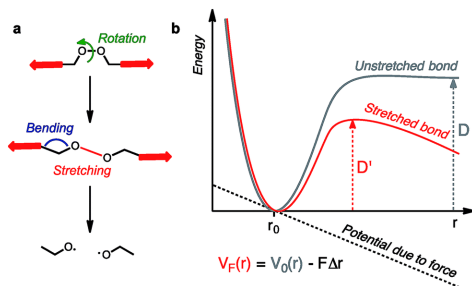


Figure 1.2: When subjected to mechanical stress, molecules may experience multiple structural distortions prior to the eventual breaking of their most vulnerable bond (a). The energy landscape associated with a bond under such tensile conditions can be effectively modeled using a Morse potential that incorporates the influence of the applied force (b) (reprinted from Ref. [23], CC BY-NC 3.0).

leagues employed controlled polymerization to precisely position mechanophores like benzocyclobutene to directly assess how chain architecture and molecular weight modulate reactivity.^[4] Regio- and stereoelectronic effects also influence reactivity. For instance, Diels–Alder adducts can be inert to force if the pulling direction does not align with the scissile bond.^[30]

With the development of computational tools, the predictive capabilities of the field have advanced majorly. This particularly aids in mechanophore design. Arguably the most popular tool is the CoGEF method (constrained geometries simulate external force),^[31] which estimates rupture forces *via* constrained quantum mechanical calculations. CoGEF has been validated across various covalent mechanophores.^[28,30,32,33] However, its accuracy decreases in complex networks or for non-covalent systems, where dynamic and entropic effects become significant.^[32,34]

Parallel and complementary to the advances in computational methods, experimental approaches have reached a high level of sophistication: Ultrasonication, an established technique to apply mechanical force to polymer chains in bulk solution, enables ensemble-level activation with real-time spectral monitoring, while single-molecule techniques provide precise, force-resolved data.^[35–37] These tools have transformed polymer mechanochemistry into a mechanism-guided platform for stress-responsive materials.

1.1.2 Conceptual Frameworks in Bulk Polymer Mechanochemistry

In bulk systems, mechanical activation of polymers occurs through macroscale deformation such as sonication, ball milling, extrusion, or shear flow rather than direct manipulation of single chains *via* atomic force microscopy (AFM). Force transmission in these environments depends on polymer chain extension under strongly non-equilibrium conditions, where collective effects and chain–chain interactions play decisive roles.^[6,38]

A central concept is the *lever-arm effect*, which describes how mechanophore positioning within the polymer backbone influences the magnitude of force transmitted to the reactive site. Mid-chain mechanophores typically experience higher loads than those placed near chain termini due to the way tension distributes along extended chains.^[2,38] In addition, chain architecture and properties such as contour length, persistence length, and entanglement density govern the efficiency of stress propagation. This determines whether mechanophores experience sufficient force to undergo activation.^[38–40] For example, entangled networks or cross-linked architectures may amplify local stresses, while shorter or more flexible chains dissipate energy more rapidly.

Theoretical frameworks for force-induced reactivity in bulk polymers often draw on extensions of the Bell–Evans model (*vide supra*).^[38] These models predict how applied force lowers the activation energy of chemical transformations and accelerate bond scission or mechanophore activation. However, persistent discrepancies between model predictions and experimental results highlight the complexity of bulk mechanochemical environments. For instance, while Bell–Evans-type models often assume uniform force application and simple exponential kinetics, real polymer systems exhibit heterogeneous stress distributions, rate-dependent viscoelastic responses, and multiple competing relaxation pathways.^[2,39] Such discrepancies are not merely quantitative deviations; they can qualitatively change the predicted mechanochemical outcome. For example, models may underestimate activation thresholds by orders of magnitude, misrepresent the role of entanglements, or fail to capture the contribution of energy dissipation mechanisms. This results in substantial challenges in

designing polymers with predictable mechanochemical behavior. Recognizing these limitations has motivated refinements in both theory and experiment, including multiscale modeling approaches that integrate molecular dynamics with continuum mechanics,^[2,39] and the use of more sophisticated force spectroscopy or *in situ* spectroscopic tools to probe mechanophore activation under realistic bulk conditions.^[6,38] Such efforts aim to bridge the gap between simplified conceptual models and the complex, non-equilibrium realities of bulk polymer mechanochemistry.

1.1.3 Applications in Bulk Systems

Having established the theoretical and experimental foundations of force-responsive polymers, these principles can now be seen translated into practical applications that exploit mechanochemical reactivity to impart new functionalities to bulk systems. Indeed, mechanochemically active polymers are essential components in a range of functional materials. For example, spiropyrans and related motifs are widely used in stress-sensing applications due to the reversible, colorimetric responses they exhibit under applied force.^[2,6,8,12,41] Another example are self-healing materials, which incorporate dynamic bonds such as Diels–Alder adducts that cleave and reform under stress, enabling autonomous repair.^[17,40] The community has also developed exciting strategies to self-strengthen polymers under load by force-induced crosslinking.^[8,9] Mechanochemical catalysis uses applied force to activate otherwise dormant catalysts within polymer matrices, which enables *in situ* reactions that occur only under mechanical load.^[14–16]

Important applications in drug delivery rely on force-triggered release systems that enable the on-demand liberation of small molecules.^[17,43] In this context, the Herrmann group has developed systems based on rolling-circle amplification (RCA) and rolling-circle transcription (RCT) that function as ultrasound-responsive cargo carriers. Importantly, these constructs form hybrid structures between the ultra-long nucleic acid strands and inorganic magnesium pyrophosphate (MgPPi),^[44] which contribute to their sonoresponsivity.^[42] Depending on the designed sequence of the resulting single-stranded DNA or RNA, periodically arranged binding domains for small molecules,^[18,45] proteins,^[46,47] or therapeutic oligonucleotides^[42] (Fig. 1.3) can be incorporated, thereby allowing these cargos to be non-covalently loaded and subsequently released upon stimulation.

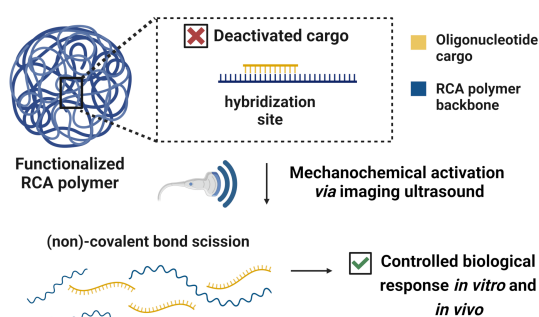


Figure 1.3: Schematic illustration of US-triggered mechanochemical release of oligonucleotides from RCA-based nucleic acid polymers, enabling controlled biological activity. (reprinted from Ref. [42], CC BY 4.0).

1.1.4 Limitations and Pitfalls

Despite the significant advances in sonication-induced mechanochemistry, several fundamental limitations constrain the efficiency, selectivity, and interpretability of mechanophore

activation in synthetic polymers. These challenges are rooted in both physical constraints of polymer structure and experimental limitations of the activation and analysis techniques.

Force Distribution and Mechanophore Placement. A central mechanistic limitation arises from the localization of tensile force at or near the polymer chain midpoint during sonication. Mechanophore activation is maximal in these regions, while end-located mechanophores typically experience insufficient force for efficient activation.^[43,48–51] Some strategies, such as block copolymer architectures, have enabled partial force transmission to near-terminal mechanophores when the inert block length is sufficiently long, though such effects are not broadly generalizable.^[51] Additionally, architectural features like branching or star-shaped topologies distribute force across multiple arms, which reduces localized tension on any individual chain segment.^[52]

Molecular Weight and Dispersity Effects. Sonication activation requires polymer chains to exceed a critical degree of polymerization (DP).^[35] Below this threshold, insufficient force is generated for mechanophore activation.^[28,35,48,49] Moreover, high dispersity ($\bar{D}$) limits activation to only the longest chains in the distribution, which reduces efficiency and reproducibility. Therefore, systems with low dispersity ($\bar{D} \leq 1.1$) are preferred to ensure consistent mechanochemical behavior.^[43,48,53]

Competing Non-Specific Chain Scission. Mechanophore activation must also compete to a certain extent with non-specific covalent bond rupture along the polymer backbone. Since offsite scission and targeted activation often occur under similar force magnitudes and timescales, selectivity remains a major concern.^[43,49,50] The relative propensity for scission is highly sensitive to polymer molecular weight, mechanophore placement, and chemical structure.^[49,50,54]

Analytical and Characterization Challenges. Standard analytical tools frequently lack the specificity needed to distinguish mechanophore activation from general chain cleavage. For example, gel permeation chromatography (GPC), whether refractive index or ultraviolet (UV)-based, cannot resolve mechanophore events without orthogonal signal contributions.^[43,50,53] Similarly, conventional spectroscopy methods often suffer from low mechanophore loadings and signal overlap with the polymer backbone, which necessitates calibration curves or kinetic approaches for reliable quantification.^[36,48,50,53] Furthermore, since bond scission and mechanophore cleavage often proceed at similar rates, extracting clean rate data is challenging without dual-probe or model-independent strategies.^[36,49,54,55]

Experimental Constraints and Physical Artifacts. A few practical issues further complicate experimental readouts interpretation. The duration of cavitation-induced force pulses is typically in the microsecond range, which may not match the assumptions of theoretical models and can misrepresent mechanochemical outcomes.^[34] Additional effects such as local heating, tip erosion, and spatial heterogeneity during sonication introduce uncontrolled force distributions, which may further complicate data interpretation.^[56] Finally, the inherent flexibility and short persistence lengths of many synthetic polymers limit the efficiency of force transduction to embedded mechanophores, though robust quantitative analysis of this effect remains underdeveloped.^[38]

1.2 Sequence-Defined and Sequence-Controlled Polymers

Beyond the design of mechanically responsive polymers, a growing area of interest in synthetic macromolecular chemistry centers on the *precise control of monomer sequences*. While polymer mechanochemistry emphasizes how stress modulates function through chain architecture and the placement of reactive motifs, it also highlights a broader trend: the desire to engineer polymers with increasingly fine-grained control over structure–function relationships. This motivation has naturally led to renewed focus on sequence-defined and sequence-controlled polymers, where not only the composition but the exact order of monomers is encoded with molecular-level precision. These systems aim to mimic the precise structure and complexity found in biological macromolecules to strive towards new possibilities in information storage, catalysis, and targeted interactions.

Over the past decade, this field has undergone significant conceptual and technical development, with explicit efforts to clarify terminologies, draw analogies to natural biopolymers such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and expand the synthetic methodologies available to researchers. A distinction exists between sequence-defined and sequence-controlled polymers: the former refers to systems in which every polymer chain carries an identical and precisely ordered monomer sequence, analogous to the templated biosynthesis of biomacromolecules. In contrast, sequence-controlled polymers encompass materials in which monomer placement is biased or directed to reduce sequence dispersity, yet without achieving single-molecule fidelity.^[57–59]

The desire to achieve sequence precision in synthetic polymer chemistry results from potential applications such as encoding programmable functions for example molecular recognition, selective self-assembly and data storage. All those applications critically depend on precise monomer order and minimal sequence dispersity. This is underscored by recent advances in therapeutic nucleic acids. One notable example is the development of messenger RNA (mRNA) vaccines for coronavirus disease 2019 (COVID-19), where clinical efficacy hinges on the exact nucleotide sequence, length, and structural features of the mRNA polymer.^[60] This case illustrates that monomer-level precision is not merely an academic ideal, but a prerequisite for advanced functional materials.

Mimicking the error-correcting and high-fidelity mechanisms of biological polymer synthesis has stimulated progress across various synthesis strategies. Initially, this involved adapting techniques from peptide and nucleic acid chemistry, particularly solid-phase iterative coupling protocols. These methods, such as phosphoramidite synthesis, enable precise monomer insertion and analytical verification of sequence identity. For example, allyl-acrylamide monomer systems and fluororous liquid-phase supports have enabled the synthesis of 10-mer and 16-mer sequence-isomers with confirmed structures *via* tandem mass spectrometry (MS).^[61] Likewise, alternating chemistries using orthogonal phosphoramidite and radical trapping reactions allow for the synthesis of binary-coded oligomers, which are readable through well-characterized fragmentation patterns.^[62]

Representative strategies for achieving sequence control through synthetic chemistry are illustrated in Fig. 1.4. Building on these foundations, more recent strategies have focused on scalability and automation. For instance, photochemical flow-based synthesis platforms utilize photo-caged diene and ene building blocks that undergo stepwise coupling in a continuous reactor, validated by in-line MS. These methods support the generation of well-defined decamer libraries with diverse sequences and architectures.^[63] Moreover, the

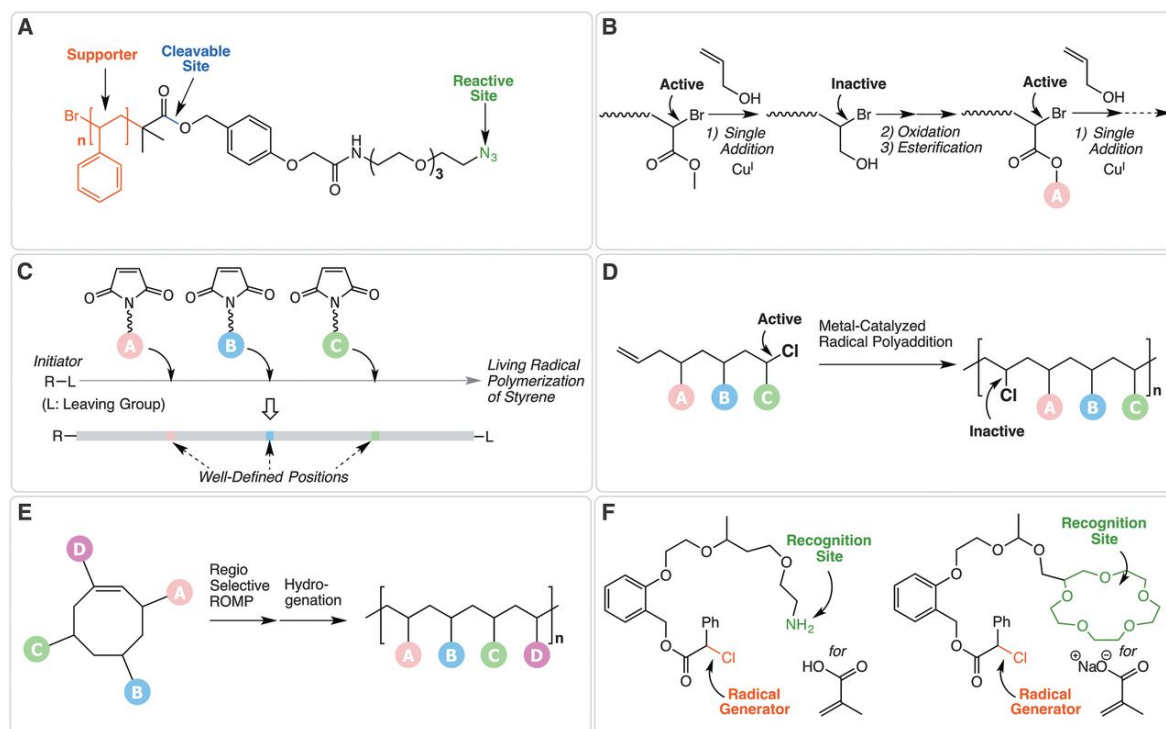


Figure 1.4: Synthetic strategies for achieving sequence control in polymers. (a) Use of a soluble polymer support for azide–alkyne click chemistry, (b) iterative addition of individual monomer units *via* allyl alcohol-mediated living radical polymerization, (c) control over monomer positioning during the polymerization of styrene, (d) metal-catalyzed radical polyaddition reactions; (e) regioselective ROMP using asymmetrically substituted cyclooctene monomers, (f) application of template-based initiators in living radical polymerization (reprinted with permission from Ref. [58], Copyright 2013, AAAS).

integration of photolithographic patterning and liquid-handling robotics has enabled parallelized construction of large oligomer libraries, particularly suited to high-throughput applications.^[57]

In parallel, efforts to integrate sequence control with chain-growth polymerization have made significant strides. For example, relay-mediated ring-opening metathesis polymerization (ROMP) utilizes macrocyclic enyne monomers and a relay mechanism to achieve monomer-by-monomer insertion while maintaining the living character of the polymerization. This approach combines the precision of stepwise synthesis with the scalability and control of chain-growth methods to increase the available toolbox of building blocks and applications.^[64]

Complementary to these chemical strategies, template-directed and enzyme-free assembly approaches offer additional means to achieve high sequence fidelity. Notably, DNA-templated translation systems have been developed where macrocyclic substrates equipped peptide nucleic acid adapters are hybridized in a sequence-defined order to guide the coupling of diverse, non-natural monomers including PEG and α - or β -peptides. These systems consistently yield well-defined polymers with high fidelity and conceptual ties to biological translation, yet without relying on enzymes or prebiotic assumptions.^[65]

Beyond synthetic technique, theoretical frameworks from information theory are also beginning to influence the field. Measures such as Shannon entropy and Kolmogorov complexity

are being explored to quantify sequence order and randomness, drawing direct parallels with the information density of biological polymers.^[57,59] Although still underutilized in experimental studies, these metrics are increasingly seen as essential for evaluating materials intended for data storage or algorithmic self-assembly.^[59,66] Likewise, kinetic and mechanistic modeling informs methods such as SUMI (single-unit monomer insertion) or relay-ROMP, though such models are typically formulated in conventional chemical terms rather than as abstract, sequence-driven systems.^[64,67]

Precise analytical methods are a consistent theme across all methodologies. MS, particularly tandem MS techniques such as matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-ToF/ToF) or electrospray ionization MS/MS (ESI-MS/MS), is the gold standard for verifying monomer sequence at the single-chain level. Complementary techniques such as nuclear magnetic resonance (NMR)—especially multidimensional and diffusion-ordered protocols—are routinely employed, particularly for shorter oligomers or to confirm functional groups and connectivity.^[57,61–63,67]

A key strength of the field lies in its diversity regarding different polymer classes. These methodologies have been successfully applied across a wide range of backbones, including phosphodiesteres, peptoids, olefinic macrocycles, and perfluorocarbon derivatives.^[57,64,65,68] Many studies emphasize the goal of mimicking natural macromolecules—not only in sequence precision but also in folding behavior, error correction, and functional complexity.^[57–59,65]

Despite substantial progress, several gaps remain. Information-theoretic and kinetic models are still infrequently applied in practical experiments. Moreover, while many synthetic demonstrations are technically rigorous, they do not always articulate broader conceptual links to biological systems or systems chemistry.^[62,63] Nonetheless, the convergence of conceptual clarity, synthetic breadth, and molecular-level validation has placed the field on a solid path toward increasingly sophisticated and functional sequence-defined materials. As synthetic approaches move closer to biological levels of sequence precision and functional complexity, it becomes increasingly valuable to examine DNA, which represents a natural, fully sequence-defined polymer whose structural and informational properties have inspired many of these efforts.

1.3 DNA Nanotechnology

DNA stands as one of the most thoroughly understood macromolecules, renowned for its dual role as the genetic material of all known living organisms and as an information-rich sequence-defined polymer. The canonical B-form DNA double helix was first described by Watson, Crick and Franklin in 1953^[69,70] and consists of two antiparallel strands winding around a common axis, stabilized by sequence-specific Watson–Crick–Franklin base pairing (A:T, G:C) and extensive base-stacking interactions (Fig. 1.5). This helix features precise physicochemical parameters: a diameter of approximately 2 nm, a helical pitch of 3.4 nm (10.5 base pairs per turn), and a persistence length near 50 nm.^[71] Those parameters inform both its biological function and later technological utilization.

Beyond genetic storage and transmission, DNA molecules serve diverse roles in cells, as they act as templates for RNA synthesis in transcription and participate actively in gene regulation, chromosomal organization, and epigenetic modifications. Consequently, disruptions in DNA structure or sequence can lead to various diseases, which range from hereditary disorders to

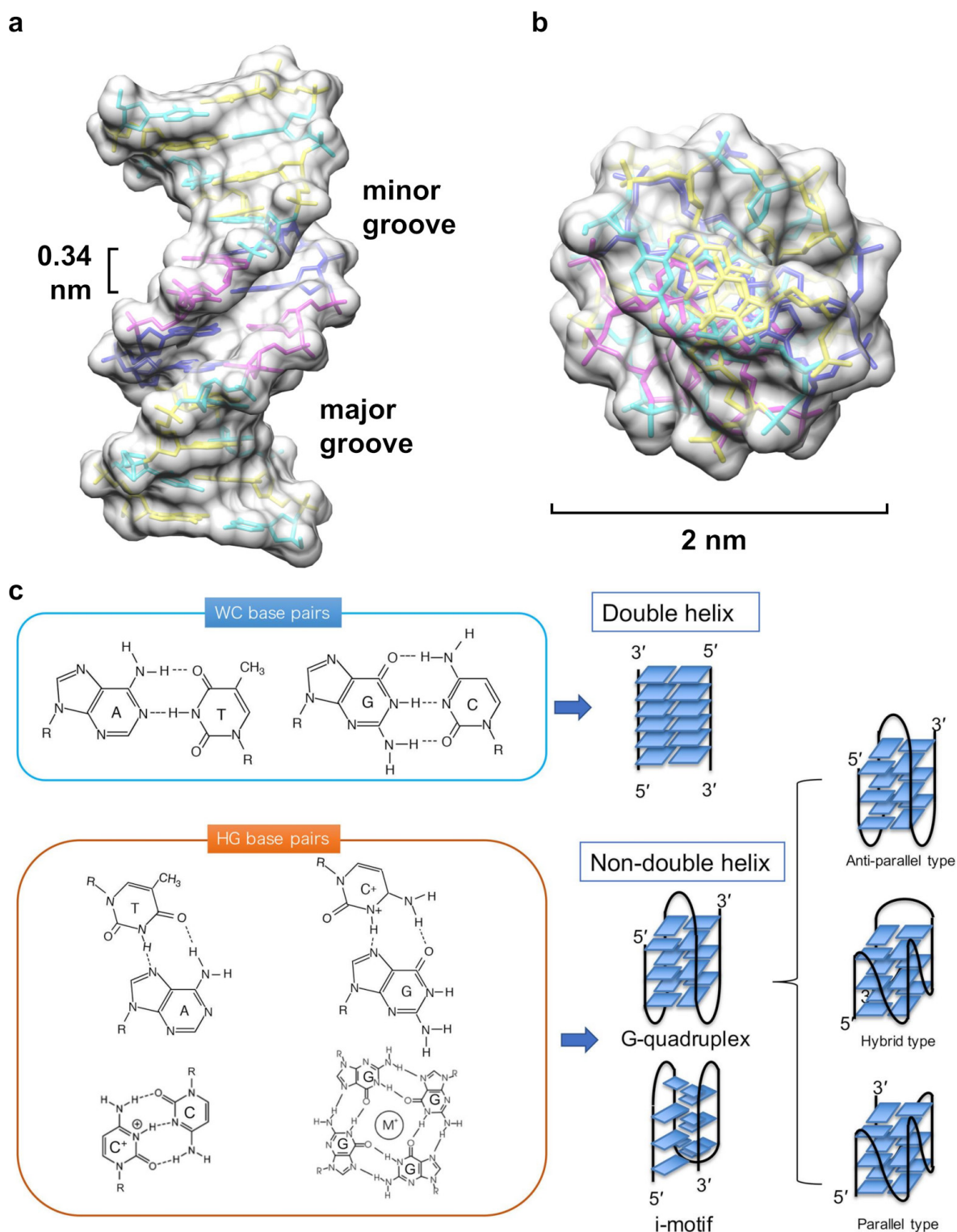


Figure 1.5: Structure and base pairings of deoxyribonucleic acid (DNA). (a) Structure of a B-form double-stranded DNA (dsDNA) dodecamer from the side and (b) from the top view. The PDB file (1BNA)^[72] was rendered with UCSF Chimera.^[73] (c) Watson–Crick–Franklin and Hoogsteen base pairing and the resulting secondary structures. Hoogsteen base pairs necessitate lower pH to protonate Cytosines or K^+ to induce G-quadruplex formation (reprinted with permission from Ref. [74], Copyright 2021, American Chemical Society).

cancers. The advent of molecular biology tools such as polymerase chain reaction (PCR),^[75] sequencing technologies,^[76] and gene-editing methods like clustered regularly interspaced short palindromic repeats with CRISPR-associated protein 9 (CRISPR-Cas9)^[77] has propelled the understanding and manipulation of DNA, which continues to transform medicine, agriculture, forensic science, and bioengineering. Hence, there is enormous potential for DNA beyond its natural biological roles through careful molecular design.

This potential has lead to the development of DNA nanotechnology, an interdisciplinary field exploiting the precise base-pairing rules of DNA to engineer nanoscale structures and devices. Conceived by Ned Seeman in the early 1980s, DNA nanotechnology employs DNA strands as building blocks for constructing intricate molecular architectures and functional devices, capitalizing on their predictable hybridization and self-assembly properties.

1.3.1 Discrete DNA Nanostructures

One of the earliest conceptualizations of discrete DNA nanostructures was proposed by Seeman in 1982, when he elucidated criteria necessary for stable nucleic acid junction formation.^[78] He emphasized the importance of unique and non-symmetric base sequences for creating immobile junctions, so-called Holliday junctions (Fig. 1.6) and preventing undesired linear duplex formation. By defining these junctions as macromolecular "valence clusters", Seeman demonstrated the potential for assembling intricate polyhedral structures and periodic lattices through specific sticky-ended ligation techniques. This laid the groundwork for subsequent developments in DNA-based structural assembly.

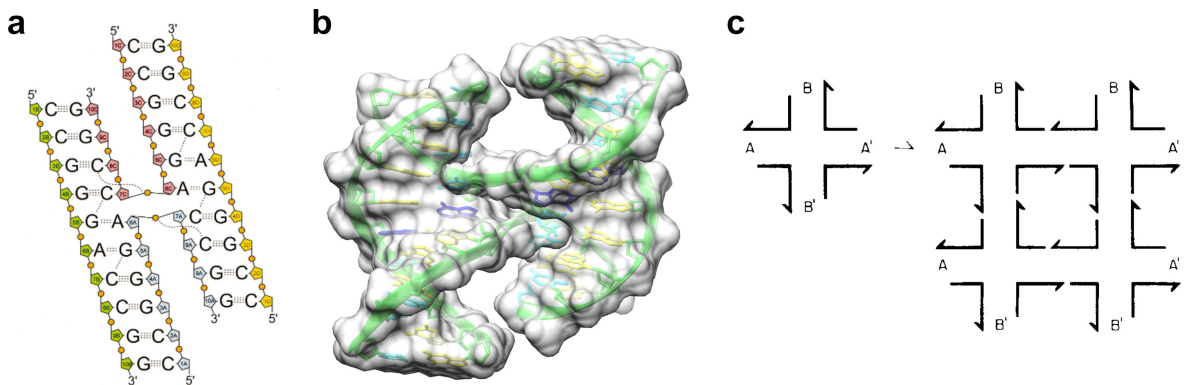


Figure 1.6: Structure and applications of the Holliday junction. (a) Schematic of the X-stacked Holliday junction with labeled strands and nucleotide positions. Nonexchanging strands are shown in green and yellow, exchanging strands in blue and red, with phosphate groups marked as orange circles (adapted with permission from Ref. [79], Copyright 1999, Nature America). (b) Crystal structure of the Holliday junction shown in (a). The PDB file (467D)^[79] was rendered with UCSF Chimera.^[73] (c) Ned Seeman's approach to form 2D lattices from immobile junctions with sticky ends. The sticky end A is complementary to A' while the same holds for B and B' (adapted with permission from Ref. [78], Copyright 1982, Elsevier).

A pioneering realization of discrete DNA nanostructures was the synthesis of a DNA cube by Junghuei Chen and Ned Seeman through carefully designed oligonucleotide strands.^[80] Each edge of the cube is composed of dsDNA, and vertices are defined by stable immobile branched junctions. The synthesis involves constructing two DNA squares initially, which

are subsequently ligated through single-stranded sticky ends. The completed structure contains unique restriction sites on each edge, allowing precise analytical verification of the cube's topology.

Expanding on these foundational principles, Goodman *et al.* in 2005 developed DNA tetrahedra that self-assemble rapidly with high efficiency into robust, rigid polyhedral structures.^[81] Each tetrahedron, formed from four oligonucleotides, assembles within seconds and achieves near-quantitative yield (Fig. 1.7). The stereochemical precision of these structures was experimentally verified using AFM and sequence-specific enzymatic digestions, confirming that the tetrahedra exhibit stable triangulated architectures with defined stereochemistry. Moreover, these DNA tetrahedra serve as versatile building blocks, capable of further integration into larger 3D assemblies through programmable DNA linkers. Less than four years later, employing cryogenic electron microscopy (cryo-EM), structural features at resolutions sufficient to distinguish the DNA double helix and major grooves were resolved for the DNA tetrahedron.^[82]

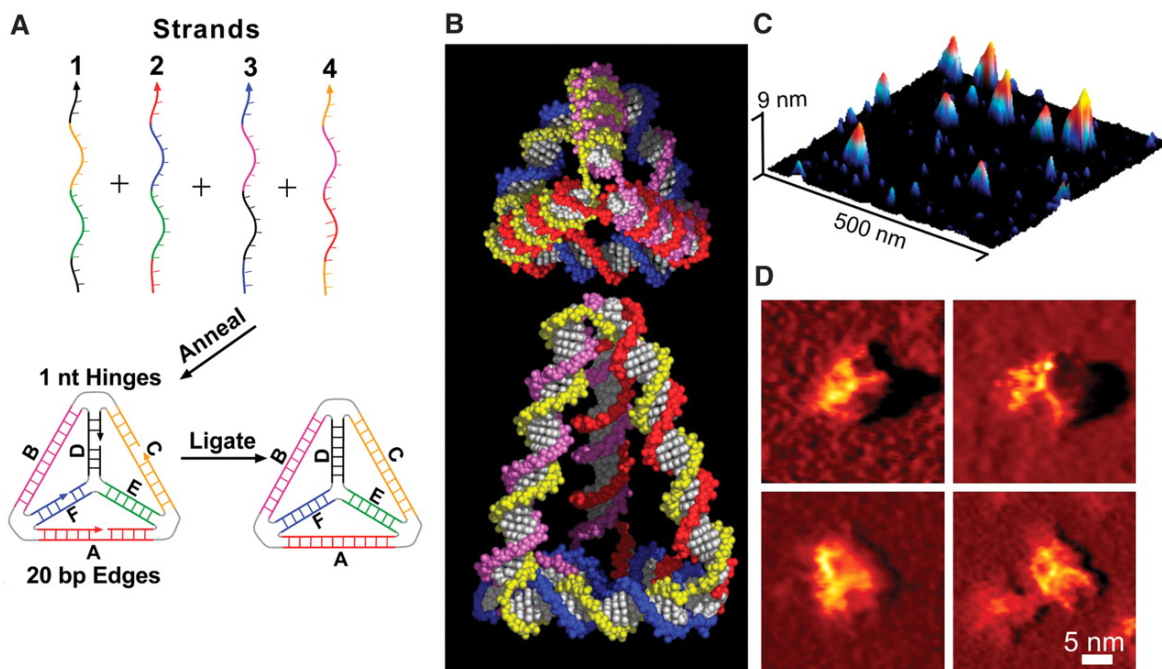


Figure 1.7: DNA tetrahedra. (a) Four oligonucleotides are annealed to form the tetrahedral structure. (b) Space-filling model with color-coded strands. (c, d) AFM images resolving the tetrahedra on a mica surface (reprinted with permission from Ref. [81], Copyright 2005, AAAS).

1.3.2 DNA Tiles, Lattices and Crystals

Transitioning from discrete structures to periodic assemblies, the double crossover (DX) DNA motif, introduced by Fu and Seeman in 1993,^[83] became a significant innovation. The DX structure provided a more robust and rigid framework compared to single crossover junctions, which paved the way for extended lattice formations. In 1998, Winfree and Seeman demonstrated the assembly of these DX tiles into 2D lattices,^[84] effectively translating discrete nano-structural motifs into periodic DNA-based crystalline arrays (Fig. 1.8).

Further advancements include the introduction of "tensegrity" triangles, rigid 3D DNA motifs developed as robust structural elements to assemble into defined 1D and 2D arrays.^[85] These triangular motifs maintain structural integrity through tension and compression forces within the DNA strands. This eventually led to the construction of the first self-assembled diffraction-grade 3D DNA crystals.^[86]

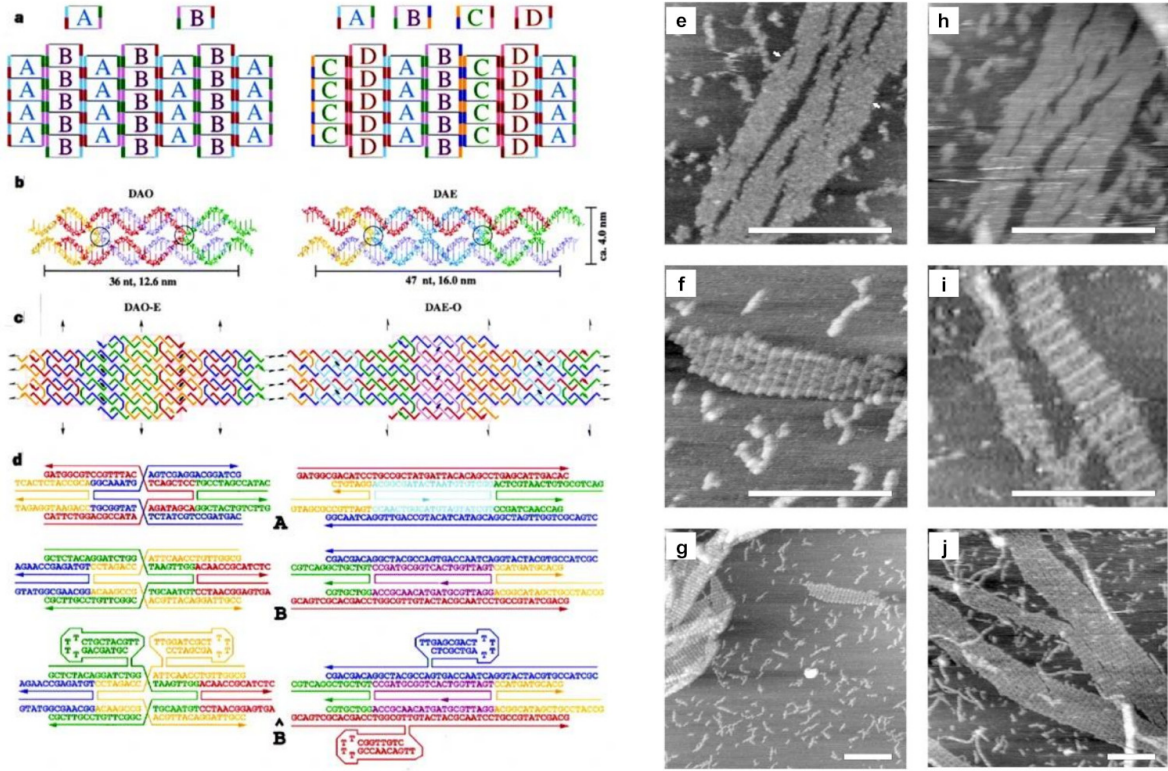


Figure 1.8: Design and imaging of DX-based 2D DNA lattices. (a) Tile schematics showing edge complementarity. (b) Model structures with crossover points. (c) Lattice topologies and symmetry elements. (d) Example oligonucleotide sequences. (e–j) AFM images of assembled lattices; all scale bars are 300 nm (adapted with permission from Ref. [84], Copyright 1998, Macmillan Magazines).

1.3.3 DNA Origami

A notable shift towards DNA origami was foreshadowed by foundational work from Yan *et al.* in 2003, who demonstrated the directed nucleation assembly of DNA tile complexes around scaffold strands to form barcode-patterned lattices.^[87] This was a key precursor to scaffold-based folding approaches. The crucial leap to folding long ssDNA scaffolds into defined nanoscale shapes was first demonstrated in 2004 by Shih and colleagues. They reported the assembly of a 1.7-kilobase ssDNA molecule into an octahedral nanostructure using strategically placed crossovers, providing a foundational proof of concept for scaffold-based folding approaches.^[88] These pioneering works set the stage for the development of DNA origami, enabling the programmable construction of intricate 3D nanostructures.

Paul Rothemund introduced scaffolded DNA origami in 2006 by showing that a single long ssDNA "scaffold"—typically the M13 bacteriophage genome—could be folded into

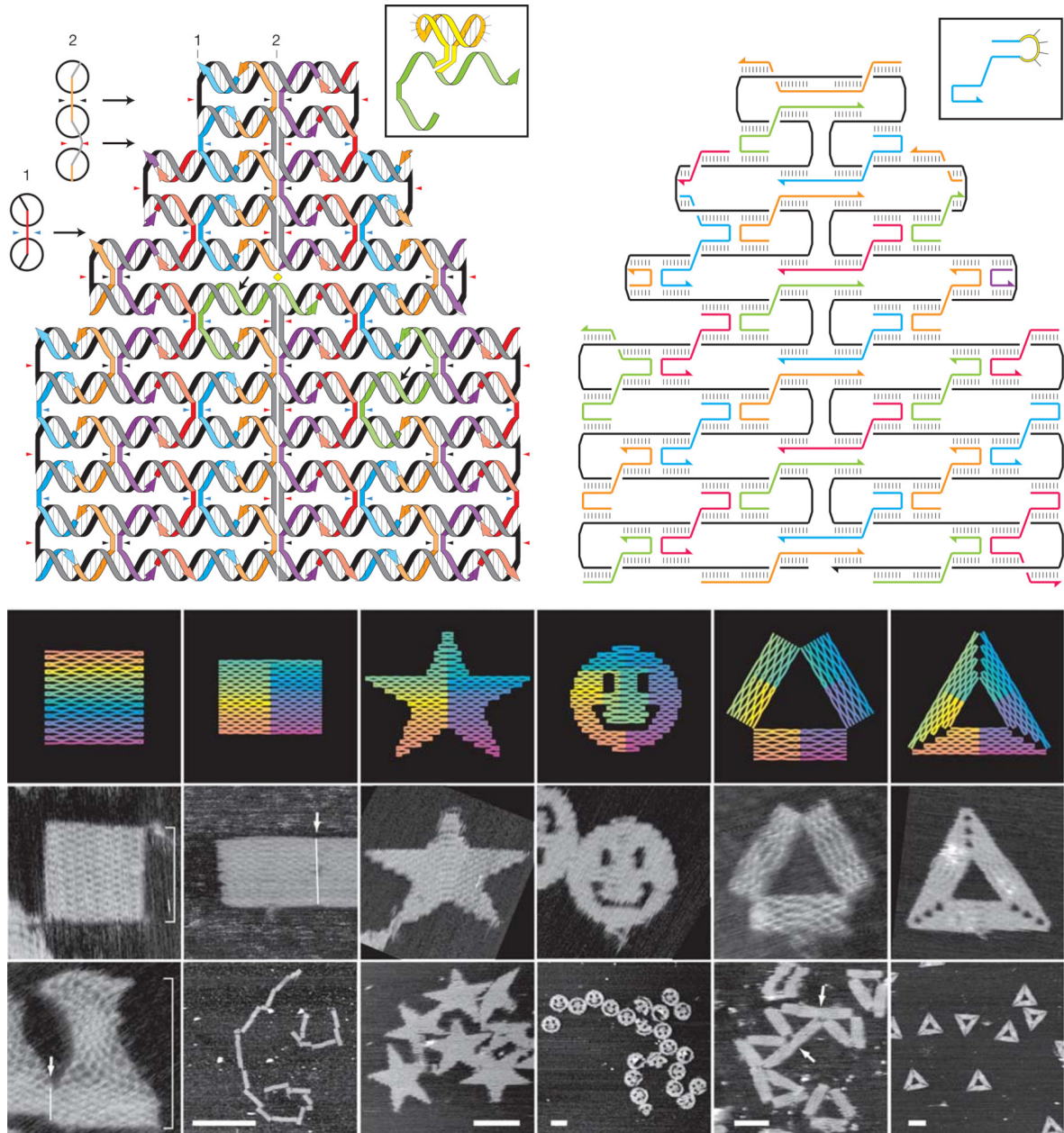


Figure 1.9: Original design and shapes of 2D DNA origami. (Top) DNA origami design showing staple crossover patterns and finished seam-bridging layout. (Bottom) AFM images of various DNA origami shapes; scale bars: 1 μm for rectangular DNA origami, 100 nm for all other shapes (adapted with permission from Ref. [89], Copyright 2006, Springer Nature).

arbitrary, user-defined 2D shapes with nanometer precision using hundreds of short, easily synthesized "staple" oligonucleotides (Fig. 1.9).^[89] This work combined rational sequence design, programmable crossover placement, and staple-directed folding into the first broadly accessible approach for construction of programmable nanoscale structures. Rothemund's protocol and design heuristics have formed the foundation for nearly every subsequent advance in the DNA origami field.

Building upon the 2D framework, Douglas *et al.* extended the scaffolded origami approach to 3D, demonstrating the self-assembly of multiple distinct nanoscale 3D shapes using a pleated honeycomb-lattice architecture.^[90] Here, a long scaffold strand was routed through a predefined path covering all target helices, stabilized by hundreds of staple strands and optimized *via* monovalent/divalent ion concentrations and elaborate thermal annealing protocols. The authors systematically assembled six rigid 3D monomers and further achieved the assembly of hierarchical multimers, such as linear tracks and wireframe icosahedra. This work represents a foundational breakthrough by enabling true control of 3D structure, architectural diversity, and hierarchical assembly in scaffolded DNA origami.

Advances in control over DNA origami's geometric and mechanical properties quickly followed. In 2009, Dietz, Douglas, and Shih reported methods for generating controlled twist and curvature in DNA origami bundles by introducing targeted base-pair insertions and deletions at specified positions along the scaffold and staples.^[91] Using programmable adjustment of crossover spacing and modular routing, they successfully engineered left- and right-handed twists and precisely bent structures down to radii of curvature as small as 6 nm. These advances allowed the construction of curved, twisted, and complex 3D origami, transcending the limitations of previous planar or linear assemblies. Notably, Douglas *et al.* also introduced an early software tool named "caDNAno" for graphical design of staple routing and helix arrangements, facilitating more sophisticated and reliable origami construction.^[92] This software is still heavily used these days and was also employed in Chapter 2 of this thesis.

The next generation of scaffolded origami research focused on architectural generalization, wireframe motifs, and computational automation. Benson *et al.* demonstrated the translation of arbitrary polyhedral meshes into DNA origami nanostructures, considerably expanding the design space to non-solid, wireframe architectures.^[93] This approach enabled the encoding of even more complex geometric information into DNA origami frameworks, opening doors for a broader range of topologies beyond merely pleated or layered solids. Simultaneously, the drive for programming increasingly complex structures motivated the development of automated computational pipelines and algorithmic design strategies. Veneziano *et al.* introduced top-down, graph-theoretic algorithms for designing wireframe DNA origami, leveraging tools such as minimum spanning trees and post-processing routines to optimize scaffold routing and crossover placement.^[94] The introduction of dual-duplex edge representations and fully automated sequence generation enabled the construction of arbitrary 2D wireframe objects, a capability further refined and empirically validated by subsequent studies employing honeycomb-edge designs and enhanced geometric analysis.^[95,96] Advances in computational methodology enabled fully autonomous scaffold routing in 2D free-form wireframe origami, as demonstrated by Jun and colleagues, who incorporated graph-based routing, optimized crossover periodicity, and algorithmic staple design into a comprehensive design platform.^[95] The development of computational approaches for 3D origami, specifically those supporting non-rasterized curvature and arbitrary surface topologies, emerged with tools such as DNAXiS,^[97] which facilitated mesh-to-scaffold mapping

and automated the placement of crossovers and staple nicks algorithmically. Recent progress has seen the integration of coarse-grained molecular dynamics modeling into origami design tools, as exemplified by MagicDNA 2.0. Pfeifer *et al.* presented a user-friendly, GUI-based CAD platform combining user-tailorable parameters with automated MD-informed routing, validating free-form 3D origami architectures experimentally and computationally.^[98] With these advances, the field has achieved rapid, reliable, and reproducible design and assembly of complex, nontrivial DNA origami shapes with minimal manual intervention.

In parallel with architectural and computational developments, foundational work addressed the scalability and practical deployment of DNA origami. The Dietz group demonstrated the gigadalton-scale assembly of programmable DNA constructs by hierarchically merging origami modules, achieving new thresholds in overall nanostructure size and complexity.^[99] Complementary progress was made in the preparation and supply of scaffolds, by establishing biotechnological methods for mass production of scaffold DNA, thereby overcoming a major bottleneck in experimental DNA origami research.^[100]

Finally, advances in scaffold composition further diversified the origami toolbox. Yang *et al.* showed that both strands of long dsDNA (such as Lambda DNA fragments) could serve as a unified scaffold for origami assembly, provided that care is taken to minimize unwanted complementarity and to control the annealing and crossover pattern to avoid spontaneous rehybridization.^[101] This approach enabled assembly of even larger origami constructs and brought new insight into the energetic and structural constraints governing scaffolded folding.

Taken together, these advances in nanoscale architectural control, automation, and scale-up form the technological backbone of scaffolded DNA origami. Continued synergy between experimental, theoretical, and software innovations has positioned the field as a leading platform for addressable nanoscale construction, programmable materials, and next-generation nanomanufacturing.

DNA Origami as a Delivery Platform

The trajectory from 2D to 3D DNA origami was not only driven by architectural ambition but also by application demand. Nowadays, 3D origami architectures became increasingly complex and are capable of dynamic motion, structural reconfiguration, and functional cargo delivery.^[102–107]

This led to key innovations include multi-layer folding, lock–key mechanisms, toehold-mediated strand displacement, and programmable hinges.^[102,108] Milestones such as the DNA “box” with a controllable lid,^[104] as well as frameworks using honeycomb lattices, wireframe bundles, and multi-scaffold assemblies, have broadened the design space for functional nanostructures.

DNA origami has proven particularly useful as a *programmable platform for molecular cargo delivery*. Enzyme encapsulation, oligonucleotide loading, and small-molecule containment have been achieved using mechanically gated structures such as nanovaults and cages.^[102,108,109] Triggers for release include toehold strand displacement, pH, enzymatic degradation, miRNA recognition, and strand-specific unlocking.^[102,105,106,110,111] These strategies have enabled delivery of small interfering RNA (siRNA), antisense oligonucleotides, and chemotherapeutics like doxorubicin, with demonstrated responsiveness to biologically relevant cues^[105–107,112] (Fig. 1.10).

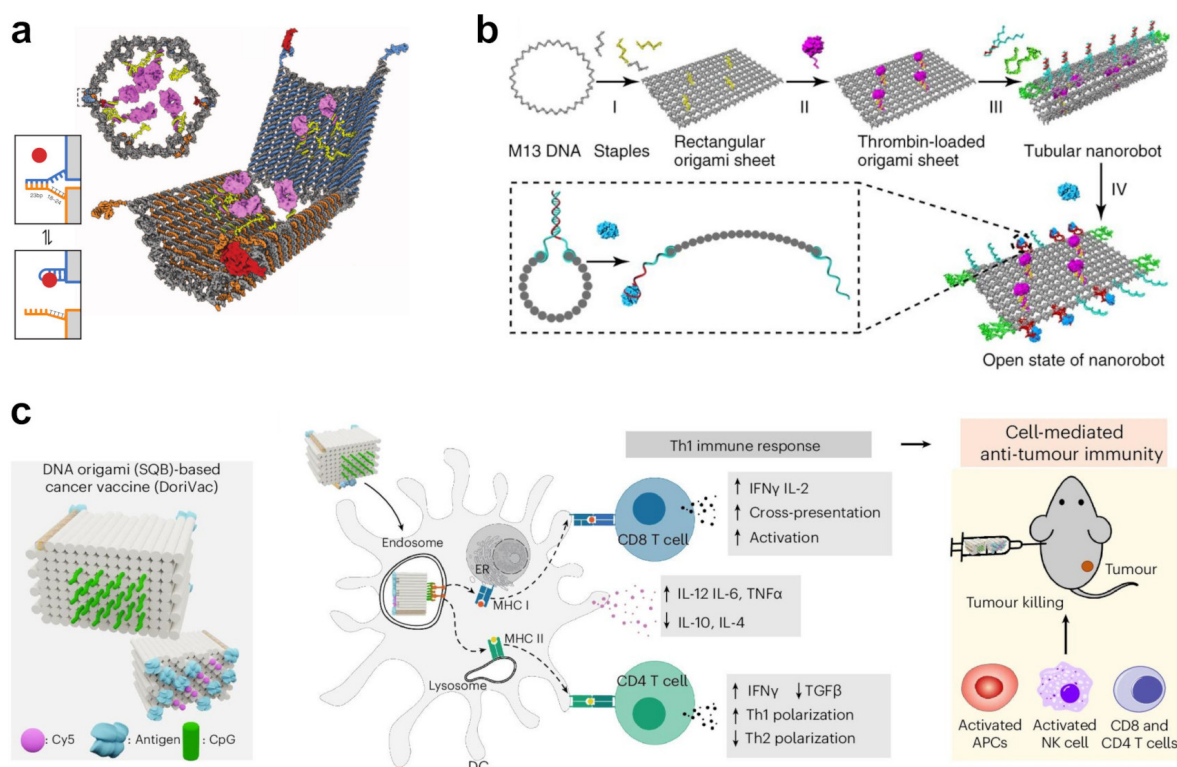


Figure 1.10: Drug delivery systems based on DNA origami. (a) Aptamer-gated DNA nanorobot with protein payloads (adapted with permission from Ref. [112], Copyright 2012, AAAS). (b) Design and characterization of a thrombin-loaded DNA nanorobot. Firstly, DNA origami folds into a sheet, thrombin is loaded *via* poly-T/poly-A hybridization, the tubular structure assembles with targeting aptamers, and finally it opens up in response to nucleolin binding to release thrombin (adapted with permission from Ref. [106], Copyright 2018, Springer Nature). (c) Design of DNA origami vaccines (DoriVac) with CpG adjuvant, OVA antigen, and Cy5 tracer. Co-delivery is realized at controlled spacing to enhance Th1-polarized immune responses (adapted with permission from Ref. [107], Copyright 2024, The Authors, under exclusive license to Springer Nature).

Cargo protection and retention remain key challenges, particularly for protein encapsulation, where leakage from partially sealed compartments limits overall efficiency.^[110] Advances in loading precision and structural sealing, guided by computational modeling and biophysical validation, continue to enhance these systems. Various surface modification strategies have been explored to improve biological stability, including protective and stimuli-responsive coatings.^[102,105,111,113]

Nevertheless, the immunogenicity and biocompatibility of DNA origami structures themselves remain incompletely understood. Although DNA is intrinsically biocompatible, foreign or chemically modified DNA nanostructures can activate innate immune pathways, notably through Toll-like receptor (TLR) engagement and subsequent cytokine release.^[114–116] Systemic administration may further provoke complement activation, rapid clearance, or off-target accumulation in organs such as the liver and spleen, complicating pharmacokinetic and safety assessments. Surface passivation, sequence optimization, and immune-tolerant coatings have shown potential in mitigating these responses, yet comprehensive *in vivo* immunotoxicity studies remain scarce.^[114–116] Standardized metrics for drug loading and

release are also still missing.^[111] Numerous reviews provide authoritative overviews of architectural progression and delivery functionalities.^[103,117–119] These resources offer insight into the practical challenges of translating DNA nanotechnology into functional materials and biomedical tools. Other works expand this view to dynamic nanodevices, stimuli-responsive elements, and hybrid systems, further enriching the design landscape.^[113,118,120] Taken together, the field currently features a robust suite of programmable delivery vehicles and functional nanostructures. Seminal experimental efforts — such as siRNA-loaded nanocarriers^[105] and nanorobots capable of molecularly triggered *in vivo* release^[106] — illustrate the promise of DNA origami as a responsive, modular platform. Continued innovation in structural refinement, stimulus-coupling strategies, and biointegration is expected to expand its utility in translational applications. Beyond its role as a nanoscale delivery vehicle, the molecular precision and mechanical robustness of DNA can also be considered from a fundamentally different perspective — that of polymer mechanochemistry.

1.3.4 DNA in Polymer Mechanochemistry

While traditional polymer mechanochemistry has established the mechanical activation of covalent bonds as a powerful means to probe and control reactivity, it remains constrained by several inherent limitations (see Section 1.1.4). Mechanophore-containing polymers typically suffer from broad molecular weight distributions, stochastic force propagation, and limited mechanistic resolution. Analytical verification of activation events often relies on indirect or ensemble-averaged methods such as GPC, end-group NMR, or fluorogenic readouts, which preclude a molecularly precise understanding of individual scission events. Moreover, the synthesis of functional mechanophores frequently demands laborious multi-step organic routes, while precise force quantification along the polymer backbone remains experimentally inaccessible.

In contrast, DNA represents a uniquely well-defined polymeric system that intrinsically overcomes many of these challenges. As a sequence-defined biopolymer, DNA features absolute monodispersity and a precisely known order of monomeric units, each corresponding to a specific nucleotide. Its stiff, double-helical structure transmits mechanical tension in a predictable manner, and both local and global responses to force can be mapped with molecular accuracy. Importantly, DNA can be interrogated through next-generation sequencing (NGS), offering an unprecedented capability to record and analyze force-induced scission or rearrangement events at single-nucleotide resolution.

Within this thesis, DNA is therefore not only employed as a structural or biological material, but also examined as a model polymer in the context of mechanochemistry. A central question addressed herein is how DNA — as a perfectly defined, sequence-encoded polymer — can contribute to the broader understanding of polymer mechanochemistry, bridging molecular precision with mechanical response.

1.4 High-Throughput DNA Sequencing Technologies

The unique contribution of this thesis is the precise tracking of *post-mortem* DNA scission distributions at the single-monomer level through NGS. To contextualize this approach, the following section outlines the historical development of sequencing technologies, highlights key differences between available platforms, and discusses their relevance to the experimental design and data interpretation strategies used herein.

The advent of high-throughput DNA sequencing has revolutionized molecular biology and genomics. This transformation was catalyzed by the completion of the Human Genome Project in 2003,^[121] which, though accomplished using the low-throughput and labor-intensive Sanger method, marked a pivotal milestone in the field. Since then, rapid innovation in sequencing technologies has dramatically increased throughput, improved accuracy, and lowered costs, enabling a broad range of applications from genome-wide association studies (GWAS) to single-cell transcriptomics. These advances have not only accelerated fundamental research but have also enabled clinical, diagnostic, and synthetic biology applications on an unprecedented scale.

1.4.1 Sanger Sequencing

Sanger sequencing, developed in the 1970s by Frederick Sanger and colleagues,^[122–125] was the first reliable method for determining the nucleotide sequence of DNA. The technique is based on the selective incorporation of dideoxynucleotides (ddNTPs), which lack a 3' hydroxyl group and thus terminate DNA strand elongation upon incorporation. This produces a series of DNA fragments of varying lengths, each ending at a specific base (Fig. 1.11).

Initially, these fragments were separated by polyacrylamide gel electrophoresis (PAGE), with separate reactions performed for each of the four bases. The products were radiolabeled—typically with ³²P-dATP—and visualized by autoradiography. The resulting band patterns were then manually read from the gel, making the process labor-intensive and time-consuming. Despite these limitations, Sanger sequencing set the gold standard for accuracy and played a pivotal role in early large-scale sequencing efforts.

Over time, several improvements enhanced the efficiency and reliability of the method. These included optimized enzymes with improved fidelity, better primer synthesis techniques, and advances in gel-based separation that extended read lengths. A major breakthrough in Sanger sequencing came with the introduction of fluorescently labeled dideoxynucleotides and the adoption of capillary electrophoresis systems, which together enabled automated, higher-throughput sequencing.^[127] These advances significantly reduced the reliance on manual gel handling and radiolabeling, thereby improving both speed and safety. As a result, Sanger sequencing became viable for large-scale genomic projects. Notably, the Human Genome Project employed hierarchical shotgun sequencing of mapped bacterial artificial chromosome (BAC) clones, with automated capillary electrophoresis serving as the primary readout method.^[121]

Nevertheless, even with automation, the method remained inherently limited in terms of scalability and throughput. As the demand for faster and massively parallel sequencing grew, Sanger sequencing was eventually supplanted by NGS technologies, which are better suited to high-throughput genomic analysis. However, it remains widely used today for

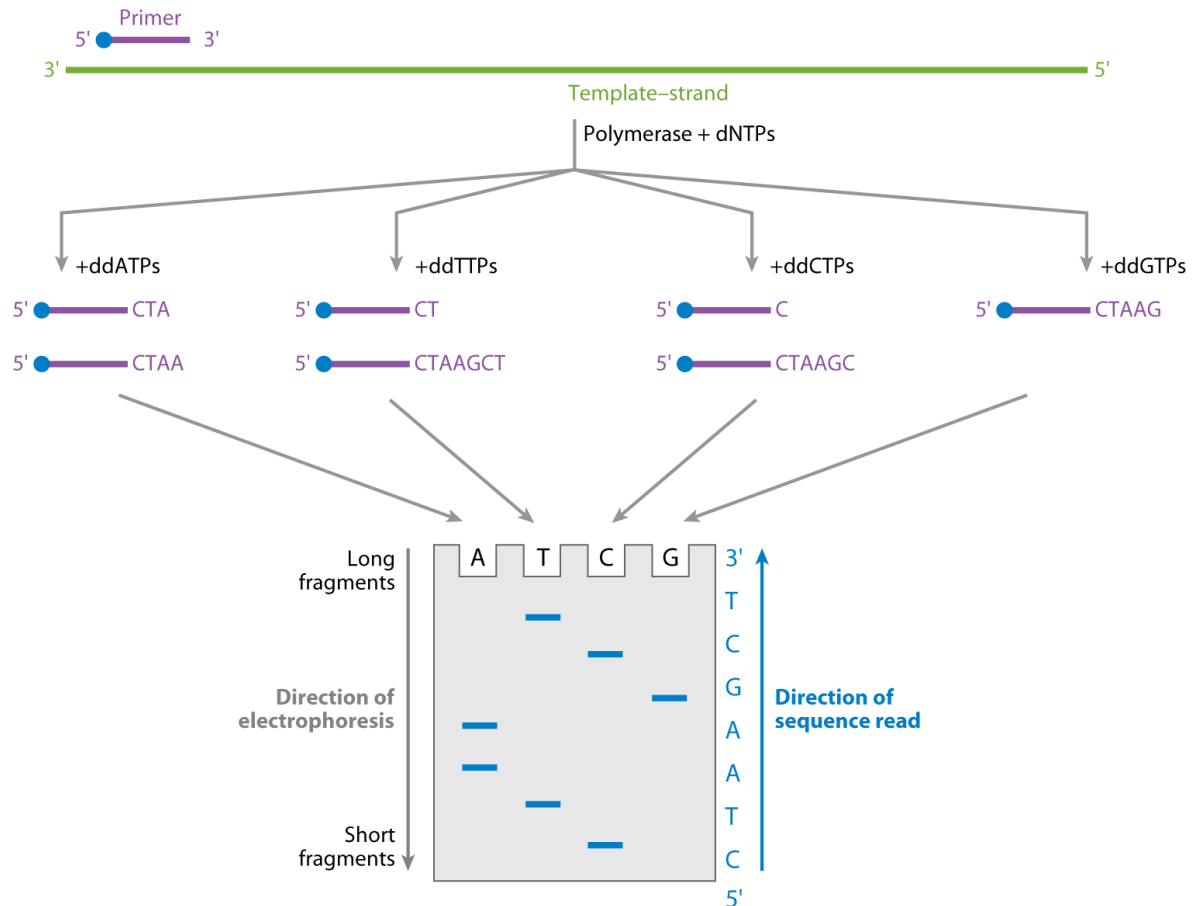


Figure 1.11: Schematic principle of Sanger sequencing. A DNA polymerase extends a template strand with a known primer, using dNTPs and chain-terminating ddNTPs, producing fragments of varying lengths that are separated by electrophoresis to infer the sequence (reprinted with permission from Ref. [126], Copyright 2013, Annual Reviews).

applications such as plasmid verification and targeted sequencing due to its high accuracy and low cost, with many companies continuing to offer Sanger sequencing services for research and industrial purposes.^[128]

1.4.2 454 Pyrosequencing

Major changes in sequencing technology surfaced with the introduction of the 454 platform. Jonathan M. Rothberg and colleagues first described high-density picolitre-scale pyrosequencing in 2005. This NGS system utilized emulsion-PCR amplification of DNA templates, allowing massive parallelization.^[129] The core of the technology involved loading beads with single DNA fragments into fibre-optic slides comprising picolitre wells and detecting pyrophosphate release during incorporation of nucleotides *via* a CCD charge-coupled imaging system, thus exploiting a sequencing-by-synthesis chemistry. The 454 platform's primary advances included a roughly 100-fold increase in raw throughput compared to Sanger sequencing, with about 25 megabases generated in a four-hour run compared to approximately 67 kilobases per hour for capillary systems. The technology demonstrated $\geq 99\%$ accuracy (Phred score ≥ 20), reduced read lengths to 100-200 nucleotides, and significantly lowered per-base costs. Notably, the publication benchmarked the new method against the established capillary Sanger sequencing using bacterial genome assemblies as a basic research test case. With 454 Pyrosequencing an important step forward was marked, which laid the groundwork for subsequent sequencing platforms in terms of scalability and automation.

Three years later, in 2008, a further milestone was achieved with the sequencing of the first complete diploid human genome using the commercial 454 Genome Sequencer FLX. Not only did this cost much less, at about 1% of the hypothetical costs if done by Sanger sequencing and BAC cloning, but also generated a 7.4-fold coverage in just two months using more than 100 million reads and over 24 gigabases of sequence.^[130]

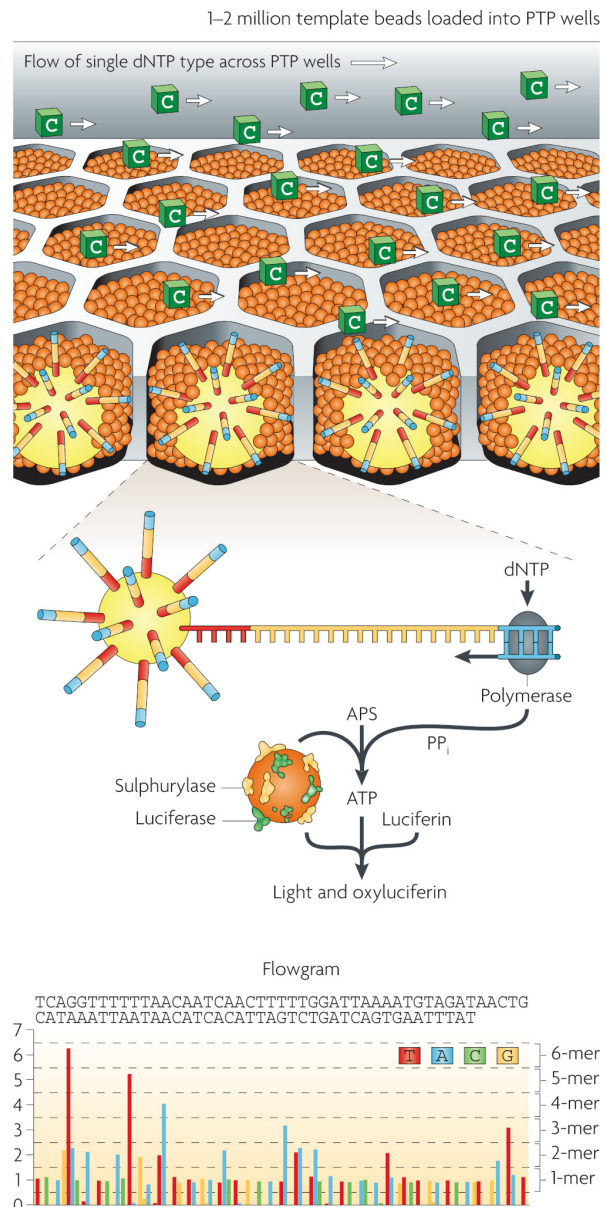


Figure 1.12: An overview of pyrosequencing on the 454 platform. DNA-bound beads are loaded into PicoTiterPlate (PTP) wells along with enzyme-coated beads containing sulfurylase and luciferase. The system delivers individual dNTPs, such as cytosine, across the wells within a flow chamber. Light generated by nucleotide incorporation is detected *via* a high-resolution camera positioned beneath the fiber-optic slide (adapted with permission from Ref. [76], Copyright 2009, Springer Nature).

1.4.3 Illumina Sequencing with Reversible Terminator Chemistry

The introduction of Illumina's® Genome Analyzer represented another major advance in massively parallel DNA sequencing. Bentley and colleagues reported the first commercial-scale demonstration of reversible-terminator chemistry in 2008.^[131] This technology utilizes 3'-O-azidomethyl fluorescent terminators in a sequencing-by-synthesis approach. Library preparation involves random genomic DNA fragmentation, followed by enzymatic end-repair, ligation onto a flow cell, and bridge amplification to produce dense clusters for sequencing (Fig. 1.13). In each cycle on the flow cell a 3'-protected base with a cleavable fluorophore attached to the nucleobase is incorporated. After each incorporation step, unreacted nucleotides are washed away, the flow cell is imaged, the 3'-end is deprotected and the fluorophore is cleaved and washed away. In their 2008 study, Illumina® was successful in sequencing of a Yoruba male genome to over 30-fold coverage, enabling the identification of roughly four million single-nucleotide polymorphisms and 400,000 structural variants. The platform achieved throughput exceeding one gigabase per run and delivered high consensus accuracy at a fraction of the cost associated with Sanger-based methods.

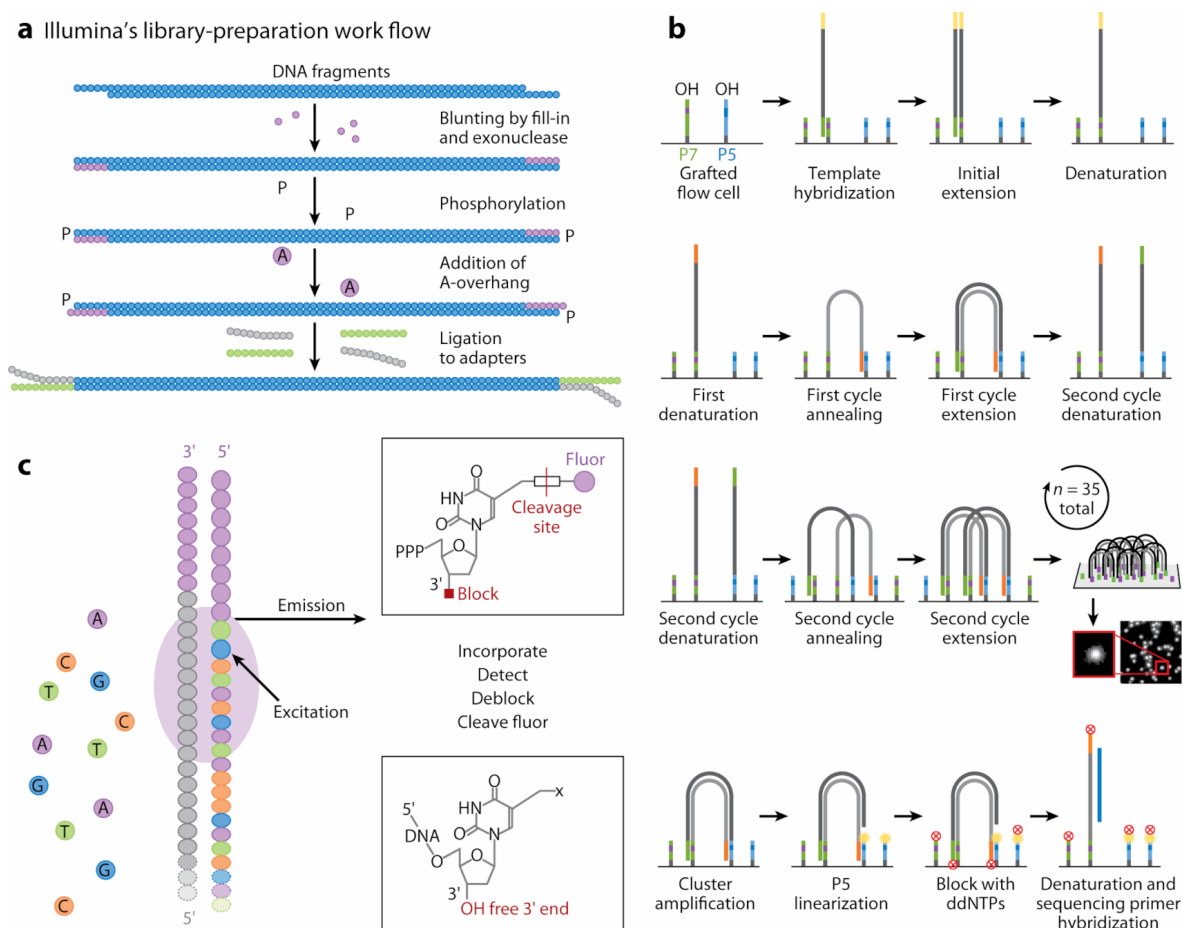


Figure 1.13: Principle of Illumina® sequencing. (a) Library preparation workflow. (b) Cluster generation by bridge amplification. (c) Sequencing by synthesis approach based on reversible dye terminator chemistry (reprinted with permission from Ref. [126], Copyright 2013, Annual Reviews).

Notably, in 2014, Illumina® became the first company to achieve the long-standing goal of the "\$1,000 genome" with the launch of its HiSeq X Ten system.^[132] Illumina's® basecalling accuracy decreases with increased nucleotide addition steps,^[126] hence the first incorporated bases have a statistically higher chance of being called correctly. This is crucial for the work described in this thesis and will be discussed further in the next chapter.

1.4.4 Semiconductor-Based DNA Sequencing with Ion Torrent

A radical shift from optical to electronic detection was introduced by the Ion Torrent platform, again led by Jonathan Rothberg. Ion Torrent is another sequencing-by-synthesis approach where base incorporation is detected directly by measuring pH changes *via* a complementary metal-oxide-semiconductor (CMOS) chip packed with over a million ion-sensitive field-effect transistor sensors (Fig 1.14).^[133] This eliminated the need for costly optics and again offered a more scalable and low-cost route to high-throughput sequencing.

Ion Torrent sequencing offers faster run times and higher throughput than the Illumina® MiSeq (up to 100 Mb/h vs. 60 Mb/h), but it produces shorter reads and has a significantly higher error rate, especially with homopolymeric regions.^[134] In contrast, Illumina MiSeq generates the highest quality reads with minimal indel errors and the lowest substitution error rate,^[134] making it more accurate for applications requiring high fidelity such as those needed within this thesis.

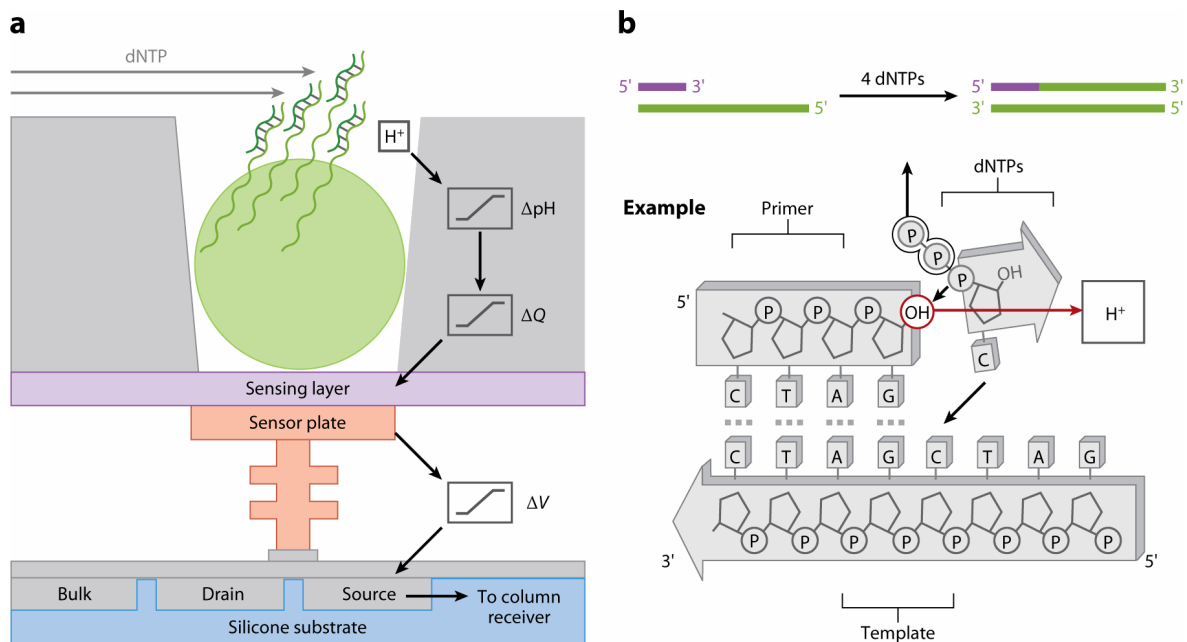


Figure 1.14: An overview of pH-based sequencing with Ion Torrent technology. (a) Schematic representation of the Ion Chip, which contains an array of wells for detecting nucleotide incorporation. (b) Detection principle based on pH change resulting from hydrogen ion release during DNA polymerase activity (reprinted with permission from Ref. [126], Copyright 2013, Annual Reviews).

1.4.5 Avidity-Based Sequencing with the AVITI Platform

Avidity sequencing, introduced by Element Biosciences and implemented on their AVITI™ platform, represents a recent and conceptually distinct advancement in short-read DNA sequencing chemistry.^[135] First disclosed in late 2022 as a preprint, this platform decouples the two core tasks of sequencing—template stepping and base identification—into independent and optimizable phases. Base identification is achieved through the use of multivalent "avidite" probes: dye-labeled, polymer-linked nucleotide ligands capable of forming stable, high-affinity complexes with engineered polymerases and clonal DNA templates (Fig. 1.15). Unlike conventional sequencing-by-synthesis methods, where incorporation and base reading are coupled in a single step, avidity sequencing first incorporates a reversible terminator nucleotide without any label. Subsequently, a separate avidite containing multiple identical nucleotides, tethered to a polymer backbone and labeled with a fluorescent dye, is introduced. These avidites bind *via* base-pairing discrimination to the incorporated base on a DNA "polony" (a localized clonal amplification site), forming a stable complex with multiple anchor points. The use of avidity—multivalent cooperative binding—reduces the effective dissociation constant K_d by more than two orders of magnitude compared to monovalent fluorescent nucleotides, ensuring persistent signal without the need for high reagent concentrations.^[135] Chemically, each avidite consists of a central fluorescently labeled protein core

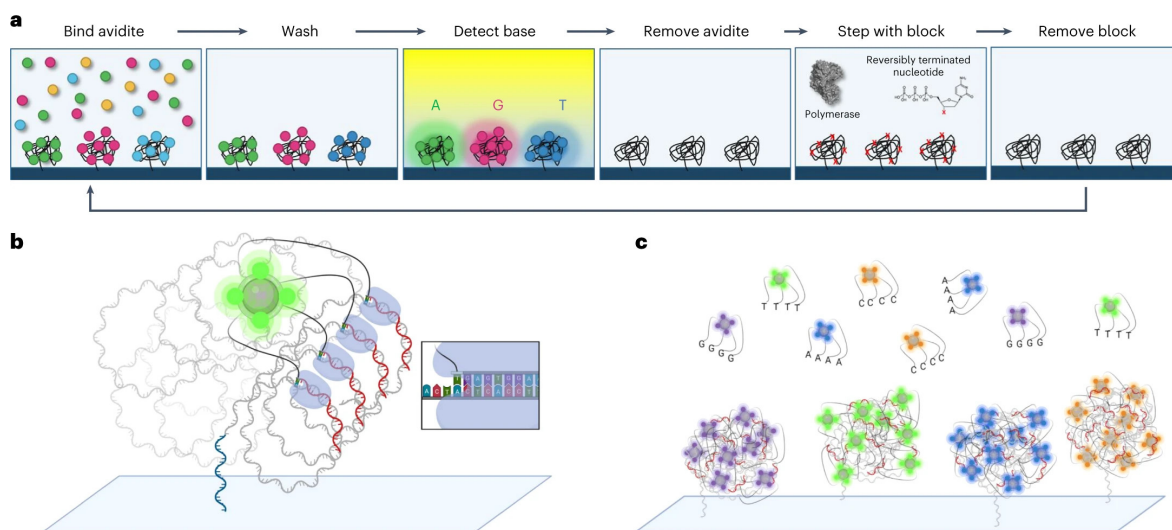


Figure 1.15: Schematic of avidity sequencing. (a) Fluorescently labeled multivalent avidites bind selectively to DNA polonies *via* engineered polymerases. (b) Each avidite connects to multiple polymerase-bound templates, enhancing signal strength and stability. (c) Multiple avidite-DNA interactions across polonies generate fluorescence for base detection (reprinted from Ref. [135], CC BY 4.0).

(often streptavidin), to which multiple nucleotide-bearing arms are attached *via* biotin–PEG linkers. The arm configuration ensures that one avidite can simultaneously interact with several complementary positions within a polony. This structural multivalency creates long-lived, noncovalent interactions that are highly resistant to dissociation, enabling accurate imaging even in the absence of free avidites in solution. Following imaging, the avidite is washed away, and an engineered polymerase incorporates the next unlabeled, blocked nucleotide, restarting the cycle. Importantly, this separation between incorporation and

detection allows each step to be chemically optimized for accuracy and efficiency. Performance metrics reported for AVITI™ sequencing include over 85% of base calls achieving Q40 or higher, corresponding to fewer than one error per 10,000 bases.^[135] Notably, the error rate remains stable even across long homopolymeric stretches, where sequencing-by-synthesis platforms typically degrade. Avidity sequencing also demonstrated strong variant-calling performance in whole-genome sequencing of the HG002 human reference genome, achieving SNP and indel F1 scores exceeding 0.995. Additionally, the chemistry proved compatible with single-cell RNA sequencing workflows and long-read extensions up to 300 cycles.^[135]

Despite its demonstrated advantages, the AVITI™ platform was not considered for this thesis, as it was neither commercially available nor published at the time sequencing work for this thesis began in autumn 2022.

1.5 Molecular Dynamics Simulations

While NGS provides powerful means to characterize DNA scission *post-mortem*, it is equally important to understand the molecular events that precede strand breakage. To gain insight into these early-stage mechanisms, molecular dynamics (MD) simulations were performed in close collaboration with the Gräter group, now based at the Max Planck Institute for Polymer Research in Mainz.

MD simulations provide a computational window into the physical behavior of molecules, capturing their atomic motions over time based on classical mechanics. Rather than relying on static structures, this approach allows us to observe how DNA responds to thermal fluctuations, mechanical stress, and interactions with its environment.

One of the earliest molecular dynamics simulations was carried out by Alder and Wainwright in 1957, who modeled the behavior of hard-sphere particles to investigate phase transitions in dense fluids.^[136] This groundbreaking study demonstrated that the collective motion of interacting particles could be tracked through time by solving Newton's equations of motion numerically, thereby providing an alternative to analytical statistical mechanics for systems where exact solutions are intractable.

Building on this foundational work, Alder and Wainwright published a general framework for MD two years later, which laid out a systematic method for computing the trajectories of hundreds of interacting particles with well-defined classical potentials.^[137] Their approach allowed for the direct calculation of both equilibrium and non-equilibrium properties, using periodic boundary conditions and simplified interaction potentials such as hard spheres or square wells.

These two papers laid the main grounds for further research by Warshel, Levitt and Karplus which has eventually been awarded with the 2013 Nobel Prize in Chemistry "for the development of multiscale models for complex chemical systems".^[138–142]

1.5.1 Principles of Molecular Dynamics

A central theoretical assumption underlying MD is the Born–Oppenheimer approximation, which posits that the motions of electrons and nuclei can be separated due to their mass difference.^[143] Electronic structure is assumed to adjust instantaneously to nuclear positions, which allows the potential energy surface governing nuclear motion to be computed

independently. In classical MD, this surface is represented by empirical force fields that approximate the potential energy V of each particle as a sum of bonded and non-bonded interactions:

$$V = V_{\text{bonded}} + V_{\text{non-bonded}} \quad (1.2)$$

$$= [V_{\text{bond}} + V_{\text{angle}} + V_{\text{dihedral}}] + [V_{\text{Lennard-Jones}} + V_{\text{Coulomb}}] \quad (1.3)$$

In practical terms, MD simulations involve assigning initial positions and velocities to atoms, then propagating their trajectories by numerically integrating Newton’s equations of motion for each particle i :

$$\mathbf{F}_i = m_i \frac{\partial^2 \mathbf{r}_i}{\partial t^2} = - \frac{\partial V_i}{\partial \mathbf{r}_i} \quad (1.4)$$

Standard integration algorithms, such as the velocity Verlet method,^[144] update positions and velocities in discrete time steps on the order of femtoseconds. The system is typically equilibrated at a target temperature and pressure using thermostats and barostats, ensuring sampling of the desired ensemble.

1.5.2 Atomistic Force Fields for DNA

For biomolecular systems such as DNA, MD simulations make it possible to observe the structural fluctuations and local stress distributions that can precede strand breakage. NGS pinpoints cleavage sites *post mortem*, but only simulation offers a time-resolved picture of how subtle torsional shifts, transient base-pair opening, solvation rearrangements and ion movements accumulate into mechanical or chemical perturbations capable of scission. Describing relevant DNA systems in MD simulations requires a force field suitable for nucleic-acids, defining the bonded and non-bonded interactions within and between DNA strands. Additionally, to describe the DNA in its native aqueous environment a sufficient parametrization of the surrounding ions and water molecules has to be selected.

Among atomistic force fields, the Assisted Model Building with Energy Refinement (AMBER) family supplies the longest continuous lineage. The original parm94/parm99 parameters supported the first explicit-solvent microsecond trajectories but proved prone to spurious α/γ conformer flips and exaggerated helical drift once longer sampling became routine. The parmbsc0 correction cured the worst α/γ instability, yet over-stabilized the BI backbone sub-state, thereby biasing backbone flexibility and twist distributions and muting the small conformational excursions thought to precede backbone cleavage.^[145] Two successor variants, bsc1 and OL15, re-tuned ϵ/ζ and χ torsions and achieved much closer agreement with NMR-derived helical parameters across diverse B-DNA duplexes, but they still struggled with stress-concentrating non-canonical forms: simulations under-populated the diagnostic ZI backbone sub-state in Z-DNA and occasionally allowed G-quadruplex channel collapse.^[146–149]

These deficiencies motivated a comprehensive re-parameterisation of glycosidic, β and sugar-pucker terms that culminated in the OL21 potential. In a 2023 benchmark spanning canonical B-DNA, A-DNA, two Z-DNA sequences and two quadruplex topologies, OL21 delivered the lowest root-mean-square deviations from crystallographic and NMR references, restored the correct ZI/ZII equilibrium and maintained quadruplex integrity throughout tens of microseconds of sampling; the alternative Tumuc1 set showed no systematic improvement over its parent parameters.^[150] OL21 also preserved the realistic elastic moduli and sequence-resolved crookedness documented for OL15 which is directly relevant to evaluating how

local bending and twisting stresses concentrate before cleavage.^[151] Together these studies identify OL21 as the most balanced AMBER description currently available for both canonical and non-canonical DNA. A more recent addition is the sugar pucker correction implemented in OL24^[152] improving the balance of DNA A and B forms. To be consistent with the part of the study that has been performed before OL24 was available, the MD simulations by our collaborator have solely been performed using OL21.

The most prominent alternative to AMBER derived forcefields is the Chemistry at Harvard Macromolecular Mechanics (CHARMM) line. CHARMM27 produced a credible description of B-DNA flexibility and, in combination with the Ascona B-DNA Consortium data set, contributed to a “consensus view” of sequence-dependent helical mechanics.^[153,154] Its successor CHARMM36 corrected specific base-stacking energetics, yet multi-microsecond tests revealed enhanced terminal fraying and gradual duplex destabilization; a Drude-polarizable extension alleviated some electrostatic imbalance but introduced new structural drift within hundreds of nanoseconds.^[147] Recent multiscale comparisons that included CHARMM27, CHARMM36 and two AMBER variants converged on the conclusion that CHARMM potentials remain less stable than the modern AMBER family on the time-scales required to probe subtle pre-damage dynamics.^[155]

The water model governs screening, hydrogen-bond lifetimes and the ion atmosphere. Transferable intermolecular potential with 3 points (TIP3P), introduced by Jorgensen in 1983,^[156] remains the reference based on practical considerations. Unlike alternatives such as TIP4P-Ew or OPC the TIP3P water model is compatible with the Joung–Cheatham ion set. Crucially, the selected force field OL21 has been benchmarked and finetuned using the combination of the TIP3P water model and the Joung–Cheatham ion set. Large-scale sequence surveys of 136 tetranucleotides found indistinguishable averages for groove width and helical rise when TIP3P and TIP4P-Ew were used with parmbsc0,^[154] and continuum-versus-explicit comparisons established that TIP3P already captures the solvent contributions most relevant to canonical stability, ZI/ZII equilibria and sequence-dependent stiffness.^[157,158] Moreover, pivotal studies of terminal fraying^[159] and force-induced elongation^[151]—both central to understanding strand-break precursors—were conducted in TIP3P, providing a direct benchmark for the present work.

For these combined theoretical and pragmatic reasons, all simulations in this thesis employ the AMBER OL21 nucleic-acid force field together with explicit TIP3P water. OL21 delivers the most accurate description available of backbone torsions, base stacking and non-canonical stability—the ingredients required to resolve the fragile conformational precursors to strand scission—while TIP3P provides a thoroughly vetted, computationally efficient solvent that preserves continuity with the experimental calibration and simulation literature on which OL21 itself is grounded.

Chapter 2

Sequence-Specific, Mechanophore-Free Mechanochemistry of DNA

2.1 Introduction

Polymer mechanochemistry explores how mechanical inputs are transduced into specific chemical outcomes along polymer backbones (see Chapter 1, Section 1.1). Traditionally, this selectivity is achieved with mechanophores—force-responsive motifs that enable functions such as mechanocatalysis, stress sensing, self-healing, and programmed depolymerization—but their synthesis, tunability, and biocompatibility can be limiting. In parallel, force-responsive biopolymers play an important role in biology. Among these, DNA is distinctive: it is readily fabricated with precise sequence and length, making it an ideal sequence-controlled polymer for materials design, nanodevices, and information storage.

Existing strategies that subject DNA to mechanical stress (*e.g.*, sonication-based fragmentation or force-triggered release from DNA nanostructures) distribute load non-selectively across many covalent and non-covalent interactions, in contrast to the targeted scission characteristic of polymer mechanochemistry. A mechanophore-free route to selective bond breakage in DNA would therefore bridge these domains and broaden biocompatible applications.

In this work, we develop a nick-programmed DNA framework that concentrates ultrasound-induced mechanical stress onto predetermined phosphodiester bonds, enabling selective strand scission without exogenous mechanophores. By strategically positioning nicks in dsDNA, we create supramolecular “sacrificial” sites that redirect force to the opposite strand. The resulting scission patterns are verified by Illumina sequencing. We further show that the immediate sequence context around a nick modulates the scission profile, providing a simple handle for programmable control *via* base design. All-atom, constant-force MD simulations support a mechanism in which localized fraying near the nick exposes a weakened single-stranded region that channels the applied load to specific bonds.

Building on this 1D framework, we extended the concept’s complexity to DNA origami during a Fulbright stay at the Biodesign Institute, Arizona State University, which demonstrates the generalizability to multi-dimensional assemblies.

Together, these findings establish nicked DNA as a tunable, biocompatible platform for selective mechanochemical activation and as a model system for probing fundamental questions in polymer mechanochemistry.

Most experimental parts of this chapter were performed together with Arjuna Selvakumar under my close supervision during his master's thesis. The algorithm and code for NGS analysis were developed together with Boris Schüpp, Gräter group (MPI-P, Mainz) as part of his master's thesis which I supervised. MD simulations were conducted independently by Boris Schüpp and are included here for completeness of the story. Illumina sequencing was conducted by the Genomics Facility, a core facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen University.

2.2 Methods

Materials and Reagents

Synthetic genes and primers required for amplifying dsDNA were ordered from Integrated DNA Technologies (IDT, USA), and the corresponding sequences are listed in Fig. 5.1-5.14 in the Appendix. Staple strands for the assembly of DNA origami were purchased from Integrated DNA Technologies (IDT, USA). Scaffold strands M13mp18 and P8064 were obtained from Bayou Biolabs. PCR amplification was carried out with Phusion High-Fidelity PCR Master Mix (Thermo Scientific™, cat. no. F531L). Site-specific nicking was achieved using the enzymes Nt.BspQI (cat. no. R0644S), Nb.BtsI (cat. no. R0707S), and Nt.BstNBI (cat. no. R0607S), all obtained from New England Biolabs (NEB). Agarose Standard (Carl Roth, cat. no. 3810.4) was employed for gel electrophoresis (GE), together with ROTI®GelStain Red (Carl Roth, cat. no. 0984.1) as the intercalating dye. Tris-acetate-EDTA (TAE) buffer was freshly prepared using Tris base (CAS 77-86-1, Fisher Bioreagents), acetic acid (CAS 64-19-7, Sigma), and disodium ethylenediaminetetraacetate dihydrate (EDTA, CAS 60-00-4, Sigma) to yield a 1× working solution containing 40 mM Tris, 20 mM acetic acid, and 2 mM EDTA. Samples were mixed with TriTrack DNA Loading Dye (6×, Thermo Scientific™, cat. no. R1161) prior to electrophoresis, and GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific™, cat. no. SM0321) was used as a molecular size marker for linear dsDNA systems. For electrophoresis involving origami samples ReadyLadder™ 1 kb PLUS™ DNA Ladder (GoldBio, cat. no. D011-500) was employed. Gel-excised DNA was recovered using the GFX™ PCR DNA and Gel Band Purification Kit (Cytiva, cat. no. 28903471). For sequencing library construction from sheared dsDNA, the NEBNext Ultra II DNA Library Prep Kit (NEB, cat. no. E7645L) was applied. Paired-end sequencing was subsequently conducted on an Illumina MiSeq platform with the MiSeq Reagent Micro Kit v2 (300 cycles; Illumina, cat. no. MS-103-1002).

Generation of Nicked DNA

Amplification of dsDNA was performed using PCR. Each reaction contained 30 ng of template DNA, forward and reverse primers at a concentration of 0.5 µM each, and 25 µL of 2× Phusion Green Hot Start II High-Fidelity PCR Master Mix. Ultrapure water was added to a total reaction volume of 50 µL. PCR conditions were as follows: initial denaturation at 98 °C for 2 min; 30 cycles of denaturation at 98 °C for 10 s, primer annealing at 67 °C for 20 s, and extension at 72 °C for 30 s; final extension at 72 °C for 7 min. Amplification products were purified either by agarose gel extraction or by solution-based cleanup. Nicks were introduced into the PCR products using the enzymes Nt.BspQI, Nb.BtsI, or

Nt.BstNBI. Each nicking reaction was set up in a final volume of 50 μL containing 1.0 μg of dsDNA, 10 U of the respective enzyme, and 5 μL of 10 $\times$ NEBuffer™ r3.1. In its final composition, the buffer provided 100 mM NaCl, 50 mM Tris·HCl, 10 mM MgCl_2 , and 500 ng recombinant albumin, adjusted to pH 7.9 at 25 $^\circ\text{C}$. Samples were incubated at 50 $^\circ\text{C}$ for 1 h to allow nicking, followed by heat inactivation at 80 $^\circ\text{C}$ for 20 min. The integrity and cleavage of nicked dsDNA were subsequently verified by agarose GE.

Ultrasound-induced Scission of DNA

For sonication experiments, 990 ng of DNA was diluted in 664.5 μL of ultrapure water. Sonication was carried out with a Q500 Sonicator (Qsonica, USA) equipped with a 3 mm probe (422-A), operating at 20 kHz. The amplitude was set to 50% of the maximum displacement (45 μm) and kept constant throughout all experiments, regardless of DNA concentration, sample viscosity, or temperature. Energy delivery during sonication (E , in joules) was recorded to determine the applied power ($P = Et^{-1}$) and the power intensity ($I_p = PA^{-1}$), where t corresponds to the effective sonication time and A to the probe tip area. Sonication was performed in pulsed mode with 2.0 s “on” and 1.0 s “off” intervals. Under these conditions, the effective power was calculated as $P = 3.76 \text{ W}$ and the corresponding power intensity as $I_p = 53.17 \text{ W cm}^{-2}$. To minimize thermal effects, samples were maintained on ice during treatment. Following sonication, DNA solutions were concentrated to volumes of 5–30 μL using a vacuum concentrator (Eppendorf Concentrator plus, 60 $^\circ\text{C}$, reduced pressure) before agarose GE. For kinetic studies, gel band intensities were quantified with ImageJ software (v1.54). The sonication setup is summarized in Fig. 2.1.

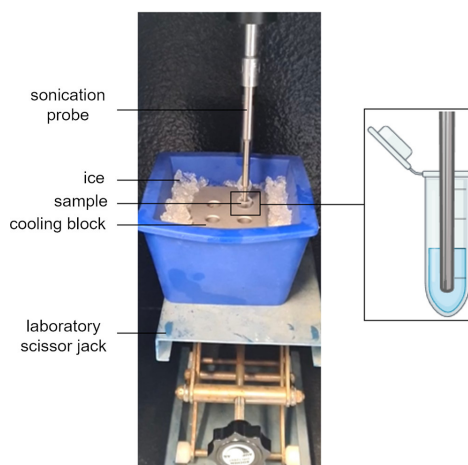


Figure 2.1: Experimental setup for the US-induced scission of DNA. Created with BioRender.com

Assembly and Purification of DNA Origami

For the assembly of 2D and 3D DNA origami structures, two different scaffolds were employed: the M13mp18 strand for the Rothemund rectangle (RRO) and the P8064 strand for the six-helix bundle (6HB). In both cases, scaffold strands (20 nM) were mixed with a 10-fold excess of staple strands (200 nM) in 1 $\times$ TAE buffer containing 12.5 mM Mg^{2+} . The total reaction volume was 100 μL , adjusted with ultrapure water. The excess of staples was used to ensure quantitative folding of the scaffold into the desired origami structure.

Annealing conditions were adapted to the design. For the RRO, the mixture was heated to 90 $^\circ\text{C}$ for 5 min in a thermal cycler, followed by slow cooling to 20 $^\circ\text{C}$ at a rate of 0.1 $^\circ\text{C}$ per 6 s.^[160] For the 6HB, the annealing protocol involved heating to 80 $^\circ\text{C}$ for 15 min, cooling to 66 $^\circ\text{C}$ at a rate of 1 $^\circ\text{C}$ per 5 min, and then gradual cooling to 30 $^\circ\text{C}$ at 1 $^\circ\text{C}$ per 30 min.^[161] After annealing, samples of the 6HB were stored at 4 $^\circ\text{C}$.

Excess staples were removed by centrifugal filtration using 100 kDa molecular weight cut-off units (Amicon Ultra-0.5, Ultracel-100 membrane, Millipore; UFC510024), following a

published procedure.^[162] Each filter was loaded with only a single folding reaction to prevent overloading, as excess staples would otherwise remain detectable in agarose GE quality checks. The washing step was repeated four times.

Purified DNA origami structures were quantified spectrophotometrically (NanoDrop, Thermo Scientific), with molar concentrations estimated using the extinction coefficient of a fully double-stranded P8064 molecule ($\epsilon = 164,568,055 \text{ M}^{-1} \text{ cm}^{-1}$).

AFM Imaging of DNA Origami

AFM was performed using a Bruker Multimode system to visualize DNA origami structures following purification and sonication. A volume of 3 μL of DNA origami solution (1–2 nM) was deposited onto freshly cleaved mica mounted on an AFM metal sample stage. To promote immobilization of the origami structures, 60 μL of 1 $\times$ TAE buffer supplemented with 12.5 mM MgCl_2 and 4 μL of 0.2 M NiCl_2 were added. Samples were imaged in fluid mode using a Bruker SCANASYST-FLUID+ cantilever. Image data were processed and analyzed with NanoScope Analysis software (Bruker).

UV Welding of DNA Origami Structures

Purified DNA origami samples were transferred into PCR tubes in a final volume of approx. 80 μL . The samples were positioned in a custom-built Styrofoam holder designed to maintain alignment between the tube and a medium power 310 nm UV LED (Boston Electronics, cat. no. VC1X1C48L3-310-v1). The LED was mounted on a heat sink (cat. no. 30.1.006770) and coupled to a fused silica lens with a 30° beam angle to ensure uniform irradiation across the sample volume. The driver kit (PS-700A6W-ND) was operated at 700 mA constant current, providing an output power of 6–7.2 W. All irradiation steps were conducted inside a cardboard enclosure to prevent light leakage, and UV-protective safety glasses were worn at all times.

Samples were irradiated for 30 minutes at room temperature, with the LED positioned directly above the PCR tube at a fixed distance determined by the geometry of the Styrofoam holder. Following irradiation, the samples were immediately removed from the holder and stored under standard buffer conditions (1 $\times$ TAE buffer supplemented with 12.5 mM MgCl_2) until further use.

The procedure resulted in covalent stabilization of DNA origami structures through UV-induced crosslinking. These welded structures were subsequently subjected to downstream assays, including heat resistance evaluation and ultrasound stability measurements, without further purification.

Illumina Sequencing

For Illumina sequencing, agarose gel bands containing ultrasound-cleaved DNA fragments were excised once the reaction reached > 90% conversion. DNA was extracted from the gel and sequencing libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit. Sequencing was performed on an Illumina MiSeqTM instrument with a MiSeq Reagent Kit v2 Micro, generating 2 $\times$ 75 bp paired-end reads with a 5% PhiX control (Illumina, cat. no. MS-103-1002).

Data processing was carried out using a custom-built algorithm (available on [GitHub](#)) and is described as Algorithm 1 (*vide infra*). Since the parent DNA sequence was known, a template-matching procedure was employed instead of the conventional *de novo* assembly in order to achieve precise assignment of the 5' termini. The workflow was divided into two stages: first, the initial x bases of each read were aligned exactly to the template, thereby fixing the absolute read position. In the second step, the remaining sequence portion was compared to the template allowing up to y mismatches in total. If either step failed for one or both members of a paired read, the entire pair was excluded from further analysis. In this study, parameters were set to $x = 20$ and $y = 10$.

From the aligned datasets, histograms of 5' positions (referred to as the “breaking distribution”) were generated for both strands. To characterize the distribution near the nicked site, the data were fitted with a Gaussian function within a ± 50 bp window, and the fit parameters μ and σ were determined using the `curve_fit` routine from the Python SciPy library.

Filtering Algorithm

The task of determining the breaking distribution of a known template sequence differs substantially from assembling sequencing reads into a continuous sequence. The latter is usually handled efficiently by standard *de novo* assemblers. In our case, however, the reference sequence was already known, which enabled the development of a dedicated approach that assigns reads directly to the template in a computationally efficient manner. Since each read originates from the reference, it can in principle be mapped unambiguously. Paired-end sequencing was applied, generating two reads per fragment (read 1 and read 2), each extending from opposite 5' termini. For each single read, the first t bases were aligned to the template sequence. If these t bases did not match any subsequence of the reference, the complete paired read was discarded. This criterion ensures error-free identification of the 5' position, which is the essential information for constructing the breaking distribution. Throughout this study, $t = 20$ was chosen, based on the observation that no subsequence of this length occurs more than once on the template, considering both strands.

Reads passing this filter were assigned to a specific coordinate on the reference. For the remaining part of each read, up to k mismatches were tolerated; reads exceeding this threshold were removed together with their mate. In this work, $k = 10$ was used, corresponding to an approximate error tolerance of 13%. This method allows efficient processing of millions of reads, though it does not correct insertions or deletions, which are consequently excluded. Only pairs for which both reads passed the filtering steps were retained for further analysis. The pseudo-code of this algorithm is provided in Algorithm 1. Conceptually, the dataset is partitioned into three categories of paired reads:

- Paired reads with a valid match in the first t bases and no more than $k = 10$ mismatches in the remaining sequence, which are retained for further analysis.
- Paired reads for which at least one single read fails to match the template within the first t bases, typically due to sequencing errors, primers, adapters, or other impurities.
- Paired reads for which at least one single read exceeds k mismatches in the remaining subsequence, usually caused by insertions or deletions relative to the template.

Algorithm 1: Filtering algorithm for paired-end reach with a given reference sequence. Enforce at least t initial matching bases and allow at most k faulty bases.

Input : Reads, reference sequence
Output : Final dataset for further analysis

```
1  $t \leftarrow 20$ ;           // Initial bases lining up exactly with the
   reference
2  $k \leftarrow 10$ ; // Maximum allowed number of mismatches in remaining
   read
3 proper_reads  $\leftarrow$  empty list
4 foreach read in Reads do
5     Select the first  $t$  bases of the read;
6     Try to find a match between the first  $t$  bases of the read and any part of the
       reference (or its complement);
7     if a match is found then
8         Align the read to the reference utilizing the starting bases;
9         Compare the remaining bases of the read with the expected bases on the
           reference;
10        Count the number of mismatches (mismatch_counter);
11        if  $\text{mismatch\_counter} \leq k$  then
12            Add the read to proper_reads;
13        end if
14    end if
15 end foreach
16 Reassemble paired-reads using sequence identifiers (paired_reads);
17 final_dataset  $\leftarrow$  empty list
18 foreach paired_read in paired_reads do
19     read_A  $\leftarrow$  paired_read[0]; // First read in the paired read
20     read_B  $\leftarrow$  paired_read[1]; // Second read in the paired read
21     if read_A is in proper_reads and read_B is in proper_reads then
22         Add paired_read to final_dataset;
23     end if
24 end foreach
25 return final_dataset;
```

2.3 Results and Discussion

2.3.1 US-induced DNA Breakage with High Precision

In order to generate a DNA framework that breaks specifically around a designated position in its backbone, a dsDNA sequence was initially designed. An important first parameter was the envisioned length, since for US-induced chain scission of polymers a certain contour length is necessary.^[35,163] The first sequence chosen was a 704 bp long dsDNA (**704**) with its sequence derived from lac phage vector M13mp18. This was done to maximize uniqueness of the sequence as many restriction sites are encoded which could, for example, work as unique primer binding sites. PCR was done as shown in Fig. 2.2 using optimized primer binding sites. PCR was done as shown in Fig. 2.2 using optimized primer sequences used throughout this whole chapter (Fig. 5.14 in Appendix).

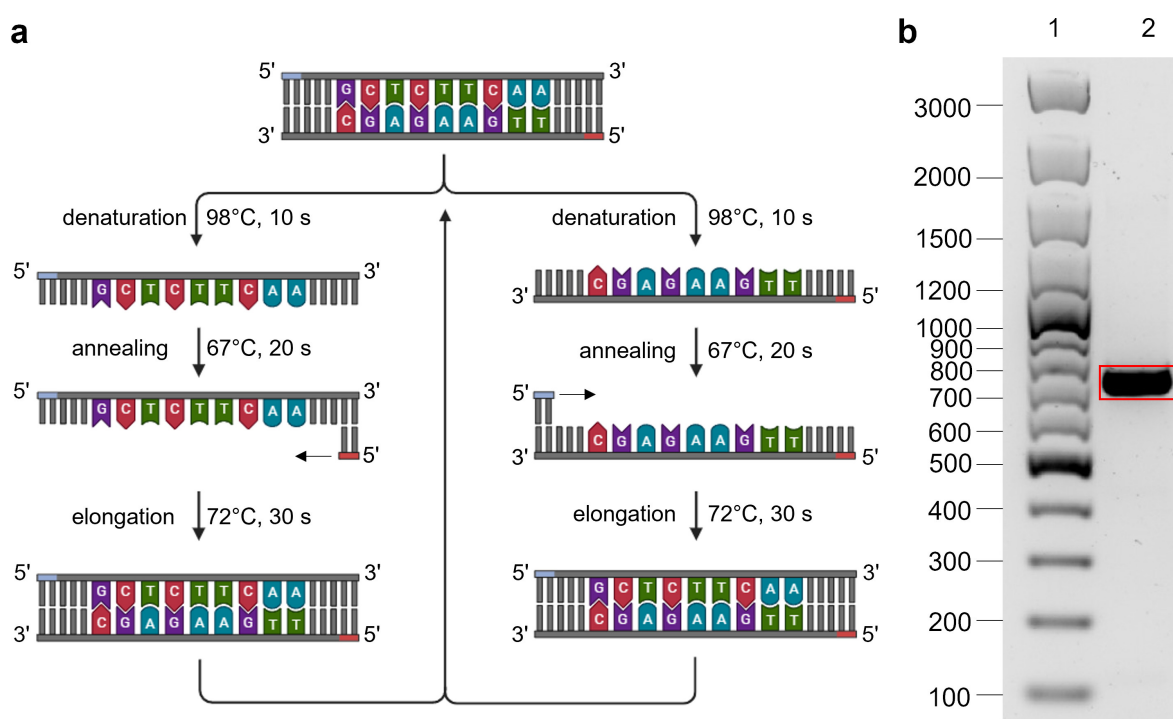


Figure 2.2: (a) Optimized PCR protocol for amplification of **704** with 30 cycles of: denaturation, primer annealing and elongation. (b) Agarose gel: lane 1: 100 bp Plus ladder; lane 2: PCR **704**; red framed: **704**. Created with BioRender.com

As shown in the agarose gel, no sideproducts could be observed. Further, the absence of leftover primer in the gel indicates quantitative conversion of primers towards extended dsDNA. Another key feature of the sequence is a central unique restriction site for a nicking endonuclease placed close to the middle of **704**. This resulted in a central nick (*) between positions 352 and 353 of the DNA to yield **704***. This mid-position was chosen to maximize the statistically averaged mechanochemical activation probability during ultrasonication.^[39,164] Subsequently, either intact **704** or nicked **704***, were subjected to 20 kHz ultrasound. Agarose GE revealed distinct fragmentation patterns: while the intact construct produced a broad distribution of fragment sizes, the nicked variant yielded a sharp band corresponding to approximately half the original length (Fig. 2.3). Moreover, the nicked DNA displayed

an accelerated cleavage rate, breaking about 23% faster than the intact control (Fig. 2.10 and 2.11).

In the case of 704*, ultrasonic scission opposite the programmed nick generated two fragments of around 352 bp each. To determine whether this nucleotide-precise cleavage event accounted for the observed electrophoretic band, we performed NGS. Illumina sequencing was selected over Oxford Nanopore sequencing, since the latter provides substantially lower quality scores for the first 130 bases.^[165] In conventional NGS library preparation, the first step typically involves DNA fragmentation. In our case, fragmentation had already occurred during ultrasonication. If cleavage is not restricted to the site opposite the nick, a mixture of blunt ends—where both strands are cleaved evenly—and sticky ends—with single-stranded overhangs—is expected to form (Fig. 2.4). Since Illumina sequencing requires blunt ends for adapter ligation, an end-repair step was introduced. In this process, a DNA polymerase removed 3' overhangs and filled in 5' overhangs, thereby generating blunt-ended fragments. Importantly, the original US-induced break positions were preserved through this step and could later be reconstructed from systematic analysis of the resulting 5' termini (Fig. 2.4). Following end-repair, adapters (short dsDNA sequences serving as amplification primers and molecular barcodes) were ligated to the fragments. Paired-end sequencing was then performed, yielding information from both 5' termini of each fragment. This enabled the construction of breaking distributions for both the nicked and intact strands of sequence 704*.

In an ideal case, each sequenced fragment corresponds to a unique cleavage position on the reference, which can be reconstructed by mapping the paired reads against the known template. Incorporation of the reference sequence into the workflow not only accelerated data processing but also ensured that sequencing outcomes could be directly interpreted in the context of DNA mechanochemistry (Fig. 2.5). For each paired read, one 5' end was assigned to position j on the upper, non-nicked strand, while the other mapped to position i on the nicked strand (Fig. 2.5). Fractional histograms of i and j were generated to quantify the relative frequency of fragment termini at each base position. As hypothesized, the nicked bottom strand protected all phosphodiester bonds of the complementary strand except the one located directly opposite the nick. Accordingly, the histogram of i confirmed that the majority of 5' termini on the lower strand aligned with the enzymatic nick. In contrast, position j , representing the US-induced scission site on the upper strand, displayed a narrow distribution centered within ± 10 nt of the nick (Fig. 2.4). This distribution could be fitted with a Gaussian, yielding mean (μ) and standard deviation (σ) parameters that describe the most probable cleavage site and the precision of the mechanochemical scission, respectively.

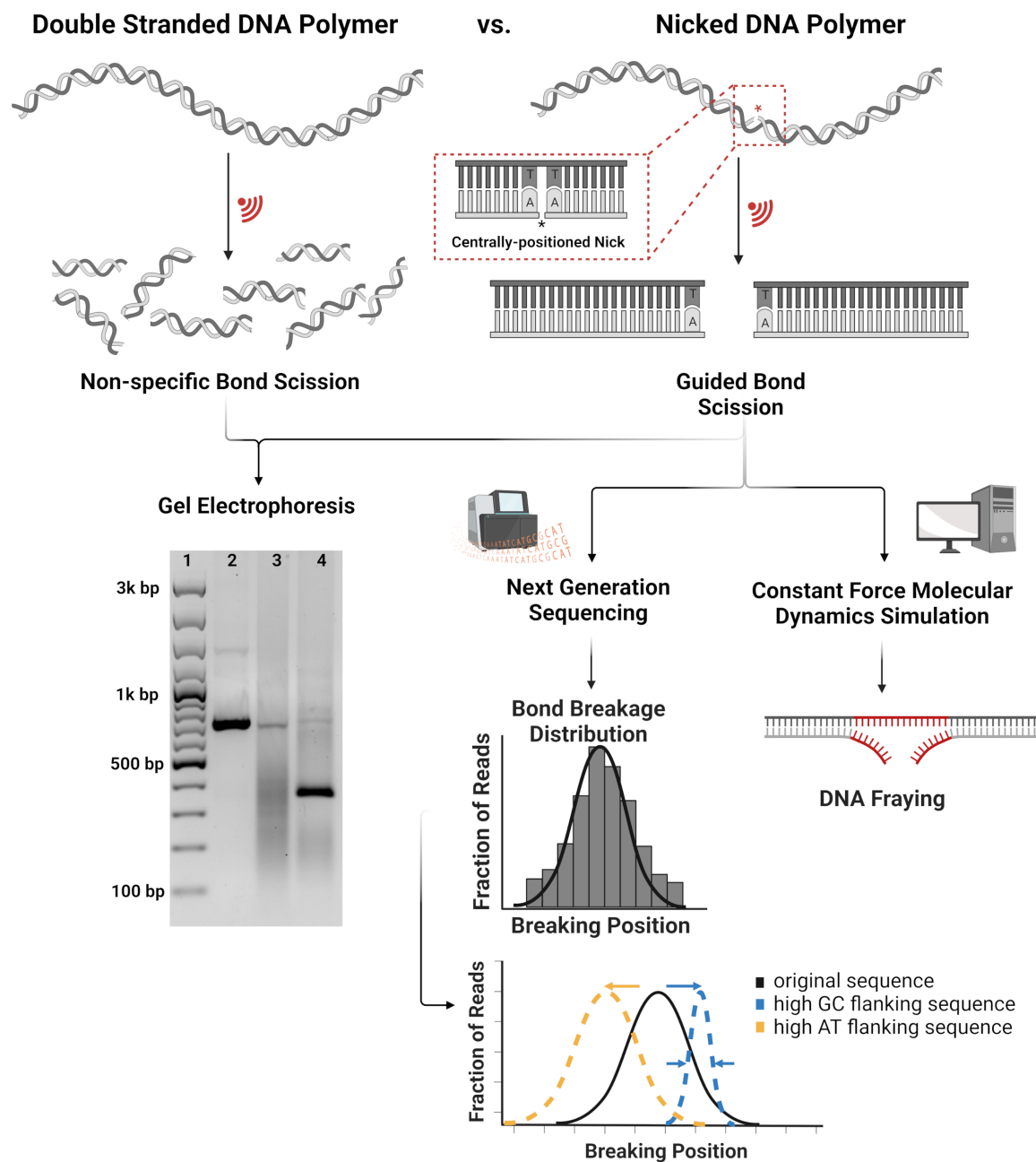


Figure 2.3: Strategic placement of nicks in dsDNA as mechanophore analogs. Sonication of intact dsDNA produced a broad distribution of fragment sizes, whereas the nicked construct of the same sequence exhibited a more defined and directed scission, as shown by agarose GE (left): lane 1, 100 bp Plus ladder; lane 2, 704; lane 3, 704 after 6 min US; lane 4, 704* after 6 min US. The mechanochemical cleavage process was investigated by two complementary approaches. Constant-force MD simulations (right) indicated local fraying as a possible origin of the observed breakage, while NGS analysis (middle) allowed the generation of a breaking distribution, which could be described by a Gaussian fit. Altering the bases flanking the nick provided control over the peak position and width of the distribution. *dsDNA containing a nick (reprinted from Ref. [166], CC BY 4.0).

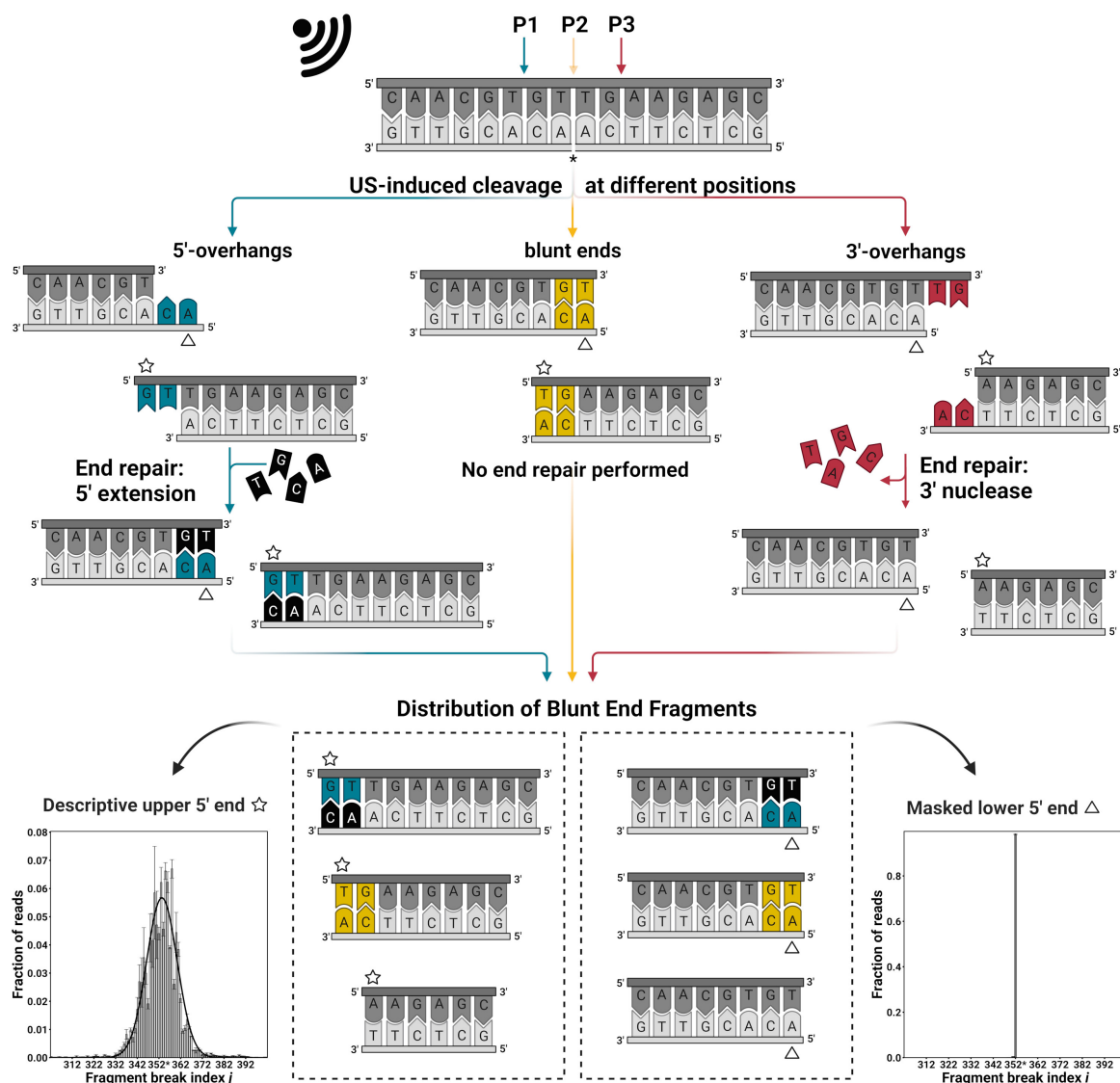


Figure 2.4: Preservation of the initially cleaved 5' ends by an end-repair mechanism after sonication and before sequencing. Ultrasound-induced cleavage of nicked dsDNA generated a mixture of blunt ends (yellow) as well as fragments with 5' overhangs (blue) or 3' overhangs (red). During the end-repair step, a polymerase with exonuclease activity removed 3' overhangs and extended 5' overhangs (black bases), thereby producing blunt-ended fragments. Importantly, the original 5' scission site was retained in all cases, enabling alignment of sequenced fragments to the known reference. This workflow allowed the construction of bond-breakage distributions that preserved the positions of the upper and lower 5' termini, denoted as j ($\star$) and i (Δ), respectively. Each bar represents the mean of three independent replicates, with error bars indicating the standard deviation. (reprinted from Ref. [166], CC BY 4.0).

2.3.2 Scission Distribution is Explained by the Magnitude of Fraying

The mechanistic origin of the observed scission distribution was initially unclear. Previous optical tweezers studies demonstrated that nicked DNA undergoes local melting near the nicked site, a process known as fraying, which exposes a short ssDNA segment.^[167] We hypothesized that such fraying also occurs in the nN force regime relevant for covalent bond rupture,^[168] thereby shaping the mechanochemical response of nicked dsDNA. To probe this, our collaborator Boris Schüpp performed fully atomistic constant-force MD simulations. Under identical forces, nicked constructs displayed greater fractional extensions than intact duplexes (Fig. 2.6), consistent with increased local flexibility from fraying (Fig. 2.8a). Quantifying the separation of complementary bases revealed a significant increase in the region adjacent to the nick (Fig. 2.8b). The degree of fraying, defined as the number of base pairs with a mean separation > 1 nm, was estimated as 9 ± 4 nt for 704*.

If forces above the scission threshold (> 1 nN)^[168] act on such a ssDNA domain, bond rupture is expected within the frayed segment.

Backbone force analysis further supported this view: C3'–O3' bonds near the nick showed a plateau of elevated forces (Fig. 2.8c). Since generally $>90\%$ of US-induced DNA breaks occur at C–O bonds^[169] and C3'–O3' bonds are most prone to scission under these conditions,^[170] this localization explained the regiospecific, but not nucleotide-precise, cleavage observed experimentally. Thus, MD simulations suggest that force-induced fraying produces a locally exposed domain of highly strained nucleotides, rationalizing the experimentally obtained breaking distributions.

To experimentally verify this model, we altered the sequence context near the nick. Given the higher thermodynamic stability of G–C relative to A–T base pairs, the nick-flanking GC content was expected to influence the extent of fraying. Constructs AT* and GC*, with 0% and 100% GC content 30 bp upstream of the nick, shifted the breaking distribution peak μ by -1 and $+1$ bp, respectively, relative to 704* (Fig. 2.9). Moreover, GC* showed a narrower distribution (σ decreased by 21%), in line with MD simulations indicating shorter fraying widths for GC-rich contexts (6 ± 3 nt) compared to AT-rich ones (12 ± 7 nt, Fig. S70). These results confirm that the stability of nick-adjacent base pairs modulates fraying and thereby governs cleavage precision.

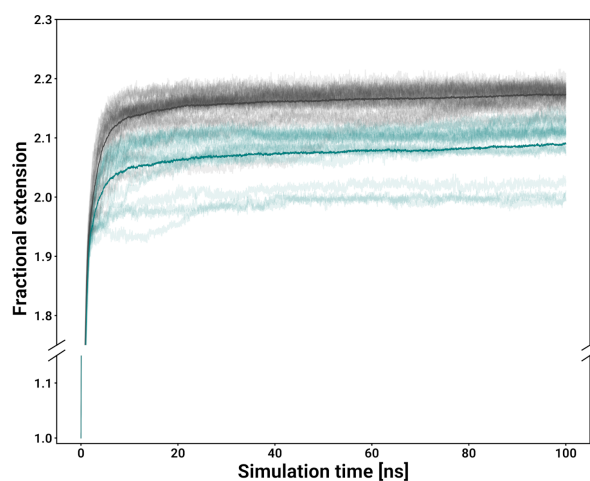


Figure 2.6: Time-dependent elongation of dsDNA under constant force. Fractional extension is plotted as the end-to-end distance over simulation time for intact (teal) and nicked (gray) duplexes. A plateau was reached after $t > 20$ ns, indicating force equilibrium. In the nicked constructs, fraying of bases adjacent to the central nick led to an increased equilibrium extension compared to the non-nicked reference. Transparent traces correspond to individual trajectories, while bold lines represent averages across all simulations (reprinted from Ref. [166], CC BY 4.0).

We next examined chain-length effects. Therefore, different lengths of dsDNA, all originating from the bacteriophage M13mp18 were amplified *via* PCR (Fig. 2.7). No side-products could be observed and the absence of a band at the height of primers indicated near-quantitative conversion. All different lengths shown in Fig. 2.7 were nicked centrally, identically to 704*. With increasing length k_{app} increases exponentially (Fig 2.10). This behavior is expected, as mechanochemical reactivity scales with the degree of polymerization.^[35] Scission products of those varying lengths were also analyzed with NGS. The hypothesis is, that if fraying magnitude dominates σ , then varying duplex length should alter the scission rate k_{app} but leave σ unchanged. Indeed, 400* cleaved 78% slower than 704* (Fig 2.10), with nearly identical σ values (Fig. 2.9). In contrast, 1500* cleaved about nine times faster but exhibited a 35% broader σ (Fig. 2.9). This suggests that for long duplexes, higher mid-chain forces increase indiscriminate fraying, producing broader distributions less dependent on local base-pair stability. Additional MD simulations across forces from 0.5–3.0 nN corroborated this interpretation. Fraying width increased only modestly at lower forces but expanded significantly at 3.0 nN (Fig. S73). Consequently, the difference in μ between 1500* and 704* (Fig. 2.9b) can be attributed to enhanced force-induced fraying, which exposes a larger ssDNA domain and broadens the scission pattern. In this regime, cleavage becomes less dependent on nick-flanking bases and more influenced by neighboring sequences and general biases observed for non-nicked dsDNA.

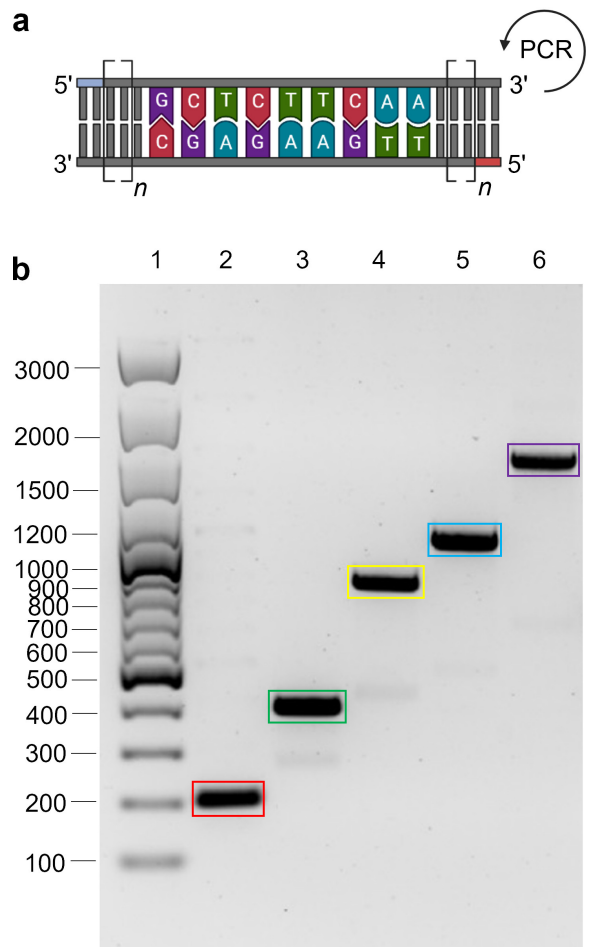


Figure 2.7: (a) Schematic representation of the different dsDNA constructs with different lengths. (b) Agarose GE: lane 1, 100 bp Plus ladder; lane 2, PCR product 200; lane 3, 400; lane 4, 800; lane 5, 1000; lane 6, 1500. Colored boxes highlight the respective fragments: red, 200; green, ds400; yellow, 800; blue, 1000; purple, 1500. Created with BioRender.com

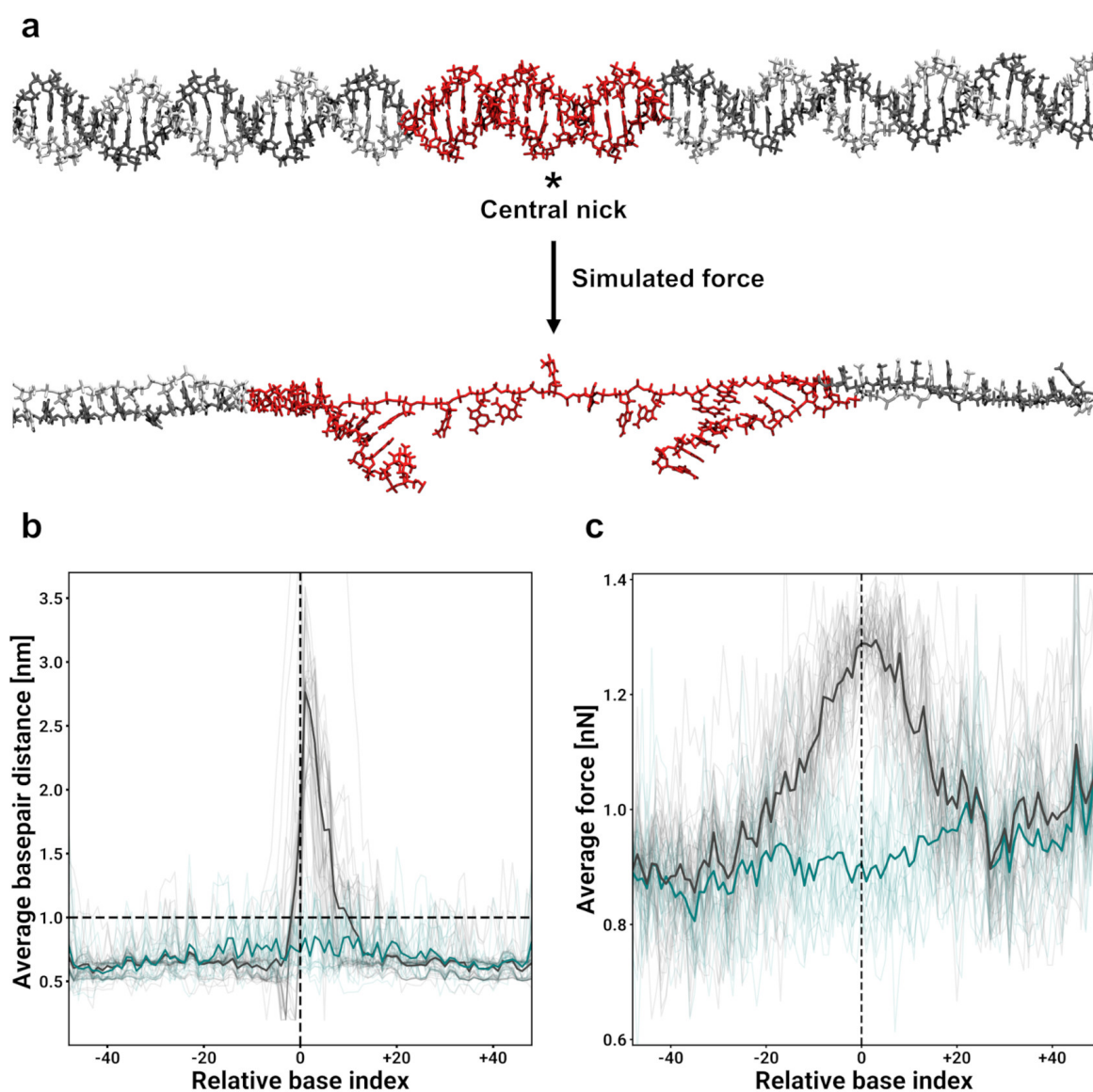


Figure 2.8: Fully atomistic constant-force MD simulations of nicked dsDNA indicate that local fraying gives rise to the observed breaking distributions. (a) Representative snapshot from a trajectory in force equilibrium ($t > 20$ ns) after applying a pulling force of 0.83 nN on both termini of a 100 bp duplex. Bases adjacent to the nick undergo partial melting, exposing a ssDNA region (red). (b) Time-averaged base-pair distances for intact (teal) and nicked (gray) dsDNA. The peak at the nick position confirms the presence of fraying in the nicked construct. (c) Average force on the C3'–O3' bonds during force equilibrium for nicked (gray) and non-nicked (teal) dsDNA. A broad force plateau centered on the nick highlights stress localization at the frayed region, where bond scission is expected. Transparent lines represent individual trajectories; bold lines denote averages over 20 simulations (reprinted from Ref. [166], CC BY 4.0).

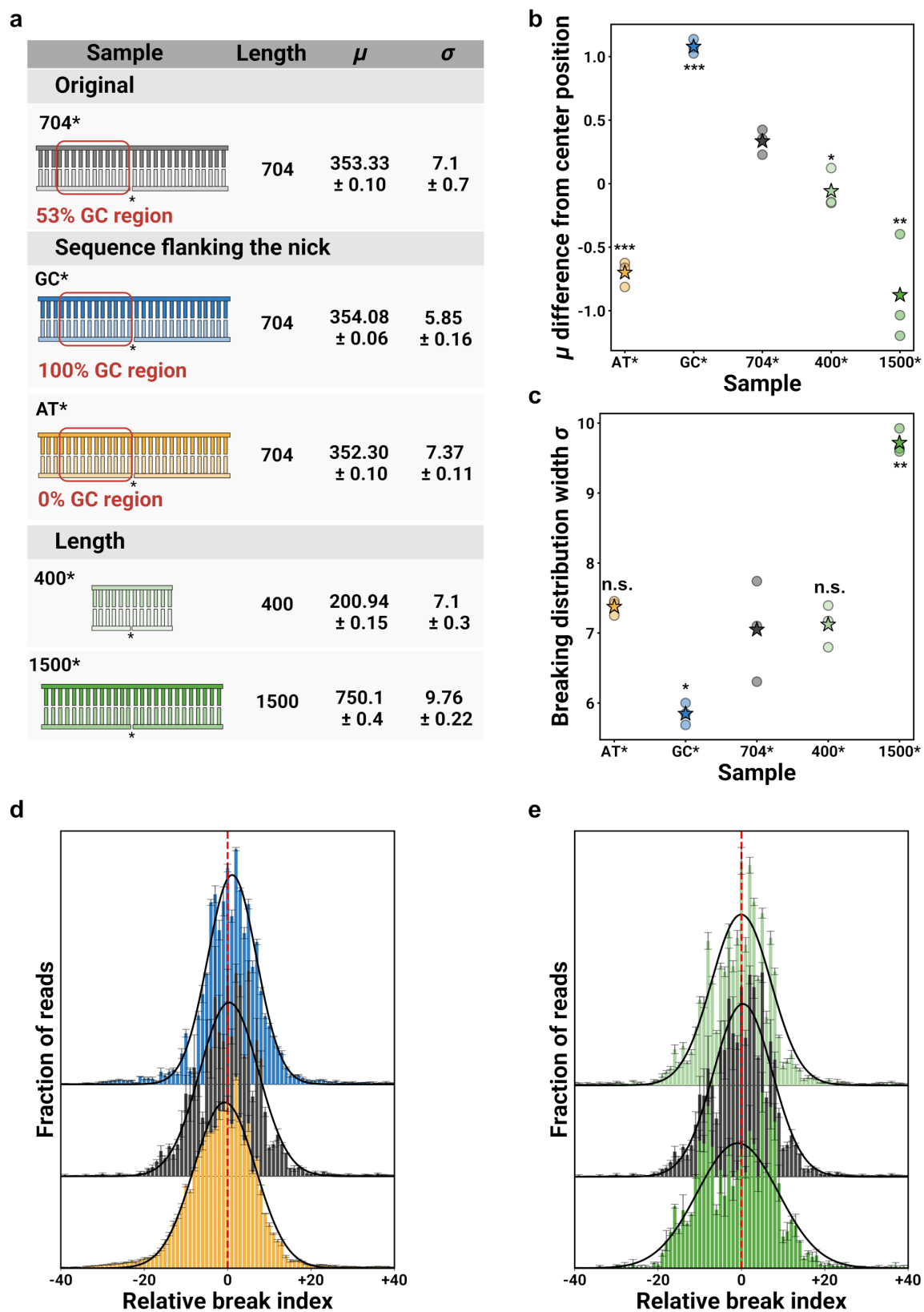


Figure 2.9: Control of DNA breaking distributions by the sequence context flanking the nick and by polymer length. (a) Schematic overview of the varied parameters, the resulting DNA

constructs, and the corresponding Gaussian fit parameters μ and σ , averaged over three independent experiments. Error bars represent the standard deviation. (b) Values of μ obtained from Gaussian fits of the breaking distributions for different nicked DNA samples. Stars indicate the mean values. (c) Values of σ obtained from Gaussian fits of the breaking distributions for different nicked DNA samples. Stars indicate the mean values. Statistically significant deviations in μ or σ compared with 704* are noted above the datasets. (d) Breaking distributions of GC* (blue), 704* (gray), and AT* (yellow), plotted as relative break index normalized to the nick position. (e) Breaking distributions of 400* (light green), 704* (gray), and 1500* (green), plotted as relative break index normalized to the nick position. For all histograms, bars represent averages of three independent experiments, and error bars denote the standard deviation (reprinted from Ref. [166], CC BY 4.0).

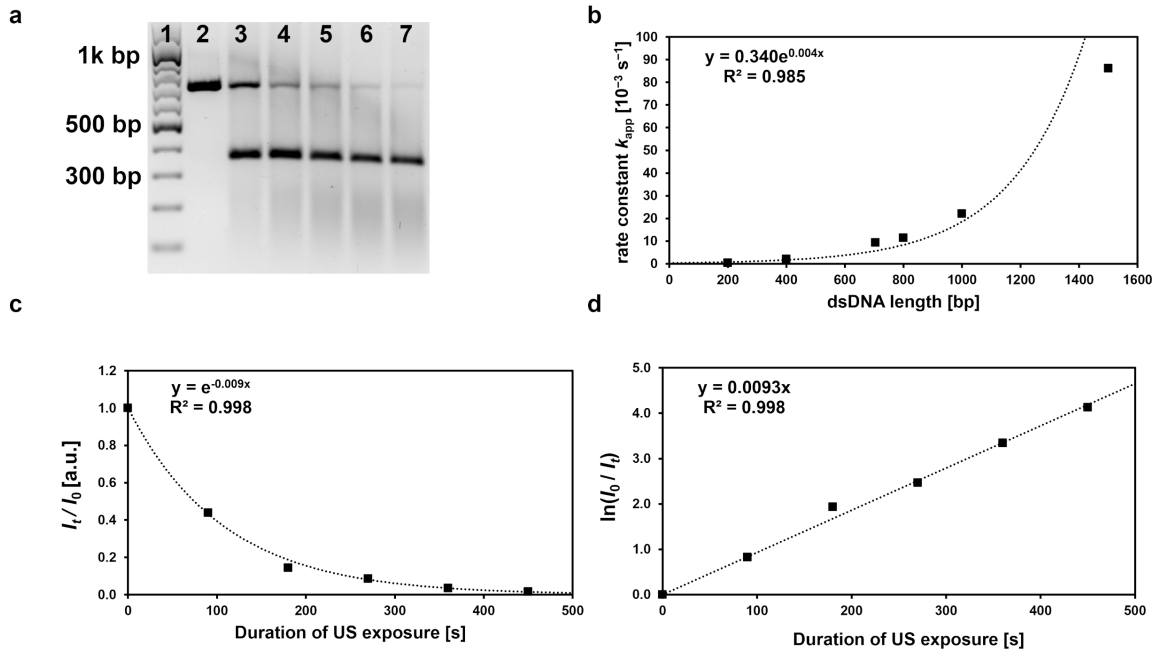


Figure 2.10: Kinetic analysis of precise bond scission in nicked dsDNA. (a) Agarose GE of 704* subjected to US at different time points. Lane 1, 100 bp Plus ladder; lane 2, untreated 704*; lanes 3–7, samples after US exposure for 90, 180, 270, 360, and 450 s, respectively. (b) Apparent rate constants (k_{app}) as a function of dsDNA length. (c) Exponential decay of normalized band intensity for 704* after six different sonication times, consistent with first-order kinetics. (d) Linear regression of $\ln(I_0/I_t)$ versus sonication time, with the slope corresponding to k_{app} . Here, I_0 is the band intensity of untreated DNA (lane 2), and I_t the intensity of the residual band after US exposure time t (lanes 3–7) (reprinted from Ref. [166], CC BY 4.0).

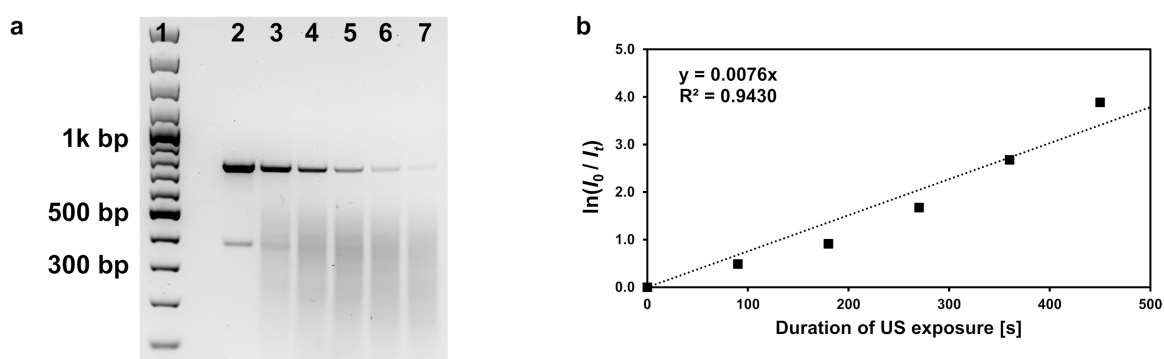


Figure 2.11: Kinetic analysis of bond scission in non-nicked dsDNA. (a) Agarose GE of 704 exposed to US at different time points. Lane 1, 100 bp Plus ladder; lane 2, untreated 704; lanes 3–7, samples after US treatment for 90, 180, 270, 360, and 450 s, respectively. The distinct band at ~400 bp in the untreated sample corresponds to single-stranded 704, which was completely degraded after 90 s of sonication. (b) Linear regression of $\ln(I_0/I_t)$ versus sonication time, yielding k_{app} as the slope. Here, I_0 is the band intensity of untreated DNA (lane 2), and I_t the residual band intensity after US exposure time t (lanes 3–7) (reprinted from Ref. [166], CC BY 4.0).

2.3.3 Tuning Fraying Width by Changing Single Nucleotides

To investigate whether the scission distribution could be modulated more precisely, we extended our strategy beyond altering only the GC content flanking the nick. Instead, we modified the immediate sequence context on both sides of the nick in a stepwise manner. For this purpose, a nicking enzyme was chosen that permitted sequence variation within ± 4 bp of the nicking site. A set of constructs, termed **ATMi0*** to **ATMi4***, was designed with mirrored sequences 20 bp upstream and downstream of the nick, where the AT content was progressively increased by one base pair within the first four positions on each side (Fig. 2.12a). In all variants, the remaining 17 bp contained a fixed AT content of 12%. This design was intended to gradually enhance fraying and thereby broaden the scission distribution by systematically varying single nucleotides.

Analysis of the resulting breaking distributions showed that the center of cleavage (μ) remained essentially unchanged with increasing AT content. Statistical regression confirmed that neither the slope nor the intercept differed significantly from zero, indicating the absence of a systematic shift in the distribution center. In contrast, the distribution width (σ) exhibited a clear linear increase with higher AT content (Fig. 2.12c), demonstrating that single-nucleotide changes directly influence the extent of fraying.

In summary, the mirrored **ATMi** sequences provide a rational method to tune the breadth of the scission distribution while maintaining the cleavage center at the nick. These results highlight the sensitivity of the fraying process to subtle differences in base-pairing stability, even at the level of a single hydrogen bond. More broadly, the approach illustrates how targeted sequence design can be used to systematically modulate fraying and thereby control mechanochemical reactivity. In future polymer architectures, this principle could be extended to integrate multiple mechanophore analogs with tailored sensitivities toward ultrasound activation.

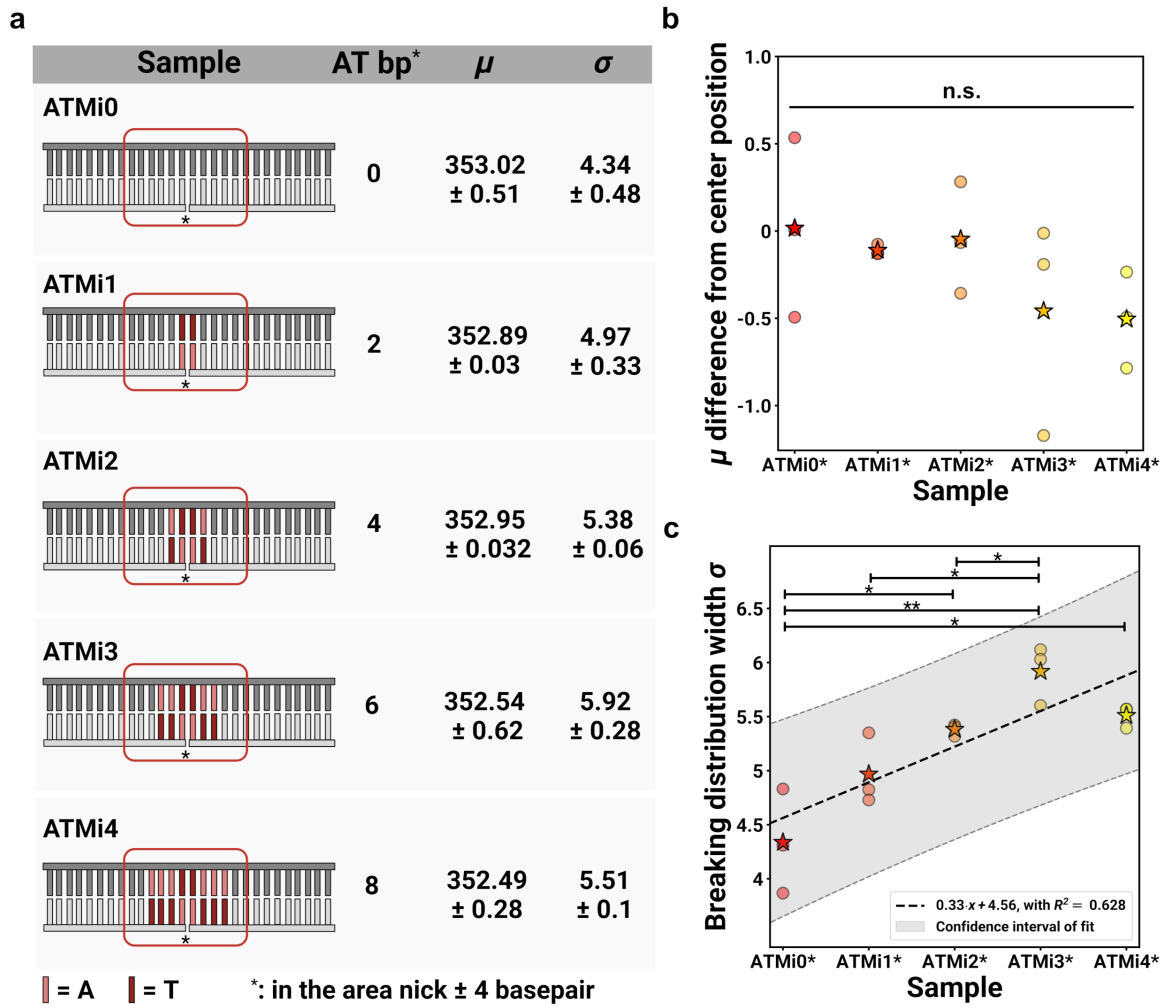


Figure 2.12: Control of breaking distributions by single-nucleotide changes. (a) Schematic illustration of the sequence modifications, the resulting constructs, and the Gaussian fit parameters μ and σ obtained from three independent experiments (mean $\pm$ SD). (b) μ values for nicked DNA samples with symmetrically increased AT content flanking the nick. Stars represent mean values; n.s. indicates no statistically significant differences between samples. (c) σ values for the same constructs. Stars denote mean values; the dashed line represents a linear regression with 95% confidence interval shown in gray. Statistically significant differences are marked with * for $p \leq 0.05$ and ** for $p \leq 0.01$ (reprinted from Ref. [166], CC BY 4.0).

2.3.4 Taking the Technique Further: Shifted Nicks and Multiple Nicks

To further explore the positional behavior of mechanophore analogs in DNA, we examined how scission selectivity changes when nicks are shifted from central to peripheral positions, and we investigated constructs containing two nicks to enable hierarchical cleavage.

In the first system, the 1500* construct was modified so that the nick was positioned between bases 280 and 281 (Fig. 2.13a). Ultrasonication of this construct produced two distinct and well-defined bands (Fig. 2.13b), demonstrating that precise cleavage could be maintained even when the nick was relocated away from the chain center. These results align with earlier observations by O'Neill and Boulatov, who showed that apparent mid-chain selectivity is primarily a statistical effect rather than a direct consequence of force distribution along the chain.^[39]

In the second system, we designed a construct with two programmed nicking sites to probe sequential scission events. Specifically, an additional nick was introduced into 1500* between bases 280 and 281, while the original nick between bases 750 and 751 was retained (Fig. 2.13c). GE after sonication revealed three distinct fragments, with cleavage occurring preferentially at the central nick (Fig. 2.13d). Upon extended sonication, the fragment carrying the peripheral nick underwent further cleavage, as reflected by the gradual loss of the 750 bp band and the appearance of two smaller products (280 and 470 bp).

These findings show that in double-nicked constructs, bond rupture proceeds hierarchically: the central nick is cleaved first, followed by cleavage at the peripheral nick. Such controlled sequential scission would be extremely challenging to reproduce in synthetic polymers, where positioning multiple mechanophores with high precision is synthetically demanding.^[171] Double-nicked dsDNA therefore represents an attractive model system for studying intrachain scission competition with a level of precision not attainable in synthetic analogues.

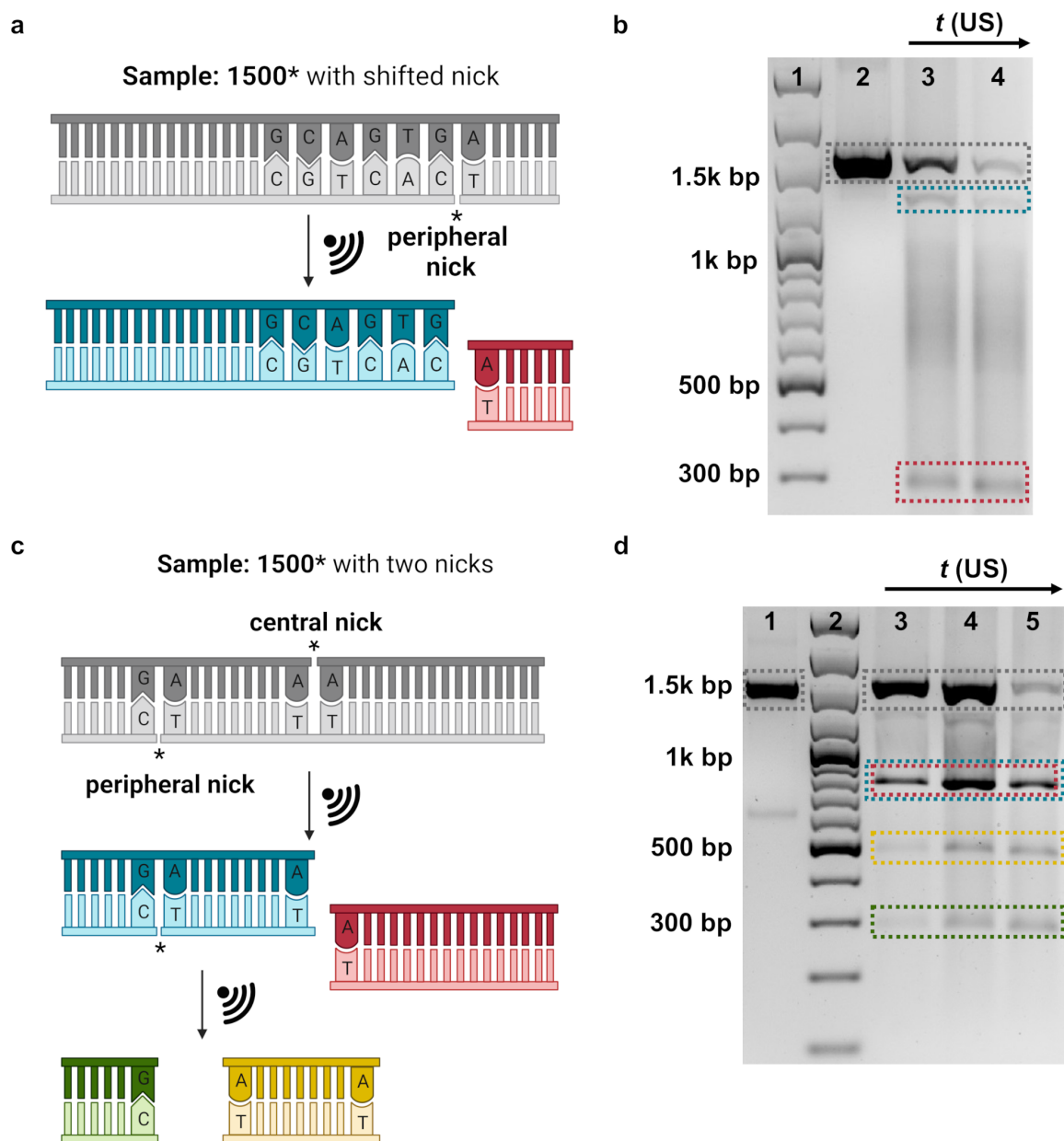


Figure 2.13: Off-center and multiple mechanophore-analog systems in dsDNA. (a) Schematic of a 1,500 bp construct with a peripheral nick introduced between bases 280 and 281. (b) Agarose GE of the construct from (a) after sonication. Lane 1, 100 bp Plus ladder; lane 2, untreated DNA; lanes 3–4, samples after 20 and 40 s US, respectively. Two major fragments (turquoise and red) were detected. The shorter fragment (red) remained stable, while the longer one (turquoise) underwent further cleavage with extended sonication. (c) Schematic of a 1,500 bp construct carrying two nicks: a central one at positions 750/751 and a peripheral one at positions 280/281. (d) Agarose GE of the construct from (c) after sonication. Lane 1, untreated DNA; lane 2, 100 bp Plus ladder; lanes 3–5, samples after 7, 15, and 35 s US, respectively. Initial scission occurred at the central nick, producing two main fragments (turquoise and red). With prolonged sonication, further cleavage of the turquoise fragment yielded additional products (green and yellow), demonstrating hierarchical bond scission (reprinted from Ref. [166], CC BY 4.0).

2.3.5 Translation to DNA Origami

Having established sequence-programmable, mechanophore-free scission in linear duplexes (*i.e.*, 1D systems), we next investigated whether the same concept could be extended to DNA origami architectures, where the complex network of crossovers and staple connections distributes mechanical forces in a non-uniform manner. Two origami designs were selected: a 2D RRO and a 3D 6HB, representing sheet-like and rod-like geometries, respectively. This expansion aimed to translate the concept from linear to multidimensional DNA systems. By default, DNA origami structures contain hundreds of staples, each introducing a nick in the phosphate backbone and thereby acting as a potential mechanophore analog. To improve control over force transmission and prevent uncontrolled strand separation, we also intended to selectively “seal” specific nicks using light-induced thymidine dimerization, effectively shielding them from mechanical activation.

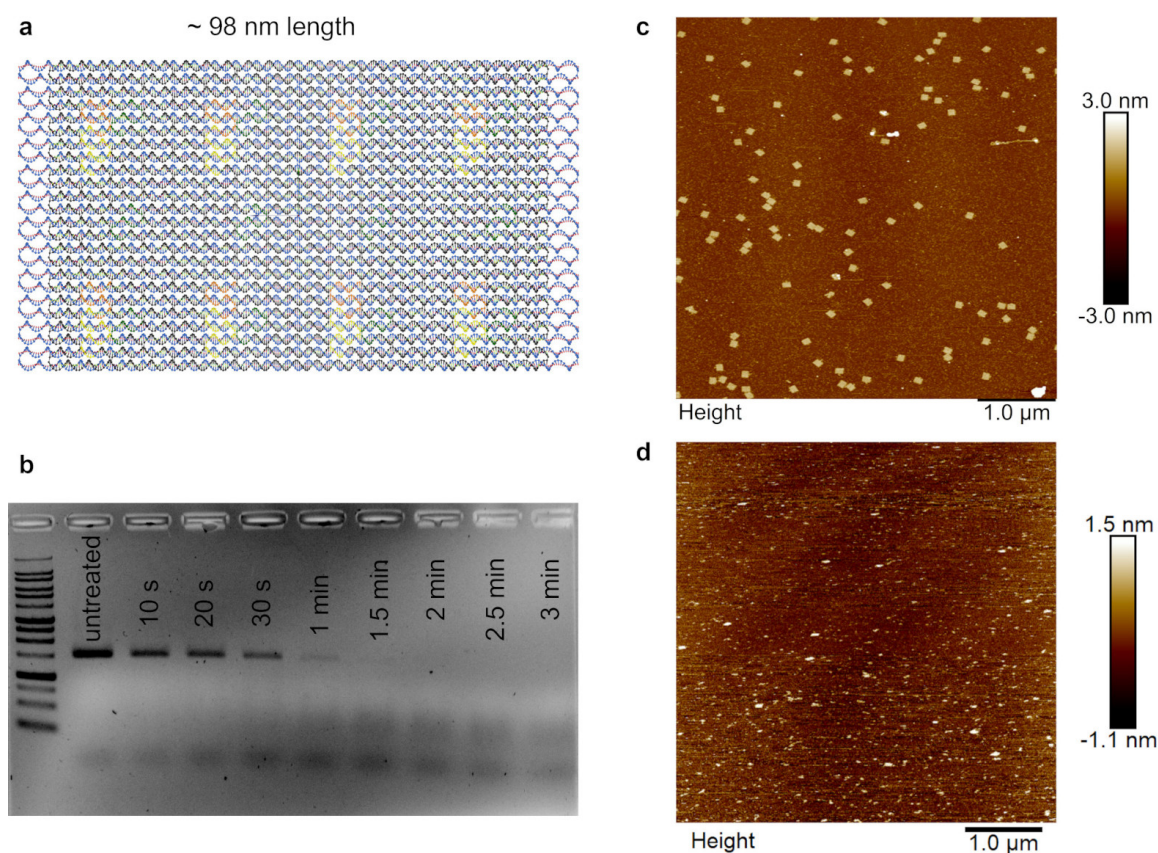


Figure 2.14: Sonication of RRO. (a) Schematic of the expected 2D sheet structure generated with oxView.^[172] (b) Agarose GE of untreated and sonicated RRO at indicated time points (10 s - 3 min). (c) AFM image of untreated RRO showing intact rectangular structures. The small protrusion corresponds to the unpaired ssDNA loop from the M13mp18 scaffold. (d) AFM image of RRO after 1 min of US showing disintegration into small DNA fragments adhered to the mica.

The origami structures were assembled following the procedure outlined in Section 2.2. Design files were kindly provided by the Hariadi group. The RRO folded successfully, as confirmed by a distinct band in the agarose gel and intact structures in AFM images (Fig. 2.14c). The absence of free staples, ensured through molecular-weight cut-off filtration,

confirmed efficient purification prior to imaging and electrophoresis. The AFM images revealed the characteristic rectangular geometry of the RRO, including the single-stranded loop of the M13mp18 scaffold visible as a small protrusion. After only one minute of 20 kHz ultrasonication, the RRO structure was completely degraded (Fig. 2.14b,d). While the AFM showed only small, undefined fragments, the agarose gel displayed a smear of short DNA fragments up to a defined threshold length, indicating non-selective breakage across multiple weak points.

Given the anisotropic shape and extended contour length of the 6HB (Fig. 2.15a), a different mechanical response under sonication was expected. The 6HB folded cleanly, as evidenced by a single sharp gel band (Fig. 2.15b) and the presence of intact rod-like structures in AFM images (Fig. 2.15c). After only 30 s of sonication, the DNA rods exhibited fragmentation approximately at half their original length (Fig. 2.15b). AFM imaging confirmed that the structures did not unfold or denature, as their height remained constant, while their length was reduced to roughly 50% (Fig. 2.15d).

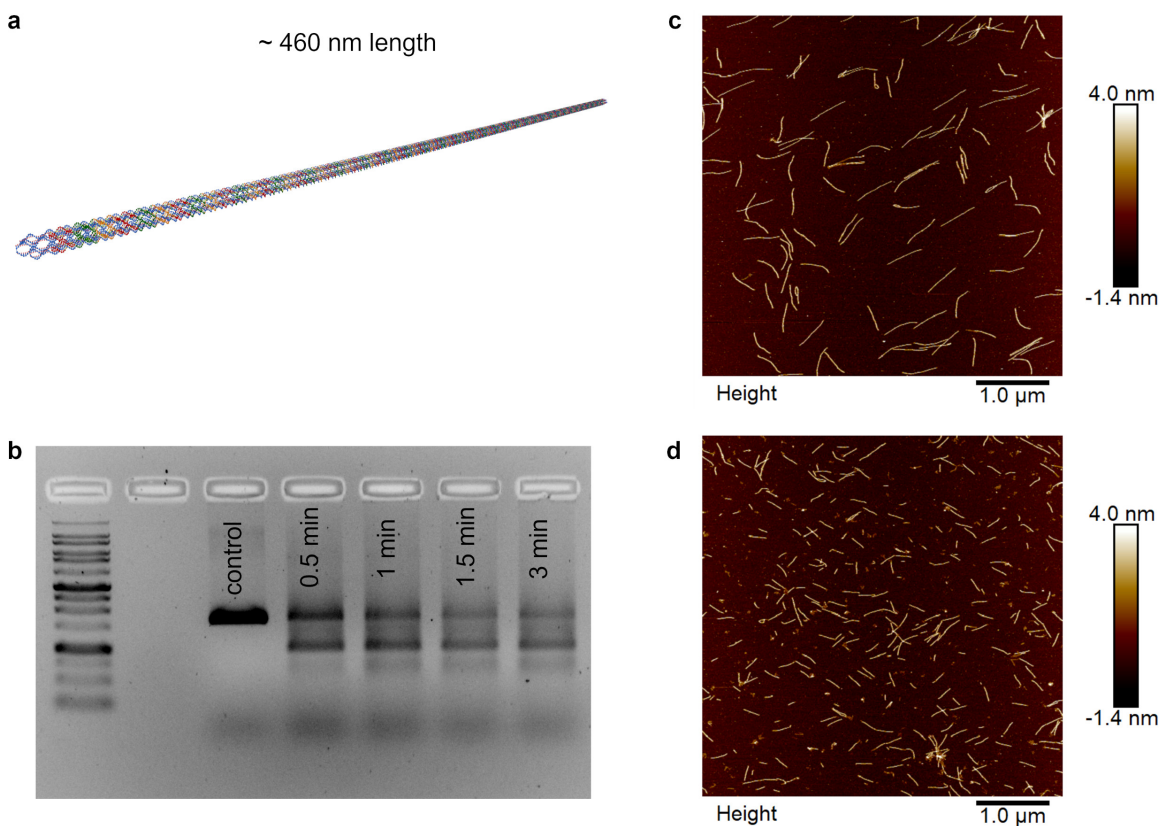


Figure 2.15: Sonication of 6HB. (a) Schematic of the expected 3D rod-shaped structure generated with oxView.^[172] (b) Agarose GE of untreated and sonicated 6HB at indicated time intervals (30 s – 3 min). (c) AFM image of intact, unsonicated 6HB structures. (d) AFM image of 6HB after 30 s of US, showing shortened but structurally intact rods.

Both DNA origami architectures contain hundreds of nicks, each of which can serve as a potential initiation site for fraying and mechanical scission. The indiscriminate breakage observed for the RRO is consistent with this, suggesting that cleavage occurs at multiple weak points across the structure. In contrast, the 6HB exhibited a more defined central cleavage pattern, resembling the “mid-chain bias” characteristic of stiff polymers with high

persistence length. Thus, despite its numerous nicks, the 6HB preferentially ruptured near its midpoint.

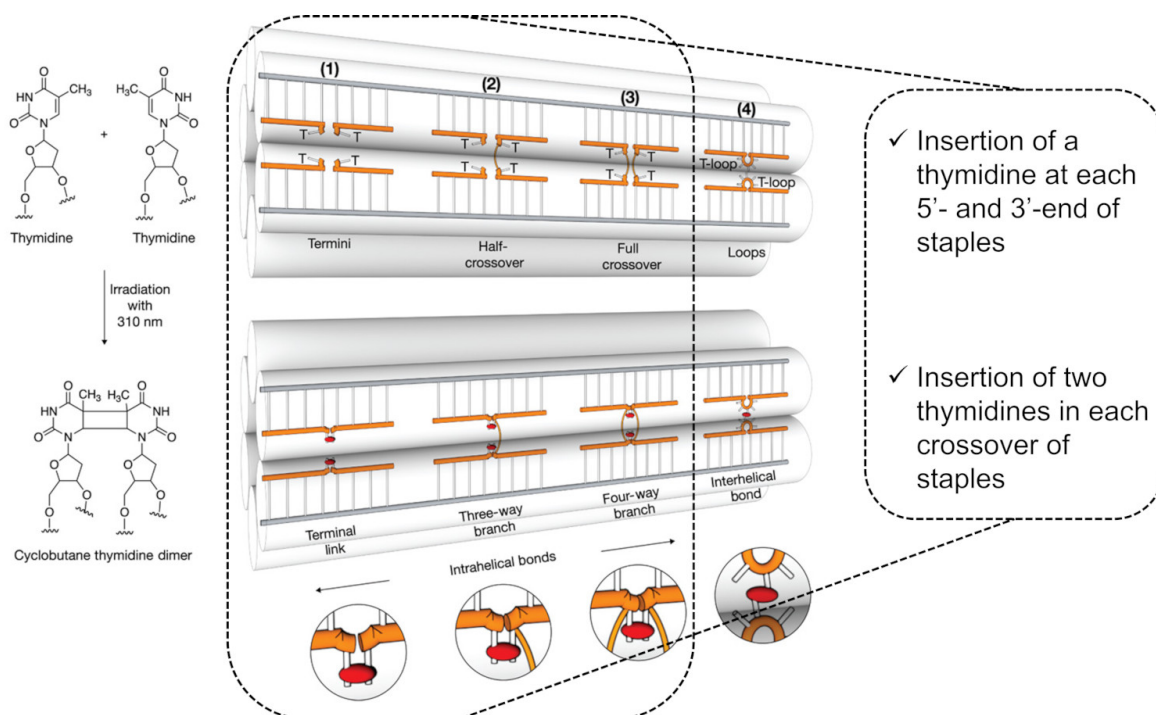


Figure 2.16: Proximal thymidines can act as photo-crosslinking sites within DNA nanostructures. Before UV irradiation, thymidines can occur at strand ends, half crossovers, full crossovers, or thymidine loops. Upon exposure to 310 nm light, cyclobutane pyrimidine dimers (CPDs) form at these sites (red ellipsoids). Motifs (1)–(3) were employed in this work (adapted from Ref. [173], CC BY-NC 4.0).

Encouraged by these findings, we sought to further improve scission selectivity and potentially shift the breaking site along the structure. Gerling *et al.* demonstrated that DNA origami can be selectively photo-crosslinked *via* thymidine overhangs at strand termini, such as at nicks where the 5' end of one staple meets the 3' end of another (Fig. 2.16).^[173] Building on this concept, we modified all 188 staples of the 6HB with terminal thymidines at both ends, as well as two additional thymidines per crossover, allowing controlled dimerization upon UV irradiation.

Following assembly, a temperature stability assay compared UV-treated and untreated samples (Fig. 2.17). After incubation at 60 °C for 30 min, untreated samples displayed significant dehybridization of staples, while UV-treated samples showed the formation of

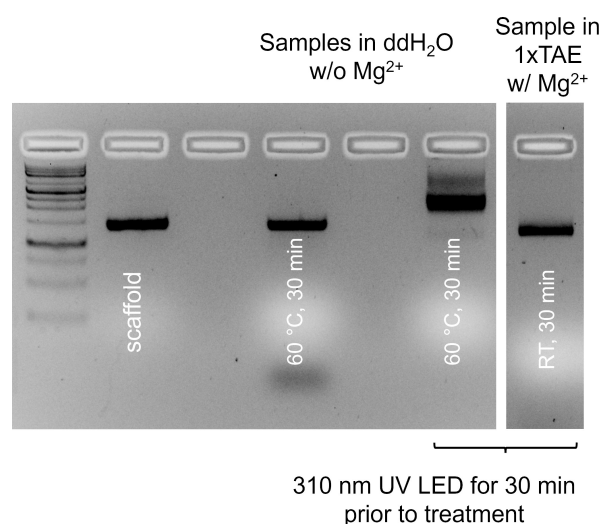


Figure 2.17: (a) Agarose GE of 6HB with and without UV treatment, followed by heating to 60 °C.

UV-treated samples showed the formation of

higher-order species (dimers and trimers) but no detectable staple dissociation. A control experiment at room temperature under UV exposure produced no dimers, confirming that the formation of dimers and trimers occurred only upon subsequent heating and not due to UV exposure. These results verified successful thymidine dimerization and crosslink formation as intended.

We next examined whether these photo-crosslinked thymidine motifs (Fig. 2.16) influenced sonication behavior. Agarose gel analysis showed that UV-treated 6HB constructs still fractured predominantly near their midpoint (Fig. 2.18a,b). However, scission occurred substantially faster—after only 1 min of ultrasound exposure, no intact structures remained, and the reaction appeared complete. Notably, the gel pattern was cleaner, with fewer by-products, indicating a more uniform fragmentation process.

To test whether the cleavage site could be shifted away from the center, we designed a variant in which four staples at approximately 25% of the total contour length were left unmodified and therefore could not form thymidine dimers (Fig. 2.18c). This design was intended to create a localized mechanical weak point. Nevertheless, sonication produced similar results to the fully modified construct, with scission still occurring at the midpoint (Fig. 2.18d). The anticipated asymmetric fragmentation into two unequal products was not achieved.

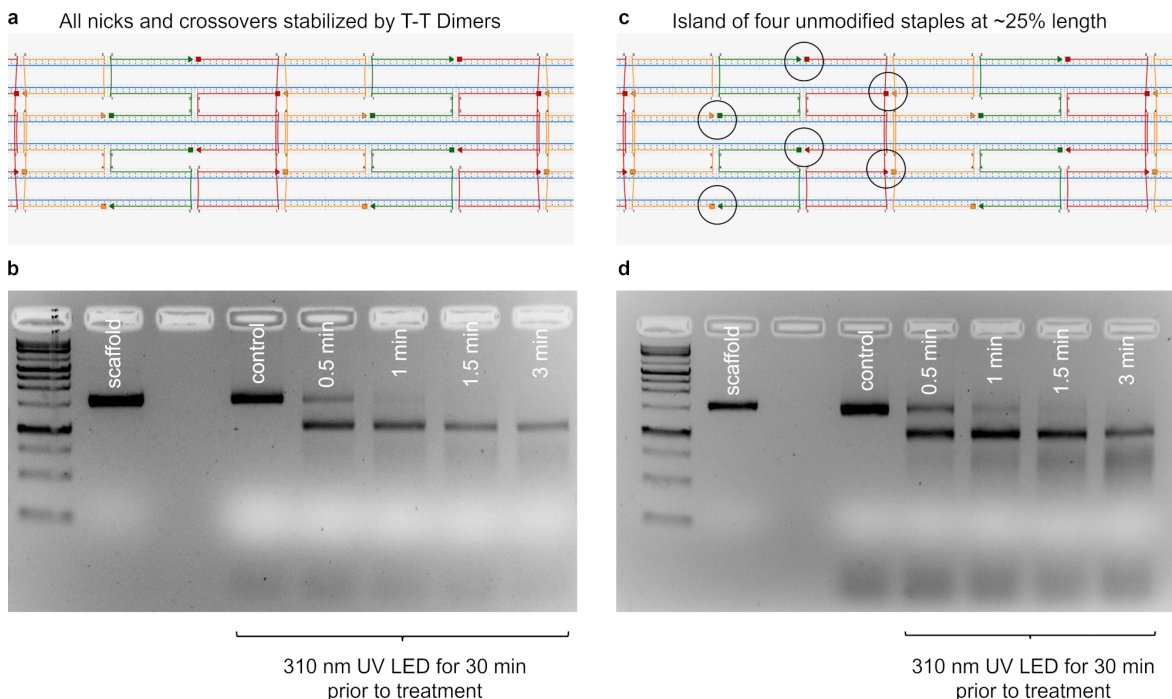


Figure 2.18: Sonication of UV-treated 6HB with thymidine-modified staples. (a) caDNAno design of fully modified 6HB, with all staples containing thymidines for UV-induced dimerization. (b) Agarose GE of UV-dimerized 6HB after increasing sonication times (up to 3 min). (c) caDNAno design of partially modified 6HB, where four staples at ~25% of the total length (green and red) were left unmodified, leaving unsealed nicks or crossovers (indicated by circles). (d) Agarose GE of the partially modified construct after sonication (up to 3 min).

The contrasting responses of the RRO and 6HB to ultrasound highlight how DNA origami architecture governs the localization of mechanical damage. The RRO, being a thin 2D sheet with extensive lateral connectivity and numerous nicks distributed across its surface, lacks a dominant axis along which tension can accumulate. As a result, the structure behaves mechanically like a network with many equivalent weak points and sonication produces highly heterogeneous breakage. The AFM images and smeared gel profile are consistent with a delocalized failure mechanism in which fraying and scission occur simultaneously at multiple sites rather than along a single force pathway.

In contrast, the 6HB behaves more like a semiflexible rod with a well-defined long axis and a high effective persistence length. Even though it contains hundreds of nicks—each, in principle, a mechanochemical initiation point—its anisotropic geometry channels tensile forces along its length. This produces a classical "mid-chain bias", reminiscent of force-induced rupture in stiff polymers, where the highest tension accumulates near the center. The observation that the 6HB shortens symmetrically while preserving its cross-section strongly suggests that backbone rupture, rather than global unfolding, is responsible for the fragmentation pattern.

The UV-induced thymidine crosslinks provide further insight into the mechanical landscape of the 6HB. Crosslinking many of the nicks strengthens the structure globally, resulting in a cleaner fragmentation pattern and faster overall scission kinetics. However, the persistence of central cleavage – even when a nominally weaker region was deliberately introduced at 25% of the contour length – indicates that force redistribution within the 6HB is dominated by its macroscopic geometry rather than by local sequence or crossover modifications. In other words, the architecture enforces a mechanical "funnel" that directs stress to the midpoint. This overpowers relatively subtle changes in local nick sealing.

Collectively, these findings suggest that in DNA origami, architectural anisotropy is a much stronger determinant of force localization than the presence or absence of individual nicks. While crosslinking modulates the uniformity and rate of scission, it does not fundamentally alter the underlying force propagation pathway. This has important implications for the rational design of mechanochemically responsive nanostructures: achieving programmable scission sites in multidimensional architectures may require more drastic geometric re-engineering. This could entail asymmetric cross-sections, tapered rod designs, or intentional gradients in stiffness, rather than only local modifications at strand junctions.

Overall, the behavior of RRO and 6HB under ultrasound underscores that extending sequence-programmable mechanochemical activation from 1D duplexes to 2D and 3D DNA architectures introduces an additional structural hierarchy that strongly influences where mechanical forces accumulate. These insights inform the design principles needed to achieve selective and predictable mechanochemical responses in complex DNA nanostructures.

2.4 Conclusion and Outlook

In this chapter, we demonstrated that a single nick in the phosphate backbone of dsDNA can act as a minimal and efficient mechanophore analog, enabling precise ultrasound-induced bond scission. Mechanical tension transmitted along the DNA backbone was focused onto the complementary strand opposite the nick, leading to highly site-specific cleavage confirmed at near-nucleotide resolution by Illumina sequencing. Constant-force MD simulations identified localized fraying of the overstretched duplex as the origin of minor

deviations from perfect scission specificity. While such melting behavior had previously been observed in optical tweezers experiments,^[167] its mechanistic role under flow-induced polymer mechanochemistry had not been shown before.

By systematically varying the nucleotide sequence surrounding the nick, we were able to tune both the precision and position of the cleavage distribution. The observed correlation between local base-pairing strength, fraying dynamics, and mechanochemical reactivity underscores the ability of DNA to function as a programmable, sequence-defined polymer for force-responsive studies. The combination of NGS and MD simulations provides a high-resolution, quantitative method to map scission distributions and directly relate local sequence context to bond reactivity.

This work establishes DNA as a powerful model polymer for studying mechanochemical processes with molecular precision, offering an elegant alternative to synthetically demanding systems in conventional polymer mechanochemistry.^[34] The integration of NGS as an analytical tool further provides a scalable, high-throughput method that surpasses traditional bulk characterization techniques such as GPC or fluorogenic assays. Beyond fundamental studies, the approach holds potential for applications in DNA-based materials where controlled mechanical response is critical. Building upon this foundation, we extended the concept to multidimensional DNA origami systems. Both the 2D RRO and the 3D 6HB were subjected to sonication to examine how complex crossover networks and connectivity influence force propagation. While the RRO exhibited non-selective fragmentation consistent with numerous equivalent nicks, the 6HB displayed a clear mid-chain scission bias, reminiscent of tension localization observed in synthetic polymers with high persistence length. This demonstrates that the mechanochemical response can persist even in topologically intricate nanostructures.

To further refine mechanical control, we employed UV-induced thymidine dimerization to covalently seal most nicks within the 6HB, thereby reducing the number of force-sensitive sites. Temperature assays confirmed successful crosslinking, and subsequent sonication revealed faster and more precise scission kinetics. Although shifting the cleavage point away from the midpoint was not achieved, the results indicate that global force focusing in stiff DNA architectures dominates over local sequence-dependent instabilities.

Looking forward, future studies could exploit this understanding to design more advanced mechanically programmable DNA systems. Leaving larger regions of staples unmodified could create extended domains of mechanical weakness, enabling controlled multi-step fracture behavior or cooperative responses. By systematically varying structure stiffness, length, and nick distribution, it may become possible to balance global and local force effects to direct scission away from the chain center. This could potentially be done by moving unsealed nicks towards ~40% of the total contour length, which hopefully surpasses the mid-chain bias.

Beyond these structural refinements, the broader outlook of this work lies in enabling mechanochemically responsive DNA nanodevices. The ability to program force localization with nucleotide precision could be leveraged for force-gated drug release, stress-reporting sensors within DNA-based materials, or mechanically triggered reconfiguration of nanoscale machines. Moreover, integrating sequence-defined mechanophore analogs into larger origami assemblies opens pathways toward hierarchical systems in which mechanical inputs propagate across multiple length scales. Such possibilities highlight the wider relevance of this work: establishing design principles for DNA architectures that convert mechanical stress into predictable molecular responses.

Chapter 3

Precise Mechanochemical Scission of DNA Guided by Secondary Structures

3.1 Introduction

Building on the concept of mechanophore-free mechanochemistry established in the previous chapter, we next sought to improve the spatial precision of force-induced DNA cleavage. While nick-programmed duplexes enabled site-selective bond rupture under ultrasound, the intrinsic base fraying around single-strand interruptions inherently limited precision at the nucleotide level.^[166] To overcome this, we turned to DNA secondary structures, specifically hairpins, as intrinsically defined, force-focusing elements capable of directing mechanical stress within the duplex.

Hairpins combine structural rigidity with programmable geometry, allowing systematic control over parameters such as loop size or stem length. To probe their potential as "biomechanophores", we designed a ~750 bp dsDNA construct with a central hairpin assembled *via* modular ligation. Variants with different stem (S), loop (L), and opposing unpaired base (N) configurations, denoted Hp(N, S, L), enabled us to examine how local structure governs scission localization.

Upon ultrasonication, these constructs yielded sharply defined fragment sizes, suggesting highly localized bond rupture near the hairpin junction. Constant-force molecular dynamics simulations confirmed that mechanical load concentrates along the strand opposite the hairpin, while NGS mapped distinct scission patterns for the two strands: a narrow, well-defined cleavage region in the lower strand and a broader, bimodal profile in the upper one. These findings reveal a two-step cleavage mechanism and establish DNA hairpins as programmable biomechanophores.

Most experimental parts of this chapter were performed together with Montgomery Labudda under my close supervision during his research internship. Yuanxu Zhou conducted PCR and restriction/digestion with established protocols during his master's thesis which was also supervised by me. Arjuna Selvakumar helped with the design of sequences and the ligation protocol. The algorithm and code for NGS analysis were developed together with Boris Schüpp, Gräter group (MPI-P, Mainz). MD simulations were conducted independently by Boris Schüpp and are included here for completeness of the story. Illumina sequencing was conducted by the Genomics Facility, a core facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen University.

3.2 Methods

Materials and Reagents

The synthetic genes for DNA_{left} and $\text{DNA}_{\text{right}}$ were obtained from Eurofins Genomics. Primers used to amplify DNA_{left} and $\text{DNA}_{\text{right}}$ were sourced from Sigma-Aldrich. HPLC-purified oligonucleotides for assembling the middle fragments (the secondary motifs) were purchased from biomers.net GmbH. Complete sequences for genes, primers, and oligonucleotides are listed in the Appendix as Fig. 5.15–5.21 and Table 5.1. DNA polymerase was supplied by Biovendis Products GmbH as Pfu/Psp polymerase (cat. no. TK-005222). dNTPs were from GeneON GmbH (cat. no. 110-012). The restriction enzymes FastDigest BstXI (cat. no. FD1024) and FastDigest Eco31I (cat. no. FD0293), as well as T4 Ligase (cat. no. EL0012), were procured from Thermo Scientific™. Agarose Standard (cat. no. 3810.4, Carl Roth) was used for agarose GE, together with ROTI®GelStain Red Eco as the intercalating dye (cat. no. 223C.1, Carl Roth). TAE buffer was prepared from Tris base (CAS 77-86-1, Fisher Bioreagents), acetic acid (CAS 64-19-7, Sigma), and disodium ethylenediaminetetraacetate dihydrate (EDTA) (CAS 60-00-4, Sigma), yielding a final 1× composition of 40 mM Tris, 20 mM acetic acid, and 2 mM EDTA. Samples were loaded using TriTrack DNA Loading Dye (6×) (cat. no. R1161) and separated against GeneRuler 100 bp Plus DNA Ladder (cat. no. SM0321), both from Thermo Scientific™. Gel bands were purified with the GFX™ PCR DNA and Gel Band Purification Kit (Cytiva, cat. no. 28903471). For library preparation of sheared dsDNA, the NEBNext Ultra II DNA Library Prep Kit (NEB, cat. no. E7645L) was employed. Illumina paired-end sequencing was performed on a MiSeq instrument using the MiSeq Reagent Micro Kit v2 (300-cycles) (Illumina, cat. no. MS-103-1002).

Generation of DNA with Central Hairpin Secondary Structure

Generation of Ligation Substrates

DNA_{left} and $\text{DNA}_{\text{right}}$ genes were amplified *via* PCR. Table 3.1 lists the components for one PCR reaction.

Table 3.1: Components for PCR amplification of DNA_{left} and $\text{DNA}_{\text{right}}$.

Components	V [μL]	c_{final}
PCR template	1	0.2 ng/ μL
Forward primer	2.75	0.688 μM
Reverse primer	2.75	0.688 μM
dNTPs	1	0.200 mM
Pfu/Psp polymerase buffer	5.0	1×
Pfu/Psp polymerase	0.4	0.04 U/ μL
Water	37.1	–
Σ	50	–

The reaction is set up in a PCR tube and then transferred to a thermocycler. In Table 3.2 the utilized PCR parameters are displayed.

Table 3.2: PCR parameters for thermocycler.

Reaction step	Cycle(s)	T [°C]	t [s]
Heat lid	–	110	∞
Initial denaturation	1	98.0	180
Denaturation	30	98.0	20
Annealing	30	66.0	30
Extension	30	72.0	60
Final extension	1	72.0	300
Storage	–	4.0	∞

The amplified DNA_{left} and $\text{DNA}_{\text{right}}$ PCR products were then purified by solution purification using the GFX PCR DNA and Gel Band Purification kit (Cytiva). The purified PCR products are then restricted by two different enzymes to generate specific sticky ends. The reaction components for the restriction digest are shown in Table 3.3. One fast digestion approach contains 1 μg of purified PCR product. The volume Y of the purified PCR product is adjusted accordingly.

Table 3.3: Restriction digest protocol for DNA_{left} (BstXI) and $\text{DNA}_{\text{right}}$ (Eco31I).

Components	V [μL]	c_{final}
PCR product (1 μg)	Y	33.3 ng/ μL
FD Green Buffer	2.40	0.8 $\times$
FD Enzyme BstXI (DNA_{left})	1.20	–
or FD Enzyme Eco31I ($\text{DNA}_{\text{right}}$)	1.20	–
Water	to 30	–
Σ	30	–

This mixture was incubated at 37 °C for 1 h. Subsequently, the enzyme was deactivated at 80 °C for 5 min. The solution was then purified from an agarose gel. The central secondary motifs (Table 5.1 in Appendix) were synthesized by targeted hybridization. The reactions were set up according to Table 3.4 and assembled by slowly cooling from 95 °C to 4 °C with 1 °C/20 s. The targeted hybridization product was used in the subsequent ligation reaction without further purification.

Table 3.4: Targeted hybridization of central secondary motifs with short oligonucleotides.

Components	V [μL]	c_{final} [μM]
Bottom strand	2.0	2.4
Top strand	2.0	2.8
T4 Ligase buffer	0.50	1 $\times$
Water	0.50	–
Σ	5.0	–

Ligation Reaction

In the first ligation central secondary motif is ligated to the left restriction product. The required reaction components for one reaction approach of this first ligation step are listed in Table 3.5. The mixture was incubated at 22 °C for 2 h and the enzyme was subsequently deactivated at 70 °C for 5 min.

Table 3.5: Ligation of central secondary motif with left restriction product.

Components	V [μL]	c_{final}
Central secondary motif	1.0	67.5 nM
Restriction product (left)	Y	100 nM
T4 Ligase buffer	2.0	1×
T4 DNA Ligase	1.5	0.38 U/ μL
Water	to 20	–
Σ	20	–

In the second ligation the right restriction product is ligated to the first ligation product. The required reaction components for one reaction approach of second ligation are listed in Table 3.6.

Table 3.6: Second ligation step to generate full-length DNA.

Components	V [μL]	c_{final}
Restriction product (right)	Y	200 nM
T4 Ligase buffer	2.0	2×
T4 DNA Ligase	0.5	0.25 U/ μL
Water	to 10	–
Σ	10	–

The reaction mixture is added directly to the first ligation reaction solution. Ligation proceeds at 8 °C for 16 h with subsequent deactivation of the enzyme at 70 °C for 5 min. To the resulting solution 3.3 μL per 30 μL 1 wt.% SDS (dilution to 0.1 wt.%) were added. Then, the solution was exposed to 60 °C for 10 min, and immediately cooled on ice. TriTrack Loading Dye (1 μL per 5 μL of sample) was quickly added, the solution was mixed thoroughly and the product was purified *via* agarose gel and GFX PCR DNA and Gel Band Purification kit (Cytiva).

Ultrasound-Induced Scission of DNA

For sonication experiments, samples were prepared by diluting 1 μg of purified full-length DNA in 30 μL of 1×

TSE buffer. The solution was then heated up to 85 °C for 5 min and subsequently hybridized by slowly cooling to 4 °C (1 °C/12 min). Sonication was carried out under ice cooling using a Q500 Sonicator (Qsonica, USA) fitted with a 3 mm diameter tip (422-A) and a single-tip horn. The operational frequency was 20 kHz and the amplitude was adjusted to 40% of its maximum (45 μm) and maintained consistently during the entire sonication process, independent of variations in concentration, temperature or viscosity of the samples. The energy input during sonication, labeled as E

(in joules), was monitored to calculate the sonication power, represented as $P = Et^{-1}$ and the power intensity, also shown as $I_P = PA^{-1}$. t is considered as the effective sonication “on” time and A as the area of the probe tip. Pulsed sonication was used, alternating between 2.0 seconds “on” and 1.0 second “off”. From this, the calculated power was $P = 2.92$ W and the power intensity was $I_P = 41.31$ W cm⁻². Subsequently, the products were concentrated (Eppendorf Concentrator plus) under reduced pressure and 60 °C to be subsequently loaded in agarose gels for purification of product bands.

Illumina Sequencing

For Illumina sequencing, the product band after US-induced scission was cut out after full conversion of the mechanochemical reaction. Subsequently, DNA was extracted from the agarose gel and afterwards library preparation was conducted using the NEBNext Ultra II DNA Library Prep Kit. Then, sequencing was performed with an Illumina MiSeq™ benchtop sequencer using a MiSeq Reagent Kit v2 Micro with 2×75 bp paired-end reads and 5 % PhiX (cat. no. FC-110-3001, Illumina). Data processing was carried out using a custom-built algorithm (available on [GitHub](#)).

Learning from the investigation in the previous chapter combined with the need for a different way of combining the reference structure with the NGS reads made improvements and changes in the algorithm necessary. Unlike in the nicked structures the secondary hairpin structures will produce fragments that cannot be clearly mapped to double stranded regions of the template. To accommodate this challenge paired reads have been decoupled and the individual single reads are mapped to either strand of the template. Additionally, high accuracy of the sequencing method allowed for the simplification of the algorithm to only consider exact matches and dropping the notion initially matched bases and permitted mismatches. As previously, we obtain a distribution of 5' ends on both strands of the template reflecting the relative probability that a fragmentation took place at a given relative base index. Compared to the previous chapter the indexing was shifted from using integers to using the true center of the sequence, meaning that the center of a DNA with an even number of nucleotides will have its zero in the bond that connects the two central residues. Finally, to better capture heavy-tailed distributions with a certain level of non-specific noise a Student's t -distribution is fitted instead of a Gaussian distribution. Instead of the standard deviation σ the interquartile range is used to describe the width of the distribution. To validate the adapted algorithm the data from the previous chapter has been rerun reproducing all qualitative findings from before.

3.3 Results and Discussion

In the search for a DNA-embeddable mechanophore with a relatively simple design that enables precise investigations of mechanical scission, the secondary structures of DNA offer a promising alternative. Owing to their unparalleled sequence controllability, structural polymorphism, and programmable functionality, these motifs present an attractive platform whose mechanophore-like behavior has, until now, remained unexplored.

We were inspired by the original crystal structure of a simple hairpin consisting of a six-nucleotide stem and a four-nucleotide loop, as reported by Chattopadhyaya *et al.*^[174] Notably, this structure lacks a complementary (template) strand on the opposite side of the hairpin. We hypothesized that, due to the orthogonal hybridization of the stem, the application of force on both strands would first lead to precise scission of the lower strand. In this case, the fraying mechanism that inherently limits the precision of mechanochemical scission, as outlined in Chapter 2, could potentially be eliminated.

However, sonication of a simple 16 nt hairpin alone would not induce mechanochemical scission, as a minimum contour length is required for efficient force transduction.^[38] Therefore, the secondary motif was incorporated into a longer DNA framework of comparable length to that used in the previously reported nicked DNA system.^[166] This was achieved using a DNA “plug and play” (DNA PnP) strategy, in which the left (DNA_{left}) and right fragments (DNA_{right}) were synthesized by PCR from commercially available, custom-designed genes (as described in Chapter 2). DNA_{left} and DNA_{right} were subsequently digested with restriction enzymes to generate specific sticky ends for ligation (Fig. 3.1).

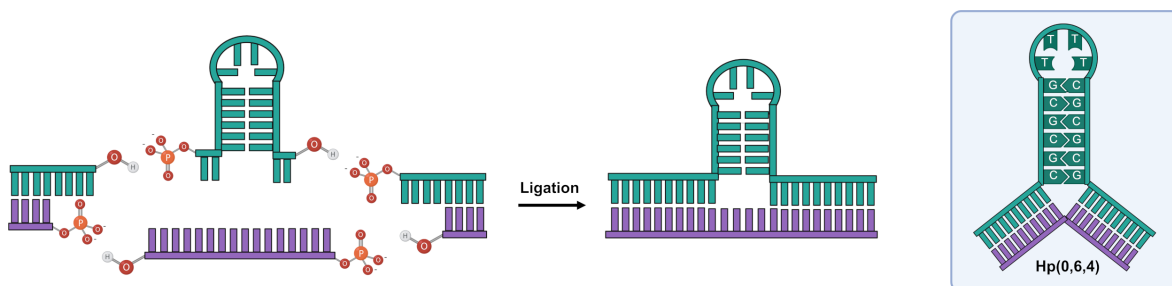


Figure 3.1: Schematic of the DNA “plug and play” (DNA PnP) system for ligating different secondary motifs into a DNA framework of sufficient length for mechanochemical activation. In this example, Hp(0,6,4) is shown, although other secondary motifs were also incorporated. Created with BioRender.com

The actual “biomechanophore” is located within a short (~70 bp) middle fragment, which was generated through targeted DNA hybridization and standard phosphoramidite solid-phase DNA synthesis. This middle fragment contains sticky-end base pairs complementary to DNA_{left} and DNA_{right}, respectively. The first step was therefore to verify that the two middle fragments hybridized correctly to form a duplex. The migration positions of Hp(0,6,4), Hp(1,6,4), and Hp(2,6,4) in the agarose gel correspond to approximately 70 bp (Fig. 3.2), consistent with the expected size of the middle fragments. As anticipated, no visible difference in electrophoretic mobility was observed among the middle fragments, since they differ only in the number of thymidines present in the bottom strand. The individual top and bottom strands applied in the gel appear at positions corresponding to ~35 bp and ~25 bp, respectively, which is approximately half of their nucleotide length. Comparison of the

starting materials with the hybridized products confirms that hybridization was successful and proceeded with high yield, as neither the top nor the bottom strand is visible in the product lanes.

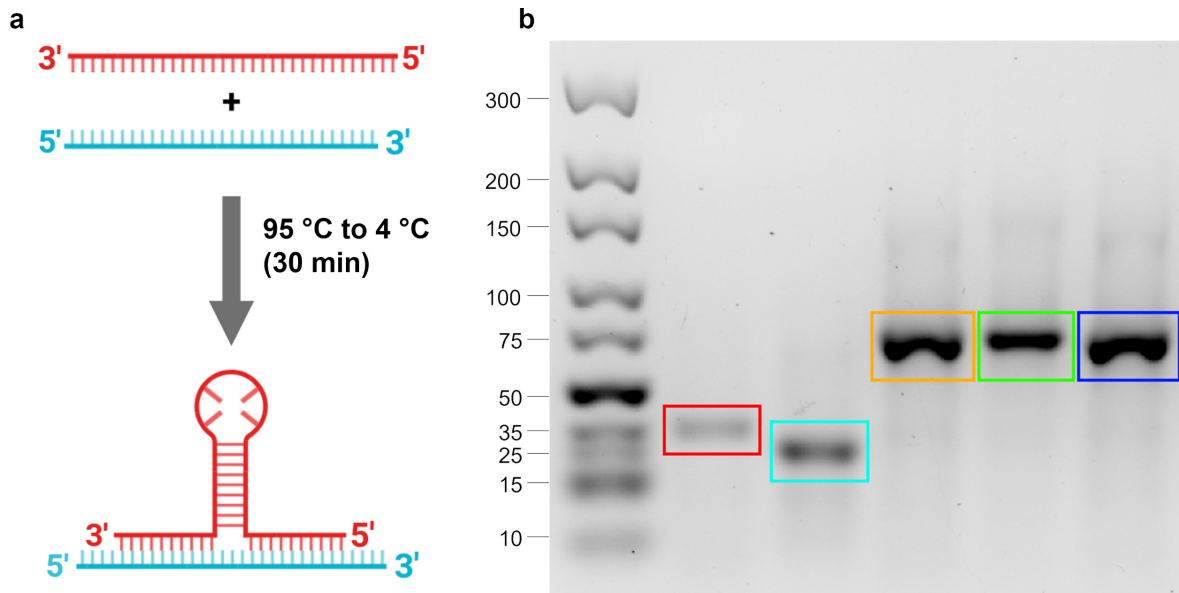


Figure 3.2: Targeted hybridization of short DNA hairpins used as middle fragments for subsequent ligation. (a) Schematic representation of the hybridization between the top strand (red) and bottom strand (cyan) forming the hairpin structure. (b) Agarose GE showing the results of the targeted hybridization of the middle fragments. Lanes from left to right: Ultra Low Range DNA Ladder, top strand (red), bottom strand (cyan), Hp(2,6,4) (orange), Hp(1,6,4) (green), and Hp(0,6,4) (blue). Created with BioRender.com

The PCR was performed using double-stranded template DNA that was intentionally designed to be slightly longer than the fragments required for ligation. Subsequent restriction digestion trimmed the amplified DNA to the desired length and generated the necessary sticky ends. The indicated fragment lengths refer to the expected total size of the final construct after complete ligation with the middle fragment (*e.g.*, $440 \times 2 + 70 = 950$ bp), from which approximately 200 bp are removed during restriction digestion. Figure 3.3 shows an agarose gel of the DNA_{left} and DNA_{right} fragments of 500, 750, and 1000 bp produced by PCR. The observed band positions are in good agreement with the expected fragment sizes.

DNA_{left} and DNA_{right} fragment of identical sizes exhibited identical lengths, with the observed bands matching the expected sizes. The 500 bp fragments migrated at approximately 300 bp, the 750 bp fragments near 450 bp, and the 1000 bp fragments around 570 bp. Minor low-intensity bands likely originated from excess single-stranded DNA due to slight primer imbalance. Overall, the PCR yielded the correct products in high purity and quantity.

The final step before connecting the three puzzle pieces into an intact construct was the restriction digestion to generate specific sticky ends using restriction enzymes. For this purpose, we employed BstXI and Eco31I, which cleave outside their recognition sites. This strategy provided the flexibility to design arbitrary sticky ends of the form NNNNN/N, where the enzyme recognition site lies upstream and downstream of the cleavage point (/). In this way, we generated the specific, non-palindromic overhangs CCTT and TCGC, which prevent self-hybridization.

Figure 3.4 shows an agarose gel containing the DNA_{left} and DNA_{right} fragments of 500, 750, and 1000 bp after restriction digestion. Compared to Figure 3.3, the corresponding bands appear at lower molecular weights, confirming the expected shortening of the fragments following digestion. Additionally, a new band is visible for each fragment at approximately 100–120 bp (colored in blue), corresponding to the cleaved-off segments generated as co-products of the restriction reaction.

The disappearance of the original, undigested PCR bands indicates that the digestion proceeded to completion with high efficiency. The expected fragment lengths after digestion are 215 bp, 340 bp, and 465 bp, which agree well with the observed values.

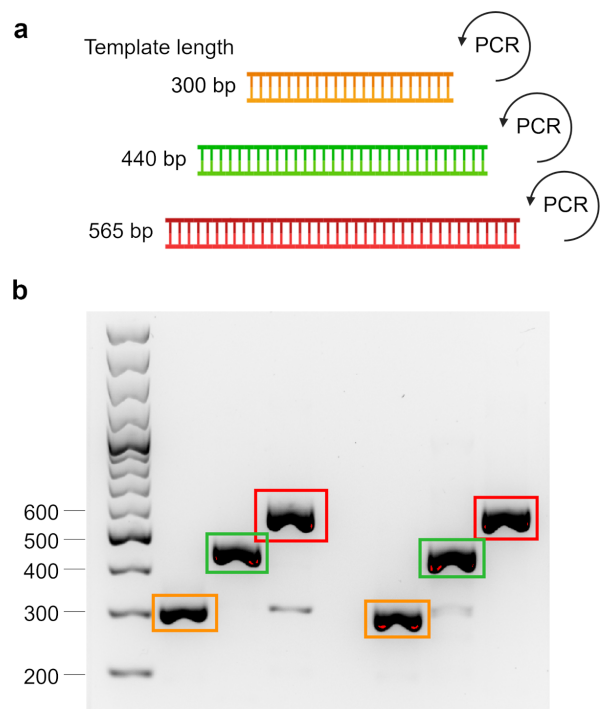


Figure 3.3: PCR amplification of differently sized DNA_{left} and DNA_{right} fragments for subsequent restriction digestion. (a) Schematic representation of template amplification at varying lengths using PCR. (b) Agarose GE of left and right fragments directly after PCR. Created with BioRender.com

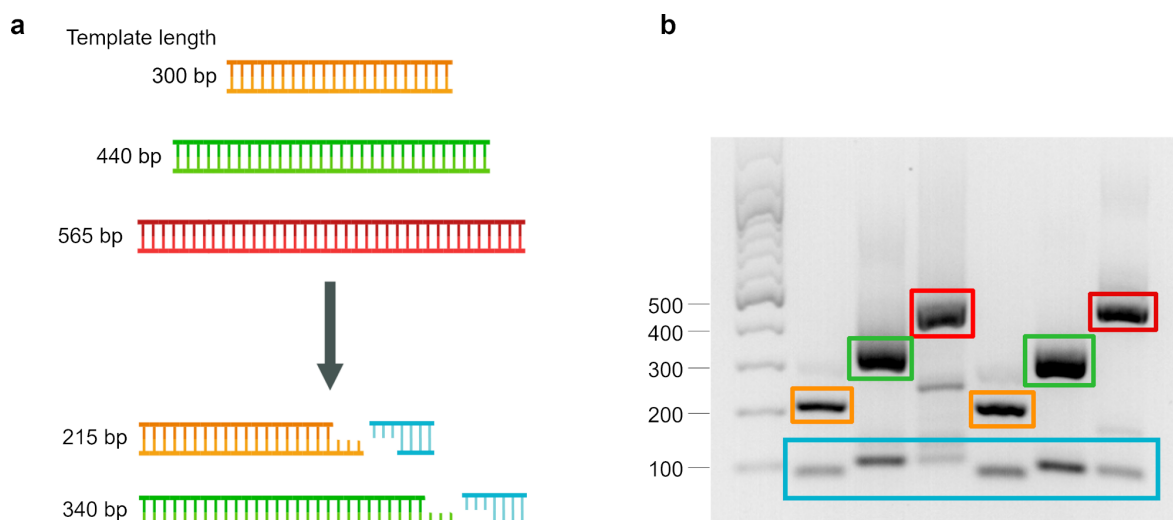


Figure 3.4: Restriction digestion of PCR-amplified fragments of three different lengths. (a) Schematic representation of the restriction digestion process. (b) Agarose GE of DNA_{left} and DNA_{right} fragments, color-coded as follows: orange (300 bp to 215 bp), green (440 bp to 340 bp), and red (565 bp to 465 bp). The blue-colored fragments are co-products of the enzymatic reaction. Created with BioRender.com

During ligation, the sticky ends of the central fragments were joined with those of DNA_{left} and DNA_{right} to obtain dsDNA constructs containing different mechanophore units and overall lengths. Figure 3.5 shows an agarose gel of the ligation products for Hp(0,6,4) with total lengths of approximately 750 and 500 bp.

Each lane displays two prominent bands. For the 750 bp construct, the main band appears at approximately 750 bp (green), and for the 500 bp construct at around 500 bp (orange). The upper bands correspond to the full-length ligation products, in which the middle fragment is successfully connected to both the left and right fragments. The lower bands represent intermediates consisting of the middle fragment ligated to only one side. The middle fragment itself, expected at around 70 bp, is barely visible, suggesting that formation of the fully ligated construct is less efficient than ligation of a single fragment.

Additional purification after the first ligation step did not significantly improve yields, although it considerably increased the overall processing time. In a comparative experiment, two parallel ligations were performed—one with and one without intermediate purification. The purified sample showed no marked improvement, likely due to DNA loss occurring during the purification step itself.

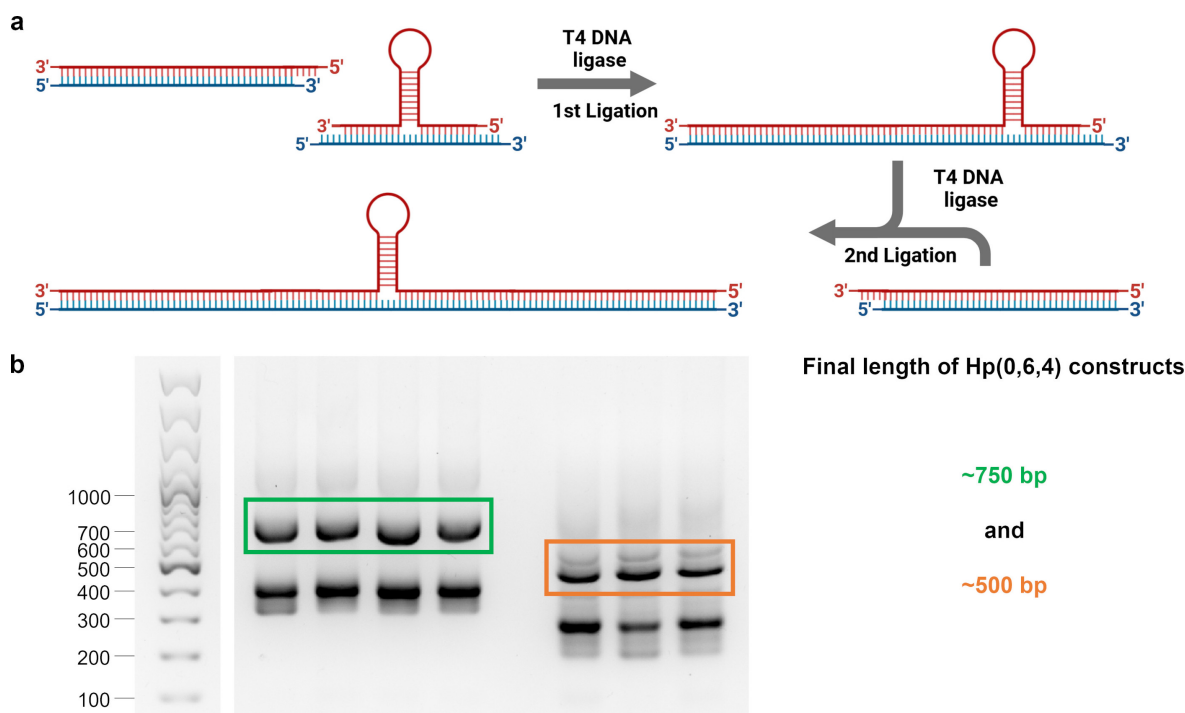


Figure 3.5: Two-step ligation process combining all three previously described DNA fragments. In the first step, DNA_{left} is ligated to the central secondary motif. Following ligation, DNA_{right} is added directly to the reaction mixture without intermediate purification to yield the complete DNA construct. (a) Schematic illustration of the ligation process. (b) Agarose GE of the ligation products corresponding to final fragment lengths of approximately 750 and 500 bp. Created with BioRender.com

Following ligation, we focused exclusively on the 750 bp constructs for all subsequent experiments, even though constructs with several other lengths were successfully synthesized. This decision was made for several reasons. First, 750 bp represents an ideal fragment length for both Illumina sequencing and efficient mechanochemical activation, as demonstrated in previous experiments. Second, maintaining a similar contour length allows for direct comparison with the nicked DNA constructs described in Chapter 2, where length-dependent effects were observed.^[166] By keeping the overall DNA length constant, we could specifically investigate the influence of secondary structure—rather than molecular length—on mechanochemical behavior.

Following ligation, it was essential to reestablish the desired dsDNA containing the hairpin structure through a targeted hybridization process. Purification using silica columns requires strongly chaotropic and denaturing environments, which can lead to partial unwinding of DNA and the appearance of single-stranded forms.^[175] Therefore, an additional annealing step is required to rehybridize the complementary strands into their correct double-stranded configuration and to repair potential structural imperfections introduced during purification. Thermal annealing facilitates this self-assembly. Upon heating, the DNA strands denature, separating into single strands, while slow cooling enables rehybridization according to sequence complementarity. This process can also resolve structural defects by allowing the system to reach thermodynamic equilibrium. From a kinetic perspective, the input of thermal energy enables the DNA to escape "kinetic traps" — local energy minima that represent metastable, non-equilibrium conformations, such as misfolded or partially hybridized

secondary structures.^[176] For subsequent sonication experiments, it is crucial that the DNA constructs occupy uniform energetic states, as variations in conformation could influence the kinetics of mechanochemical scission. However, excessive heating can lead to complete strand separation, especially in complex architectures such as DNA hairpins. Once the strands are fully dissociated, they can diffuse away from one another and compete with alternative, non-productive intermolecular pairings in solution. Therefore, the denaturation temperature must be optimized to provide sufficient thermal energy to release kinetic traps while avoiding total denaturation.

To identify suitable conditions, self-assembly experiments were performed using equal amounts of Hp(0,6,4) at temperatures ranging from 70 to 95 °C. Figure 3.6 shows that above 90 °C, the intensity of the main bands decreases markedly, accompanied by increased smearing in the gel. This smearing indicates the formation of higher-molecular-weight aggregates derived from denatured DNA strands. Based on these observations, 85 °C was selected as an optimal annealing temperature, providing sufficient energy to promote correct self-assembly while minimizing irreversible denaturation.

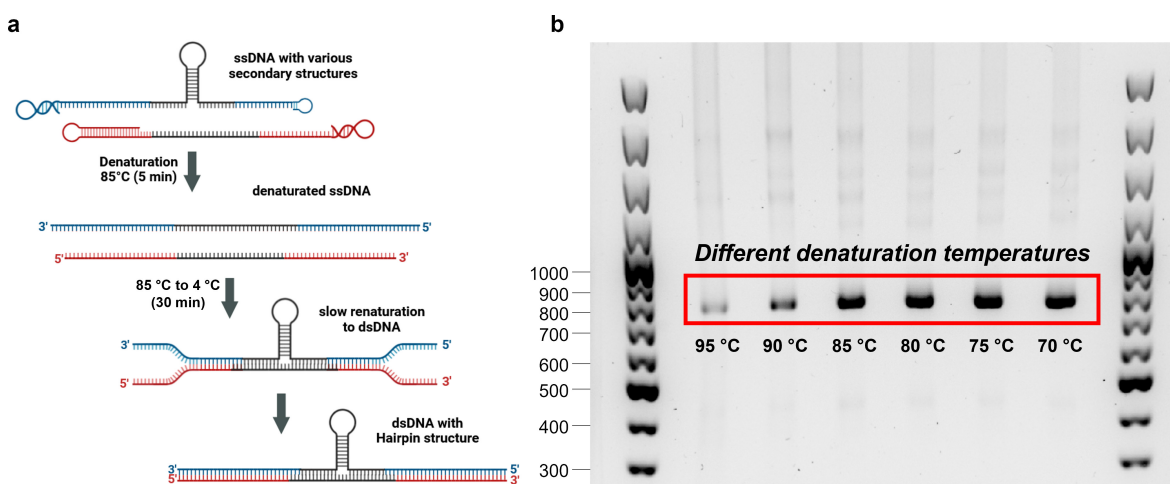


Figure 3.6: Targeted hybridization of the full-length Hp(0,6,4) structure after gel purification of the ligation product. (a) Schematic representation of denatured ssDNA fragments occurring during DNA adsorption onto a silica column. (b) Agarose GE of self-assembled Hp(0,6,4) with a total length of approximately 750 bp, analyzed at different denaturation temperatures. Created with BioRender.com

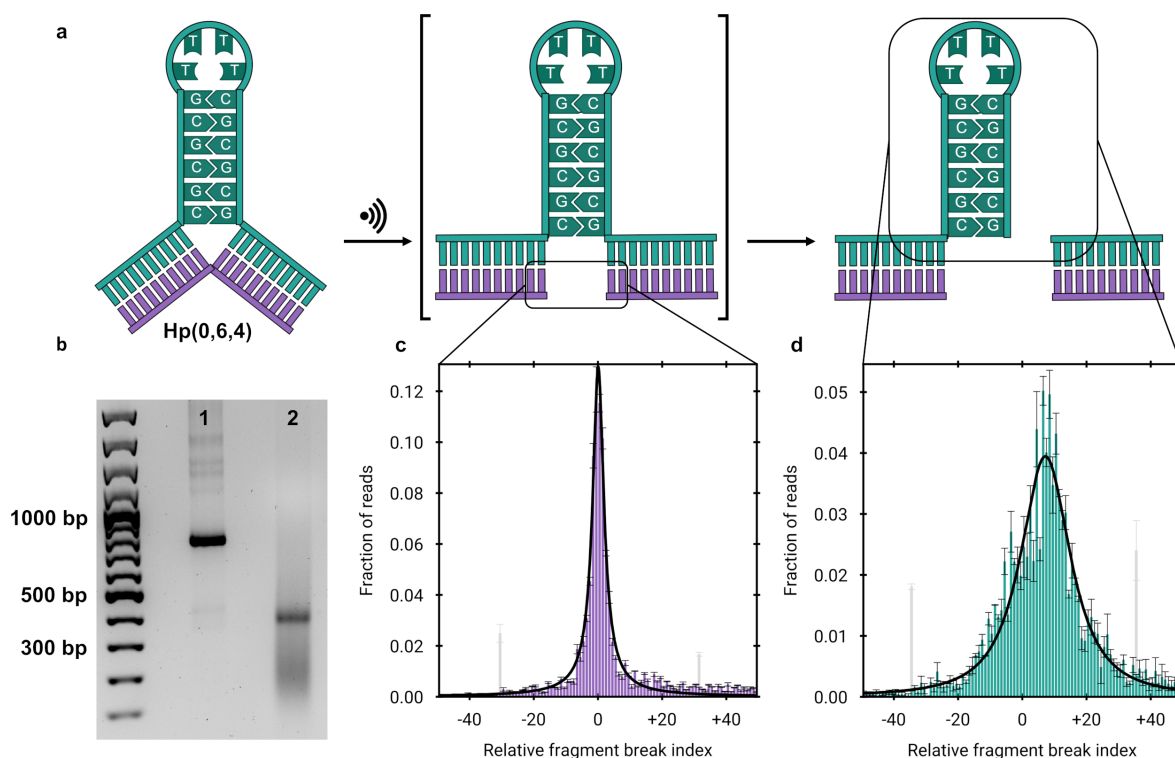


Figure 3.7: Mechanochemical scission of Hp(0,6,4) demonstrated by agarose GE and NGS. (a) Schematic representation of the proposed scission mechanism of the central secondary structure within a 750 bp dsDNA construct. (b) Agarose GE of the sonicated sample shown in (a). Lane 1: untreated Hp(0,6,4); Lane 2: Hp(0,6,4) after 8 min of sonication. A sharp band appears at approximately 375 bp, indicating precise central scission. (c) NGS-derived scission distribution of the lower strand, yielding an interquartile range of 5.4 ± 0.3 nt. (d) NGS-derived scission distribution of the upper strand, indicating less precise cleavage. Created with BioRender.com

To assess the precision of strand scission, Hp(0,6,4) was sonicated for 8 min (Fig. 3.7a), resulting in a sharp band at approximately half the size of the original dsDNA (Fig. 3.7b). To further evaluate this phenomenon at a nucleotide-level resolution, Illumina sequencing was performed following the same procedure described previously, enabling precise identification of the US-induced scission points as the 5'-ends of the lower (Fig. 3.7c) and upper strands (Fig. 3.7d). A narrow scission distribution was observed for the lower strand, whereas the upper strand exhibited a substantially broader distribution.

Notably, both strands displayed minor additional signals, resembling "antennas", which deviated from the main cleavage pattern at approximately ± 30 nucleotides (shown greyed out). These artifacts arise from incomplete ligation during construct assembly, as T4 DNA ligase does not achieve full conversion due to thermodynamic limitations. As a control, an identical construct was assembled by enzymatically digesting two dsDNA molecules with λ -exonuclease to produce two long ssDNA fragments, which were subsequently rehybridized. Sonication of this control yielded scission distributions identical to those of the original construct but without the spurious antenna signals (Fig. 3.8).

The width of the scission distribution on the lower strand was up to twice as precise as that previously reported for the nicked DNA system (see comparison in Fig. 3.11e). This increase in precision is particularly remarkable, given that the hairpin constructs—unlike the

nicked DNA—require two concurrent and accurately positioned scission events on both DNA backbones. Thus, the mechanochemical behavior of our construct as a biomechanophore arises solely from the hybridization of its secondary structure.

To test the proposed mechanism, fully atomistic, constant-force MD simulations were performed in two steps by our collaborator Boris Schüpp. In the first step, a 2.0 nN force was applied to the unmodified hairpin to obtain a force profile along the relevant backbone bonds. In the second step, the residue identified as most likely to cleave in step one was pre-cleaved, and the resulting structure was simulated under the same 2.0 nN force. Structural analysis (Fig. 3.9a) shows that the hairpin remains largely intact during the first step but fully opens in the second, exposing a single-stranded bridge. Time-resolved end-to-end distance data (Fig. 3.9b) at a lower force of 0.1 nN reveal two major opening events: an initial relaxation of the junction at the beginning of the simulation, followed by the loss of hairpin base pairing after approximately 30 ns. Subsequent increases in end-to-end distance arise from bond stretching and the transition to zip-DNA, an extended ladder-like conformation.^[177]

The C3'–O3' bond was considered most relevant for US-induced scission,^[169,170] and time-averaged forces were computed for both steps. Prior to the first scission (Fig. 3.9c), the highest forces are localized near the hairpin junction on the non-hairpin strand, consistent with the experimentally observed precise cleavage at that position. Before the second scission, the force is transmitted primarily through the single-stranded bridge, creating a broad region of elevated forces on the hairpin strand within residues that previously participated in base pairing. This force-enriched region is notably wider than in the first step, explaining the broader scission distribution observed experimentally for this strand. In the control system, Hp(4,0,4), which lacks strand asymmetry, no significant accumulation of force was observed on either strand or region (Fig. 3.9e), consistent with its uniform scission distributions (Fig. 3.10). Overall, the MD simulations successfully reproduce the experimental results and strongly support the proposed two-step scission mechanism.

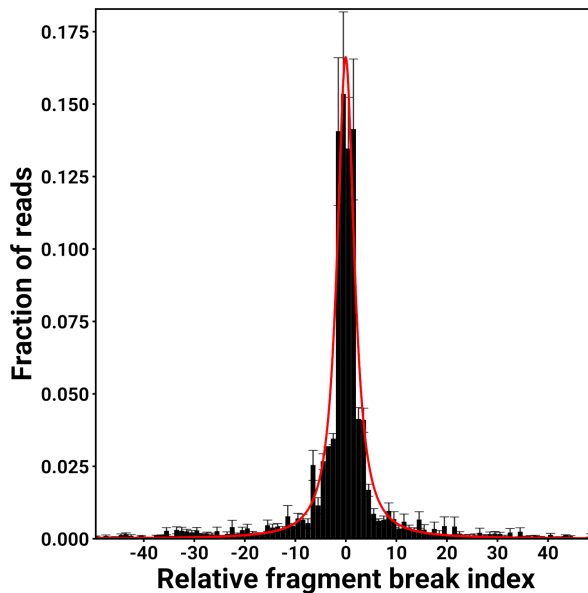


Figure 3.8: Average scission distribution of the lower strand of self-assembled Hp(0,6,4). Error bars represent standard deviations ($n = 2$). The red line corresponds to a Student's t -distribution fit.

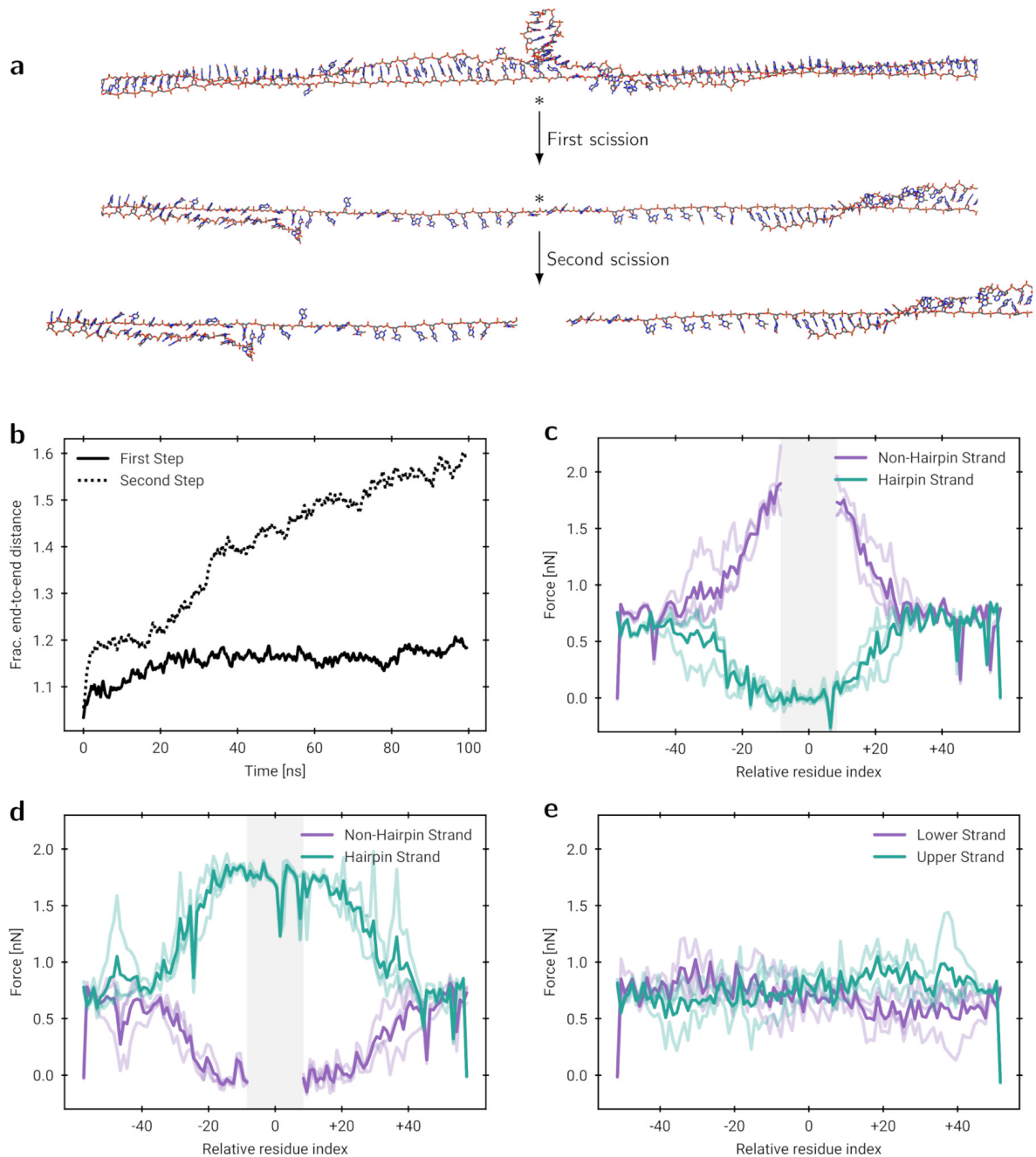


Figure 3.9: Two-step mechanism of DNA hairpin cleavage from MD simulations. (a) Representative MD snapshots under load illustrating the two scission steps, with fragments shown after the second cleavage. Stars mark the most probable break points. (b) Time evolution of the fractional end-to-end distance (running average over 50 frames) at 0.1 nN, showing hairpin opening during the second step. (c) Time-averaged C3'-O3' bond force per residue for Hp(0,6,4) prior to the first scission under 2.0 nN, revealing higher forces on the non-hairpin strand. (d) Same analysis prior to the second scission, showing elevated forces on the hairpin strand. (e) Control system Hp(4,0,4) prior to the first scission, showing no strand-specific force enrichment.

To further elucidate the secondary-structural features responsible for precise cleavage (Fig. 3.7) and force concentration on the bottom strand (Fig. 3.9), we examined a hairpin variant lacking a stem, designated Hp(0,0,4). We hypothesized that if stem formation is critical for effective force transduction and localized scission, then the absence of a stem would significantly reduce cleavage precision. Consistent with this hypothesis, sonication of Hp(0,0,4) produced a markedly broader scission distribution compared to constructs containing even minimal stem regions (Fig. 3.11b,c). Notably, the inclusion of only four base pairs in the stem was sufficient to reduce the distribution width by more than half, underscoring the crucial role of stem stability in mechanical force focusing. To determine whether longer stems further improve precision, we compared constructs with 4, 6, and 10 bp stems. Statistical analysis revealed no significant differences among these variants (all $p > 0.05$), indicating that cleavage precision does not increase with stem length. Instead, a minimal stem of 4 bp is sufficient to achieve the observed level of regiospecificity, with only the no-stem construct Hp(0,0,4) differing significantly from the others. To further support these experimental observations, constant-force MD simulations were performed on the same series of motifs, and the time-averaged force on the C3'–O3' bonds of the non-hairpin strand prior to the first scission was analyzed (Fig. 3.11d). Across all systems, forces peaked at or near the hairpin junction, indicating a localized scission opposite the hairpin. Although this general pattern persisted, Hp(0,0,4) exhibited a distinctly flatter force profile with a lower maximum, consistent with the reduced cleavage precision observed experimentally. These results suggest that structural asymmetry between strands—even in the absence of a hairpin—can induce a degree of localization in scission; however, the highest precision is achieved when force transmission at the junction is reinforced by a hairpin stem of at least four base pairs. Comparison to previous results on nicked-DNA structures,^[166] which require a precise enzymatically-produced break to yield sharply defined fragments (Fig. 3.11e), indicates that incorporating a hairpin element provides an additional level of control over the exact site of mechanochemical cleavage.

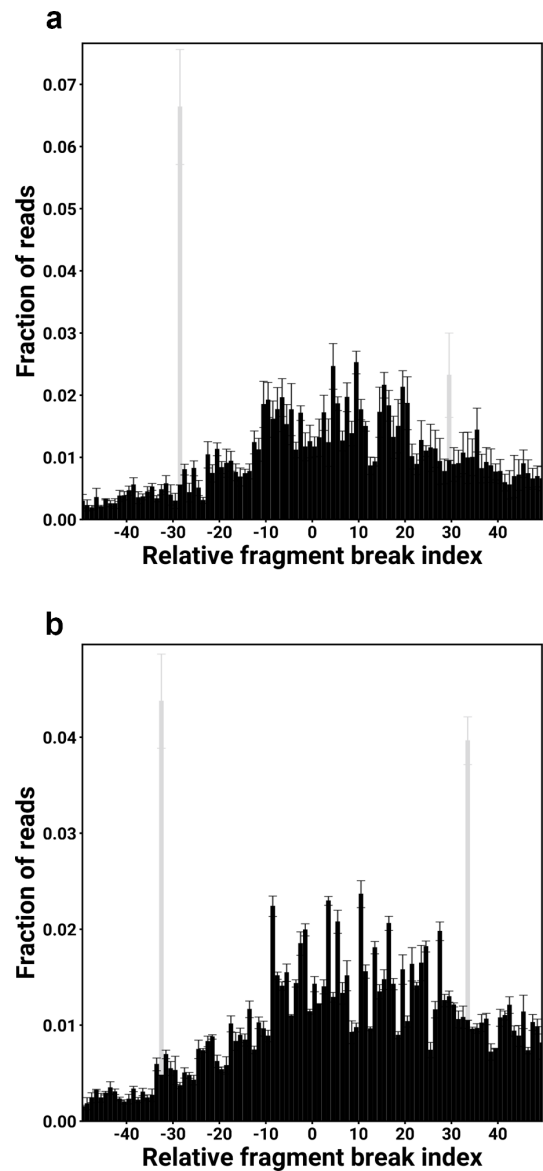


Figure 3.10: Average scission distribution of the upper (a) and lower (b) strand of Hp(4,0,4). Error bars represent standard deviations ($n = 3$).

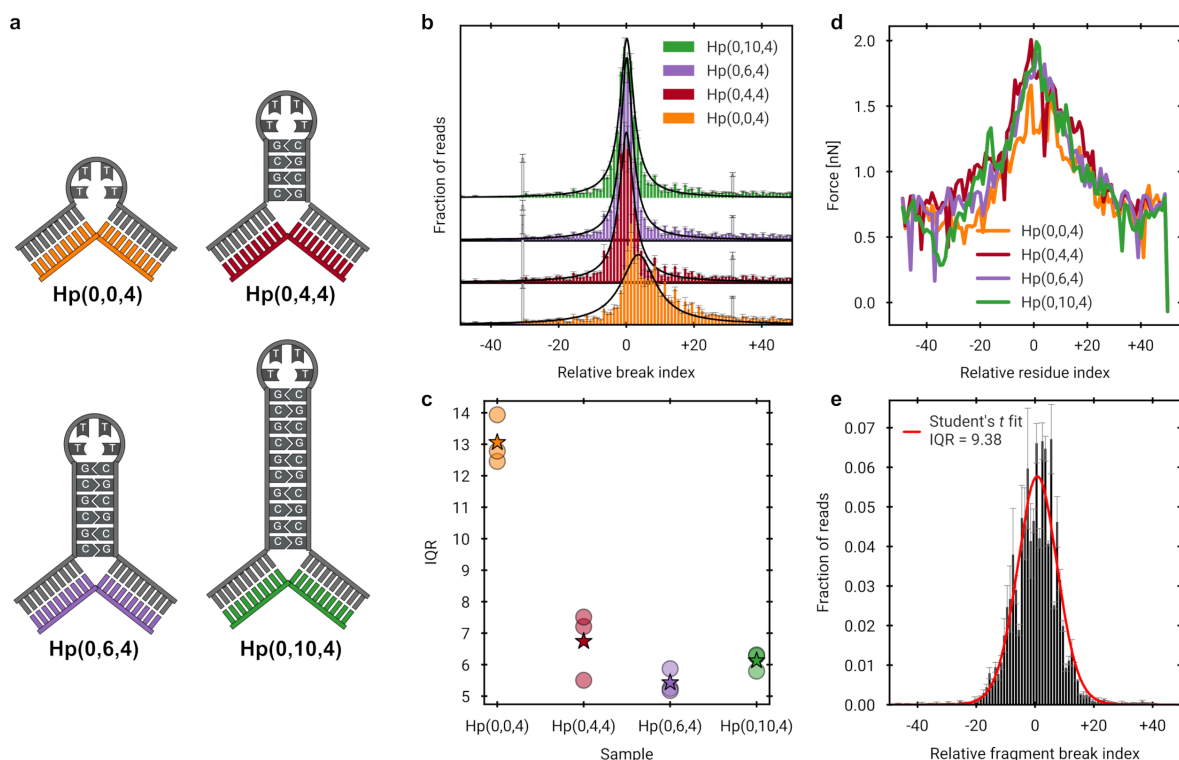


Figure 3.11: Inclusion of a stem is crucial for scission precision. (a) Schematic representation of the central secondary-structure motifs incorporated within a 750 bp dsDNA construct. (b) Scission distributions of the lower strand obtained by NGS, each fitted with a Student's *t*-distribution; the interquartile range (IQR) serves as a measure of distribution width. (c) IQR values of the scission distributions. Hp(0,0,4) exhibits a markedly broader IQR than the other constructs. (d) Time-averaged C3'-O3' bond force per residue for systems with varying hairpin stem lengths, evaluated prior to the first scission under a constant 2.0 nN load. The overall force profiles are similar, with Hp(0,0,4) showing a distinct deviation. (e) Experimental scission distribution on the non-nicked strand of a previously reported nicked-DNA system.^[166] The IQR is larger than for the hairpin structures (except Hp(0,0,4)), indicating lower scission precision. Created with BioRender.com

3.4 Conclusion and Outlook

This chapter establishes DNA secondary structures—specifically hairpins—as programmable “biomechanophores” that direct US-induced forces to predetermined bonds with high regioselectivity. By integrating a central hairpin into a ~750 bp duplex and systematically varying the stem length, we achieved sharply localized cleavage near the hairpin junction. NGS resolved strand-specific scission maps, revealing a narrow, well-defined distribution on the strand opposite the hairpin and a broader distribution on the hairpin strand. Together with fully atomistic, constant-force MD simulations, this data supports a two-step mechanism: (i) force focusing across the junction drives precise rupture on the non-hairpin strand; (ii) subsequent opening creates a single-stranded bridge that transmits load across a wider region of the hairpin strand, broadening its break distribution. Controls lacking a stem produced substantially wider distributions, whereas minimal stems (4 bp) restored precision to near-nicked-DNA levels and, on the lower strand, surpassed them.

Relative to the nick-programmed system presented in Chapter 2, where base fraying limits precision of scission,^[166] hairpins provide an intrinsic, sequence-defined geometry that concentrates mechanical stress without introducing exogenous mechanophores. Notably, the hairpin construct attains high precision despite requiring two coordinated scission events on opposite backbones, underscoring that secondary-structure-guided force routing can compensate for fraying-related dispersion. Methodologically, the combination of modular ligation (“DNA PnP”), US activation, NGS readout, and MD analysis furnishes a general workflow to quantify bond-selective mechanochemical reactivity with nucleotide-level resolution.

The present findings open several avenues. First, design space: extending beyond canonical hairpins to bulges, internal loops, three-way junctions, or G-quadruplex inserts could map how local geometry, stiffness, and stacking modulate force focusing. Systematic scans of stem length, loop entropy, and targeted mismatches will enable predictive “force lenses” that tune precision and threshold energies. Second, environment and loading: varying salt, cosolvents, temperature/annealing histories,^[176] and US parameters (frequency, duty cycle, pulse envelopes) should disentangle intrinsic structural effects from extrinsic damping and chain-tension propagation. Third, architecture: multi-hairpin arrays or periodically spaced secondary motifs may yield cooperative force funneling or sequential cleavage cascades, enabling programmable “mechanochemical circuits” in DNA. Fourth, analytics: integrating real-time reporters (*e.g.*, fluorogenic gates) with *post-mortem* NGS could correlate kinetics with final scission maps; Bayesian deconvolution and ML models trained on sequence–structure–force features may further refine positional predictions. Finally, translation: embedding biomechanophores into DNA origami or hybrid polymer–DNA materials could realize stress-gated release, reconfiguration, or signal amplification under benign stimuli, advancing biocompatible mechanochemistry without synthetic mechanophore installation.

In sum, secondary-structure engineering in DNA affords an experimentally simple yet mechanistically rich route to precise, bond-selective mechanochemical activation. By coupling modular sequence design with quantitative readouts (NGS) and atomistic modeling (MD), this framework should accelerate hypothesis-driven exploration of force transduction in soft matter and inform the construction of responsive nucleic-acid–based devices with tunable mechanical function.

Chapter 4

How DNA Feels Force During Freezing of Water

4.1 Introduction

Since the earliest reports of mechanical stress arising from water freezing, the fracture of macromolecules during ice formation has remained a well-known yet fundamentally unresolved phenomenon. Despite decades of study, the precise nature and magnitude of the forces responsible for this degradation remain poorly characterized at the molecular level. Understanding these processes is not only of academic interest but also of considerable practical relevance, as freezing-induced damage to biomacromolecules, cells, tissues, and organs^[178–180] poses one of the central challenges in cryopreservation, which is an essential technique in both life sciences and food storage.

The destructive effects of freezing can broadly be divided into two categories. The first is *physical degradation*, which is the focus of this chapter, encompassing phenomena such as osmotic imbalance, high ionic concentration, ice recrystallization, and the mechanical stress arising from rapid ice growth and expansion.^[181] The second category is *chemical degradation*, largely attributed to the generation of reactive oxygen species and free radicals during freezing and thawing.^[182] Both forms of damage compromise biological and synthetic macromolecules, leading to structural breakdown and loss of functionality.

In natural environments, numerous organisms have evolved sophisticated mechanisms to survive subzero conditions. Among the most remarkable are antifreeze proteins (AFPs), which regulate ice nucleation and growth, effectively minimizing the formation of large, damaging ice crystals. Inspired by these biological strategies, research into biomimetic cryoprotectants has yielded promising alternatives to conventional agents such as dimethyl sulfoxide (DMSO), glycerol, and trehalose, offering lower toxicity and enhanced efficiency.^[183–186] Alongside this, the fundamental physical chemistry of water freezing—encompassing processes such as supercooling, critical nucleus formation, and crystal growth—has been intensively studied through both theoretical modeling and experimental approaches.^[187,188]

However, despite these advances, the molecular mechanisms underlying the mechanical degradation of biomacromolecules during ice formation remain largely unexplored. Most investigations have focused on macroscopic or functional outcomes, such as enzyme inactivation or cellular membrane rupture,^[184,189,190] rather than the microscopic forces acting at the molecular scale. As a result, while cryopreservation research has elucidated many

aspects of ice–cell interactions, the precise physical mechanisms governing polymer and biopolymer scission under freezing stress remain poorly understood.

This knowledge gap is particularly striking for linear biomacromolecules such as DNA, which serve as ideal model systems for studying polymer mechanics. DNA offers precise sequence control, defined mechanical properties, and a well-characterized double-helical structure, making it uniquely suited for quantitative mechanochemical investigations. Yet, while significant progress has been made in understanding DNA behavior under flow,^[191] tension,^[192] or ultrasound,^[170] its response to freezing-induced mechanical stress has not been systematically characterized.

Beyond the fundamental interest, this question carries practical relevance for fields that rely on high-integrity nucleic acids, such as transcriptomics and clinical genomics. Many workflows in these areas inherently involve freeze–thaw cycles, long-term storage, or cryogenic transport, yet the potential mechanical impact of ice formation on DNA and RNA quality is rarely considered.^[193–195] Even subtle strand damage or fragmentation can influence sequencing-based readouts.^[194] Despite this, the mechanical component of freeze–thaw–induced degradation has received little systematic attention,^[193,196] leaving a critical gap between cryopreservation practices and the molecular fidelity required in modern nucleic-acid–based analyses.

This chapter therefore harnesses the principles of *polymer mechanochemistry*^[2,23,40] to uncover the molecular nature of mechanical forces generated during water freezing. By employing DNA as a sequence-defined polymeric model,^[166] we establish a framework that allows mechanochemical processes to be quantitatively monitored and spatially resolved at single-monomer precision. Introducing well-defined mechanical weak points, *i.e.* single-strand interruptions (nicks), into the DNA backbone enables the controlled study of mechanochemical activation under freezing stress, transforming DNA into a molecular force sensor.

Through this mechanochemical lens, we aim to answer key open questions: How do the forces generated during water freezing compare to those induced by elongational flow or sonication? What roles do parameters such as temperature, ice–container interface, and material stiffness play in governing stress transmission? And how does macromolecular architecture such as contour length influence susceptibility to freezing-induced degradation? Addressing these questions not only advances our molecular understanding of freezing-induced stress but also provides actionable insights for cryopreservation science. The mechanochemical framework introduced here opens a new avenue for studying how mechanical forces arise, propagate, and act upon macromolecules in frozen environments. This has the potential to lay the groundwork for the rational design of next-generation cryoprotective strategies that minimize damage at its molecular origin.

Most experimental parts of this chapter were performed together with Marvin Rauschen under my close supervision during his master’s thesis. Illumina sequencing was conducted by the Genomics Facility, a core facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen University.

4.2 Methods

Materials and Reagents

All genes and primers used for the preparation of nicked dsDNA were obtained from Integrated DNA Technologies (IDT), and their sequences are listed in the Appendix as Fig. 5.22-5.25 and Table 5.2. Pfu DNA Polymerase (cat. no. TK-005222) was purchased from Bioventis. The nicking endonuclease Nt.BspQI (cat. no. R0644S) was acquired from New England Biolabs (NEB). Standard Agarose (cat. no. 3810.4, Carl Roth) was used for agarose GE, in combination with ROTI[®] GelStain Red Eco (cat. no. 223C, Carl Roth) as the intercalating dye. TAE buffer (1×) was freshly prepared from Tris base (CAS 77-86-1, Fisher Bioreagents), acetic acid (CAS 64-19-7, Sigma) and disodium EDTA dihydrate (CAS 60-00-4, Sigma), resulting in final concentrations of 40 mM Tris, 20 mM acetic acid, and 2 mM EDTA. Samples were loaded using TriTrack DNA Loading Dye (6×) (cat. no. R1161, Thermo Scientific[™]) and resolved against GeneRuler 100 bp Plus DNA Ladder (cat. no. SM0321, Thermo Scientific[™]). DNA fragments excised from gels were purified using the GFX[™] PCR DNA and Gel Band Purification Kit (cat. no. 28903471, Cytiva). Capillary electrophoresis was performed on a Qsep1 system (BiOptic Inc.) equipped with high-resolution cartridges (cat. no. C105202, NIPPON Genetics). For library preparation from fragmented dsDNA, the NEBNext Ultra II DNA Library Prep Kit (cat. no. E7645L, NEB) was employed. Illumina paired-end sequencing was performed on a MiSeq platform using the MiSeq Reagent Micro Kit v2 (300 cycles) (cat. no. MS-103-1002, Illumina).

Generation of Nicked dsDNA

DNA fragments were amplified by PCR. Each 50 µL reaction contained 30 ng of template DNA, 0.5 µM of forward and reverse primers, and 0.4 µL of Pfu DNA Polymerase (5 U µL⁻¹), with ultrapure water added to a final volume of 50 µL. The thermal cycling protocol consisted of an initial denaturation at 98 °C for 2 min, followed by 30 cycles of denaturation (98 °C, 20 s), primer annealing (65 °C, 30 s), and extension (72 °C, 1.5 min), and concluded with a final elongation at 72 °C for 5 min. PCR products were purified by agarose gel extraction or using a solution-based DNA purification kit.

Site-specific nicking of the amplified dsDNA was achieved using Nt.BspQI. Each 50 µL reaction contained 1.0 µg of dsDNA, 10 U of Nt.BspQI, and 5 µL of 10× NEBuffer[™] r3.1, providing final concentrations of 100 mM NaCl, 50 mM Tris·HCl, 10 mM MgCl₂, and 500 ng recombinant albumin at pH 7.9 (25 °C). Samples were incubated at 50 °C for 1 h, followed by enzyme inactivation at 80 °C for 20 min.

Freeze–Thaw Experiments

Nicked DNA samples (ds400*, ds800*, ds1500*) were prepared by diluting 5 µg of DNA in 3 mL ddH₂O from nicking reaction solutions (1 µg DNA, 5 µL 10× NEBuffer[™] r3.1, and 45 µL ddH₂O per batch). Unnicked and hairpin DNA samples (ds800, hp10, hp20, hp30) were prepared similarly, with 5 µg of DNA, 25 µL 10× NEBuffer[™] r3.1, and ddH₂O added to a final volume of 3 mL. Samples underwent 90 freeze–thaw cycles, and 20 µL aliquots were collected after 0, 15, 30, 60, and 90 cycles. Each cycle consisted of immersion in liquid nitrogen (1–3 min) until thermal equilibration, followed by complete thawing in a 45 °C

water bath (2–4 min), with durations adjusted to vial material and size. Hairpin DNA aliquots were heated to 90 °C for 10 min and snap-cooled on ice, whereas dsDNA aliquots were heated to 70 °C and slowly cooled to 4 °C over 90 min. Each aliquot received 10 µL of a reference solution containing 3 ng µL⁻¹ ds80 DNA.

Processed samples were analyzed *via* capillary electrophoresis using an S1 High-Resolution DNA cartridge, with injection at 8 kV for 30 s (dsDNA) or 20 s (hpDNA) and separation at 6 kV for 300 s (dsDNA) or 150 s (hpDNA). Raw data were exported and processed in Python. Reference peak intensities were normalized, and sample peak elution times were aligned. Peak areas were integrated using the `scipy.integrate.simpson` method, and sample-to-reference ratios were normalized to the zero-cycle control.

Illumina Sequencing

For Illumina sequencing, the entire gel lane—excluding the intact, non-cleaved DNA fraction—was excised following 90 freeze–thaw cycles. DNA was recovered from the agarose gel and processed for sequencing using the NEBNext Ultra II DNA Library Prep Kit. Sequencing was carried out on an Illumina MiSeq[™] platform with the MiSeq Reagent Kit v2 Micro for 2×75 bp paired-end reads, including a 5% PhiX spike-in (cat. no. FC-110-3001, Illumina). Sequencing data was analyzed as previously described to obtain scission distributions. Here, the original version of the algorithm described in Chapter 2 was used.^[166]

4.3 Results and Discussion

In Chapter 2 of this thesis, we demonstrated that DNA can serve as a precise analytical platform for probing covalent bond scission in macromolecules, enabling monomer-resolved characterization of *post-mortem* scission distributions.^[166]

Building on this framework, we investigated whether nicked DNA can act as a model system to elucidate the nature of mechanical forces acting on macromolecules subjected to repeated freeze–thaw (FT) cycles. For this purpose, we analyzed 800 bp double-stranded DNA containing a central nick (**ds800***) across varying numbers of FT cycles (Fig. 4.1). **ds800*** was amplified by PCR and enzymatically nicked as described in Chapter 2. The observed linear trend supports the hypothesis that an increasing number of FT cycles proportionally amplifies the mechanical stress exerted on non-crosslinked macromolecules.

To further examine this behavior, we compared the scission efficiency of nicked and non-nicked 800 bp duplexes. After 90 FT cycles, the nicked construct exhibited approximately 12 % cleavage, whereas only about 3 % of the non-nicked counterpart underwent strand breakage (Fig. 4.2a,b). This finding is consistent with the markedly higher rate constant observed for ultrasound-activated nicked DNA relative to its intact analog.^[166] Notably, Illumina sequencing revealed that strand cleavage occurred predominantly opposite the nick, confirming regioselective bond rupture (Fig. 4.2c). However, the FT-induced scission profile could not be satisfactorily described by a Gaussian fit, as significant off-center fragmentation was also apparent. It therefore remains an open question whether the fraying mechanism previously proposed for dsDNA scission^[166] also governs cleavage under these conditions. For the non-nicked DNA, scission events were evenly distributed along the sequence, showing no mid-strand preference. This uniform fragmentation contrasts with the characteristic central breakage observed in flow-induced polymer mechanochemistry, where overstretched chains statistically rupture near their midpoint.^[39,164]

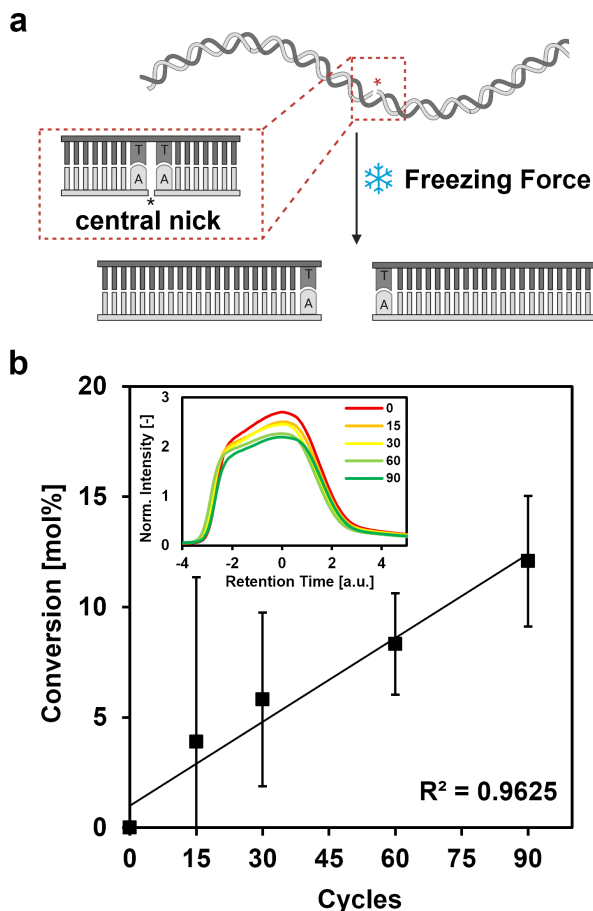


Figure 4.1: (a) Schematic representation of the covalent bond cleavage occurring opposite to the nick in double-stranded DNA (dsDNA) during repeated freeze–thaw (FT) cycling (adapted from Ref. [166], CC BY 4.0). (b) Mechanochemical conversion of substrate peak areas for **ds800*** as a function of FT cycle number, up to 90 cycles. Error bars represent standard deviations from three technical replicates, and the solid line corresponds to a linear regression fit.

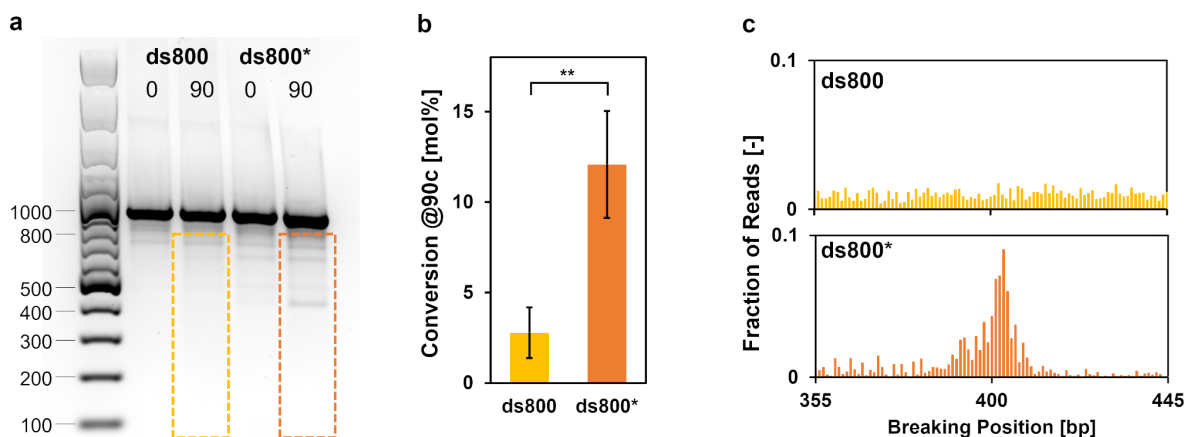


Figure 4.2: Comparison of nicked and non-nicked dsDNA subjected to repeated freeze-thaw (FT) cycles. (a) Agarose GE of **ds800** and **ds800*** after 0 and 90 FT cycles. The dotted boxes mark the gel regions excised for subsequent NGS analysis. (b) Conversion of the DNA substrates after 90 FT cycles. (c) NGS-derived scission distributions of the upper DNA strand from **ds800** and **ds800*** after 90 FT cycles.

To probe potential activation of the mechanophore-analog DNA across a broad size range, we selected two systems: short DNA hairpins (10, 20, and 30 bp) and long dsDNA fragments (400, 800, and 1500 bp). The hairpin DNA (hpDNA) constructs are self-assembled, closed-ended duplexes containing a central nick, formed from single-stranded precursors (Fig. 4.4a). The closed ends promote kinetic refolding upon melting, accounting for their low melting temperatures (30–62 °C). Comparing the activation of DNA with different molecular weight resembles the observation of scission for both very short as well as longer chains (Fig. 4.4b). Remarkably, even the short hpDNA construct hp20 showed up to 12 % conversion, whereas it remained stable under elongational flow (Fig. 4.4c). These findings evidence an activation mode that does not require a certain contour length for mechanochemical activation during freezing. We also hypothesized, that the exerted thermal stress is influenced by the interactions between ice and the surrounding substrate. Indeed, we observed a consistent effect when increasing the Young's modulus of the substrate (glass vs. stainless steel, ca. 190 GPa). The mechanochemical conversion of **ds800*** increased markedly at higher Young's modulus (Fig. 4.4d). Changing the temperature during freeze-thaw cycles revealed no activation of the DNA **ds800*** at –25 °C while activation at –196 °C is observed (Fig. 4.3). This indicates that the nature of force (pulling, stretching, shear) is necessarily different, depending on the lowest temperature reached during FT cycling.

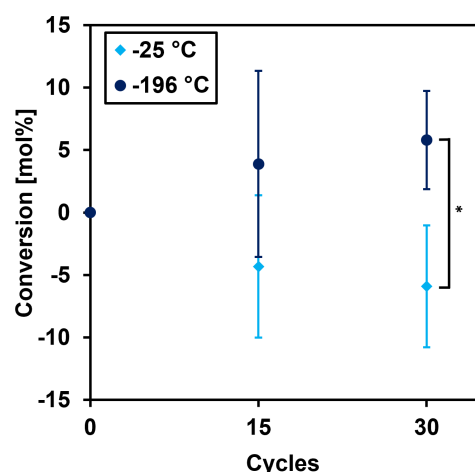


Figure 4.3: Mechanochemical conversion of **ds800*** after 0–30 freeze-thaw cycles at –196 °C vs. –25 °C.

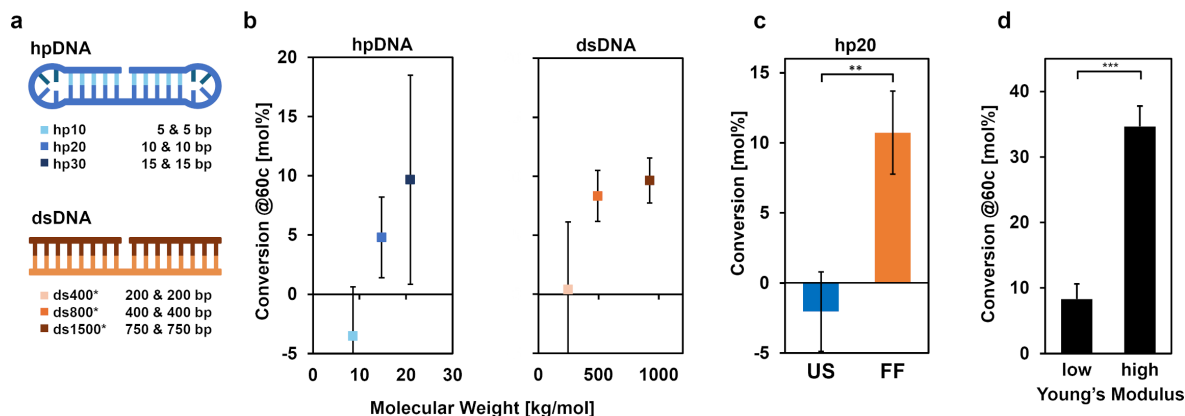


Figure 4.4: Effect of DNA length and Young's modulus of container material on susceptibility to freezing-induced mechanical stress. (a) Schematic representation of the investigated DNA constructs and their fragment sizes, with hpDNA samples (**hp10**, **hp20**, **hp30**) shown in blue and dsDNA samples (**ds400***, **ds800***, **ds1500***) shown in orange. (b) Mechanochemical conversion after 60 FT cycles for all six constructs depicted in (a), grouped by structural type. Error bars represent standard deviations from three technical replicates. (c) Mechanochemical conversion of **hp20** after 30 min of 20 kHz US or 180 FT cycles. Notably, **hp20** remains unaffected by US but exhibits more than 10 % degradation following FT cycling. (d) Mechanochemical conversion of **ds800*** after 60 FT cycles for low and high Young's moduli of the sample container.

4.4 Conclusion and Outlook

This chapter applied the concepts of polymer mechanochemistry to elucidate mechanical forces exerted on DNA during water freezing. By introducing a single-strand interruption (nick) as a mechanophore analog into the DNA backbone, we created a defined mechanical weak point that enabled site-specific activation under freezing-induced stress. In contrast, intact DNA lacking a nick exhibited considerably slower bond rupture, confirming the requirement of a predetermined scission site for mechanochemical activation in this context. Although FT cycling generates mechanical stress less frequently than elongational flow, the local forces achieved are sufficiently high to induce covalent bond cleavage. Temperature-dependent activation revealed that the minimum temperature reached during FT cycles critically influences DNA breakage, suggesting distinct force generation mechanisms that depend on the freezing profile. Moreover, we identified a strong correlation between container surface mechanics and the extent of DNA activation: materials with a higher Young's modulus and lower thermal expansion coefficient promoted greater stress accumulation in the ice, resulting in enhanced DNA cleavage. Remarkably, even short-chain macromolecules—traditionally considered mechanochemically inactive—were affected by freezing, as evidenced by the response of very short hairpin DNA constructs.

Collectively, these findings demonstrate that polymer mechanochemistry provides a powerful molecular-level framework to probe freezing-induced degradation in biomacromolecules. Understanding the mechanistic interplay between thermal, interfacial, and mechanical effects during ice formation not only deepens our insight into cryo-induced molecular stress but also suggests strategies to minimize such damage. This mechanochemical approach

thus establishes a foundation for the rational design of cryopreservation techniques that mitigate destructive forces during freezing.

Future studies could expand this methodology to explore a broader range of DNA contour lengths, bridging the gap between hpDNA and dsDNA constructs and extending beyond 1500 bp to capture effects arising from intramolecular folding or entanglement at higher molecular weights. Furthermore, systematic variation of vial geometry, heat-transfer characteristics, and cooling rates may clarify how macroscopic freezing dynamics influence microscopic stress distributions. Independent investigations of surface chemistry—such as nucleation-site density, electrostatic interactions, and hydrophilicity—would further disentangle the role of interfacial forces from bulk ice mechanics.

In addition, incorporating cryoprotective agents (CPAs) and ice-modulating proteins could reveal how molecular additives redistribute or dampen mechanical stress, thereby improving cryostability. Locating the precise spatial and temporal onset of mechanochemical activation, potentially through time-resolved spectroscopic or imaging techniques, may enable real-time mapping of stress propagation during freezing.

Perhaps most intriguingly, the demonstrated activation of short-contour-length mechanophores, which are otherwise unresponsive to ultrasound, suggests a new route toward compact, low-threshold mechanochemical systems. Such advances could ultimately eliminate the need for long polymer backbones in force-activated chemistry and inspire the development of molecular devices or responsive materials that harness freezing-induced stresses for functional transformations.

Chapter 5

Conclusion and Outlook

This thesis establishes DNA as a powerful, sequence-defined model polymer for studying mechanochemical phenomena with high spatial precision. By combining well-controlled architectures with NGS, the work bridges a long-standing gap between polymer mechanochemistry and molecular-level force analysis. The results show that structural features in DNA—nicks, secondary motifs, and contour length—act as tunable mechanochemical elements that govern where and how mechanical energy is converted into bond scission. This approach achieves scission localization within ± 4 –6 nucleotides, representing an analytical resolution not previously reached in polymer mechanochemistry.

In **Chapter 2**, we introduced a minimal mechanophore analog in the form of a nick and showed that even a simple backbone discontinuity enables site-selective mechanochemical activation under ultrasound. Mechanical tension was funneled onto the opposite strand, which produced precise cleavage with near-nucleotide resolution. Constant-force MD simulations linked the slight broadening of the scission distribution to localized fraying, directly connecting molecular dynamics with experimental observables. This chapter established the fundamental principles of force focusing in DNA and demonstrated a mechanophore-free strategy for probing covalent bond rupture.

Chapter 3 extended this concept by embedding hairpin motifs as programmable "biomechanophores". These secondary structures acted as intrinsic force lenses that sharpened scission precision beyond what is achievable with nicks alone. Systematic variation of stem length enabled controlled modulation of cleavage localization. NGS revealed complementary strand-specific scission patterns—narrow and well-defined on the strand opposite the hairpin, broader on the hairpin strand—consistent with a two-step mechanism supported by MD simulations. These results demonstrate that structural programming of local geometry provides an effective design principle for sequence-dependent mechanochemical control. Having established that DNA responds predictably to ultrasound, the next step was to test whether this mechanochemical sensitivity extends to different mechanical stimuli, such as freezing-induced stress.

In **Chapter 4**, we expanded the platform to FT-induced mechanochemistry and uncovered the molecular-scale effects of ice formation on DNA. By using nicks as predefined weak points, we showed that water freezing exerts sufficient mechanical stress to trigger site-specific bond cleavage. Activation depended on minimum temperature, number of FT cycles, and container mechanics. Remarkably, even short hairpin constructs—largely inert under ultrasound—were activated by freezing, revealing a new form of cryomechanochemical reactivity. These results provide molecular insight into ice-induced stresses and highlight

mechanochemistry as a quantitative framework for understanding and mitigating freezing-related biomolecular damage.

Together, these findings establish DNA as a modular, analyzable, and architecturally programmable platform for mechanochemistry. The combination of controlled molecular design, scalable experiments, and quantitative readout redefines how force–reactivity relationships can be interrogated and engineered. The mechanophore-free activation strategy introduced here, relying entirely on sequence design rather than synthetic chemistry, marks a shift toward simpler and more predictive approaches in polymer mechanochemistry.

Building on this foundation, several directions emerge. From a *conceptual* perspective, expanding to more complex secondary and tertiary motifs—bulges, G-quadruplexes, multi-junction structures—may reveal new modes of force focusing and cooperative activation. Combining multiple biomechanophores in a single architecture could enable DNA-based “mechanochemical circuits” capable of sequential or logic-like responses to mechanical input. From a *mechanistic* perspective, time-resolved spectroscopy or single-molecule force microscopy could provide kinetic information on scission events, enabling models that link stress propagation to rupture probabilities. Varying environmental parameters such as temperature, viscosity, ionic strength, and co-solutes would further clarify how external conditions modulate force transduction.

From an *application-oriented* perspective, embedding programmable weak points into DNA-based materials, hydrogels, or hybrid polymers could yield systems that undergo stress-triggered transformations, self-healing, or signal generation. The insights gained here also inform cryopreservation strategies: understanding how cooling profiles, container mechanics, and ice–interface dynamics contribute to damage enables more rational protection of biomolecules. The activation of short DNA constructs under freezing suggests new routes to initiate mechanochemical reactivity at low temperatures, potentially enabling cryogenic catalysis or controlled release.

Finally, coupling DNA-based mechanochemistry with data-driven modeling may accelerate predictive control over force–reactivity landscapes. Training machine-learning models on large datasets of sequence, structure, and scission patterns could enable computational design of mechanically programmed polymers with tailored breaking behaviors.

In summary, merging the precision of DNA nanotechnology with the principles of polymer mechanochemistry enables detailed control over molecular-scale force responses. This integration of analytical resolution, structural programmability, and mechanistic understanding lays the groundwork for next-generation force-responsive materials, mechanochemical diagnostics, and improved cryoprotective strategies rooted in molecular design.

Appendix

DNA Sequences

Sequence-Specific, Mechanophore-Free Mechanochemistry of DNA

```
1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  GAGTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTCATGAAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGGTTACGAT  GCGCCCATCT  ACACCAACGT
351 G+TTGAAGAGC ATTACGGTCA  ATCCGCCGTT  TGTTCACCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAATTTAA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTGGGG  CTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTTACG  ATTACGTTT
651 ATCGATTCTC  TTGTTTGCTC  CAGACTCTCA  GGCAATGACC  TGATAGCCTT
701 TGTA
```

Figure 5.1: Sequence **704** in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 354-360), marked in red. The nicking enzyme will create a nick between bases 352 and 353 (marked in green) on the complementary strand, which leads to sequence **704***.

```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  GAGTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTTCATGAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGATATATTT AATAATAAAT TTTAATATAA
351 TATGAAGAGC ATTACGGTCA  ATCCGCCGTT  TGTTCCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAAATTTA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTGGGG  CTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTACG  ATTACCGTTC
651 ATCGATTCTC  TTGTTTGCTC  CAGACTCTCA  GGCAATGACC  TGATAGCCTT
701 TGTA

```

Figure 5.2: Sequence AT in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 354-360), marked in red. The nicking enzyme will create a nick between bases 352 and 353 on the complementary strand, which leads to sequence AT*. The 30 bases upstream (bold) have 100% AT content.

```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  GAGTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTTCATGAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGCCCCCGG GCGGCGGGG GCGGCGGGG
351 GGTGAAGAGC ATTACGGTCA  ATCCGCCGTT  TGTTCCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAAATTTA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTGGGG  CTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTACG  ATTACCGTTC
651 ATCGATTCTC  TTGTTTGCTC  CAGACTCTCA  GGCAATGACC  TGATAGCCTT
701 TGTA

```

Figure 5.3: Sequence GC in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 354-360), marked in red. The nicking enzyme will create a nick between bases 352 and 353 on the complementary strand, which leads to sequence GC*. The 30 bases upstream (bold) have 100% GC content.


```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  TACTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTTCATGAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGAAATTAGC  GACGCCCGGG  GGGCTCAGCG
351 CCCCGCGACT  CGGGGGGGCC  GCTATAAACA  GATTCCCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAAATTTA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTTGGGG  CTTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTTACG  ATTACCGTTC
651 ATCGATTCTC  TTGTTTGCTC  CACACTATCA  GGCAATGACC  TGATAGCCTT
701 TGTA

```

Figure 5.4: Sequence **ATMi0** in 5' → 3' direction. It contains the recognition site of Nt.BstNBI, which lies in the complement of the subsequence GACTC (bases 357-361), marked in red. The nicking enzyme will create a nick between bases 352 and 353 on the complementary strand, which leads to sequence **ATMi0***.

```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  TACTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTTCATGAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGAAATTAGC  GACGCCCGGG  GGGCTCAGCG
351 CAACGCGACT  CGGGGGGGCC  GCTATAAACA  GATTCCCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAAATTTA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTTGGGG  CTTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTTACG  ATTACCGTTC
651 ATCGATTCTC  TTGTTTGCTC  CACACTATCA  GGCAATGACC  TGATAGCCTT
701 TGTA

```

Figure 5.5: Sequence **ATMi1** in 5' → 3' direction. It contains the recognition site of Nt.BstNBI, which lies in the complement of the subsequence GACTC (bases 357-361), marked in red. The nicking enzyme will create a nick between bases 352 and 353 on the complementary strand, which leads to sequence **ATMi1***.

```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  TACTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTTCATGAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGAAATTAGC  GACGCCCGGG  GGGCTCAGCG
351 TAATTCGACT  CGGGGGGGCC  GCTATAAACA  GATTCCCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAAATTTA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTGGGG  CTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTACG  ATTACCGTTC
651 ATCGATTCTC  TTGTTTGCTC  CACACTATCA  GGCAATGACC  TGATAGCCTT
701 TGTA

```

Figure 5.6: Sequence **ATMi2** in 5' → 3' direction. It contains the recognition site of Nt.BstNBI, which lies in the complement of the subsequence GACTC (bases 357-361), marked in red. The nicking enzyme will create a nick between bases 352 and 353 on the complementary strand, which leads to sequence **ATMi2***.

```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  TACTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTTCATGAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGAAATTAGC  GACGCCCGGG  GGGCTCAGCT
351 TAATTCGACT  CGGGGGGGCC  GCTATAAACA  GATTCCCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAAATTTA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTGGGG  CTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTACG  ATTACCGTTC
651 ATCGATTCTC  TTGTTTGCTC  CACACTATCA  GGCAATGACC  TGATAGCCTT
701 TGTA

```

Figure 5.7: Sequence **ATMi3** in 5' → 3' direction. It contains the recognition site of Nt.BstNBI, which lies in the complement of the subsequence GACTC (bases 357-361), marked in red. The nicking enzyme will create a nick between bases 352 and 353 on the complementary strand, which leads to sequence **ATMi3***.

```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  TACTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GAAAACCCCTG
101 GCGTTACCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGATCGC  CCTTCCCAAC  AGTTGCGCAG
201 CCTGAATGGC  GAATGGCGCT  TTTTCATGAA  AAGTCTTTAG  TCCTCAAAGC
251 CTCTGTAGCC  GTTGGAGTGC  GATCTTCCTG  AGGTGACATA  CGTCGTCGTC
301 CCCTCAAAC  GGCAGATGCA  CGAAATTAGC  GACGCCCGGG  GGGCTCAGTT
351 TAATTTGACT  CGGGGGGGCC  GCTATAAACA  GATTCCCACG  GAGAATCCGA
401 CGGGTTGTTA  CTCGCTCACA  TTTAATGTTG  ATGAAAGCTG  GCTACAGGAA
451 GGCCAGACGC  GAATTATTTT  TGATGGCGTT  CCTATTGGTT  AAAAAATGAG
501 CTGATTTAAC  AAAAAATTTA  TGCGAATTTT  AACAAAATAT  TAACGTTTAC
551 AATTTAAATA  TTTGCTTATA  CAATCTTCCT  GTTTTTGGGG  CTTTTCTGAT
601 TATCAACCGG  GGTACATATG  ATTGACATGC  TAGTTTTACG  ATTACCGTTC
651 ATCGATTCTC  TTGTTTGCTC  CACACTATCA  GGCAATGACC  TGATAGCCTT
701 TGTA

```

Figure 5.8: Sequence **ATMi4** in 5' → 3' direction. It contains the recognition site of Nt.BstNBI, which lies in the complement of the subsequence GACTC (bases 357-361), marked in red. The nicking enzyme will create a nick between bases 352 and 353 on the complementary strand, which leads to sequence **ATMi4***.

```

1  AATTCGAGCT  CGGTACCCGG  GGATCCTCTA  GAGTCGACCT  GCAGGCATGC
51  AAGCTTGGCA  CTGGCCGTCG  TTTTACAACG  TCGTGACTGG  GACAACTGTT
101 TGAAGAGCCA  ACTTAATCGC  CTTGCAGCAC  ATCCCCCTTT  AGCGAGCTGG
151 CTTAATAGCG  AAGAGGCCCG  CACCGAGGCA  ATGACCTGAT  AGCCTTTGTA

```

Figure 5.9: Sequence **200** in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 102-108), marked in red. The nicking enzyme will create a nick between bases 100 and 101 on the complementary strand, which leads to sequence **200***.

```

1  AATTCGAGCT  CGGTACCCGG  CCGATCGCCC  TTCCCAACAG  TTGCGCAGCC
51  TGAATGGCGA  ATGGCGCTTT  TCATGAAAAA  GTCTTTAGTC  CTCAAAGCCT
101 CTGTAGCCGT  TGGAGTGCGA  TCTTCTGAG  GTGACATACG  TCGTGTGCC
151 CTCAAACCTG  CAGATGCACG  GTTACGATGC  GCCCATCTAC  ACCAAGCTGT
201 TGAAGAGCAT  TACGGTCAAT  CCGCGTTTG  TTCCACGGA  GAATCCGACG
251 GGTTGTTACT  CGCTCACATT  TAATGTTGAT  GAAAGCTGGC  TACAGGAAGG
301 CCAGACGCGA  ATTATTTTIG  ATGGCGTTC  TATTGGTTAA  AAAATGAGCT
351 GATTTAACAA  AAATTTAATG  CGAATAGGCA  ATGACCTGAT  AGCCTTTGTA

```

Figure 5.10: Sequence **400** in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 202-208), marked in red. The nicking enzyme will create a nick between bases 200 and 201 on the complementary strand, which leads to sequence **400***.

```

1  AATTCGAGCT  CGGTACCCGG  TGAGCGGATA  ACAATTTTCAC  ACAGGAAACA
51  GCTATGACCA  TGATTACGGG  ATCCTCTAGA  GTCGACCTGC  AGGCATGCAA
101 GCTTGCGACT  GGCCGTCGTT  TTACAACGTC  GTGACTGGGA  AAACCCTGGC
151 GTTACCCAAC  TTAATCGCCT  TGCAGCACAT  CCCCCTTTAG  CGAGCTGGCT
201 TAATAGCGAA  GAGGCCCGCA  CCGATCGCCC  TTCCCAACAG  TTGCGCAGCC
251 TGAATGGCGA  ATGGCGCTTT  TCATGAAAAA  GTCTTTAGTC  CTCAAAGCCT
301 CTGTAGCCGT  TGGAGTGCGA  TCTTCTGAG  GTGACATACG  TCGTCGTCCC
351 CTCAAACTGG  CAGATGCACG  GTTACGATGC  GCCCATCTAC  ACCAACGTGT
401 TGAAGAGCAT  TACGGTCAAT  CCGCCGTTTG  TTCCACGGA  GAATCCGACG
451 GGTGTGTTACT  CGCTCACATT  TAATGTTGAT  GAAAGCTGGC  TACAGGAAGG
501 CCAGACGCGA  ATTATTTTIG  ATGGCGTTCC  TATTGGTTAA  AAAATGAGCT
551 GATTTAACAA  AAATTTAATG  CGAATTTTAA  CAAAATATTAA  ACGTTTACAA
601 TTTAAATATT  TGCTTATACA  ATCTTCTGT  TTTTGGGGCT  TTTCTGATTA
651 TCAACCGGGG  TACATATGAT  TGACATGCTA  GTTTTACGAT  TACCGTTCAT
701 CGATTCTCTT  GTTTGCTCCA  GACTCTCGAT  CTCTCAAAAA  TAGCTACCCCT
751 CTCGGGCATT  AATTTATCAG  CTAGAAGGCA  ATGACCTGAT  AGCCTTTGTA

```

Figure 5.11: Sequence **800** in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 402-408), marked in red. The nicking enzyme will create a nick between bases 400 and 401 on the complementary strand, which leads to sequence **800***.

```

1  AATTCGAGCT  CGGTACCCGG  GCGGGCAGTG  AGCGCAACGC  AATCGATTTA
51  TAATGICTAC  TCGGATAGGC  ACCCCAGGCG  TTTGTCCTTC  GATCGTTCGG
101 TAAGTTCACT  TGTGGAAAAG  ATTACGTGTG  AGACTTTCAC  ACAGGAAACA
151 GCTATGACCA  TGATTACGGG  ATCCTCTAGA  GTCGACCTGC  AGGCATGCAA
201 GCTTGCGACT  GGCCGTCGTT  TTACAACGTC  GTGACTGGGA  AAACCCTGGC
251 GTTACCCAAC  TTAATCGCCT  TGCAGCACAT  CCCCCTTTAG  CGAGCTGGCT
301 TAATAGCGAA  GAGGCCCGCA  CCGATCGCCC  TTCCCAACAG  TTGCGCAGCC
351 TGAATGGCGA  ATGGCGCTTT  TCATGAAAAA  GTCTTTAGTC  CTCAAAGCCT
401 CTGTAGCCGT  TGGAGTGCGA  TCTTCTGAG  GTGACATACG  TCGTCGTCCC
451 CTCAAACTGG  CAGATGCACG  GTTACGATGC  GCCCATCTAC  ACCAACGTGT
501 TGAAGAGCAT  TACGGTCAAT  CCGCCGTTTG  TTCCACGGA  GAATCCGACG
551 GGTGTGTTACT  CGCTCACATT  TAATGTTGAT  GAAAGCTGGC  TACAGGAAGG
601 CCAGACGCGA  ATTATTTTIG  ATGGCGTTCC  TATTGGTTAA  AAAATGAGCT
651 GATTTAACAA  AAATTTAATG  CGAATTTTAA  CAAAATATTAA  ACGTTTACAA
701 TTTAAATATT  TGCTTATACA  ATCTTCTGT  TTTTGGGGCT  TTTCTGATTA
751 TCAACCGGGG  TACATATGAT  TGACATGCTA  GTTTTACGAT  TACCGTTCAT
801 CGATTCTCTT  GTTTGCTCCA  GACTCTCGAT  CTCTCAAAAA  TAGCTACCCCT
851 CTCGGGCATT  AATTTATCAG  CTAGAAGGCA  TGAATATCAT  ATTGATGGTG
901 ATTTGACTGT  CTCGGCCTT  TCTCACCTT  TTGAATCTTT  ACCTACACAT
951 TACTCAGGCA  TTGCATTTAA  AATATAGGCA  ATGACCTGAT  AGCCTTTGTA

```

Figure 5.12: Sequence **1000** in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 502-508), marked in red. The nicking enzyme will create a nick between bases 500 and 501 on the complementary strand, which leads to sequence **1000***.

```

1  AATTCGAGCT  CGGTACCCGG  TTCTTTTGAT  TTATAAGGGA  TTTTGCCGAT
51  TTCGGAACCA  CCATCAAACA  GGATTTTTCG  CTGCTGGGGC  AAACCAGCGT
101 GGACCGCTTG  CTGCAACTCT  CTCAGGGCCA  GGCGGTGAAG  GGCAATCAGC
151 TGTGCCCCGT  CTCCTGGTGG  AAAAGAAAAA  CCACCTGGC  GCGCAATACG
201 CAAACCGCCT  CTCCCGCGC  GTTGGCCGAT  TCATTAATGC  AGCTGGCACG
251 ACAGGTTTCC  CGACTGGAAG  GCGGGCAGTG  AGCGCAACGC  AATCGATTTA
301 TAATGTCTAC  TCGGATAGGC  ACCCCAGGCG  TTTGTCTTTC  GATCGTTCCG
351 TAAGTTCACT  TGTGGAAAAG  ATTACGTGTG  AGACTTTCAC  ACAGGAAACA
401 GCTATGACCA  TGATTACGGG  ATCCTCTAGA  GTCGACCTGC  AGGCATGCAA
451 GCTTGGCACT  GGCCGTGCTT  TTACAACGTC  GTGACTGGGA  AAACCCTGGC
501 GTTACCCAAC  TTAATCGCCT  TGCAGCACAT  CCCCTTTAG  CGAGCTGGCT
551 TAATAGCGAA  GAGGCCGCA  CCGATCGCCC  TTCCAACAG  TTGCGCAGCC
601 TGAATGGCGA  ATGGCGCTTT  TCATGAAAAA  GTCTTTAGTC  CTCAAAGCCT
651 CTGTAGCCGT  TGGAGTGCGA  TCTTCTGAG  GTGACATAAG  TCGTCGTCC
701 CTCAAAGTGG  CAGATGCACG  GTTACGATGC  GCGCATCTAC  ACCAAGCTGT
751 TGAAGAGCAT  TACGGTCAAT  CCGCCGTTTG  TTCCACGGA  GAATCCGACG
801 GGTTGTTACT  CGCTCACATT  TAATGTTGAT  GAAAGCTGGC  TACAGGAAGG
851 CCAGACGCGA  ATTATTTTGG  ATGGCGTTCC  TATTGGTTAA  AAAATGAGCT
901 GATTTAACAA  AAATTTAATG  CGAATTTTAA  CAAAATATTA  ACGTTTACAA
951 TTTAAATATT  TGCTTATACA  ATCTTCTGT  TTTTGGGGCT  TTTCTGATT
1001 TCAACCGGGG  TACATATGAT  TGACATGCTA  GTTTTACGAT  TACCGTTTAT
1051 CGATTCTCTT  GTTTGCTCCA  GACTCTCGAT  CTCTCAAAAA  TAGCTACCT
1101 CTCGGGCATT  AATTTATCAG  CTAGAACGGT  TGAATATCAT  ATTGATGGTG
1151 ATTTGACTGT  CTCGGCCTT  TCTCACCTT  TTGAATCTTT  ACCTACACAT
1201 TACTCAGGCA  TTGCATTTAA  AATATATGAG  GGTTCTAAAA  ATTTTATCC
1251 TTGCGTTGAA  ATAAAGGCTT  CTCCGCAAA  AGTATTACAG  GGTGATAATG
1301 TTTTGGTAC  AACCATTATA  GCTTTATGCT  CTGAGGCTTT  ATTGCTTAAT
1351 TTTGCTAATT  CTTTGCCTTG  CCTGTATGAT  TTATTGGATG  TTAATGCTAC
1401 TACTATTAGT  AGAATTGATG  CCACCTTTTC  AGCTCGCGCC  CCAAATGAAA
1451 ATATAGCTAA  ACAGGTTATT  GACCAAGGCA  ATGACCTGAT  AGCCTTTGTA

```

Figure 5.13: Sequence 1500 in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 752-758), marked in red. The nicking enzyme will create a nick between bases 750 and 751 on the complementary strand, which leads to sequence 1500*. The recognition site of Nb.BtsI lies in the complement of the subsequence GCAGTG (bases 275-280), marked in blue. Here, the nicking enzyme will create a nick between bases 280 and 281.

```

1  AATTC  GAGCT  CGGTA  CCCGG
1  TACAA  AGGCT  ATCAG  GTCAT  TGCTT

```

Figure 5.14: Primer sequences in 5' → 3' direction.

Precise Mechanochemical Scission of DNA Guided by Secondary Structures

```

1  GTGCTCGTAG AACGTCCACG GAGTCTTTAG TCCTCAAAGC CTCTGTAGCC
51  GTTGGATGGC GATCTTCCTG AGGTGACATA CGTCGTGTC CCCTCAAAC
101 GGCAGATGCA CGGTTACGAT GCGCCCATCT ACACCAACGT GGTACGAGA
151 ATTACGGTCA ATCCGCCGTT TGTTCCACG GAGAATCCGA CGGGTGTGTC
201 TACGCCTCCA TCCTCTGGA CCCAACTTAA TCGCCTTGCA GCACATCCCC
251 CTTTAGCGAG CTGGCTTAAT AGCGAAATGT TCCGATAGTC CAGTCACCAC

```

Figure 5.15: Sequence DNA_{left}(500) in 5' → 3' direction. It contains the recognition site of BstXI (red), which, after restriction, exposes the sticky end CCTT (blue) which is needed for subsequent ligation to the middle part.

```

1  GTGCTCGTAG AACGTCCACG GAACGGCCAT CCGAGCATGC ACAGTCGCCG
51  CACCTAAGAC ATAGCGCTCC TCTGTTCGTT AGTGTTCGCC GAGACCATAC
101 GGTCAATCCG CCGTTTAGAT GAACTTATTC ACCGCTTCCT GATAGATCTA
151 ATGTTCTTAT GTGGTACATC AAGTATTACC TGATTCTCAT GTTCTATATG
201 GTAGCTTAAG ACGAAATAGA TGAATTATAG TTCAACGCTG ATGTCACCTG
251 GTCGTTGATG ATTAGTACAT CTCCTTATGT TCCGATAGTC CAGTCACCAC

```

Figure 5.16: Sequence DNA_{right}(500) in 5' → 3' direction. It contains the recognition site of Eco31I (red), which, after restriction, exposes the sticky end TCGC (blue) which is needed for subsequent ligation to the middle part.

```

1  GTGCTCGTAG AACGTCCACG GACCTGCTTC TATTCGCGGA AGTGAATGGA
51  TGCCACGTCG CGCATACGTC CAGTCTGGCT CCTGTTATCT TGGTACCTGG
101 CCGGCTCGCC CGCGGTGCTG CCTTCTGTGC ATCGTCGGTA ACCATAAGTC
151 TTTAGTCTC AAAGCCTCTG TAGCCGTTGG ATGGCGATCT TCCTGAGGTG
201 ACATACGTCG TCGTCCCCTC AAAGTGGCAG ATGCACGGTT ACGATGCGCC
251 CATCTACACC AACGTGGTTA CGAGAATTAC GGTCAATCCG CCGTTGTGTC
301 CCACGGAGAA TCCGACGGGT TGTCTACGC CTCATCCTT CTGGACCCAA
351 CTTAATCGCC TTGCAGCACA TCCCCCTTAA GCGAGCTGGC TTAATAGCGA
401 AGAGGCCCGC ACCGATATGT TCCGATAGTC CAGTCACCAC

```

Figure 5.17: Sequence DNA_{left}(750) in 5' → 3' direction. It contains the recognition site of BstXI (red), which, after restriction, exposes the sticky end CCTT (blue) which is needed for subsequent ligation to the middle part.


```

1  GTGCTCGTAG AACGTCCACG GCCTGTTCCA CATCATAACG GCCATCCGAG
51  CATGCACAGT CGCCGCACCT AAGACATAGC GCTCCTCTGT TCGTTAGTGT
101 TCGCCGAGAC CATAACGGTCA ATCCGCCGTT TAGATGAACT TATTCACCGC
151 TTCCTGATAG ATCTAATGTT CCTATGTGGT ACATCAAGTA TTACCTGATT
201 CTCATGCGCT ATATGGTAGC TTAAGACGAA ATAGATGAAT TATAGTTCAA
251 CGCTGATGTC ACTTGGTCGT TGATGATTAG TACATCTCTT TAATATTAAG
301 ATAGCAGAAC CTGTAAACATG TTCAGTTCTT TACGTCCGCT CATCAGCTCG
351 TAGGGCGAAG TGAAGAGAAT AGATAACTAA CTTGTGATGT GTTACATTTA
401 ATCATCCAGC TTGTTTATGT TCCGATAGTC CAGTCACCAC

```

Figure 5.18: Sequence DNA_{right}(750) in 5' → 3' direction. It contains the recognition site of Eco31I (red), which, after restriction, exposes the sticky end TCGC (blue) which is needed for subsequent ligation to the middle part.

```

1  GTGCTCGTAG AACGTCCACG GTACCCGGGC GGGCAGTGAG CGCAACGCAA
51  TCGATTTATA ATGTCTACTC GGATAGGCAC CCACGGCGTT TGTCTTTTGA
101 TCGTTCCGTA AGTTCACTTG TGGAAAAGAT TACGTGTGAG ACTTTACCT
151 GCTTCTATTC GCGGAAGTGA ATGGATGCCA CGTCGCGCAT ACGTCCAGTC
201 TGGCTCCTGT TATCTTGGTA CCTGGCCGGC TCGCCCGCGG TCGTGCCTTC
251 TGTGCATCGT CGGTAACCAT AAGTCTTTAG TCCTCAAAGC CTCTGTAGCC
301 GTTGGATGGC GATCTTCCTG AGGTGACATA CGTCGTGCTC CCCTCAAAC
351 GGCAGATGCA CGGTTACGAT GCGCCCATCT ACACCAACGT GGTTACGAGA
401 ATTACGGTCA ATCCGCCGTT TGTTOCCACG GAGAATCCGA CGGGTTGTTC
451 TACGCCTCCA TCBTCTGGA CCAACTTAA TCGCCTTGCA GCACATCCCC
501 CTTTAGCGAG CTGGCTTAAT AGCGAAGAGG CCGCACCGA TATGTTCCGA
551 TAGTCCAGTC ACCAC

```

Figure 5.19: Sequence DNA_{left}(1000) in 5' → 3' direction. It contains the recognition site of BstXI (red), which, after restriction, exposes the sticky end CCTT (blue) which is needed for subsequent ligation to the middle part.

```

1  GTGCTCGTAG AACGTCCACG GCCTGTTCCA CATCATAACG GCCATCCGAG
51  CATGCACAGT CGCCGCACCT AAGACATAGC GCTCCTCTGT TCGTTAGTGT
101 TCGCCGAGAC CATAACGGTCA ATCCGCCGTT TAGATGAACT TATTCACCGC
151 TTCCTGATAG ATCTAATGTT CCTATGTGGT ACATCAAGTA TTACCTGATT
201 CTCATGCGCT ATATGGTAGC TTAAGACGAA ATAGATGAAT TATAGTTCAA
251 CGCTGATGTC ACTTGGTCGT TGATGATTAG TACATCTCTT TAATATTAAG
301 ATAGCAGAAC CTGTAAACATG TTCAGTTCTT TACGTCCGCT CATCAGCTCG
351 TAGGGCGAAG TGAAGAGAAT AGATAACTAA CTTGTGATGT GTTACATTTA
401 ATCATCCAGC TTGTTTTACC CGGGCGGGCA GTGAGCGCAA CGCAATCGAT
451 TTATAATGTC TACTCGGATA GGCACCCAG GCGTTTGTCT TTCGATCGTT
501 CCGTAAAGTTC ACTTGTGGAA AAGATTACGT GTAGGACTTT CATGTTCCGA
551 TAGTCCAGTC ACCAC

```

Figure 5.20: Sequence DNA_{right}(1000) in 5' → 3' direction. It contains the recognition site of Eco31I (red), which, after restriction, exposes the sticky end TCGC (blue) which is needed for subsequent ligation to the middle part.

1 GTGCT CGTAG AACGT CCACG G
1 GTGGT GACTG GACTA TCGGA ACAT

Figure 5.21: Primer sequences in 5' → 3' direction.

Table 5.1: DNA oligonucleotide strands used to assemble the central secondary motifs.

Strands	Sequence in 5' → 3' direction
TopHp	ACGATGCGCCCATCTACACCAACGTGTCGCGCGTTCGCGCGT- GAAGAGCATTACGGTCAATCCGCCGT
Top0S	ACGATGCGCCCATCTACACCAACGTGTTTTTTGAAGAGCATTAC- GGTCAATCCGCCGT
Top4S	ACGATGCGCCCATCTACACCAACGTGTCGCGTTCGCGTGAAG- AGCATTACGGTCAATCCGCCGT
Top10S	ACGATGCGCCCATCTACACCAACGTGTCGCGCGCGCGTTCGCG- GCGCGCGTGAAGAGCATTACGGTCAATCCGCCGT
Top0L	ACGATGCGCCCATCTACACCAACGTGTCGCGCGCGCGCGTGAAG- AGCATTACGGTCAATCCGCCGT
Top8L	ACGATGCGCCCATCTACACCAACGTGTCGCGCGTTCGCGCGTTCGCG- GCGTGAAGAGCATTACGGTCAATCCGCCGT
BotHp0	GCGAACGGCGGATTGACCGTAATGCTCTTCAACACGTTGGTGTA- GATGGGCGCATCGTAAGG
BotHp1	GCGAACGGCGGATTGACCGTAATGCTCTTCATACACGTTGGTGT- AGATGGGCGCATCGTAAGG
BotHp2	GCGAACGGCGGATTGACCGTAATGCTCTTCATTACACGTTGGTG- TAGATGGGCGCATCGTAAGG
BotHp4	GCGAACGGCGGATTGACCGTAATGCTCTTCATTTTACACGTTGG- TGATAGATGGGCGCATCGTAAGG
BotHp5	GCGAACGGCGGATTGACCGTAATGCTCTTCATTTTACACGTTG- GTGTAGATGGGCGCATCGTAAGG

How DNA Feels Force During Freezing of Water

```

1  AATTCGAGCT  CGGTACCCGG  CCGATCGCCC  TTCCCAACAG  TTGCGCAGCC
51  TGAATGGCGA  ATGGCGCTTT  TCATGAAAAA  GTCTTTAGTC  CTCAAAGCCT
101 CTGTAGCCGT  TGGAGTGCGA  TCTTCTGAG  GTGACATACG  TCGTCGTCCC
151 CTCAAACCTGG  CAGATGCACG  GTTACGATGC  GCCCATCTAC  ACCAACGTGT
201 TGAAGAGCAT  TACGGTCAAT  CCGCCGTTTG  TTCCACGGA  GAATCCGACG
251 GGTTGTTACT  CGCTCACATT  TAATGTTGAT  GAAAGCTGGC  TACAGGAAGG
301 CCAGACGCGA  ATTATTTTIG  ATGGCGTTCC  TATTGGTTAA  AAAATGAGCT
351 GATTTAACAA  AAATTTAATG  CGAATAGGCA  ATGACCTGAT  AGCCTTTGTA

```

Figure 5.22: Sequence **400** in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 202-208), marked in red. The nicking enzyme will create a nick between bases 200 and 201 on the complementary strand, which leads to sequence **400***.

```

1  AATTCGAGCT  CGGTACCCGG  TGAGCGGATA  ACAATTTTAC  ACAGGAAACA
51  GCTATGACCA  TGATTACGGG  ATCCTCTAGA  GTCGACCTGC  AGGCATGCAA
101 GCTTGGCACT  GGCCGTGCTT  TTACAACGTC  GTGACTGGGA  AAACCCTGGC
151 GTTACCCAAC  TTAATCGCCT  TGCAGCACAT  CCCCTTTAG  CGAGCTGGCT
201 TAATAGCGAA  GAGGCCGCA  CCGATCGCCC  TTCCCAACAG  TTGCGCAGCC
251 TGAATGGCGA  ATGGCGCTTT  TCATGAAAAA  GTCTTTAGTC  CTCAAAGCCT
301 CTGTAGCCGT  TGGAGTGCGA  TCTTCTGAG  GTGACATACG  TCGTCGTCCC
351 CTCAAACCTGG  CAGATGCACG  GTTACGATGC  GCCCATCTAC  ACCAACGTGT
401 TGAAGAGCAT  TACGGTCAAT  CCGCCGTTTG  TTCCACGGA  GAATCCGACG
451 GGTTGTTACT  CGCTCACATT  TAATGTTGAT  GAAAGCTGGC  TACAGGAAGG
501 CCAGACGCGA  ATTATTTTIG  ATGGCGTTCC  TATTGGTTAA  AAAATGAGCT
551 GATTTAACAA  AAATTTAATG  CGAATTTTAA  CAAAATATTA  ACGTTTACAA
601 TTTAAATATT  TGCTTATACA  ATCTTCCTGT  TTTTGGGGCT  TTTCTGATTA
651 TCAACCGGGG  TACATATGAT  TGACATGCTA  GTTTTACGAT  TACCGTTCAT
701 CGATTCTCTT  GTTTGCTCCA  GACTCTCGAT  CTCTCAAAAA  TAGCTACCCCT
751 CTCGGGCATT  AATTTATCAG  CTAGAAGGCA  ATGACCTGAT  AGCCTTTGTA

```

Figure 5.23: Sequence **800** in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 402-408), marked in red. The nicking enzyme will create a nick between bases 400 and 401 on the complementary strand, which leads to sequence **800***.

```

1  AATTCGAGCT CGGTACCCGG TTCTTTTGAT TTATAAGGGA TTTTGCCGAT
51  TTCGGAACCA CCATCAAACA GGATTTTTCG CTGCTGGGGC AAACCAGCGT
101 GGACCGCTTG CTGCAACTCT CTCAGGGCCA GGCGGTGAAG GGCAATCAGC
151 TGTTGCCCGT CTCACTGGTG AAAAGAAAAA CCACCTGGC GCCAATACG
201 CAAACCGCCT CTCCCGCGC GTTGGCCGAT TCATTAATGC AGCTGGCACG
251 ACAGGTTTCC CGACTGGAAG GCGGGCAGTG AGCGCAACGC AATCGATTTA
301 TAATGTCTAC TCGGATAGGC ACCCCAGGCG TTTGTCTTTC GATCGTTCCG
351 TAAGTTCACT TGTGGAAAAG ATTACGTGTG AGACTTTCAC ACAGGAAACA
401 GCTATGACCA TGATTACGGG ATCCTCTAGA GTCGACCTGC AGGCATGCAA
451 GCTTGGCACT GGCCGTGCTT TTACAACGTC GTGACTGGGA AAACCCTGGC
501 GTTACCCAAC TTAATCGCCT TGCAGCACAT CCCCTTTAG CGAGCTGGCT
551 TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCAACAG TTGCGCAGCC
601 TGAATGGCGA ATGGCGCTTT TCATGAAAAA GTCTTTAGTC CTCAAAGCCT
651 CTGTAGCCGT TGGAGTGCGA TCTTCTGAG GTGACATAAG TCGTCGTCC
701 CTCAAAGTGG CAGATGCACG GTTACGATGC GCCCATCTAC ACCAACGTGT
751 TGAAGAGCAT TACGGTCAAT CCGCCGTTTG TTCCACGGA GAATCCGACG
801 GGTTGTTACT CGCTCACATT TAATGTTGAT GAAAGCTGGC TACAGGAAGG
851 CCAGACGCGA ATTATTTTIG ATGGCGTTCC TATTGGTTAA AAAATGAGCT
901 GATTTAACAA AAATTTAATG CGAATTTTAA CAAAATATTA ACGTTTACAA
951 TTAAATATT TGCTTATACA ATCTTCTGT TTTTGGGGCT TTTCTGATT
1001 TCAACCGGGG TACATATGAT TGACATGCTA GTTTTACGAT TACCGTTCAT
1051 CGATTCTCTT GTTTGCTCCA GACTCTCGAT CTCTCAAAAA TAGCTACCT
1101 CTCGGCATT AATTTATCAG CTAGAACGGT TGAATATCAT ATTGATGGTG
1151 ATTTGACTGT CTCGGCCTT TCTCACCTT TTGAATCTTT ACCTACACAT
1201 TACTCAGGCA TTGCATTTAA AATATATGAG GGTTCTAAAA ATTTTATCC
1251 TTGCGTTGAA ATAAAGGCTT CTCCGCAAA AGTATTACAG GGTGATAATG
1301 TTTTGGTAC AACCGATTAA GCTTTATGCT CTGAGGCTTT ATTGCTTAAT
1351 TTTGCTAATT CTTTGCCTTG CCTGTATGAT TTATTGGATG TTAATGCTAC
1401 TACTATTAGT AGAATTGATG CCACCTTTTC AGCTCGCGCC CCAAATGAAA
1451 ATATAGCTAA ACAGGTTATT GACCAAGGCA ATGACCTGAT AGCCTTTGTA

```

Figure 5.24: Sequence 1500 in 5' → 3' direction. It contains the recognition site of Nt.BspQI, which lies in the complement of the subsequence GAAGAGC (bases 752-758), marked in red. The nicking enzyme will create a nick between bases 750 and 751 on the complementary strand, which leads to sequence 1500*. The recognition site of Nb.BtsI lies in the complement of the subsequence GCAGTG (bases 275-280), marked in blue. Here, the nicking enzyme will create a nick between bases 280 and 281.

```

1  AATTC GAGCT CGGTA CCCGG
1  TACAA AGGCT ATCAG GTCAT TGCTT

```

Figure 5.25: Primer sequences in 5' → 3' direction.

Table 5.2: DNA oligonucleotide strands hp10, hp20 and hp30 and ds80.

Strands	Sequences in 5' → 3' direction.
hp10	ACACGTTTTTCGTGTTGAAGTTTTCTTCA
hp20	ACACGTTGTCTTTTGACAACGTGTTGAAGAGCCATTTTGGCT- CTTCA
hp30	ACACGTTGTCCCAGTTTTTACTGGGACAACGTGTTGAAGAGCC- AACTTATTTTAAAGTTGGCTCTTCA
ds80 Bot	GCGAACGGCGGATTGACCGTAATGCTCTTCACGCGCGAAAACG- CGCGACACGTTGGTGTAGATGGGCGCATCGTAAGG
ds80 Top	ACGATGCGCCCATCTACACCAACGTGTCGCGCGTTTTTCGCGCG- TGAAGAGCATTACGGTCAATCCGCCGT

List of Abbreviations

AFM	atomic force microscopy
AFPs	antifreeze proteins
AT	adenine–thymine
B-DNA	B-form deoxyribonucleic acid
bp	base pairs
CAS	Chemical Abstracts Service
CCD	charge-coupled device
CE	capillary electrophoresis
CMOS	complementary metal–oxide–semiconductor
CPAs	cryoprotective agents
CPDs	cyclobutane pyrimidine dimers
ddH ₂ O	double-distilled water
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
dNTPs	deoxynucleoside triphosphates
dsDNA	double-stranded DNA
EDTA	ethylenediaminetetraacetic acid
EM	electron microscopy
EtBr	ethidium bromide
FD	FastDigest
FT	freeze–thaw
GC	guanine–cytosine
GE	gel electrophoresis
GFX	glass fiber extraction
GPa	gigapascal
GPC	gel permeation chromatography
HB	hydrogen bond
hpDNA	hairpin DNA
HPLC	high-performance liquid chromatography
<i>i.e.</i>	<i>id est</i> (Latin), meaning "that is" or "in other words."
IQR	interquartile range

LED	light-emitting diode
LIG	ligation
LN ₂	liquid nitrogen
M13mp18	filamentous bacteriophage M13-derived ssDNA scaffold
MD	molecular dynamics
mRNA	messenger ribonucleic acid
MW	molecular weight
NEB	New England Biolabs
NEBuffer	NEB buffer system (r3.1)
NGS	next-generation sequencing
NMR	nuclear magnetic resonance
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDB	Protein Data Bank
Pfu	Pfu DNA polymerase
PhiX	Illumina PhiX calibration library
PnP	plug and play
Q500	Qsonica 500 ultrasound processor
QM/MM	quantum mechanics/molecular mechanics
QY	quantum yield
RMSD	root mean square deviation
RRO	Rothemund rectangle origami
RNA	ribonucleic acid
ROS	reactive oxygen species
RT	room temperature
SDS	sodium dodecyl sulfate
SCM	single-chain mechanophore
SPP	single-point projection (in MD)
ssDNA	single-stranded DNA
TAE	Tris–acetate–EDTA
TEM	transmission electron microscopy
TIP3P	transferable intermolecular potential 3-point water model
TSE	Tris–sodium chloride–EDTA

T_m	melting temperature
Tris	tris(hydroxymethyl)aminomethane
UV	ultraviolet
US	ultrasound
VIS	visible light
μ	mean value of Gaussian fit
σ	standard deviation of Gaussian fit

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List of Publications

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