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Active DNA Olympic Hydrogels Driven by Topoisomerase Activity

Brad A. Krajina[1], Audrey Zhu[1], Sarah C. Heilshorn[2], Andrew J. Spakowitz[1,2,3]
1. Department of Chemical Engineering, Stanford University, Stanford, CA, 94305 USA
2. Department of Materials Science and Engineering, Stanford University, Stanford, CA, 94305 USA
3. Department of Applied Physics, Stanford University, Stanford, California 94305, USA

Biological systems are equipped with a diverse repertoire of proteins that regulate DNA topology with precision that is beyond the reach of conventional polymer chemistry. Here, we harness the unique properties of topoisomerases to synthesize Olympic hydrogels formed by topologically interlinked DNA rings. Using dynamic light scattering microrheology to probe the viscoelasticity of DNA topological networks, we show that topoisomerase II enables the facile preparation of active, ATP-driven Olympic hydrogels that can be switched between liquid and solid states on demand. Our results provide a versatile system for engineering switchable topological materials that may be broadly leveraged to model the impact of topological constraints and active dynamics in the physics of chromosomes and other polymeric materials.

Four decades ago, Pierre-Gilles de Gennes envisioned the possibility of synthesizing polymeric gels from topologically interlinked ring polymers, coined "Olympic gels", rather than chemical or physical bonds [36]. Despite long-standing interest in topological bonds across the fields of chemistry, nanotechnology, and polymer physics, the synthesis of Olympic gels has remained an enduring challenge for polymer chemists [37–43]. To date, the synthesis of topologically interlinked (catenated) ring polymers using conventional synthetic chemistry has been limited to very simple topologies, such as catenated dimers [39, 44–47]. Nevertheless, theoretical studies [48] and the experimental realization of other topological materials, such as slide ring gels [49] and poly[n]catenanes [43], suggest that the molecular mobility uniquely afforded to topological bonds may confer a variety of unusual material properties, such as nonlinear elasticity [48], high toughness and strechability [50], and stimuli-responsive molecular flexibility [43].

Biological systems are masters of topology. In particular, chromosome regulation requires a tremendous amount of genomic material to be packaged into a confined space while orchestrating dynamic biological processes, such as gene expression, replication, and chromosome segregation [51, 52]. Consequently, biological systems have evolved a family of enzymes, known as topoisomerases, which are exquisite regulators of DNA topology. Topoisomerases perform a diverse repertoire of functions, including disentangling chromosomes, topologically linking and unlinking DNA rings, and twisting or untwisting DNA to modulate its supercoiled state.

The existence of extended catenated DNA networks resembling an Olympic gel was discovered in kinetoplast mitochondrial DNA (left panel of Fig. 1) [53], which is maintained in a single network of thousands of rings. Although DNA base-pairing has been extensively exploited to engineer a variety of DNA-based materials, including 2D and 3D nanostructures [40, 54] and base-pair cross-linked hydrogels [55, 56], the unrivaled control over DNA topology endowed to topoisomerases remains under-explored as a synthetic tool.

The ability of topoisomerases to orchestrate facile synthesis of Olympic hydrogels was recently demonstrated through the use of topoisomerase II inhibitors that arrest topoisomerase II in a closed protein clamp during its strand passage cycle, thereby producing catenated gels from concentrated solutions of DNA rings [57]. Although that study highlights the unique molecular specificity of topoisomerases for topological transactions, another powerful feature that remains under-explored is the ability of topoisomerases to actively and reversibly fluidize DNA topology. The reversible and dynamic nature of topoisomerase-mediated reactions holds the potential to develop a variety of switchable, active materials with properties beyond the reach of statically catenated networks.

Here, we harness the intrinsic capabilities of topoisomerases to synthesize biologically inspired, active, and switchable Olympic hydrogels formed by DNA rings. Our strategy directly circumvents barriers to synthesizing Olympic gels that are inherent to templated cyclization techniques [41, 42, 44, 46, 59], which require dilute solutions to favor intramolecular ring closure over intermolecular chain elongation. We show that dynamic topological transactions mediated by topoisomerases enable facile preparation of hydrogels whose topology can be actively transformed between non-catenated, topologically dynamic, and statically catenated states based solely on the presence of enzyme and the availability of catalytic co-factors (right panels of Fig. 1). We envision that this material platform will present broad opportunities for directing self-assembly and active dynamics in topological materials, owing to the unique properties of DNA and the vast array of molecular motors and enzymes that act upon it.

Our synthesis of DNA Olympic hydrogels is inspired by the biological roles of type II topoisomerases in resolving topological links during DNA replication and segregation [51, 52]. We hypothesized that Olympic gel formation would be promoted during topoisomerase-mediated,

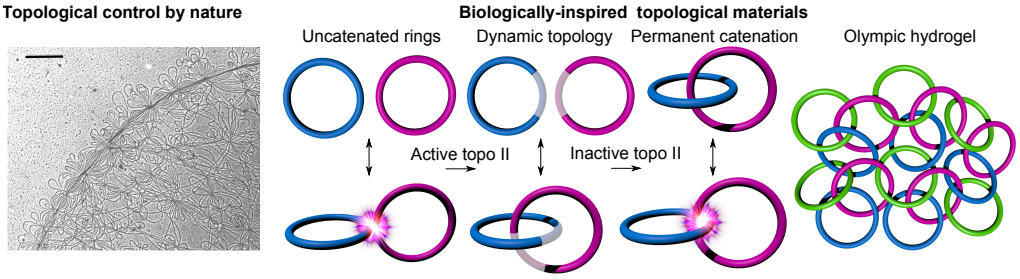

FIG. 1. Synthesis of active topological networks inspired by nature. *Left*: Electron micrograph of kinetoplast mitochondrial DNA, which is maintained in a topologically linked (catenated) network of rings by topoisomerases (scale bar = 500 nm) [58]. *Right*: Topoisomerase II (topo II) enables the synthesis of DNA ring topological networks that can be actively switched between three topological states based on the presence and catalytic state of enzyme: uncatenated rings in which linking reactions are topologically forbidden, dynamic topological networks fluidized by topo II-mediated active linking/unlinking reactions, and permanently catenated networks (Olympic gels), in which unlinking reactions are topologically forbidden. The electron micrograph of kinetoplast DNA was adapted with permission from Ref. [58].

random strand passage reactions in crowded solutions of non-supercoiled (i.e. torsionally relaxed) rings with high molecular weights, which we expected to entropically drive the formation of topologically interpenetrating rings. Thus, we began with relatively large (22.6 kb) DNA plasmid rings prepared as uncatenated solutions well above the critical overlap concentration (6 mg/mL). Since our plasmids were replicated in *Escherichia coli* (*E. coli*) in a supercoiled form, we torsionally relaxed the rings using a singe strand nicking restriction endonuclease. In the presence of human topoisomerase II-α (topo II) at a final concentration of ≈ 0.4 homodimers per DNA molecule (155 nM), a catenated network was formed that exhibited a dynamic topological state due to ATP-dependent active strand passage reactions that reversibly catenate and decatenate overlapping DNA rings, resulting in network fluidization.

To interrogate the topological state of our DNA networks, we implement dynamic light scattering microrheology (DLSμR), which probes the viscoelastic behavior of soft materials by monitoring passive fluctuations of tracer particles embedded within the material. Our recent work on DLSμR [60] enables us to non-destructively access a broad hierarchy of time scales (10^{-6} s to 10 s) associated with molecular relaxations in materials and is particularly well-suited for soft materials, such as the DNA networks considered here.

Our DLSμR measurements on DNA topological networks confirm that strand-passage reactions catalyzed by topo II fluidize network topology to produce an active Olympic hydrogel formed by catenated DNA rings (Fig. 2). The frequency dependence of the shear modulus G^* reveals a hierarchy of molecular relaxation processes (illustrated in Fig. 2), ranging from the thermal bend fluctuations of individual semiflexible DNA chains to the many-body dynamics of the topologically dynamic network. These distinct molecular relaxation processes dominate the viscoelastic behavior over different time-scale regimes, which are indicated by the shaded regions in Fig. 2 (annotated "A","B","C",and "D" in order of longest to shortest time-scale).

At the longest time scales probed by our measurements ($\omega < 2$ s^{-1}, region "A"), the viscoelastic behavior is governed by dynamic catenation and decatenation reactions mediated by topo II. Our DLSμR measurements reveal a characteristic cross-over between the storage and loss moduli (G' and G'', respectively) at a frequency $\omega = \tau_T^{-1} \approx 2\ s^{-1}$ below which the network transitions to more "liquid-like" behavior. This is a rheological signature of a polymeric solution in which polymers are able to escape local confinement from linked chains over the span of the topological relaxation time of the network τ_T.

At time-scales faster than the strand passage rate of topo II ($2\ \text{s}^{-1} < \omega < 2 \times 10^2\ s^{-1}$, region "B"), rings in the active Olympic hydrogel are constrained by topological links with their neighbors. This is reflected in our DLSμR measurements by an elastic plateau in which $G' > G''$, and G' exhibits a weak frequency dependence. The magnitude of G' in this regime enables estimation of the density of topological links. The magnitude of G' in the elastic plateau regime is related to the number density of effective elastic modes n_e by $G' = k_b T n_e$ [61]. If each effective elastic mode represents one topological link, then each ring in the network is catenated with about 3 other neighboring rings.

On shorter time-scales ($\omega > 2 \times 10^2$ s^{-1}, regions "C" and "D"), the DNA chains have yet to encounter topological entrapment by the surrounding network, and viscoelasticity is governed by relaxation of effective elastic chains. Over time-scales that are longer than the bend relaxation time of the DNA and shorter than the time required for effective elastic chains to experience topological confinement (2×10^2 s$^{-1} < \omega < 5 \times 10^3$ s^{-1}, region "C"), the active Olympic hydrogel exhibits a power-law scaling in which $G^* \sim \omega^{\frac{1}{2}}$, which is consistent with the entropic relaxation of long polymer segments that behave

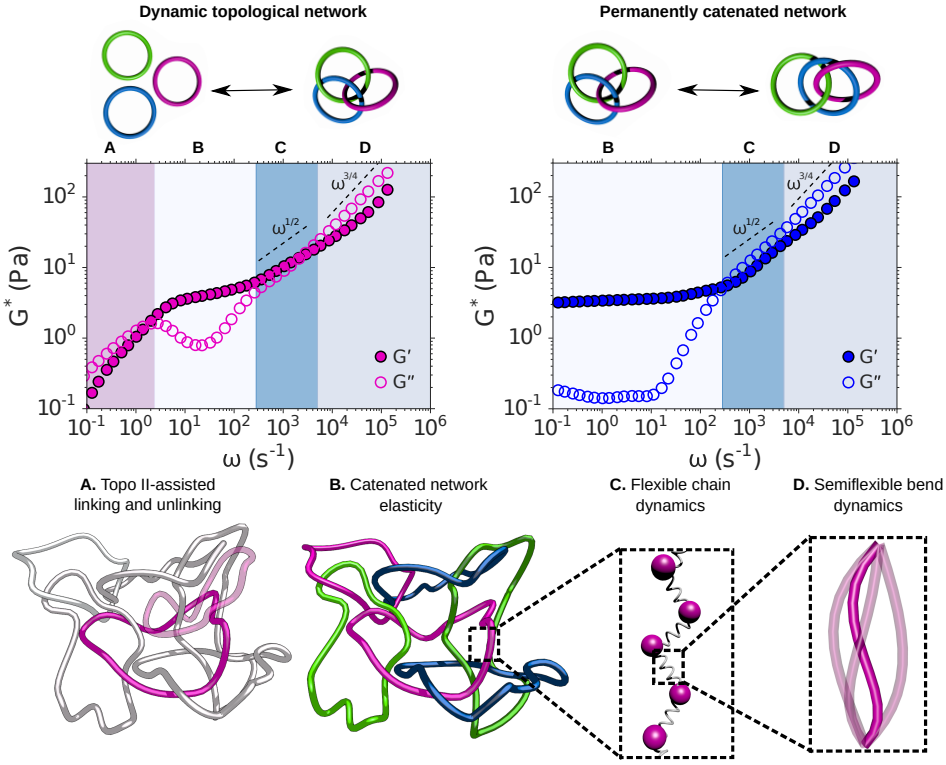

FIG. 2. Topo II enables the synthesis of Olympic hydrogels that can be switched between dynamic topological networks and permanently catenated networks based on the catalytic activity of topo II. Plots provide the shear modulus G^* as a function of angular frequency ω in the presence of active topo II (left) and inactivated topo II (right). The dashed lines indicate scaling laws for a Rouse polymer ($G^* \sim \omega^{1/2}$) and a freely-draining wormlike chain ($G^* \sim \omega^{3/4}$), respectively. Shaded regions in the plots labeled "A","B","C", and "D" demarcate time-scale regimes associated with the molecular relaxations illustrated in the bottom schematics.

like flexible Rouse polymers [61]. At shorter time scales ($\omega > 5 \times 10^3$, region "D"), the finite bending stiffness of the DNA double helix emerges, and the viscoelastic response transitions to the expected scaling behavior for a wormlike chain, in which $G^* \sim \omega^{\frac{3}{4}}$ [62, 63].

We hypothesized that inactivating topo II after permitting it remodel the network would "freeze" network topology and produce a permanently catenated Olympic hydrogel. Topo II was permitted to remodel network topology for 1 hour and subsequently inactivated by chelating divalent cations with EDTA. The resulting viscoelastic spectrum (right plot in Fig. 2) confirms the presence of a permanently catenated Olympic hydrogel. The terminal relaxation regime (region "A") is abolished, and instead the elastic plateau regime (region "B") extends to the longest time-scales accessed by DLSμR. This behavior starkly contrasts with classical polymer reptation in entangled networks of linear chains, in which true topological constraints do not exist. However, the catalytic state of topo II only substantially impacts network viscoelasticity on frequencies on the order of and lower than the intrinsic turnover rate for strand passage reactions of human topoisomerase II (measured to be $1 - 4\ \mathrm{s}^{-1}$ [64, 65]). The magnitude of G' in the elastic plateau regime (region "B") is unaltered by topo II inactivation, as are the time-scales associated with the Rouse and wormlike chain regimes (regions "C", and "D", respectively).

The relationship between topo II turnover rate and network fluidization is highlighted by comparing the rheological behavior of the active topo II catenated network to the precursor solution of uncatenated DNA rings. Prior to addition of topo II, the uncatenated ring solution exhibits viscoelastic signatures of a physically entangled polymer network in which rings are confined by entanglements with surrounding polymers, but are able to escape local entanglements over the course of the disentanglement time of the network τ_D (Fig. 3). This is manifested by an elastic plateau appearing at intermediate time scales and a transition to more viscous behavior at time scales longer than τ_D.

Addition of topo II enhances translocation of DNA rings through the surrounding polymers by permitting chains to effectively pass through one another, producing topological network relaxation on time scales faster than the disentanglement time of uncatenated rings by about a factor of 4 (Fig. 3). After extended incubation with topo II, network relaxation slows, which may be due

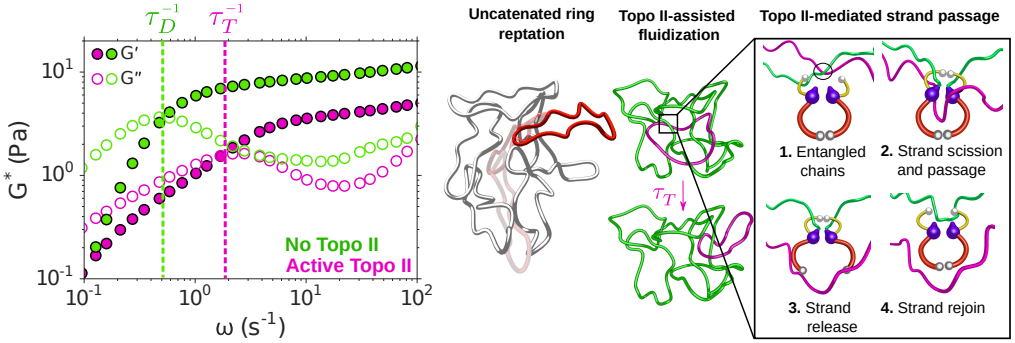

FIG. 3. Topo II-mediated linking and unlinking enhances network fluidity compared to uncatenated solutions of entangled rings. *Left*: DLSμR measurements of the frequency ω dependence of the shear modulus G^* of uncatenated solutions of entangled rings with no topo II (green) and dynamic catenated topological networks with active topo II (magenta). Magenta and green dashed lines indicate the time scales associated with the disentanglement time of the uncatenated solution τ_D and the topological relaxation time of the network after addition of topo II τ_T, respectively. *Middle*: Illustration of reptation of ring polymers in uncatenated solutions of transiently entangled rings. *Right*: Illustration of topo-II mediated translocation of rings through dynamic catenated networks, and the underlying strand-passage reactions that enable network fluidization.

to consumption of ATP (Supporting Fig. 1). Changes in overall network topology are accompanied by a reduction in the magnitude of G' in the elastic plateau regime by a factor of approximately 2.25.

To further demonstrate the existence of catenated structures in our DNA networks, we performed an agarose gel electrophoresis assay of topological structures released by digestion of Olympic hydrogels containing varying fractions of a plasmid bearing a HINDIII recognition sequence. Electrophoresis revealed increasing prevalence of high molecular weight structures that were retained in the wells of an agarose gel as the fraction that was susceptible to linearization was decreased (Supporting Fig. 2). These observations are consistent with a network that is maintained through topological links, rather than transient polymer entanglements.

The ability of topo II to simultaneously capture two DNA strands presents the possibility for it form protein cross-links, even in its catalytically inactive state. To address this possibility, we treated quenched Olympic hydrogels with proteinase K to digest the topo II and performed DLSμR. After extended digestion with proteinase K, the plateau modulus of the Olympic hydrogel was found to decrease by $\approx 50\%$, but the material retained qualitative rheological signatures of a catenated hydrogel network, rather than a physically entangled network (Supporting Fig. 3). This change in rheological behavior is consistent with a topologically cross-linked hydrogel that contains additional reinforcing protein cross-links in which each topo II homodimer participates in a cross-link that contributes 1 k_BT per unit volume to the plateau modulus.

Gelation of DNA rings has been previously demonstrated through *irreversible* catenation by topo II in the presence of either a non-hydrolyzable ATP analog (AMP-PNP) in place of ATP or a topo II inhibitor (ICRF-193) [57]. In contrast, network fluidization in our dynamic topological network requires dynamic strand passage reactions (Fig. 2). Thus, the dynamic topological networks possess a linking topology that enables the material to switch between liquid and solid states based on the catalytic state of topo II.

Previous microrheology studies on entangled solutions of *linear* DNA have similarly confirmed the ability of topo II to transiently reduce network relaxation time in an ATP-dependent fashion [66]. However, in linear DNA solutions, no permanent topological remodeling occurs, so the rheological profile of the network was fully restored to its initial state after depletion of ATP.

The capacity of topo II to alter DNA network rheology may have important implications for its biological function. Micromechanical manipulation experiments on mitotic chromosomes have shown that condensed chromatin behaves like a polymer gel containing topological cross-links that can be removed by topo II, supporting the *in vivo* function of topo II as a decatenase during chromosome segregation [67]. Topo II has been historically proposed to disentangle chromosomes by leveraging its intrinsic preference for decatenation relative to topological equilibrium [68, 69]. Our DLSμR measurements also support a role for topo II in transiently resolving topological cross-links. However, our results suggest it may more broadly function to fluidize the topological state of DNA in order to support either catenation or decatenation, depending on local molecular driving forces.

The synthesis of Olympic gels by topoisomerases is not limited to type II topoisomerases, which enact ATP-dependent strand passage reactions through *double-stranded* breaks in DNA. Type I topoisomerases, which introduce ATP-independent *single-stranded* breaks in DNA, are also capable of catenating and decatenating DNA in the presence of pre-existing single-stranded

nicks [70]. We find that in the presence of *E. coli* topoisomerase I (topo I), Olympic hydrogels are formed from the same single-strand nicked, uncatenated ring precursor solutions and exhibit similar viscoelastic properties as the topo II-synthesized permanently catenated networks (Supporting Fig. 4).

In summary, in this work we demonstrate the facile synthesis of topologically linked, active Olympic gels that exploits the mastery of DNA topology inherent to biological enzymes. Although the synthesis of topological materials remains a formidable challenge, this biologically-inspired strategy highlights the unique ability of DNA-based materials to circumvent challenges that remain for the fabrication of Olympic hydrogels using synthetic polymers. These biomimetic materials may be leveraged to explore a variety of phenomena that remain elusive using synthetic systems. Our strategy is similar to a previously reported synthesis of DNA Olympic hydrogels using topoisomerase catalytic inhibitors [57]. However, in contrast to the previous synthesis of DNA Olympic hydrogels, we also demonstrate the potential for topoisomerase-based DNA Olympic hydrogels to serve as active ATP-driven materials whose fluidity can be switched on command. In the future, we envision that these DNA topological networks can be further augmented using the unique molecular recognition properties of DNA, and the vast array of molecular motors that act upon it, to synthesize active, switchable materials beyond the reach of conventional chemistry.

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