

Physical Confinement Regulates Transitions in Nematode Motility

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How do worms navigate their complex natural surroundings? Laboratory studies using nematodes such as *Caenorhabditis elegans* interrogate motility patterns as an indicator of their behavioral responses using liquid cultures or two-dimensional (2D) agar pads. However, *C. elegans* typically inhabit three-dimensional granular niches—such as soft soil and decomposed biological matter—with spatiotemporally varying viscoelastoplastic material properties that impose physical constraints on their motion. Here, by directly visualizing worm motility in transparent 3D granular matrices with tunable mechanical properties, we find that the propulsive speed of nematodes shows a nonmonotonic dependence on the yield stress of their microenvironment. Biophysical measurements reveal that this nonmonotonicity emerges as nematodes optimize for efficient navigation under higher confinement by matching their forward propulsive speed to the wave speed along their body. This regulation of locomotory behavior is purely dictated by the physical interaction of the nematode with its environment without involving soft-touch sensory neurons. Remarkably, our predictions from a slender body theory of undulatory motion in yield stress environments exactly capture the scaling behavior for both efficiency and mode of motion as obtained from the experimental data. Further, in a nondimensionalized phase space defined by propulsive efficiency and timescale of motility, we show that increasing confinement leads to a sharp gait transition from poorly efficient thrashing to highly efficient crawling. Our work thus establishes a biophysical paradigm wherein the physical interaction of worms with their mechanical microenvironment leads to a transition between distinct modes of undulatory motion, entirely as a consequence of 3D physical confinement.

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I. INTRODUCTION

Undulatory motion is a widespread phenomenon in the biological world and is commonly employed by worms, especially nematodes, living in environments such as soil, fruit pulp, and host tissues, to efficiently navigate their complex and disordered surroundings [1,2]. Such niches are also routinely subject to spatiotemporally dynamic agents such as temperature, moisture content, and chemical degradation, which significantly alter their structural properties—forcing inhabitant nematodes to constantly adapt their motility patterns in response to the prevailing mechanical regimes [2]. Understanding such motility patterns has proven instrumental

for research on adaptation, ageing, developmental progression, neurological disorders, and mechanosensory responses [3–7] using model organisms such as *Caenorhabditis elegans* [1]. These nematodes accomplish motion through undulatory contortions of their bodies, which range from thrashing in liquids to surface-level crawling on agar pads [7,8]. However, such observations are largely derived from two-dimensional (2D) plates and homogeneously stirred liquids, which only span a subset of the mechanical complexity encountered in natural environments [9,10]. Although past efforts have quantitatively described how altered rheological properties alter undulatory motion, these remain constrained to fluidlike physical regimes [11–18]. Prior attempts at mimicking 2D soft solidlike matrices have employed packings of glass beads, while fruit pulp and paper stacks have been used to generate 3D scaffolds [19–22]. A fundamental limitation of such systems is that even though they allow for burrowing behavior, the process of nematode motion itself destroys the structural integrity and alters the material properties of these matrices. Furthermore, meaningful biophysical measurements of motility require precise observation and tracking of nematode movement in 3D space—which cannot be afforded by such

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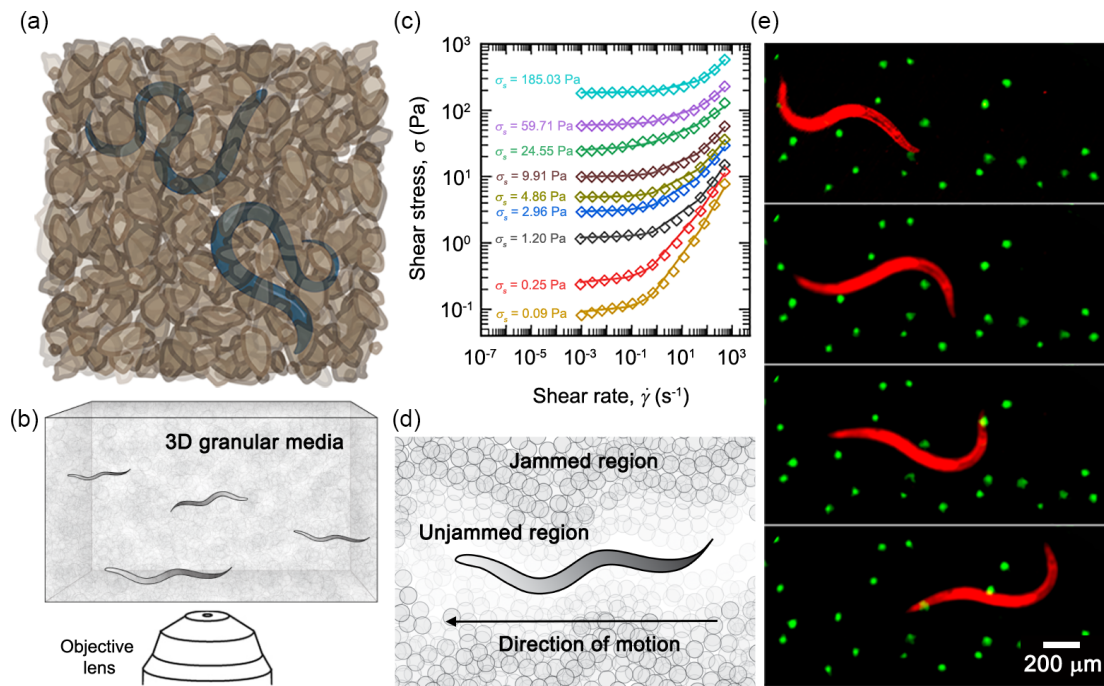


FIG. 1. Engineering an optically transparent, mechanically tunable, 3D yield stress matrix for observing undulatory nematodes. (a) The natural habitats of nematodes, such as soil, are granular and porous 3D environments that are also opaque in nature, making it difficult to directly observe and track them in 3D space. (b) Schematic representation of the nematode *C. elegans* in the optically transparent 3D microgel growth matrix. The matrix is comprised of highly swollen hydrogel granules dispersed in liquid nutrient broth, giving rise to an internally disordered pore-space geometry. Each granule is a randomly cross-linked long-chain polymer with nanometer-scale internal mesh sizes which allow for unimpeded nutrient diffusion throughout the bulk matrix. (c) Characterization of the 3D matrix, as reported using shear rheology. The tunable mechanics of the system enables reproducible engineering of precisely defined mechanical regimes—representing altered degrees of physical confinement—indicated here by the varying yield stresses of different matrices. (d) The yield stress nature of the system allows nematodes in motion to transiently fluidize the matrix by displacing the hydrogel granules surrounding them, which subsequently recovers in the wake of the nematode motion, thereby conserving the material properties of the system. (e) Nematode motion in a 3D microgel matrix with embedded fluorescent beads, showing that worms only deform their immediate surroundings without causing far-field perturbations—only beads directly in the path of an undulating worm get displaced, whereas beads in the surrounding regions remain at rest.

opaque building materials that preclude direct visualization [Fig. 1(a)]. Consequently, how undulatory microswimmers such as nematodes navigate a truly 3D, granular, and disordered environment across different mechanical regimes has not been systematically explored.

Interrogating undulatory motion in 3D space requires optically transparent, self-healing, biocompatible, 3D, soft solidlike matrices which stably retain their material properties following repeated rounds of mechanical shear. We address these requirements by introducing an optically transparent, self-healing, viscoelastic granular matrix that presents a porous and disordered 3D microenvironment for nematodes. The material itself comprises jammed packings of hydrogel granules swollen in an M9-based nutrient broth, which can be elastically deformed and reversibly displaced by the nematode's motion. Importantly, this material exhibits shear-dependent rheology, by transitioning from a soft solidlike state at rest to being locally and reversibly yielded by the nematode's motion—above a minimal critical stress level termed the yield stress—before immediately recovering the original material properties once the nematode moves away. The highly swollen nature of the individual granules makes the matrix optically transparent due to its low solid fraction, allowing us direct visualization of nematode motion inside a 3D

granular media. Further, we leverage the tunable mechanical properties of this matrix by varying the packing fraction of the hydrogel granules to precisely regulate the degree of physical confinement, which is manifested by an alteration in the material's yield stress. Tracking nematode motility reveals that the propulsive speed varies nonmonotonically with the increase in 3D physical confinement. Combining experimental findings with predictions from slender body theory, we show that the underlying basis for these trends is a maximization of the worm's forward propulsive efficiency under higher physical confinement.

Across these physical regimes, we observe a progressive transition between gaits, from a thrashing motion in low yield stress regimes—similar to that observed in homogeneous liquid—to a crawling motion in high yield stress regimes—akin to that observed on 2D surfaces. Remarkably, using genetically mutated nematode strains, we find that both the nonmonotonic trends in propulsive speed and the optimization of propulsive efficiency are independent of soft-touch mechanosensory responses. Rather, we establish that the rheological properties of the nematode's microenvironment directly enable context-specific modes of motility. Transforming these results into a phase space defined by the nondimensionalized efficiency of propulsive motion and

a nondimensionalized timescale between the undulatory frequency of the nematode body and relaxation time of the granular matrix, we observe a transition in nematode gait occurs as higher efficiency of motion emerges with increasing physical confinement. Together, these results explain how nematodes modulate their undulatory patterns to optimize for the most efficient form of forward motion as a function of the material properties of 3D granular media. Our work shows that motion in viscoelastic 3D disordered environments has unexplored facets, capturing and modeling which requires leveraging experimental platforms that allow for direct visualisation of active matter in biomimetic complex mechanical regimes. These findings hold significant implications towards reshaping our ideas of motion in 3D space and suggest important considerations for characterizing the behavioral patterns of undulatory microswimmers. Finally, we view this as an impetus for revisiting our canonical understanding of motility-associated phenotypes as readouts for the regulatory processes controlling development, mechanotransduction, and cognition.

II. RESULTS

A. An optically transparent 3D growth matrix for studying nematode motion

To structurally mimic the complex 3D natural habitats of nematodes, we pack together micron-sized hydrogel particles above jamming concentrations to generate an optically transparent, granular, and porous 3D matrix [23,24] [Fig. 1(b)]. Each such microparticle comprises of randomly cross-linked long-chain polymers which are highly swollen in the liquid culture media at neutral pH, rendering them optically transparent [Fig. 1(b)]. We precisely control the rheological properties of the matrix by altering the solid fraction of the polymer, as evidenced by their tunable shear moduli [25]. When unperturbed, the material exhibits soft solidlike properties, which are assessed using rheological measurements by applying small (1%) amplitude shear over varying time periods. Herein, we observe that the elastic storage modulus of the material (G')—which denotes an elastic solidlike nature, corresponding to the energy stored within the material while undergoing deformation—is significantly higher than the viscous loss modulus (G'')—which represents the viscous liquidlike nature, corresponding to the energy lost by frictional forces and heat generated during deformation (see Fig. S1 of the Supplemental Material [26]). This implies that the material exhibits solidlike behavior at rest. Importantly, we do not observe any significant change in the G' across varying shear frequencies, which demonstrates its time-invariant material properties.

In natural niches such as soil, nematodes locally rearrange the particles during motion, whereas at rest, they remain embedded within a granular microenvironment. This aspect of the nematode habitat is captured by our jammed granular microgel systems; as the microparticles themselves are discrete noncovalently bonded entities, they can be readily displaced by the application of a shearing force which locally yields the material. Importantly, the particles recover this deformation in the wake of the shearing agent, whereby, the mechanical

properties of the overall matrix remain conserved despite repeated deforming stresses. To quantitatively characterize this, we subject the matrices to unidirectional shear at varying rates and record the shear stress responses corresponding to each shear rate [Fig. 1(c)]. Under high shear rates, we observe a shear-dependent stress response, which subsequently plateaus under low shear rates. This behavior implies a self-healing property (Fig. S2A) [26], wherein the 3D granular matrix reversibly transitions from solidlike behavior under low shear regimes—when the microparticles remain at rest—to fluidlike behavior under high shear regimes—when the microparticles are transiently displaced [Fig. 1(d)]. This crossover from solidlike behavior to fluidlike behavior is set by the specific yield stress limit of a given microgel formulation, which determines how much deforming stress the nematode needs to generate to fluidize the surrounding matrix during its movement. To verify this, we observe nematode motion in a 3D microgel bath embedded with homogeneously dispersed fluorescent beads. Indeed, beads embedded in the vicinity of a moving nematode are displaced, whereas beads positioned away from the nematode remain at rest during motion [Fig. 1(e) and Figs. S2B and S2C]. Hence, the undulatory movement only locally and reversibly deforms these matrices, followed by immediate recovery in the wake of a propagating nematode (Fig. S3) [26]. We also note that such rearrangements only minimally alter the positions of individual beads, the overall displacements of which largely remain within a range of two to three bead diameters from their starting positions (Fig. S3) [26]. Furthermore, our microgel growth medium does not mechanically deform or compress the nematode bodies, thereby ruling out any experimental artefacts due to aberrant organismal physiology (Fig. S2D) [26].

B. Increasing physical confinement leads to nonmonotonic trends in propulsive speed, but not in sideways speed

Leveraging the optically transparent nature of the 3D microgel matrix, we directly visualize and track nematodes embedded within these using a custom-built imaging chamber. We prepare these chambers by placing a 200- μm -deep well—hollowed out from a thick plastic film—sandwiched between a coverslip and a microscopy slide. More precisely, each such imaging well is first placed on a microscopy slide and then filled with the jammed granular microgel matrix, within which a small number of nematodes are carefully embedded. Subsequently, a coverslip is used to seal off this thin slab of the microgel matrix, creating a 3D housing for the nematodes to swim around. Using confocal microscopy, we capture individual nematode motion within this setup as time-lapse images at a high frame rate [Fig. 2(a)]. Further image processing workflows to threshold and segment the nematode body allow us to quantitatively analyze the nematode motion as a sinusoidal wave propagation [Fig. 2(b)], from which we obtain the components of amplitude (A), frequency (f), and wavelength of motion (λ). We further use these to determine the sideways speed (fA) and wave speed ($f\lambda$). Collectively, these parameters allow us to quantitatively describe nematode motion within 3D granular media.

To interrogate whether the undulatory propulsive patterns of nematodes depend on the material properties of their

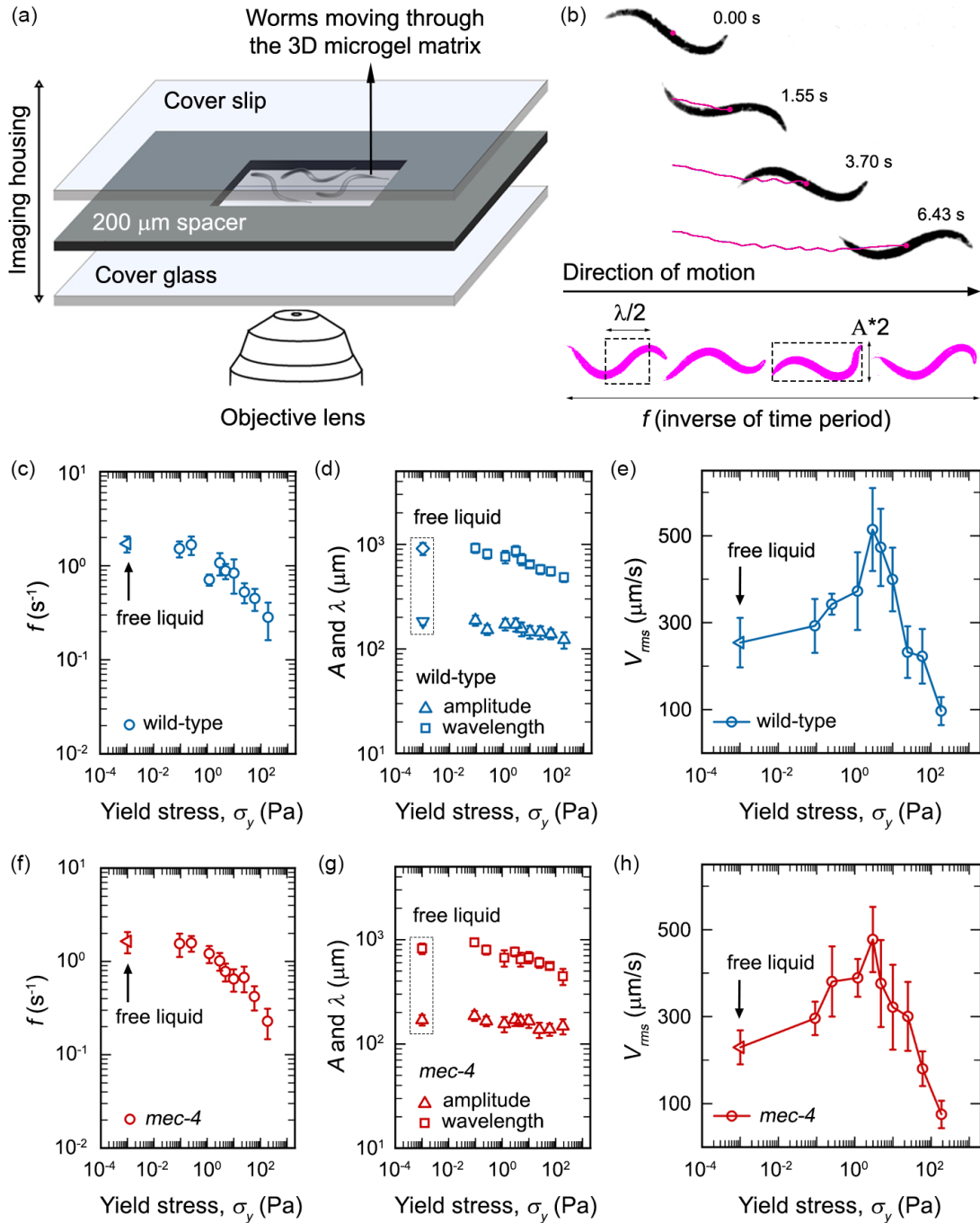


FIG. 2. Quantitative analysis of nematode motion reveals a non-monotonic dependence of the forward propulsive speed on matrix yield stress. (a) Schematic representation of the experimental setup for visualizing nematode motion in the 3D microgel matrix. (b) Nematode motion is idealized as a propagating sinusoidal wave, with the specified descriptors of wavelength, frequency, and amplitude of each undulatory motion obtained through quantitative analysis of time-lapse imaging performed on individual nematodes. (c),(d) While the frequency of undulatory motion shows a progressive decline with increasing matrix yield stresses, both the amplitude and wavelength remain less affected. (e) The forward propulsive speed, V_{rms} , as measured from the centroid displacement of the nematode, shows an unexpected nonmonotonic dependency on the matrix yield stress—initially increasing with an increase in physical confinement, followed by a sharp decline under higher degrees of confinement. (f),(g),(h) Experiments using soft touch-insensitive mutant *mec-4* strains mirror the trends observed with the wild-type strain, indicating that the observed patterns of yield stress-regulated motility are not specifically driven by this mechanotransduction-based pathway. Since free liquid does not have a yield stress, an arbitrary value of 10^{-3} Pa (viscosity of water divided by one second) is assigned to include these data on the same plot for panels (c)–(h). All error analyses have been performed as described in Sec. IV F on Statistical Analysis.

surroundings, we first observe how fundamental descriptors of nematode motion—viz., the frequency, amplitude, and wavelength of individual sinusoidal undulations—are altered across different degrees of physical confinement, represented by varying yield stresses of the 3D matrix. While we observe a progressive decay in the frequency of undulations with increasing yield stress [Fig. 2(c)], both the amplitude and wavelength remain largely conserved across different mechanical regimes [Fig. 2(d)]. This suggests that elevated physical confinement has a more pronounced effect on the rate at which the nematode executes undulations, as opposed to the nature of these undulatory patterns. Hence, it is likely that a nematode's propulsive speed through 3D granular media is strongly influenced by environmental mechanics. To test this, we first quantify the nematode's overall displacement by tracking the body centroid to obtain its mean-square displacement (Fig. S4A) [26]. From the mean-square displacements, we measure root-mean-square speed (V_{rms}) as a function of lag time (τ), which represents the forward propulsive speed (Fig. S4B) [26]. Next, to fix an appropriate timescale for comparing V_{rms} in different matrices, we analyze the nematode body undulations to identify characteristic frequencies and time periods of motion. We compare the V_{rms} at lag times corresponding to the time period of motion in a given matrix. Extending this analysis over different regimes of physical confinement—defined by the yield stress of the system—we find that the V_{rms} shows a nonmonotonic dependence on the yield stress of the system [Fig. 2(e)]. Surprisingly, an increase in confinement appears to provide a propulsive advantage to these undulatory swimmers up until a threshold yield stress, beyond which the expected effects of confinement in reducing the speed of motion manifest. To further verify that this experimental setup truly captures 3D phenomena, we independently assess worm motion in 3-mm-deep chambers filled with the microgel growth media across all ten mechanical regimes and find an excellent agreement across all kinematic parameters describing worm motion between the 200- μm -deep and 3-mm-deep systems (Fig. S5) [26].

In our experiments, the nematode moves through a bath of granular particles. Hence, it is conceivable that its motion is regulated by physical interaction with the particles and touch sensation, which actively effect behavioral changes leading up to an alteration in the nematode motion. We test this hypothesis by employing a gentle-touch sensory mutant (*mec-4*), which carries a genetic aberration rendering it insensitive to soft mechanical pressures [27]. Given the low shear moduli of our microgels in comparison to 2D agar/plastic/glass substrates, gentle-touch mechanosensation represents a putative module for environmental perception by nematodes moving in granular 3D media. Surprisingly, we find no significant differences between the wild-type and *mec-4* mutant strains for any of the descriptors of undulatory motion across the entire range of physical confinement interrogated by our study [Figs. 2(f) and 2(g)]. Indeed, the V_{rms} of soft touch-insensitive mutant nematodes also exhibit a nonmonotonic dependence on yield stress, mirroring the trend observed for the wild-type strain [Fig. 2(h)]. Furthermore, the nonmonotonic nature of the forward propulsive speed across different mechanical regimes remains robustly conserved for both wild-type and *mec-4* strains, irrespective of the time period of motion

(Figs. S4C and S4D) [26]. Together, these observations suggest that the mechanism via which the forward propulsive speed of nematodes is modulated in a yield stress-dependent manner is likely not driven by active neuronal perception and consequent biochemical response to altered mechanical regimes. Rather, we hypothesize that such trends reflect how 3D confinement effects a physical regulation of undulatory motion.

C. A slender body theory-inspired formulation predicts the dependence of wave speed on yield stress

To quantitatively understand how different degrees of physical confinement imposed by the 3D microgel matrix regulate nematode motion, we use predictions from a slender body theory to describe the interactions involved in this process. We adopt a previously described formulation [28] to conceptualize nematode motion through a yield stress matrix, which can be modeled as a Herschel-Bulkley fluid obeying the following relation:

$$\sigma = \sigma_y + K\dot{\gamma}^n$$

for shear stress σ , yield stress σ_y , consistency K , index n , and the strain rate $\dot{\gamma}$. We consider $n = 0.5$ throughout, which is in good agreement with our experimental medium. This assumption is informed by both an average value of n derived from Herschel-Bulkley model fitting to the experimentally obtained rheological measurements (Table S1) [26], as well as prior reports on similar jammed microgel-based granular media [25]. Further, we incorporate the sinusoidal nature of the worm body undulations by modeling its propagation using the idealized waveform

$$X_{(z,t)} = A \sin \left[2\pi \left(\frac{z}{\lambda} - ft \right) \right].$$

Given such a waveform, a resistive-force theory approach allows us to determine the forward speed V and power input P as functions of the waveform (i.e., A , λ , and f), and the rheology of the matrix. Our approach [28,29] relies on the assumption that deformation is relatively localized to the long and thin organism (details are provided in the Supplemental Material [26]). The importance of the yield stress can be characterized by the dimensionless Bingham number, B , as

$$B = \frac{\sigma_y R^n}{KV^n}.$$

Given a value of B and a body form—defined as the ratio between amplitude and wavelength (A/λ)—our theoretical formulation returns the forward speed V and the power P . A simple measure of efficiency is provided by the Strouhal number—defined as the ratio between the sideways component of motion (fA) and forward speed (V)—the magnitude of which inversely correlates with the efficiency of forward motion with respect to the lateral displacement. Interestingly, we find a nonmonotonic behavior in the Strouhal number as a function of the body form (A/λ), which, along with a screening for the optimal A/λ ratio across different regimes of B , implies that optimal conditions favoring forward motion occur within a range of $0.1 \leq A/\lambda < 0.35$ [Fig. 3(a) and Fig. S6A]. Together, these results indicate that efficient propulsive

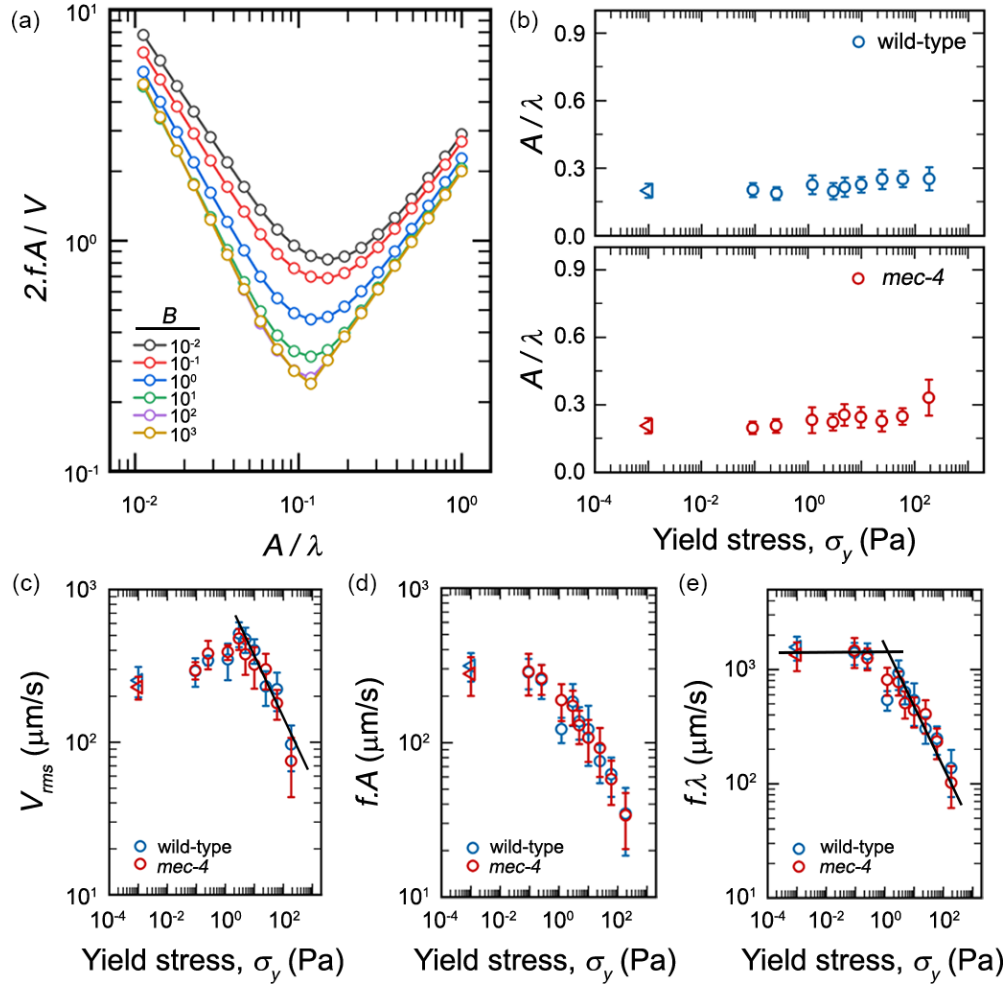


FIG. 3. The undulatory motion of nematodes in high yield stress regimes is characterized by small A/λ ratios as well as a power law dependency of the propulsive and wave speed on yield stress. (a) Numerical solutions comparing the Strouhal number against different body forms (A/λ ratio) show a generalized nonmonotonic behavior across different mechanical regimes (varying B). (b) Experiments reveal that nematodes maintain their body form (A/λ ratio) at a small magnitude ($0.1 \leq A/\lambda \leq 0.35$), as predicted by the theoretical model, across different degrees of 3D physical confinement. (c) Pooled datasets of the propulsive speed (V_{rms}) show a distinct power-law dependence on the matrix yield stress under higher degrees of physical confinement, which decays as $V_{rms} \propto \sigma_y^{-1/2}$. (d) The sideways component of motion (fA) undergoes a progressive and sustained decrease with increasing physical confinement. (e) The wave speed ($f\lambda$) of the undulatory motion remains initially constant before exhibiting a decay with increasing yield stress, which follows a scaling behavior similar to that of the propulsive speed, given by $f\lambda \propto \sigma_y^{-1/2}$. The experimentally observed scaling behavior of both V_{rms} and $f\lambda$ are in good agreement with predictions from the theoretical model. In all the plots of panels (b)–(e), the triangular symbols represent data from nematodes moving through free liquid. Since free liquid does not have a yield stress, an arbitrary value of 10^{-3} Pa (viscosity of water times one second) is assigned to include these data on the same plot. All error analyses have been performed as described in Sec. IV F on Statistical Analysis.

behavior in yield stress environments representative of our experimental system requires a pattern of undulatory motion consisting of comparatively small amplitudes—indicative of sideways displacement—relative to the wavelength of undulation—indicative of forward displacement—to achieve efficient forward motion while minimizing lateral movement. Furthermore, we test whether the key assumptions underlying this formulation remain conserved within our experimental paradigm. Encouragingly, for both wild-type and soft-touch insensitive mutant *mec-4* strains, we observe that in high yield stress regimes, the body form (A/λ) for the nematode's undulatory motion remains small, falling within the $0.1 \leq A/\lambda < 0.35$ range, which mirrors the exact constraints necessitated by the theoretical model [Fig. 3(b)].

Next, we plot all experimental propulsive speed (V_{rms}) data from both the wild-type and soft touch-insensitive mutant strains, and we observe that the speeds of nematodes follow a universal scaling with the yield stress of the matrix. More precisely, in high yield stress regimes, the propulsive speed shows a power-law dependence on the yield stress [Fig. 3(c)]. We find that with increasing yield stress, the V_{rms} decays with a power-law exponent of the order of approximately $-1/2$, such that

$$V_{rms} \propto \sigma_y^{-1/2}.$$

Interestingly, however, the nonmonotonic trend holds true only for the forward propulsive speed, V_{rms} . A comparison of the sideways component of motion, given by the product of

frequency and amplitude (fA), instead shows a progressive decline with an increase in the yield stress of the 3D matrix, even under regimes of low physical confinement [Fig. 3(d)]. This contrast between both the V_{rms} and the sideways speed indicates that even within a small range of increase in yield stress, motion within the 3D matrix manifests distinctly different effects on forward propulsion and sideways motion; while the former shows a transient increase followed by a sustained decline, the latter almost immediately undergoes a gradual decrease with increasing yield stress.

The theoretical formulation predicts a relationship between the waveform parameters A , λ , and f , the forward speed (V), and the power (P) expended by the nematode. In general, there is no analytical expression for this relationship, but for amplitudes in the optimum range outlined above and for large enough yield stress, previous reports have arrived at numerical solutions as [28,29] (elaborated in the Supplemental Material [26])

$$V \propto c \propto \left(\frac{P}{\sigma_y R} \right),$$

where c , given by $c = f\lambda$, represents the wave speed along the worm body.

Thus, the experimental observation that the propulsive speed (V_{rms}) decays as the square root of the yield stress immediately implies that the power is increasing as $P \propto \sigma_y^{1/2}$, and the wave speed of motion in the solidlike mechanical regime ($B > 1$) is

$$c = \lambda f \propto \sigma_y^{-1/2}.$$

Remarkably, we find that our experimentally measured data on the wave speed ($f\lambda$) of nematodes across different mechanical regimes also exhibit a similar scaling behavior under high yield stress [Fig. 3(e)]. This strong agreement between the theoretical prediction and the experimental data clearly indicates that the observed modulation of nematode motility across different degrees of physical confinement is dependent on the matrix yield stress properties. Furthermore, the correspondence between both wild-type and soft touch-insensitive mutant nematodes underscores that the phenomena reported herein are driven by generalizable interactions between the worm motion and their environment, rather than touch-specific neuronal signaling.

It is interesting to consider the limits within which power generation by the undulating worm may be modulated in response to the degree of 3D confinement. Prior measurements of muscle power in response to varying biomechanical loads suggest that worms repartition their power expenditure based on the external mechanical milieu [15,30]. Under low external mechanical loads, worms expend more power towards contorting their bodies (corresponding to swimming/thrashing-like behavior), whereas higher external loads redirect more power towards overcoming mechanical resistance (corresponding to crawling-like behavior). Along similar lines, we expect that as the degree of confinement in our experiments increases from more fluidlike to solidlike mechanical regimes, worms likely repartition an increasing fraction of their power expenditure towards yielding their surroundings to achieve propulsive motion. It is conceivable

that under sufficiently high degrees of confinement, worms would eventually reach an upper limit of power generation, beyond which the power exerted to yield the system (P) does not increase with increasing external mechanical resistance. This would consequently lead to both locomotory speed and wave speed decreasing with increasing confinement, likely decaying on the order of σ_y^{-1} . However, this theoretical scaling is in practice only attainable for very large degrees of confinement, and it may simply be that the experiments here have yet to explore these high yield stress limits. Rather, both our theoretical model and experiments operate within yield stress ranges wherein P shows a dependence on the degree of confinement as $P \propto \sigma_y^{1/2}$. As shown above, this also helps explain why both locomotory and wave speed also decay on the order of $\sigma_y^{-1/2}$.

D. Nematodes optimize their forward propulsive efficiency under higher confinement, wherein wave speed matches the propulsive speed

Following the observations from our theoretical framework and experimental findings above, we next ask how nematode motion is mechanistically regulated to accomplish efficient forward propulsion under high yield stress regimes. To elucidate this, we first numerically solve for the efficiency of forward motion, as captured by the Strouhal number, at different mechanical regimes represented by varying B . We find that even small variations in the body form (A/λ ratio) influence efficiency of forward motion [Fig. 4(a)]. As expected, the overall efficiency turns out to be inversely dependent on the A/λ ratio, since the Strouhal number is directly proportional to amplitude. Interestingly, however, beyond $B > 1$, which indicates a solidlike regime (since the yield stress component outweighs the viscous shear applied by the nematode), the efficiency of forward motion plateaus regardless of the body form, suggesting that a more solidlike mechanical regime enables the conservation of the forward propulsive efficiency. We further validate this by considering the Lighthill efficiency (Fig. S6B) [26], which gives the relation between the movement speed and expended power—the higher the index, the more effective the nematode's motion. Strikingly, our numerical results predict that in solidlike mechanical regimes ($B > 1$), Lighthill efficiency also becomes independent of Bingham number, indicating that peak propulsive efficiency is both enabled and constrained by elevated physical confinement (Fig. S6B) [26]. Together, our findings indicate that a decrease in the Strouhal number captures an increase in propulsive efficiency as depicted by the Lighthill parameter. Hence, from hereon, we will consider the Strouhal number as the metric for the efficiency of propulsive motion in complex environments.

Interestingly, the experimental measurement of the Strouhal number for wild-type as well as *mec-4* mutants across different degrees of confinement both quantitatively and qualitatively captures the theoretical predictions. More precisely, the experimental Strouhal number shows an initial decline with an increase in yield stress, followed by a subsequent stable plateau, akin to the numerical results [Fig. 4(b)]. We also note that the average body form (A/λ) in our experiments remains within the range of 0.1–0.35, with a value of approximately 0.25. Remarkably, for this regime, our

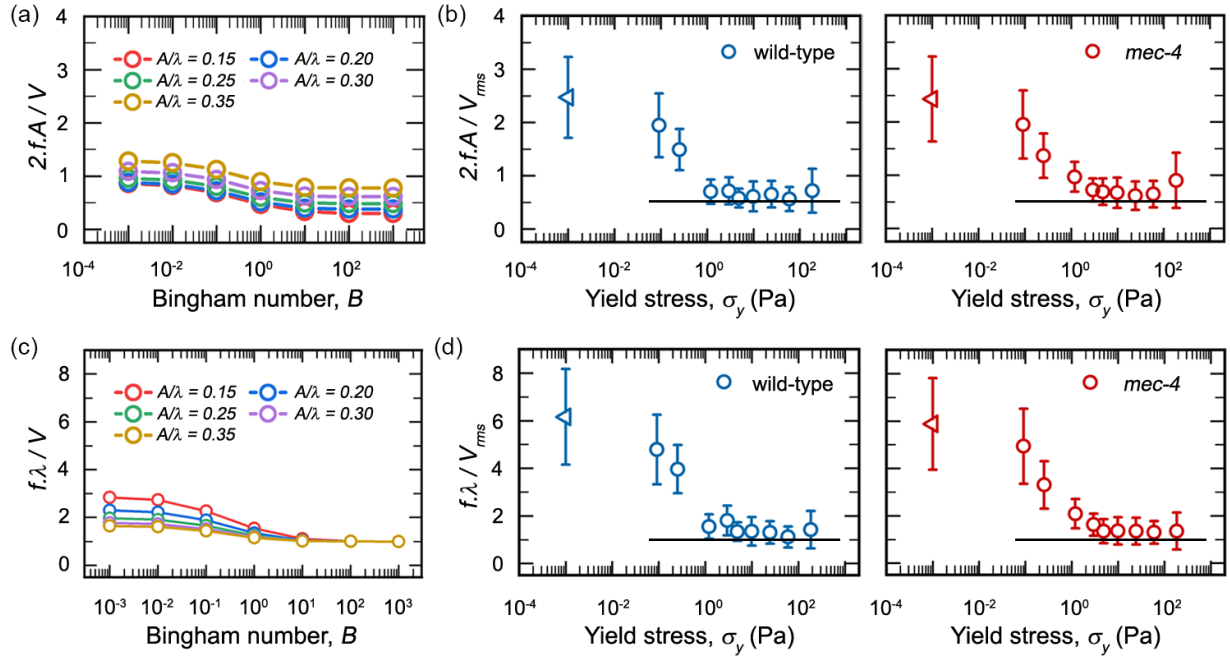


FIG. 4. Nematodes optimize their motion in response to elevated physical confinement by minimizing sideways motion, whereby the wave speed along their body matches their forward propulsive speed. (a) Numerical analysis shows that the Strouhal number attains a plateauing behavior under solidlike regimes ($B > 1$) for all different body forms (A/λ). (b) Under high yield stress regimes, both wild-type and *mec-4* soft-touch mechanosensory mutant strains optimize for effective forward propulsion by minimizing their sideways component of motion (fA) in comparison to their forward propulsive speed (V_{rms}). (c) Under solidlike mechanical regimes, numerical analysis shows that nematodes match their wave speed ($f\lambda$) to their forward speed (V), across all different body forms. (d) Within high yield stress matrices, the wave speed along nematode bodies matches their propulsive speed, for both wild-type and *mec-4* strains. In all the plots of panels (b) and (d), the triangular symbols represent data from nematodes moving through free liquid. Since free liquid does not have a yield stress, an arbitrary value of 10^{-3} Pa (viscosity of water times one second) is assigned to include these data on the same plot. All error analyses have been performed as described in Sec. IV F on Statistical Analysis.

theoretical predictions of Strouhal number match identically with our experimental findings for both the wild-type and *mec-4* strains [indicated by the guidelines in Fig. 4(b)], which shows that propulsive efficiency is directly regulated by the interaction between microenvironment mechanics and bodily undulations. Interestingly, the transition point where the stable Strouhal number regime commences matches the yield stress that gives the maximal V_{rms} . This experimental regime corresponds to a Bingham number of $O(1)$ (Fig. S7) [26], which in turn mirrors trends in the numerical analysis observed when $B > 1$. Together, these results solidify our hypothesis that nematodes optimize their motion to achieve maximal forward propagation while undergoing minimal sideways displacement upon an increase in the degree of physical confinement.

Next, we employ the theoretical model of worm motion to understand how the ratio between the wave speed ($f\lambda$) and the forward speed (V) depends on the environmental mechanics (B). We find that as the mechanical regime becomes increasingly solidlike ($B > 1$), the forward speed of the nematode does appear to match its wave speed, regardless of the body form (A/λ)—exactly as predicted by our model above [Fig. 4(c)]. We next examine the experimental data and find that the ratio between the wave speed and the propulsive speed also shows an initial decline, followed by plateauing at critical yield stress, beyond which both remain approximately equal—perfectly mirroring our theoretical predictions [Fig. 4(d)]. Similar to the motility efficiency as captured by

the Strouhal number, the onset of this transition occurs at the exact yield stress wherein the propulsive speed (V_{rms}) is maximal and the Bingham number is of the $O(1)$ (Fig. S7) [26]. For additional consistency, we also plot the experimentally derived parameters describing nematode motion against the experimentally determined Bingham number and observe similar trends (Figs. S7 and S8) [26]. Hence, we infer that nematodes optimize their forward propulsive efficiency under elevated physical confinement when the forward propulsive speed matches the wave speed along their body.

E. Increasing physical confinement alters nematode gait from thrashing to crawling, making forward propulsive motion more efficient in solidlike regimes

Our present consensus on *C. elegans* motility demarcates two distinct forms of motion, varying from a thrashing motion in bulk fluids to crawling on 2D surfaces. However, these models do not clearly account for how physical confinement within granular 3D materials alters the propulsive behavior. Further, while past work using different species of worms has looked at burrowing behavior within sediment beds [31,32], our understanding of how different degrees of physical confinement enable or hamper specific gaits of motion (viz. thrashing or crawling) remains incomplete. Here, considering the stark differences between the trends in propulsive speed and sideways speed with increasing environmental yield

stress, we hypothesize that confinement-induced alteration to the nematode's motion might help explain these patterns. To test this, we generate time projections of nematode motion for identical durations across different degrees of physical confinement [Fig. 5(a)]. Comparison between these reveals a stark difference testifying to our quantitative analysis: within low yield stress materials, nematodes execute a thrashing motion while gradually transitioning to a crawling motion with an increase in yield stress, which is set by the degree of 3D confinement. These observations also fall in line with our measurements of nematode body motion; while the sideways speed—maximal when purely thrashing, minimal when purely crawling—decreases with increasing yield stress, the propulsive speed becomes adjusted to the wave speed along the nematode body (as enabled by crawling behavior) under higher degrees of confinement. Together, these observations underscore that the physical confinement-driven regulation of nematode motility is dependent on the gait transitions induced by the yield stress of the 3D matrix.

This begs the question—why exactly is the yield stress property of the material influencing nematode gait and forward propagation? Using shear rheology, we previously observed that our 3D growth matrices show yield stress behavior, transitioning from a viscous fluidlike behavior under high shear rate to a more elastic solidlike behavior under low shear. The transition between these regimes is characterized by the yield stress and its corresponding crossover shear rate, which shows a correlated behavior [Figs. 5(b) and 5(c)]. Specifically, the crossover shear rate represents the shear rate at which both the viscous and elastic components of the shear stress are equal, representing the solid to liquid transition. This implies that higher confinement matrices recover faster from mechanical shear as opposed to low confinement matrices. Since nematodes moving through the 3D matrix also generate deforming shear stresses on the material, we directly compare the timescales of the nematode-generated stress against the matrix crossover shear rates to better understand the microenvironmental mechanics in the vicinity of a moving nematode. For this, we define T^* , as the ratio between the matrix crossover shear rate and the frequency of nematode undulation, as

$$T^* = \frac{\dot{\gamma}_c}{f}.$$

Effectively, $T^* < 1$ implies that the nematode body undulates faster than the matrix relaxation time, which makes the nematode experience a fluidlike environment. By contrast, $T^* > 1$ makes the nematode experience a solidlike environment since the nematode undulation is slower than the material relaxation time. To quantitatively assess if the variation in matrix properties leads to a transition in motion, we define a phase space described by nondimensional efficiency (Strouhal number) and a nondimensional timescale of motility (T^*). Herein, we capture four distinct zones: (I) the red zone, wherein highly undulatory motions (akin to thrashing) give rise to less efficient motility under low confinement; (II) the grey zone, wherein we would hypothetically expect efficient propulsion under low confinement; (III) the white zone, wherein we would hypothetically expect poorly efficient propulsion under high confinement; and finally (IV) the green

zone, wherein crawlinglike behavior enables highly efficient motion under high confinement [Fig. 5(d)]. Transforming our experimental findings into this phase space reveals that our data lie squarely within the (I) red zone and the (IV) green zone. Importantly, we observe a sharp transition between the zones when the nematode's propulsive efficiency increases, which also coincides with the gait transition from thrashing to crawling. It is noteworthy that this occurs within a narrow range of increasing matrix yield stresses—going from just about ~ 1.2 Pa to ~ 3 Pa during this transition. Collectively, these analyses quantitatively capture the transition from a more undulatory and less efficient motion (red zone) to a less undulatory and more efficient motion (green zone) with an increase in the degree of physical confinement. This is also validated by the fact that a comparison between T^* and sideways speed shows a monotonic decay in the sideways locomotory component with increasing T^* (Fig. S9) [26]. Together, these phase spaces describing nematode motility beautifully capture a crossover from thrashing to crawling behavior as a function of the physical confinement, whereby worms optimize their motion for greater forward propulsive efficiency within increasingly solidlike environments.

III. DISCUSSION

Our work introduces the first-ever experimental platform to directly observe, track, and measure nematode motility within an optically transparent, mechanically tunable, viscoelastic, 3D granular matrix. We report that under increasing degrees of physical confinement, forward propulsion shows a nonmonotonic trend, whereas sideways motion monotonically decreases. Importantly, the nematodes undertake these alterations to optimize their efficiency of forward propulsion under increasing confinement, which is achieved when their propulsive speed matches the wave speed along their body. These effects manifest in the form of a gait change from thrashing behavior in low yield stress regimes to crawling within higher yield stress matrices. Interestingly, in a phase space between propulsive efficiency and a dimensionless timescale of motion, T^* —set by the ratio between matrix relaxation time and nematode body undulation frequency—the motility transitions between two distinct regimes: one, wherein inefficient forward motion dominates under fluidlike environments, and two, wherein increasingly efficient forward motion correlates with increasingly solidlike environments. Our work hence establishes a mechanistic basis for the regulation of undulatory motility by 3D physical confinement.

Gait patterns in nematode motion represent a dynamic behavioral trait, which can be regulated by both neuromuscular feedback and the external mechanical environment. Biochemical investigations have shown how dopamine-serotonin signaling controls the transition between crawling and swimming/thrashinglike gaits—representing a neuronal mechanism for controlling gait transitions [4]. Complementarily, repartitioning of muscle power between elastic deformations of the worm body and overcoming external mechanical resistance represents a physical mechanism of gait control [30]. Prior studies of worm motility in viscoelastic fluids have analyzed locomotory behavior as a function of the Deborah number (De) which captures the relation between undulatory

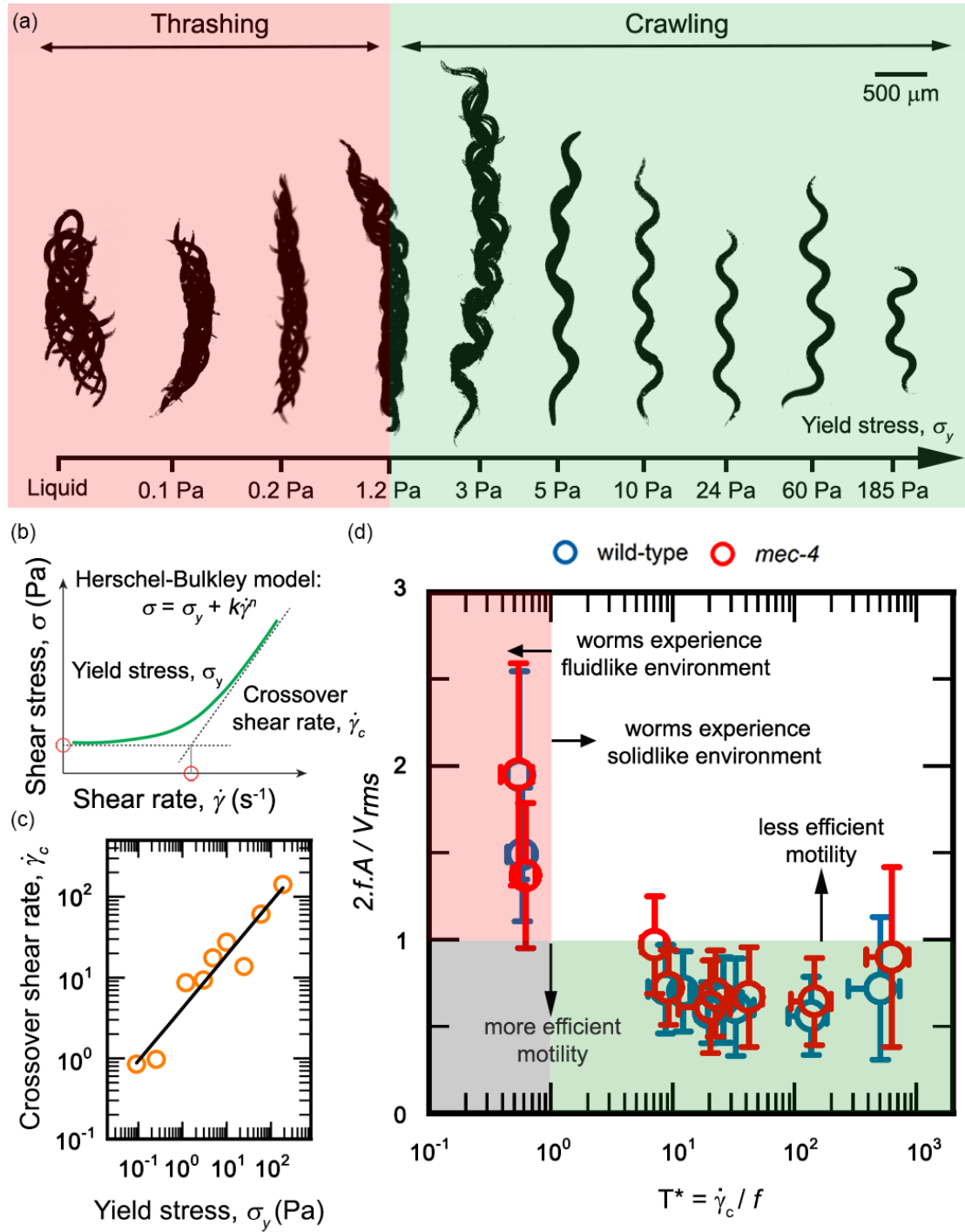


FIG. 5. Nematodes exhibit a progressive gait transition in response to increasing physical confinement, which underlies the optimization for forward motion in high yield stress regimes. (a) The undulatory motion of nematodes in the 3D matrix shows a progressive gait transition from thrashinglike behavior in fluidlike regimes to crawlinglike motion in more solidlike regimes. All gait projections shown here are for identical durations of time (~ 5 s). (b) The yield stress nature of the 3D microgel matrix follows a Herschel-Bulkley fluidlike behavior, showing shear-dependent rheological properties. (c) Each such matrix is characterized by a specific crossover shear rate ($\dot{\gamma}_c$), which indicates the timescale of elastic recovery following shear-induced solid to liquid transition—higher $\dot{\gamma}_c$ indicates faster recovery of solidlike elastic properties, and vice versa. (d) A 2D phase space defined between the Strouhal number (indicating the efficiency of forward motion) and T^* (a measure of the matrix recovery time under nematode motion-induced shear, relative to the rate of nematode undulations) shows two distinct regions: in more fluidlike environments (low T^*), nematodes exhibit inefficient forward motion (high Strouhal number value), whereas in more solidlike environments (high T^*), nematodes increase the efficiency of forward motion (low Strouhal number value). All error analyses have been performed as described in Sec. IV F on Statistical Analysis.

frequency (f) and an inverse of the fluid relaxation time (t), given by $De = ft$ [11,33]. Separately, worm motion within solidlike environments such as sediment beds has been experimentally interrogated [31,32]—albeit lacking a direct extrapolation to models developed for analyzing fluidlike environments. However, as we show in this work, gaits progressively transition from thrashing to crawling as we move from liquidlike to solidlike regimes, rather than manifesting as abrupt jumps between these states. Hence, how worms adapt to such a change in material properties cannot be captured using existing platforms.

By contrast, our work bridges distinctly different material properties by exploring an expansive mechanical regime spanning more than three orders of magnitude, from purely fluid to solidlike degrees of confinement. Similar to the Deborah number, this is captured by our dimensionless timescale of motion (T^*). Unlike the De , however, T^* relates the solid-to-fluid transition timescale of the 3D matrix to the worm's undulatory frequency. Further, by maintaining identical chemical compositions of the experimental medium across all conditions, as well as employing both wild-type and *mec-4* soft-touch sensory mutant worm strains, we exclude the possibility of differential chemical signaling or biological sensing. Rather, we show that gait optimization from inefficient thrashing under low confinement to efficient crawling under high confinement emerges as a consequence of the worms achieving efficient propulsive motion. It is likely that the organism employs multiple control strategies to effect these outcomes. For instance, enhanced power allocation towards yielding the external environment under high confinement regimes could be linked to dopamine-driven engagement of the appropriate muscle groups. It will be interesting to explore how the interfacing between these regulatory modules is influenced by 3D viscoelastic milieus, and whether hitherto unexplored classes of internal control mechanisms may be engaged in such complex environmental settings.

Specifically, in their natural settings, undulatory microswimmers traverse a diverse range of mechanically complex habitats which are granular 3D environments. It is conceivable that sensory perception and subsequent responses within these milieus are governed by a specialized set of neuronal networks and associated biochemical signaling modules. To date, these have remained largely unidentified due to technical limitations precluding direct observation of worm motion within mechanically tunable 3D systems. The unique structural properties of the 3D granular microgel-based platform introduced in this work position it as an excellent candidate for the identification of such putative biological effectors. It is also worth noting that environmental niches may feature a prominent element of state/activity-dependent material properties—for instance, both the moisture content (state) as well as burrowing behavior (activity) of worms would likely alter the rheological behavior and packing density of granular matter such as soil. To incorporate such environmental hysteresis as part of the experimental paradigm, future efforts could make use of modified 3D growth media, such as those manufactured using jammed packings of flash-solidified agarose granules [34]. Leveraging this, a broad range of heterogeneous material properties may be accessed by varying parameters such as the degree of jamming, the solid fraction

of agarose, particle sizes, and the particle morphology—potentially enabling a more biomimetic recapitulation of natural habitats.

Parallely, behavioral responses to chemotactic cues are widely used as a readout of neuronal activity and physiological functionality. However, past work on this typically employs flat agar pads with constrained two degrees of freedom. It is an intriguing prospect to explore how both chemoattractant/repellent gradients in 3D alter detection and consequent responses, and how the behavioral changes induced by chemical signaling coordinately regulate the nematode's motion alongside different degrees of physical confinement—effectively, a mechanochemical regulatory framework of worm motion. This is particularly intriguing, given our observation that nematode gait is directly influenced by the matrix yield stress. This also has broader implications for studying activities such as foraging for food (based on chemotactic detection of bacterial prey) and collectively coordinated motion by strains exhibiting social behavior. Moreover, this sets up the stage for exploring how genetically encoded behavioral patterns in nematodes can undergo orthogonal modulation by the mechanical properties of their microenvironment.

In general, such a conceptualization is not without precedent. Indeed, past work with short-term bodily conformations of crawling nematodes has used a dynamical systems approach to analyze the resultant eigenworm data across time, yielding predictive phase spaces of worm behavior [35]. A crucial insight here is that the state of a nematode's body determines not only its locomotory patterns but also the observed variability therein. This implication—that body mechanics capture behavioral patterns [36]—strongly echoes through our present work as well, albeit with a key difference. While past work has been restricted to data from 2D surfaces or homogeneous liquid media, our exploration of a granular, viscoelastic 3D environment provides new insights into how yield stress properties and material elasticity govern bodily gait and locomotory efficiency. Apart from this, a class of 3D behaviors such as roll maneuvers have been previously reported by tracking worm body undulations within liquid media [37]. It is interesting to consider whether viscoelastic 3D regimes also feature similar behavioral modes. However, investigating this will require sophisticated multicamera setups enabling fast volumetric imaging, which can capture the entire set of 3D coordinates corresponding to the worm backbone. Future work along these directions can help answer a fundamental question—are previously reported phase spaces of nematode behavior distinct from their state in 3D space, or do both reside within a universal paradigm of behavioral regulation mediated by bodily conformations and microenvironmental mechanics? Resolving this has profound implications for how we seek to model dynamic biological activity in complex environments.

Our present work hence extends two key advancements, both technical and conceptual. The experimental platform introduced here will be of broad interest to the worm community, by enabling the direct interrogation of nematodes in tunable biomimetic 3D settings. Further, we have identified a mechanistic basis for the biophysical regulation of motility, purely based on environmental constraints. This opens

up future opportunities for exploring the axes between genetic regulation, behavioral responses, and microenvironment mechanics-induced feedback.

IV. MATERIALS AND METHODS

A. Preparation of 3D growth matrix

We manufacture optically transparent 3D growth matrices by dispersing granules of Carbomer-980 (Ashland, Inc.) in liquid M9 nutrient broth. Following homogeneous dissolution of the granules, we adjust the pH of the suspension to neutral pH 7 using 10N NaOH, which causes the hydrogels to significantly swell and form a jammed 3D packing [38]. The low solid fraction used to manufacture these microgels (between 0.6% and 5% of the total weight) ensures that the system is highly optically transparent. By altering the packing fraction and degree of swelling of the microgel granules, we exercise precise control over the rheological properties of the 3D matrix [39]. We further characterize the viscoelastic properties of these matrices as described below to represent different degrees of confinement.

B. Rheological characterization

Using shear rheology performed on an Anton Paar MCR302e rheometer with a 50 mm roughened cone plate tool, we determine the physical properties of different 3D microgel matrices. By unidirectionally shearing the samples at varying shear rates and recording the consequent shear responses as well as viscosities, we find that the material exhibits shear-dependent rheological properties as well as yield stress behavior—i.e., above a threshold shear rate, the material behaves like a fluid (shear stress has a power-law dependence on shear rate), whereas below the threshold shear rate, the material behaves like a soft solid (shear stress is independent of shear rate) [34]. We additionally apply the Herschel-Bulkley model for yield stress fluids to determine the characteristic yield stress and associated crossover shear rates for each of these microgel matrices (Table S1) [26]. We also report the bulk stiffnesses of these matrices by subjecting them to small amplitude ($\sim 1\%$) oscillatory shear strain over varying frequencies and record the resultant elastic storage modulus (G' , indicating solidlike behavior) as well as viscous loss modulus (G'' , indicating fluidlike behavior). From this, we also infer that the rheological properties of our 3D matrix remain conserved irrespective of the time period over which deforming shear is applied—indicating time-independent material properties.

C. Nematode culturing protocols

Caenorhabditis elegans strains used in this study were sourced from the *Caenorhabditis* Genetics Centre (CGC, University of Minnesota, Minneapolis). The specimen denoted as wild-type belongs to the N2 strain (Bristol variety), while the soft touch-insensitive mutant *mec-4* is strain CB1229 [genotype: *mec-4(e1339)* X]. Maintenance cultures of nematodes are carried out on NGM (nematode growth medium) agar plates (2–2.5% w/v agar), which are seeded with the *E. coli* strain OP50 (a uracil auxotroph) as a food source.

Plates with nematodes are maintained at 21 °C, with regular transfers to fresh plates such as to prevent starvation and overcrowding-induced stresses. The M9-based nutrient broth comprises 0.6% (w/v) disodium hydrogen phosphate, 0.3 (w/v%) monopotassium phosphate, 0.5% sodium chloride, and 0.1% ammonium chloride dissolved in double-distilled water and autoclaved before use. Twenty-four hours before all assays, we transfer healthy nematodes at late L4-stage to fresh NGM agar plates and allow them to grow into adult nematodes with a body length of approximately $1\text{ mm} \pm 100\text{ }\mu\text{m}$. Approximately 4–5 nematodes per assay (to minimize the interactions among the nematodes) are first washed using the respective yield stress gel to remove possible bacterial coatings from the nematode's body surface and eliminate chemotactic responses, following which these are transferred to the imaging setup containing 50–100 μL of the 3D microgel matrix, wherein we allow for a 15-min incubation to acclimatize the nematodes. To visualize the flow field around a nematode in motion, we uniformly disperse 10-micron-diameter polystyrene green fluorescent beads in a microgel matrix and add nematodes to this before imaging. For enhanced visualization, we pseudo-color the worm bodies in micrographs depicting these experiments [Figs. 1(e), S2B-C, and S3]. Bead displacements are tracked using the built-in TrackMate manual tracking ImageJ plugin.

D. Imaging setup

200-micron-deep assay setups are prepared by gluing together two transparent 100-micron-thick A4 sheets with UV-curable Norland Optical Adhesive 81 to generate 200-micron-thick slabs. A hole is punched into this slab, to one side of which we affix a cover glass slide. This generates a confined space of $<100\text{ }\mu\text{L}$ within which the nematode-laden microgel mix is loaded, and the chamber is sealed with a cover glass with excess gel being scrapped off. Images are acquired using a Nikon Eclipse Ti2 resonant-scanning microscope with a $4\times$ objective, ~ 20 min after loading inside the imaging chamber, for a period of 1–2 min per image, and all experiments are conducted within approximately 10–15 min. At least 20 nematodes are analyzed for all experimental conditions, across both strain types. For experiments involving 3-mm-thick 3D microgel growth media samples, we 3D print hollow moulds which are 3 mm in height, with one face being sealed off using a cover glass slide, and the other being left open for loading the gel and worms. Given that the individual microgel granules are approximately 5–10 microns in size [25], these platforms hence establish an approximately 30-layer-deep (for the 200-micron-thick samples) and 400-layer-deep (for the 3-mm-thick samples) microgel growth matrix. Images are acquired using a Thorlabs CS165MU1/M-Zelux 1.6MP Monochrome CMOS Camera.

E. Quantitative image analysis

Brightfield images from all time-lapse data are processed using ImageJ. Using background subtraction methods, we obtain binary images which show the nematode body in motion through the microgel matrix. From these, we separate out frames capturing the undulatory motion of individual

nematodes, over time windows within which they execute at least one complete sinusoidal motion. Idealizing the nematode body as a sinusoidal wave, we measure the amplitude, wavelength, and frequency of motion as outlined in Fig. 2(b). Subsequently, we obtain the characteristic time period of motion for different degrees of physical confinement. Using custom MATLAB scripts, we track the nematode body centroid, from which we measure the mean-square displacement (MSD) as

$$\text{MSD}(\tau) = \langle [r(t + \tau) - r(t)]^2 \rangle_t,$$

where r is position and τ is lag time. The root-mean-square speed of forward motion is calculated from the MSD as

$$V_{\text{rms}}(\tau) = \frac{1}{\tau} \sqrt{\text{MSD}(\tau)}.$$

We then compare the characteristic time period of motion as measured by manual image analysis (described above) and find the root-mean-square speed most closely corresponding to this time interval, which we assign as the V_{rms} for each particular microgel matrix.

F. Statistical analysis

In general, for any term $h(x, y)$ that comprises two independent variables x and y each with their own mean values and errors (dx and dy), the propagated error of the overall term (dh) can be written as

$$dh = \sqrt{\left[\left(\frac{\partial h}{\partial x} \right) dx \right]^2 + \left[\left(\frac{\partial h}{\partial y} \right) dy \right]^2}.$$

For a general quantity

$$g = ab$$

the propagated error dg will be as

$$dg = \sqrt{\left[\left(\frac{\partial g}{\partial a} \right) da \right]^2 + \left[\left(\frac{\partial g}{\partial b} \right) db \right]^2},$$

which reduces to

$$dg = \sqrt{(bda)^2 + (adb)^2}.$$

Multiplying and dividing the right-hand side (RHS) by g , we get

$$dg = g \sqrt{\left(\frac{da}{a} \right)^2 + \left(\frac{db}{b} \right)^2}.$$

Similarly, if

$$g' = a/b$$

the propagated error dg will be as

$$dg' = \sqrt{\left(\frac{da}{b} \right)^2 + \left(-\frac{adb}{b^2} \right)^2}.$$

Multiplying and dividing the RHS by g' , we get

$$dg' = g' \sqrt{\left(\frac{da}{a} \right)^2 + \left(\frac{db}{b} \right)^2}.$$

Using this, we calculate the propagated error for each of A/λ , fA , $f\lambda$, $\frac{f\lambda}{V_{\text{rms}}}$, and $\frac{2fA}{V_{\text{rms}}}$ as

$$d(A/\lambda) = (A/\lambda) \sqrt{\left(\frac{dA}{A} \right)^2 + \left(\frac{d\lambda}{\lambda} \right)^2},$$

$$d(fA) = (fA) \sqrt{\left(\frac{df}{f} \right)^2 + \left(\frac{dA}{A} \right)^2},$$

$$d(f\lambda) = (f\lambda) \sqrt{\left(\frac{df}{f} \right)^2 + \left(\frac{d\lambda}{\lambda} \right)^2},$$

$$d\left(\frac{f\lambda}{V_{\text{rms}}}\right) = \left(\frac{f\lambda}{V_{\text{rms}}}\right) \sqrt{\left(\frac{d(f\lambda)}{f\lambda} \right)^2 + \left(\frac{dV_{\text{rms}}}{V_{\text{rms}}} \right)^2},$$

$$d\left(\frac{2fA}{V_{\text{rms}}}\right) = \left(\frac{2fA}{V_{\text{rms}}}\right) \sqrt{\left(\frac{d(fA)}{fA} \right)^2 + \left(\frac{dV_{\text{rms}}}{V_{\text{rms}}} \right)^2}.$$

Experimentally, we define the Bingham number as

$$B = \frac{\sigma_y R^n}{KV_{\text{rms}}^n}.$$

Obtaining a partial derivative for each term in this, we have

$$\frac{\partial B}{\partial \sigma_y} = \frac{R^n}{KV_{\text{rms}}^n},$$

$$\frac{\partial B}{\partial K} = -\frac{\sigma_y R^n}{K^2 V_{\text{rms}}^n},$$

$$\frac{\partial B}{\partial V_{\text{rms}}} = -\frac{\sigma_y n R^n}{KV_{\text{rms}}^{n+1}},$$

$$\frac{\partial B}{\partial n} = \frac{\sigma_y R^n}{KV_{\text{rms}}^n} [\ln R - \ln(V_{\text{rms}})].$$

Using the formula for propagated error, we have

$$dB = \sqrt{\left(\frac{R^n}{KV_{\text{rms}}^n} d\sigma_y \right)^2 + \left(-\frac{\sigma_y R^n}{K^2 V_{\text{rms}}^n} dK \right)^2 + \left(-\frac{\sigma_y n R^n}{KV_{\text{rms}}^{n+1}} dV_{\text{rms}} \right)^2 + \left(\frac{\sigma_y R^n}{KV_{\text{rms}}^n} [\ln R - \ln(V_{\text{rms}})] dn \right)^2}.$$

This can be simplified as

$$dB = \frac{\sigma_y R^n}{KV_{\text{rms}}^n} \sqrt{\left(\frac{d\sigma_y}{\sigma_y} \right)^2 + \left(\frac{dK}{K} \right)^2 + \left(\frac{n}{V_{\text{rms}}} dV_{\text{rms}} \right)^2 + ([\ln R - \ln(V_{\text{rms}})] dn)^2},$$

$$dB = B \sqrt{\left(\frac{d\sigma_y}{\sigma_y} \right)^2 + \left(\frac{dK}{K} \right)^2 + \left(\frac{n}{V_{\text{rms}}} dV_{\text{rms}} \right)^2 + ([\ln R - \ln(V_{\text{rms}})] dn)^2}.$$

For calculating the crossover shear rate, $\dot{\gamma}_c$, we refer to its definition as

$$\dot{\gamma}_c = \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}}.$$

The partial derivatives for all terms on the RHS will be as

$$\begin{aligned} \frac{\partial \dot{\gamma}_c}{\partial \sigma_y} &= \frac{1}{n\sigma_y} \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}}, \\ \frac{\partial \dot{\gamma}_c}{\partial K} &= -\frac{1}{nK} \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}}, \\ \frac{\partial \dot{\gamma}_c}{\partial n} &= -\frac{1}{n^2} \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}} \ln \left(\frac{\sigma_y}{K} \right). \end{aligned}$$

Hence, the propagated error $d\dot{\gamma}_c$ will be as

$$d\dot{\gamma}_c = \sqrt{\left(\frac{1}{n\sigma_y} \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}} \partial \sigma_y \right)^2 + \left(-\frac{1}{nK} \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}} \partial K \right)^2 + \left(-\frac{1}{n^2} \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}} \ln \left(\frac{\sigma_y}{K} \right) \partial n \right)^2},$$

which further reduces to

$$d\dot{\gamma}_c = \left(\frac{\sigma_y}{K} \right)^{\frac{1}{n}} \sqrt{\left(\frac{1}{n\sigma_y} \partial \sigma_y \right)^2 + \left(-\frac{1}{nK} \partial K \right)^2 + \left(-\frac{1}{n^2} \ln \left(\frac{\sigma_y}{K} \right) \partial n \right)^2}$$

Finally, we calculate the T^* as

$$T^* = \frac{\dot{\gamma}_c}{f}$$

wherein, the propagated error is given as

$$dT^* = T^* \cdot \sqrt{\left(\frac{d\dot{\gamma}_c}{\dot{\gamma}_c} \right)^2 + \left(\frac{df}{f} \right)^2}.$$

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T.B. conceptualized the study. S.D. and S.K., with help from A.B.A. and M.S., performed the experiments. M.S. and S.K. analyzed the experimental data. M.S. curated all data. D.H. performed the numerical simulations. T.B. with help from M.S. derived the formulation based on D.H.'s original theory. M.S. prepared the figures. M.S. with help from T.B. wrote the manuscript. T.B., S.D., and S.T. developed the methodology. S.P.K. and S.T. contributed reagents, protocols, and infrastructural support. T.B. secured funding and supervised the project.

The authors declare that they have no competing interests.

DATA AVAILABILITY

The data that support the findings of this article are not publicly available upon publication because it is not technically feasible and/or the cost of preparing, depositing, and hosting the data would be prohibitive within the terms of this research project. The data are available from the authors upon reasonable request.

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