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Mapping molecular-level dynamics to mesoscale mechanics in composite DNA-based biomaterials

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2. Abstract

Most naturally occurring biomaterials, such as cytoskeleton, are heterogeneous biopolymer blends (composites) that display captivating and useful scale-dependent viscoelastic properties that are completely controlled by polymer topology, stiffness, size and concentration. Thus, biopolymer blends are powerful platforms for developing dynamic, hierarchical, multifunctional materials. However, understanding the underlying macromolecular dynamics, interactions, and stress propagations that lead to the unique macroscale mechanics is critical to precisely spatiotemporally tuning composites to have desired material properties.

The PI will design blends of DNA and proteins with systematically varied topologies, stiffnesses, sizes and concentrations. Characterizing and linking together molecular dynamics and mechanical properties of these blends will determine the molecular properties necessary to achieve specific novel material properties, thereby enabling a bottom-up approach to biomaterial design. Using DNA, one of the most well-controlled and tunable polymer platforms in existence, will allow for precise modulation of mechanics over an unparalleled parameter space.

The PI will introduce new techniques, Scalable Active-Passive Molecular Rheology (SAPMR), to directly connect stresses induced in biopolymer blends to corresponding molecular deformations and network rearrangement with unprecedented temporal (~millisecond), spatial (~nanometer) and molecular (~basepair, monomer) resolution and control. The PI will investigate two biologically inspired DNA-based composites: (i) ring and linear DNA and (ii) DNA and cytoskeleton proteins.

3. Objectives

1. **Develop a multifunctional microscale experimental platform, Scalable Active-Passive Molecular Rheology (SAPMR), that performs active microrheology measurements while simultaneously tracking conformational dynamics of single molecules** to probe mechanical properties of biomaterials over an unprecedented parameter space of dynamics, lengthscales, timescales, and molecular/material properties. Develop analysis software to directly link induced stress to molecular strain in real-time, quantify strain distribution and propagation dynamics, and precisely map macromolecular structures and dynamics from the molecular-level to the mesoscale to near-macroscopic scales.

2. **Use Aim 1 technique to measure the localized force exerted by ring-linear DNA composites to resist strain induced by an optically driven micro-transducer while simultaneously tracking induced deformations of DNA sensors.** Characterize strain-induced resistive forces, network rearrangements, molecular deformations, and relaxation dynamics. Systematically vary strain characteristics to span near-equilibrium to nonlinear regimes and nanoscopic to mesoscopic lengthscales. Systematically vary the lengths and fractions of each polymer in the blend.

3. **Repeat Aim 2 for composites** of flexible DNA and stiff cytoskeleton proteins (actin and microtubules).

4. Findings – Findings for each objective, including any changes in direction these findings may have compelled you to take, and all major accomplishments

Objective 1. The focus of Obj 1 was on developing SAPMR. The key findings using SAPMR are described in Objs 2 and 3 below. However, over the course of the grant we greatly expanded the scope of the original multi-scale fluorescence optical tweezers rheological platform to incorporate differential dynamic microscopy (DDM), particle-tracking microrheology, Brownian Dynamics simulations, Large Amplitude Oscillatory Strain (LAOS) measurements and theory, and fixed-trap linear microrheology measurements. We also developed software packages for these methods to enable collaborations with other labs and broad use by undergraduate students. The key findings from the papers that focus on these methods advances are described below.

A. **I wrote a Viewpoint article for ACS Macro Letters – Chosen as an ACS Editors' Choice Publication** - describing some of the optical trapping microrheology methods we developed as part of SAPMR (**https://doi.org/10.1021/acsmacrolett.8b00498**) to enable **broad dissemination and adoption of the platform by other researchers**. As I describe in the Viewpoint, over the past few decades, microrheology has emerged as a widely used technique to measure the mechanical properties of soft viscoelastic materials. Optical tweezers offer a powerful platform for performing microrheology measurements and can measure rheological properties at the level of single molecules out to near macroscopic scales. Unlike passive microrheology methods, which use diffusing microspheres to extract rheological properties, optical tweezers can probe the nonlinear viscoelastic response, and measure the space- and time-dependent rheological properties of heterogeneous, non-equilibrium materials. To facilitate use of this platform by other researchers, I described the basic principles underlying optical tweezers microrheology, the instrumentation and material requirements, and key applications to widely studied soft biological materials. I also describe several sophisticated approaches that are built into SAPMR that include coupling optical tweezers to fluorescence microscopy and microfluidics. The described techniques can robustly characterize non-continuum mechanics, nonlinear mechanical responses, strain-field heterogeneities, stress propagation, force relaxation dynamics, and time-dependent mechanics of active materials.

B. **We collaborated with fellow AFOSR-funded researchers to develop multi-scale rheology techniques** to show that Chaetopterus mucus exhibits scale-dependent material properties governed by three intrinsic lengthscales (**doi:10.1371/journal.pone.0176732**). We used optical tweezers microrheology and bulk rheology to characterize the scale-dependent rheology and structure of mucus produced by the ubiquitous *Chaetopterus* marine worm. Specifically, we sinusoidally drive microspheres of various sizes (2–10 μm) and measure the resulting force acting on the probe. From these measurements we determine the storage modulus, G', loss modulus, G'', and complex viscosity, η of the mucus. We find three rheologically distinct regimes for $l_1 < 4$ μm, $l_2 \sim 4$–10 μm, and $l_3 > 10$ μm. For l_1 the stress response of the mucus is comparable to water, suggesting that the probes move largely within the pores of the mucus network. For l_2, mucus exhibits mechanics indicative of a continuum material with loose entanglements and predominantly viscous response properties. The macroscopic elastic gel properties measured at the macroscale only arise for l_3, where stiffer structures force the probes out of the optical trap. These collective results suggest that the mucus can be modeled as a coupled two-fluid system comprised of (i) a loosely entangled polymer mesh coupled to (ii) a larger more elastic scaffold. While mesh (i) is responsible for the enhanced viscosity of the mucus at lengthscales above l_1, mesh (ii) provides the macroscale elasticity of the mucus at l_3. This model can explain how mucus is able to simultaneously perform a multitude of functions across varying lengthscales. For example, l_1 is necessary for the passage of nutrients while l_2 is needed for trapping larger pathogens and parasites. Finally, the macroscopic gel-like properties necessary for coating organs is provided by l_3.

C. **We developed new experimental techniques and theoretical framework** to map the dynamics of micro-scale inhomogeneities and non-uniform flow fields resulting from local nonlinear strains (**https://doi.org/10.1103/PhysRevLett.123.038001**). Optical tweezers microrheology (OTM) offers a powerful approach to probe the nonlinear response of complex soft matter systems, such as networks of entangled polymers, over wide-ranging spatiotemporal scales. OTM can also uniquely characterize the microstructural dynamics that lead to the intriguing nonlinear rheological properties that these systems exhibit. However, the strain in OTM measurements, applied by optically forcing a microprobe through the material, induces network inhomogeneities in and around the strain path, and the resultant flow field complicates the measured response

of the system. Through a robust set of custom-designed OTM protocols, coupled with modeling and analytical calculations, we characterize the time-varying inhomogeneity fields induced by OTM measurements. We show that homogenization following strain does not interfere with the intrinsic stress relaxation dynamics of the system, rather it manifests as an independent component in the stress decay, even in highly nonlinear regimes such as with the microrheological large-amplitude-oscillatory-shear (MLAOS) protocols we introduce. Our specific results show that Rouse-like elastic retraction, rather than disentanglement and disengagement, dominates the nonlinear stress relaxation of entangled polymers at micro- and mesoscales. Thus, our study opens up possibilities of performing precision nonlinear microrheological measurements, such as MLAOS, on a wide range of complex macromolecular systems.

D. **We developed particle-tracking microrheology methods** to measure the viscoelastic properties of clingfish secretion beneath the suction discs of clingflish (**doi: 10.1021/acsami.0c10749**). This work, in collaboration with AFOSR-funded researcher Dimitri Deheyn, demonstrates our ability to expand and adapt SAPMR to measure rheological properties of diverse biomaterials. Our measurements demonstrated that the secretion was viscous, rather than viscoelastic, with a viscosity ~6x that of water.

Objective 2. The focus of Obj 2 was on applying SAPMR techniques to understand the rheological and dynamical properties of blends of ring and linear DNA. We expanded our SAPMR measurement and analysis suite to achieve this objective by incorporating single-molecule microfluidics experiments, Brownian Dynamics simulations, and Differential Dynamic Microscopy (DDM). Below we summarize our key findings and accomplishments.

A. **Transient Threading and Large Fluctuations of Ring DNA in Semidilute Linear DNA Solutions (https://doi.org/10.1038/s41467-019-09627-7)**. Understanding the dynamics of ring polymers is a particularly challenging yet interesting biomaterials problem. Despite recent progress, a complete understanding of the nonequilibrium behavior of ring polymers has not yet been achieved. We studied the relaxation dynamics and transient stretching behavior of single ring DNA polymers (45 kbp) in solutions of semidilute unentangled 48.5 kbp linear DNA. Our results show that ring polymers exhibit large conformational fluctuations in extensional flow, even long after the initial transient stretching process has terminated. Remarkably, this behavior occurs even at low background concentrations of linear DNA, down to $0.025\,c^*$. These large conformational fluctuations are not observed in the steady-state stretching of linear DNA in the same background solutions, or in the steady-state stretching of ring polymers in dilute solutions ($10^{-5}c^*$) under similar flow conditions. We hypothesize that these large fluctuations in ring polymer extension arise due to transient threading of linear polymers through partially open rings due to stretched conformations in flow.

B. **Threading leads to Long-Lived Self-Entanglements in Ring Polymers (10.1103/PhysRevLett.123.048002).** We use single molecule DNA experiments to study the dynamics of self-entangled ring polymers, which serve as a minimal system for studying chain entanglements. We perform experiments to induce self-entanglements in relaxed circular DNA with electric fields and gain understanding of the entangled state by examining the expansion dynamics back to equilibrium. Our results demonstrate experimentally, for the first time to our knowledge, that threadings give rise to entanglements in ring polymers and lead to a slow-down in polymer dynamics. Circular molecules are less easily driven into an arrested, entangled state compared to linear molecules. However, strongly entangled circular molecules exhibit long-lived compact states that do not relax on timescales greater than ~2000 relaxation times.

C. **Semidilute blends of supercoiled and ring DNA exhibit unexpected entanglement dynamics (https://doi.org/10.1039/C9SM01767D).** Blends of polymers of different topologies, such as ring and supercoiled, naturally occur in biology and often exhibit emergent viscoelastic properties coveted in industry. However, due to their complexity, along with the difficulty of producing polymers of different topologies, the dynamics of topological polymer blends remains poorly understood. To address the problem, we combined passive and active microrheology to determine the linear and nonlinear rheological properties of blended solutions of ring and supercoiled DNA. We showed that these blends exhibit surprising signatures of classical polymer entanglements at concentrations much lower than similar monodisperse systems of linear or ring polymers. Specifically, linear microrheology revealed a crossover at *c** from semidilute dynamics to those that align with entangled linear polymers. At the same time, nonlinear microrheology uncovered unique sustained

elasticity and multiple relaxation modes not expected at these modest concentrations. Interestingly, while blends exhibit linear viscoelasticity comparable to those of entangled linear polymers, nonlinear response characteristics align more closely with predictions for entangled rings. We interpret these differences as arising from strain-induced network rearrangements that alter the entanglement density and disrupt interactions between topologically-distinct polymers. These emergent properties demonstrate that topological blends can be exploited to create robust and stiff materials with much lower concentrations than monodisperse systems. As such, our results are not only of fundamental importance to polymer physics but also provide a route for designing low-mass high-strength viscoelastic materials.

D. **Viscoelastic properties of ring-linear DNA blends exhibit non-monotonic dependence on blend composition (https://doi.org/10.1103/PhysRevResearch.2.023213).** Entangled ring polymers, along with blends of ring and linear polymers, continue to be a topic of great interest and debate due to conflicting experimental results as well as the difficulty of producing entangled synthetic rings. We created blended solutions of entangled ring and linear DNA with varying mass fractions of linear DNA φ_L. We used optical tweezers microrheology to reveal a strong nonmonotonic dependence of linear viscoelastic properties on φ_L, with a pronounced maximum when the mass fractions of rings and linear chains are comparable. We argue that this nonmonotonicity is a result of threading of ring polymers by linear chains coupled with the relative ineffectiveness of rings to self-entangle compared to linear polymers. Pervasive threading events at $\varphi_L = 0.68$ leads to a higher elastic plateau as well as more pronounced shear-thinning compared to the pure linear system ($\varphi_L = 1$). Our nonlinear microrheology results revealed that ring-linear threading is robust to modest nonlinear strains but can be disrupted at very high strain rates, causing entangled linear DNA to build up in front of the moving probe while rings, which are less effective at stretching and orienting in the direction of the strain, slide past the probe into the wake. This process results in the linear polymers playing the principal role in the nonlinear relaxation dynamics of ring-linear blends. Our results—which highlight the importance of dynamic threading events to the rheology of ring-linear blends—not only address the dearth of experimental data on the microrheology and nonlinear response of ring-linear polymer blends, but will likely prompt new theoretical investigations of the response of topological polymer blends across wide- ranging spatiotemporal scales. Finally, the emergent strong viscoelastic response that ring-linear blends exhibit, along with the ability to finely tune the rheological properties of these blends by varying the relative fractions of each topology, suggests important industrial applications.

E. **Direct Observation of Ring Polymer Dynamics in the Flow-Gradient Plane of Shear Flow (doi: 10.1021/acs.macromol.0c01362).** Ring polymers are a unique class of macromolecules that lack free ends and show qualitatively different dynamics compared to linear chains. Despite recent progress, the non-equilibrium flow behavior of ring polymers is not fully understood. We studied ring polymer dynamics in steady shear flow using a combination of single-molecule experiments and Brownian dynamics (BD) simulations. In particular, we characterized the dynamics of DNA ring polymers in the flow-gradient plane of shear by using a custom flow apparatus that allows for direct observation of ring stretching and tumbling dynamics using single-molecule fluorescence microscopy. Using this approach, we determined the average fractional polymer extension in the flow direction $\langle x \rangle / Lc$ and the average orientation angle with respect to the flow axis $\langle \theta \rangle$ for ring DNA in dilute solution shear flow as a function of dimensionless flow strength (Weissenberg number, Wi). Interestingly, our results showed that ring and linear DNA exhibit similar average fractional extensions, orientation angle, and dimensionless gradient thickness over a wide range of flow strengths ($0 \leq Wi \leq 250$). However, ring DNA polymers show qualitatively different probability distributions of molecular chain extension compared to linear chains in steady shear, which arises due to the circular chain architecture that limits the conformational phase space. We used power spectral densities of polymer orientation angle and cross-correlations between fractional chain extension and gradient-direction thickness to determine tumbling frequency as a function of flow strength Wi, and compare probability distributions of molecular chain extension of linear and ring DNA in shear flow. Overall, these results provide a new understanding of the nonequilibrium dynamics of ring polymers in shear flow.

F. **Topological Tuning of DNA Mobility Entangled Solutions of Supercoiled DNA (DOI: 10.1126/sciadv.abf9260).** Ring polymers in dense solutions are among the most intriguing problems in polymer physics. Because of its natural occurrence in circular form, DNA has been extensively used as a proxy to study the fundamental physics of ring polymers in different topological states. Yet, torsionally constrained—such as supercoiled—topologies have been largely neglected so far. To characterize the effect of DNA supercoiling on the rheology of entangled

DNA solutions, we performed large-scale molecular dynamics simulations to show that while isolated DNA plasmids typically display a collapse with increasing levels of supercoiling, entangled DNA polymers increase their average size with supercoiling. Surprisingly, despite this swelling, larger supercoiling is accompanied by an enhanced mobility of the plasmids - in marked contrast with standard polymer systems in which larger polymer sizes correlate with slower diffusion. We experimentally validated this speedup performing differential dynamic microscopy (DDM) experiments on entangled DNA with different supercoiling degrees. Last, we used minimal surface construction and primitive path analysis (PPA) to quantify the abundance of threadings and entanglements between plasmids in solution and find that larger supercoiling decreases both of these topological constraints, in turn explaining the enhanced mobility. Our results will be key to enabling the design of complex fluids with rheology that can be precisely tuned using a combination of DNA length, concentration, topology, and supercoiling. Beyond providing blueprints for realizing the next generation of biomimetic DNA-based materials, our results can also shed light into the dynamics of DNA in vivo.

Objective 3. The focus of Obj 3 was on applying SAPMR techniques to understand the rheological and dynamical properties of composites of DNA and stiff cytoskeleton proteins (actin and microtubules). We expanded our SAPMR measurement and analysis suite to achieve this objective by incorporating DDM, BD simulations, fluorescence confocal microscopy, and spatial image autocorrelation analysis. Below we summarize our key findings and accomplishments.

A. **Crowding induces entropically-driven changes to DNA dynamics that depend on crowder structure and ionic conditions (doi: 10.3389/fphy.2018.00053).** Macromolecular crowding plays a principal role in a wide range of biological processes including gene expression, chromosomal compaction, and viral infection. However, the impact that crowding has on the dynamics of DNA remains a topic of debate. To address this problem, we used SAPMR to investigate the impact of varying macromolecular crowding conditions on the transport and conformational dynamics of large DNA molecules. We crowded 115 kbp DNA with widely used synthetic crowders that can be categorized into linear (dextrans) and branched (Ficoll, PEG) structures of small (10 kDa) and large (~500 kDa) molecular weights. We found that linear crowders entropically drive DNA to elongate while branched crowders compact DNA to maximize their entropy. Elongated conformations undergo larger conformational fluctuations than random coils, while compacted configurations are more spherical and access a smaller range of conformational states. These findings are largely independent of crowder concentration and molecular weight. Despite the marked difference in conformational dynamics that DNA exhibits in the presence of different crowders, we found that for both crowder types DNA diffuses faster than expected based on the increasing viscosity of the solutions. We attribute this enhanced diffusion to a combination of entropically-driven conformations that reduce the volume of the DNA as well as a reduced local viscosity surrounding the DNA arising from optimized packing of the crowders. We also found that DNA dynamics exhibit a complex interplay between salt concentration and crowding. In buffer conditions, DNA mobility decreases and the range of conformational states increases with increasing salt (2 to 50 mM NaCl). However, upon crowding, DNA diffusion and conformational changes exhibit an emergent non-monotonic dependence on salt concentration, with DNA diffusing the fastest and exhibiting the most extreme compaction or elongation in 10 mM NaCl compared to lower and higher salt concentrations. We hope that our results, which reveal several complex and unexpected phenomena that are highly relevant to polymer physics and cell biology, spur new theoretical investigations to fully understand them.

B. **Synergistic interactions between DNA and actin trigger emergent viscoelastic behavior (10.1103/PhysRevLett.121.257801).** Composites of flexible and rigid polymers are ubiquitous in biology and industry alike, yet the physical principles determining their mechanical properties are far from understood. We used SAPMR to elucidate the unique viscoelastic properties of custom-engineered blends of entangled flexible DNA molecules and semiflexible actin filaments. We showed that composites exhibit enhanced stress stiffening and prolonged mechanomemory compared to systems of actin or DNA alone, and that these nonlinear features display a surprising nonmonotonic dependence on the fraction of actin in the composite. Our BD simulations revealed that these counterintuitive results arise from synergistic microscale interactions between the two biopolymers. Namely, DNA entropically drives actin filaments to form bundles that stiffen the network but reduce the entanglement density, while a uniform well-connected actin network is required to reinforce the

DNA network against yielding and flow. The competition between bundling and connectivity triggers an unexpected stress response that leads equal mass DNA-actin composites to exhibit the most pronounced stress stiffening and the most long-lived entanglements. While substantial changes in viscoelasticity in composites are often attributed to large-scale phase separation and structural rearrangement, we showed that molecular-level interactions and entanglements between two distinct polymers can give rise to emergent dynamics. Our collective results reveal new physical phenomena of composite systems, demonstrate the complex interplay between microscale polymer interactions and material properties, and provide a robust biopolymer platform for investigating the physics of polymer composites.

C. **Coupling DDM, single-molecule conformational tracking and light-sheet microscopy bridges the spatiotemporal scales of macromolecular transport in crowded DNA-cytoskeleton composites (doi: 10.1039/C8SM02023J).** Crowding plays a key role in the transport and conformations of biological macromolecules. Gene therapy, viral infection, and transfection require DNA to traverse the crowded cytoskeletal network. Given the complexity of cytoskeletal crowding, the dynamics of biological macromolecules can be highly dependent on the spatiotemporal scale probed. We developed a powerful platform that spans molecular and cellular scales by coupling single-molecule conformational tracking (SMCT) and selective-plane illumination differential dynamic microscopy (SPIDDM) to directly measure crowding-induced conformations and trajectories of single molecules as well as ensemble-averaged transport properties across a large range of length and time scales. We elucidated the transport and conformational properties of large DNA, crowded by custom-designed networks of actin and microtubules, to link single-molecule conformations with ensemble DNA transport and cytoskeleton structure. We revealed that actin crowding leads to DNA compaction and suppression of fluctuations, combined with subdiffusion and heterogeneous transport, whereas microtubules have much more subdued impact across all scales. In composite networks of both filaments, scale-dependent effects emerged such that actin dictates ensemble DNA transport while microtubules influence single-molecule dynamics. We showed that these intriguing results arise from a complex interplay between network rigidity, mesh size, filament concentration, and DNA size. This complex behavior highlights the importance of characterizing dynamics across wide-ranging scales to paint a complete picture of macromolecular transport in complex biomimetic environments.

D. **Topology-dependent anomalous dynamics of ring and linear DNA are sensitive to cytoskeleton crosslinking (DOI: 10.1126/sciadv.aay5912).** We coupled SMCT with DDM to characterize the dynamics of linear and ring DNA molecules crowded by entangled and crosslinked cytoskeletal networks. Our results revealed the critical role that DNA topology plays in cytoskeleton transport and how altering cytoskeleton con- nectivity can enable a myriad of conformational and transport dynamics of biopolymers across scales. Specifically, we showed that DNA exhibits biphasic subdiffusion and slow fluctuations between a broad range of swollen conformational states and corresponding transport modes. Conversely, linear DNA undergoes faster single-mode diffusion and more compact conformations with a narrow distribution of dynamical modes. Further, while crosslinking suppresses ring DNA diffusion, it enhances the diffusion of linear DNA. Lastly, ensemble analysis revealed that, unlike linear DNA, rings undergo highly heterogeneous transport that cannot be fit to standard models of diffusion. These collective results suggest that threading—inaccessible to linear chains—plays a key role in the transport of ring DNA within cytoskeleton networks. Beyond the importance of our results to biological processes such as transfection, infection, and gene therapy, our results provide key insights into the poorly understood physics of entangled and crowded ring polymers and topological polymer blends important to materials engineering and industrial applications.

E. **Maximally stiffening composites require maximally coupled rather than maximally entangled polymer species (DOI: 10.1039/C9SM01461F).** We designed solutions of flexible DNA and semiflexible actin filaments with physical properties that can be tuned over a wide parameter space. We tackled the vast multi-dimensional parameter space of our previously introduced custom-engineered actin-DNA composites to elucidate the role that DNA length, number of entanglements, and dynamics play in their emergent rheological properties. Using our combined SAPMR approach - coupling large-scale BD simulations with OTM –we elucidated important and a priori unexpected physical principles for the design of composites made of flexible and rigid polymers. Intriguingly, we showed that these systems are tunable yet robust at the same time. They are robust because they display a non-monotonic dependence of the mechanical response on the mass composition that is broadly

preserved across the entire parameter space. They are tunable because the details, such as the amplitude of the non-monotonic response, degree of actin bundling, and mass composition at which maximal elasticity is achieved, can be altered by varying the length of the flexible species. The most important and surprising finding was that composites in which the two polymer species are maximally coupled, rather than maximally entangled, yield the most pronounced stress stiffening and elasticity. Our results – which revealed previously unappreciated physical phenomena at play in composite systems such as microscale phase separation, dynamical rearrangements and competition between bundling and pervasiveness – further elucidate the interactions between distinct polymer species in naturally occurring and biomimetic polymer composites and pave the way for the systematic design of next-generation multi-functional composite materials.

F. **DNA topology and network crosslinking dictates anomalous and heterogeneous DNA transport in cytoskeleton networks (DOI: 10.1039/d0sm00544d).** The transport of DNA, as well as other macromolecules and complexes, through the cytoskeleton plays critical roles in numerous biological processes. Yet, the complexity of the cytoskeleton and wide variation in properties of diffusing macromolecules leaves our understanding of transport phenomena limited. We elucidated the role that DNA topology plays in the transport of large DNA molecules in crosslinked composites of actin and microtubules. By altering the actin and microtubule crosslinking motif, and specifically creating composites with homologous and non-homologous crosslinking, we investigated how the mobility and connectivity of composites affects DNA dynamics. We demonstrated that threading controls the dynamics of ring DNA nearly completely, irrespective of the crosslinking motif. On the other hand, the crosslinking motif plays a significant role in linear DNA dynamics, with non-homologous crosslinking leading to the most heterogeneous dynamics. Beyond the implications our results have for understanding macromolecular transport in crowded environments, they demonstrate how biomimetic material properties and transport phenomena can be altered by tuning crosslinking motifs.

G. **DNA topology dictates strength and flocculation in DNA-microtubule composites (10.21203/rs.3.rs-498534/v1).** Polymer topology has been shown to play a key role in tuning the dynamics of complex fluids and gels. At the same time, polymer composites, ubiquitous in everyday life, have been shown to exhibit emergent desirable mechanical properties not attainable in single-species systems. Yet, how topology impacts the dynamics and structure of polymer composites remains poorly understood. We created composites of rigid rods (microtubules) polymerized within entangled solutions of flexible linear and ring polymers (DNA) to show that subtle changes in polymer topology (free or closed ends) can have dramatic effects on the structure and mechanics of polymer composites. We coupled OTM with confocal microscopy and scaled particle theory to show that composites of linear DNA and microtubules exhibit a strongly non-monotonic dependence of elasticity and stiffness on microtubule concentration due to depletion-driven polymerization and flocculation of microtubules. In contrast, composites of ring DNA and microtubules show a much more modest monotonic increase in elastic strength with microtubule concentration, which we demonstrate arises from the increased ability of rings to mix with microtubules. Our results, which shed important new light on the role that topology plays in the rheology and structure of polymer composites, have broad reaching implications in biology, chemical engineering and materials applications. The realization of polymer composites through in-situ polymerization of fillers has further advantages in wide variety of applications such as in vivo tissue engineering and in-situ polymerization for 3D printing.

5. Supported Personnel

Karthik Peddireddy, PhD, postdoctoral researcher
Manas Khan, PhD, postdoctoral researcher
Jonathan Garamella, PhD, postdoctoral researcher
Ashley Messmore, undergraduate student
Warren Mardoum, undergraduate student
Megan Lee, undergraduate student
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Shea Ricketts, undergraduate student
Carl Kyrillos, undergraduate student
Juexin Marfai, undergraduate student
Ankit Macherla, high school student
Ryan Clairmont, high school student
Karina Kadia, high school student

6. Collaborations – Anyone you collaborated with that helped further your fundamental research (within the scope of this grant)

Prof. Davide Michieletto, University of Edinburgh
Prof. Ryan McGorty, University of San Diego
Prof. Charles Schroeder, University of Illinois, Urbana-Champagne
Prof. Greg McKenna, Texas Tech University
Prof. Patrick Doyle, Massachusetts Insitute of Technology:
Prof. Dimitri Deheyn, Scripps Institute of Oceanography
Prof. Jeffrey Urbach, Georgetown University
Dr. Daniel Blair, Georgetown University

7. Publications (undergraduate, *postdoc mentored by Robertson-Anderson)

18. *KR Peddireddy, D Michieletto, G Aguirre, *J Garamella, P Khanal, **RM Robertson-Anderson**. DNA topology dictates strength and flocculation in DNA-microtubule composites. Under review at *ACS Macro Letters* (2021).

17. Y Zhou, CD Young, M Lee, S Banik, D Kong, GB McKenna, **RM Robertson-Anderson**, CE Sing, CM Schroeder. Dynamics and Rheology of Ring-Linear Blend Semidilute Solutions in Extensional Flow: Single Molecule Experiments. *Journal of Rheology*, 65, 729, doi:10.1122/8.0000219 (2021).

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8. Interactions/Transitions – Anyone (other labs, institutions, businesses) who have picked up your fundamental research to extend it past the scope of your research and/or expand it into the applied realm

1. Prof. Gregory Holland, San Diego State University
2. Prof. Charles Schroeder, University of Illinois Urbana-Champagne
3. Prof. Patrick Doyle, Massachusetts Institute of Technology
4. Prof. Alex Klotz, CSU Long Beach
5. Prof. Dimitri Deheyn, Scripps Institute of Oceanography
6. Prof. Daniel Blair, Georgetown University
7. Prof. Jeffrey Urbach, Georgetown University