Invited Articles

# Colloidal hydrodynamics of biological cells: A frontier spanning two fields

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One of the ultimate goals of science is an understanding of biological cells so complete that one can construct a living cell from its constituent molecules, control its dynamics, and repair its machinery. Advances in experimental and computational biology techniques over the past 30 years have led to landmark progress toward this goal, from atomistic models of proteins to synthesis of entire bacterial genomes. However, the current frontier in operational mastery of cells arguably resides at the interface between biology and fluid physics: cellular processes that operate over colloidal length scales, where continuum fluid mechanics and Brownian motion underlie whole-cell-scale behavior. It is at the colloidal scale that much of cell machinery operates and where reconstitution and manipulation of cells is most challenging. This operational regime is centered between the two well-understood regimes of structural biology and systems biology, where the former focuses on atomistic-scale spatial resolution with little time evolution and the latter on kinetic models that abstract space away. Low-Reynolds-number colloidal hydrodynamics modeling bridges the divide between these regimes by unifying the disparate length scales and timescales of solvent molecule and colloidal dynamics and may hold the key to operational mastery of cells. Bridging the divide between the two disciplines of biology and fluid physics is as much a part of the way forward as are developing new tools and asking new questions. In this paper we highlight the central and nontrivial roles played by low-Reynolds-number hydrodynamics and colloidal-scale motion that appear in common across cell functions, types, and conditions.

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## I. INTRODUCTION

The next frontier in "biocolloidal" discovery is operational mastery of biological cells. Operational mastery in the broadest sense implies the ability to construct or repair a cell; in the context of scientific inquiry, such mastery is an important complement to understanding, which can be fraught with ambiguity. The litmus test for operational mastery of cells is the ability to construct a living cell from nonliving components: self-assembly across multiple length scales and timescales in a crowded watery compartment. Success can range from combining DNA and other macromolecular assemblies inside a vesicle to starting with only the constituent atoms, followed by spontaneous organization into a living cell. However, nature itself does not reconstitute life from individual molecules; rather, it relies on physical compartmentalization, spatial organization, and transport across multiple length scales to convey genetic information and growth material. The spatial organization that accompanies cell function across all stages from growth to reproduction suggests that physical organization is itself a living process. Thus, the colloidal physics framework

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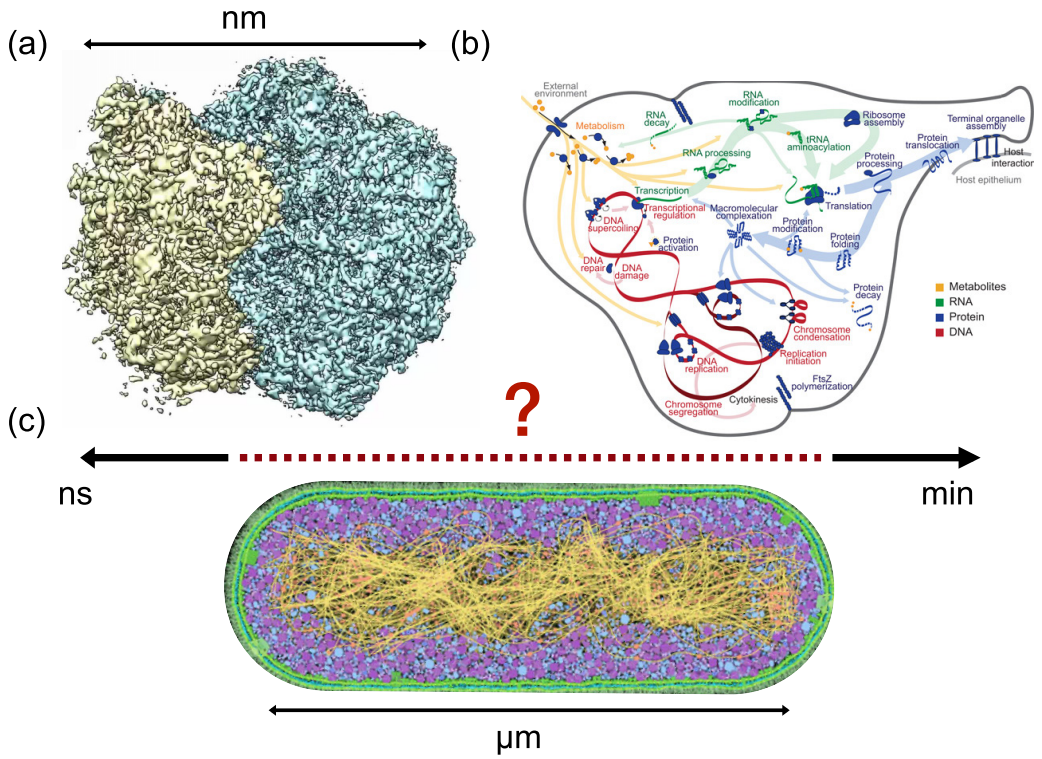


FIG. 1. State-of-the-art modeling of biological cells is successful at two distinct spatial and temporal regimes. (a) Structural biology models represent the dynamics and function of individual nanometer-scale macromolecules at atomistic spatial resolution but typically across nanosecond timescales (a ribosome is shown, from [1], with permission). (b) Systems biology models represent cells using kinetic networks, where biological functions span minutes but with limited or no spatial resolution (figure from [2], with permission). (c) A biocolloidal regime bridges microscopic physical forces of individual molecules and whole-cell biological function across the two regimes, but its biological operating principles remain largely a mystery (an illustration of *E. coli* is shown, adapted from [3], with permission).

provides one promising path to achieving operational mastery of cells and shedding light on the origins of life. Here we highlight the many opportunities for the fluid dynamics and suspension mechanics communities to make an immediate and long-term impact at the frontier of operational mastery of biological cells.

## II. A TALE OF TWO REGIMES

State-of-the-art efforts to understand life at the level of biological cells are successful in the two disparate regimes of structural biology and systems biology (Fig. 1). Decades of work have yielded numerous landmark results focusing on these two well-separated length scales, which fluid mechanicians would recognize as the bookends to colloidal-scale hydrodynamics. At one end of the spectrum, atoms and molecules undergoing reaction and diffusion interact with solvent molecules individually. At the other end of the spectrum, fluid-filled whole cells or fluid-suspended cell populations interact with a continuum aqueous phase.

At the atomistic scale, the realm of structural biology, examples of successful mechanistic mastery include mapping of the structure of DNA and its role in information transfer in living material [4,5]; structure and function of the ribosome, the factory that produces polypeptide chains

that go on to form the majority of cell machinery [6–8]; elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP), the fundamental unit of energy in a cell [9,10]; and the discovery of water and ion channels and how ATP powers their selectivity to maintain cell homeostasis [11,12]. In structural biology experiments, atomistic resolution of macromolecular assembly reveals structure-function relationships by the analysis of ensembles of instantaneous snapshots [13–15]. Corresponding computational models [16–18] partly fill the dynamics gap. For example, molecular dynamics simulations approaches such as LAMMPS [19] and NAMD [20] resolve spatial context to the atomic scale, representing each individual solvent molecule alongside biological molecules to produce exquisite representations of proteins folding [21,22] or binding to other molecules [20,23]. However, steep computational complexity limits temporal evolution to time intervals so short that mechanistic insight is still capped at function and self-assembly of individual macromolecules.

At the opposite extreme, systems biology abstracts away space substantially [24,25] or entirely [26–28]. Examples familiar across many fields include biochemical signaling [29,30], gene expression [31,32], and kinetics-based whole-cell models that encode known information about every gene and molecule within a cell [2,26,33]. For example, the work of Karr *et al.* impressively incorporates what is known about genes and molecules in *Mycoplasma genitalium* into kinetic networks to predict whole-cell scale biology. This work enables prediction of temporal occupancy dynamics of DNA-binding proteins across the entire genome, cell-to-cell variation in cell-cycle duration, and cell phenotypes after single-gene knockouts, i.e., turning off a gene to produce a changed cell state. However, discovery of governing processes outside the known pathways being modeled is limited by such approaches, even with seemingly simple questions about the functional importance of spatial positioning of genes in cells [34]. Thus, despite these impressive beginnings, we are still unable to model disease processes that implicate spatial organization, such as the formation of phase-separated aggregates associated with progressive age-related diseases like Alzheimer's, leaving a major gap in operational mastery of cells. Overall, the strength of the abstracted systems biology approach is that it bypasses staggering complexity, but largely at the cost of understanding the physical context in which biology occurs.

Nevertheless, there are important examples in which systems biology has been instrumental in mapping biology onto physics. This is exemplified in Arkin's work on the lambda lysis-lysogeny model [35]. Lambda phages are viruses that infect bacteria for the purpose of self-replication; once the virus enters, it executes one of two starkly different attacks, but the mechanistic determination of this decision was for many years a mystery. In a leap of concept, Arkin proposed that the decision is stochastic. This modeling of the lysis vs lysogeny decision triggered a shift of systems biology from continuous temporal chemical kinetics processes to discrete reaction events. Arkin leveraged the Gillespie algorithm for stochastic processes [36] to construct one of the first mappings of physics-inspired mathematical modeling to a cellular biological process, promoting the idea that biological processes could be represented in largely physical terms. While there was still no actual physical size or spatial configuration, just considering individual molecules with event-based simulation led to the fundamental discovery of stochastic regulation and decision-making in biological cells.

The key idea is that a simple physical approach opened up a whole new way of thinking about biology that laid the groundwork for two decades of productive research. Indeed, while experiments provoked by the model ultimately demonstrated that the lambda lysis-lysogeny decision has both deterministic and stochastic character [37], stochastic regulation proved to be an important mechanism in other cell functions, producing substantial biological insights into gene expression [38–41], metabolism [42,43], and more [44,45], as well as spurring the engineering of genetic circuits in the field of synthetic biology [46,47]. Stepping back, we see a physics-based mathematical connection between single-molecule behaviors and whole-cell function successfully modeled as a physical process. However, similar to Brown's initial picture of the wiggling motion of pollen grains as caused by a life force [48] and Einstein's attribution of it to a physics-based mechanism, colloidal-scale physics may provide leaps in intracellular mechanistic insight, and perhaps come full circle back to complete Brown's connection to life itself.

## III. COLLOIDAL PHYSICS INSIDE THE CELL

In between the two limits of single-molecule biophysics and whole-cell kinetics resides colloidal-scale biophysics, a biocolloidal regime (a "middle world" [49]) emerging from ever-growing evidence that microscopic forces and processes coordinate and regulate whole-cell functions. The vast separation in length scales and timescales between the two limits has historically hindered formation of mechanistic connections between atomistic-scale processes and whole-cell functions. However, this separation simultaneously makes colloidal hydrodynamics an ideal bridge to connect them, where a hybridization of biology, biological engineering, fluid mechanics, and suspension dynamics is paving the way forward toward full operational mastery of biological cells. Indeed, a robust body of experimental literature demonstrates that colloidal-scale physics orchestrate intracellular functions. A few examples of such orchestration are examined below, with a view toward familiar concepts in colloidal hydrodynamics.

### A. Active motion induces flow that regulates biological function in cells

It is well known that macromolecular motors drive life-essential intracellular processes, including ATP synthetase, a rotary motor that converts mechanical work (using a proton gradient) into chemical energy (ATP) and, in the process, produces water.[1] Another two examples are RNA polymerase, a torsional motor that transcribes DNA to RNA by mechanical ratcheting, and the ribosome, considered a molecular motor owing to its GTP-powered ratcheting of an mRNA chain as it constructs proteins, the building blocks of life. While these active motions take place at the molecular scale, more recent work has shown that these motions can propagate via solvent-mediated interactions to affect whole-cell function. For example, stress fluctuations arising from active motion can be stronger than the thermal energy $kT$ of Brownian motion, where $k$ is Boltzmann's constant and $T$ is the absolute temperature. It has been proposed that these fluctuations can increase the overall "temperature" inside a cell [50].

This leads naturally to the question of whether active motion inside cells serves to regulate other nonactive cellular processes. The word "active" takes on several meanings in the soft matter and fluid dynamics fields, contemporaneously applied to describe the motion of self-propelled whole microorganisms or to denote the motion of a probe driven by an external force of strength greater than $kT$ in active (nonlinear) microrheology [51]. Here we use the term to describe the motion of macromolecular motors, converting chemical energy (e.g., ATP or GTP) into mechanical motion, which can produce flow over length scales ranging from molecular to cellular. The impact of active motion can be quantified via the Péclet number Pe, which gives the strength of advective flow relative to the strength of diffusion, and thus sets the deterministic versus stochastic character of particle motion. A motor of size $a$ moving at a characteristic speed $U$ (which can be quite transient) can set fluid into motion and entrain passive macromolecules with characteristic diffusivity $D$, giving $\mathrm{Pe} = Ua/D$. Studies of motor-induced advective flow in large (centimeter-scale) cells convincingly argue that large values of Pe enhance transport speeds of biomolecules involved in metabolism and possibly homeostasis [52,53] and can lead to cargo localization [54]. Central to these ideas is a predicted value of the Péclet number ranging from $\mathrm{Pe} \sim 10$ to $\mathrm{Pe} \sim 1000$, depending on the size of the passive macromolecules entrained in the flow [52,53]. However, a closer look at the effects of crowding on the diffusion coefficient could significantly increase this value. In addition, many studies utilize the characteristic size of the overall cell $R$, instead of the motor size $a$, which can produce orders of magnitude change in the Péclet number. Related theoretical models advanced to explain such cytoplasmic streaming [52,53] often bypass physically representing the finite size of macromolecules or biocolloidal particles, instead abstracting the cytoplasm as a continuum fluid.

[1]While it is separately known that aquaporins pump out excess water from the cell, thus far there is no connection between these two processes.

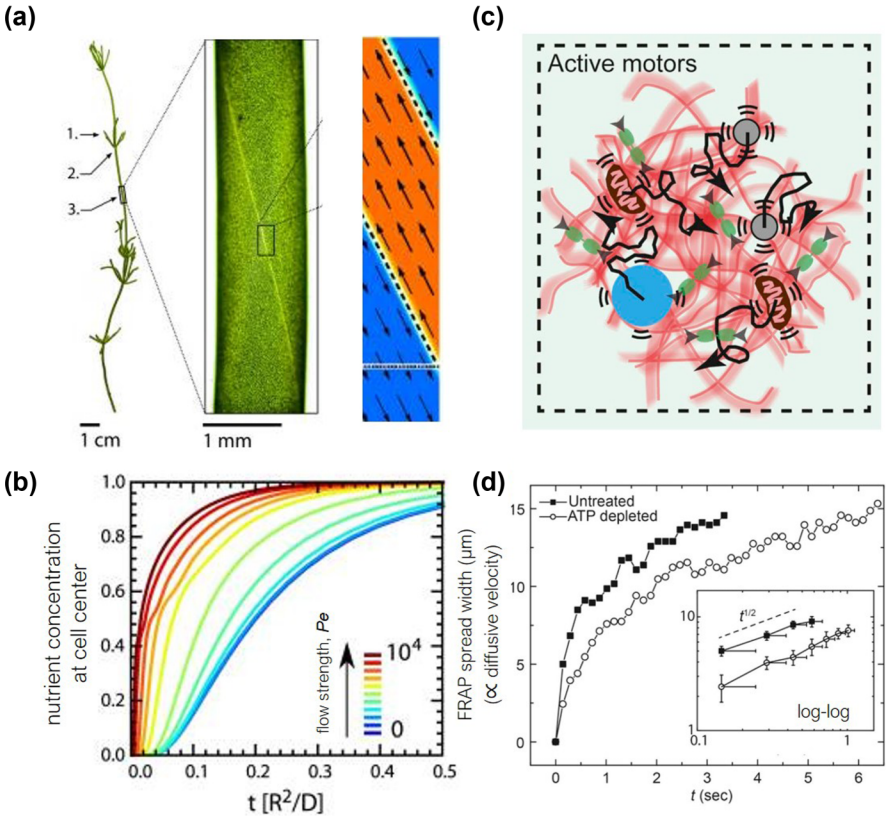


FIG. 2. Active motion regulates transport in cells. (a) In cells of the plant *Chara corallina*, an active motor network climbs a chiral network of actin filaments generating flow that (b) enhances the rate of nutrient uptake to the center of the cell ($R$ is the radius of the cylindrical cell and $D$ is the diffusion coefficient of nutrients). (c) In smaller animal cells, structured networks cause randomly directed motor protein motion; thus, active motors lead to fluctuating disturbance flows in the cytoplasm that are stronger than thermal fluctuations. These flows (d) enhance movement of surrounding macromolecules in the cytoplasmic milieu. This is illustrated by measuring diffusive speed (via FRAP) of a fluorescent protein Dendra2 in melanoma cells. Diffusion is faster in fed cells (in the presence of ATP) [figures (a) and (b) are from [52] (Copyright (2008) National Academy of Sciences U.S.A.) and (c) and (d) are from [56], with permission].

Fluid recirculation is recovered in such models, but because there are no actual particles represented, one cannot study crowding effects, association times, diffusion, effects of colloidal attractions or repulsions on phase behavior and self-assembly, or hydrodynamic interactions. Being able to model the spatial resolution of biocolloidal particles and their interactions would vastly extend the utility of such models in suggesting new hypotheses for the role of physical interactions in cell functions.

While the physics underlying these observations, i.e., the convolution of active motion of individual molecules and cell-spanning flow, are conserved across many length scales and cell types, the motion-flow coupling can differ substantially across cell types. For example, in plants, cytoplasmic structure constrains protein motion that leads to chiral flow [55] [Fig. 2(a)], and this coherent motion dramatically speeds up nutrient uptake [Fig. 2(b)]. In contrast, in many animal or bacterial cells, motor-protein motion is randomly directed, producing stronger-than-thermal fluctuating forces [Fig. 2(c)]. Brangwynne *et al.* showed the latter convincingly in Chinese hamster ovary cells (a model animal cell) by demonstrating that fluctuations caused by active motion shift the undulations of microtubules from subdiffusive to diffusive [57,58]. Such a transition may increase

the rates of biochemical reactions across the cell, in compelling support of the idea of physical regulation of whole-cell functions. The work of Guo *et al.* further supports this idea, focusing on the diffusion of a fluorescent probe protein Dendra2, of nominal size 4 nm [Fig. 2(d)]. Over a period of a few seconds, its diffusivity doubles when in the presence of ATP-activated motor proteins. Interestingly, they also showed that GFP, a fluorescent protein with nearly the same size, does not exhibit this behavior (but was only monitored for fractions of a second) [56]. The spatial context of these observations is virtually unexamined, e.g., the effects of local structure or confinement, timescales of measurement, and effects of surface charge on hydrodynamic entrainment, to name a few, offering fertile ground for hydrodynamicists to explore.

These connections of physics to biology are preserved broadly across cell types: Even in prokaryotes, directed motion is ubiquitous, for example, ribosomes translocating along mRNA or RNA polymerase translocating along DNA, but the impact of directed motion on the dynamics of the surrounding milieu is virtually unknown [59]. A decisive demonstration that active physical processes regulate cell function at the whole-cell scale is thus a green field, whether via increased cytoplasmic fluctuations, altering the rheology of the cytoplasm, or hydrodynamic recruitment of macromolecules.

Modeling has begun to provide some insight into the effect of active motion on subcellular processes however. As an example, in an impressive combination of hydrodynamics and polymer physics, Saintillan *et al.* modeled chromatin (the DNA-protein bundle packed into the nucleus of eukaryotic cells) as long flexible Brownian polymers and showed that active extensile forces produce fluid motion that leads to large-scale organized dynamics. Qualitative agreement with experimental measurements supported the idea that this physics-based hydrodynamic phenomenon can regulate gene expression [60]. Similarly, Shelley and co-workers elucidated the fluid dynamics and forces involved in the positioning of the mitotic spindle during cell division of eukaryotes [61–64]. From those observations one can infer that these flows exert influence on other whole-cell processes.

### B. A colloidal glass transition in cells is triggered by transitions in biological state

Growing evidence suggests that the cytoplasm of cells can undergo a liquid-to-glass phase transition when exposed to conditions not ideal for growth, but the underlying physical mechanisms, as well as biological implications, for such a transition remain largely unknown. Recent studies report such transitions as being colloidal in nature. For example, Jacobs-Wagner and co-workers starved *Caulobacter crescentus* cells, thus minimizing their metabolic activity, and observed that cells enter a reversible glasslike state. This was done by introducing a native protein, crescentin (a colloidal probe), as well as $\mu$NS (a foreign probe) into a cell and tracking their motion over time. Increasing probe size slowed probe diffusion and, at sufficiently large size, probe dynamics became non-Gaussian, exhibiting caging effects emblematic of glassy behavior [59] (Fig. 3).[2] The simplest explanation for this result is simple steric entrapment in a viscoelastic network that may stiffen under starvation conditions. This is a very different "transition" from the glass transition of particles suspended in a purely viscous suspending medium, where particle-particle interactions alone thwart a well-defined liquid-to-crystal transition [65]. This latter type of transition mechanism is perhaps the most interesting due to its connection to fundamental colloidal physics [66], and the authors attempt to connect to it. The authors of the *C. crescentus* study hypothesize that the largest particles become trapped in a cage of nearest neighbors, rather than in a network. They assert that this proposed mechanism of particle caging is justified in bacteria, which lack the extensive cytoskeletal

[2]The authors identify glassy dynamics only in starved cells. However, particles in fed cells also show a dynamics plateau, prompting a more nuanced reading: Data show possible confinement at the cellular length scale for fed conditions and confinement at the particle scale for starved conditions, illustrating an opportunity for contribution from a colloid science perspective.

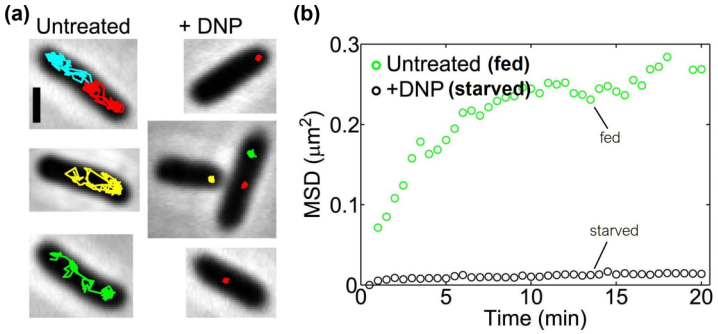


FIG. 3. Glass transitions can control dynamics in cells. The mobility of plasmid DNA (a circular DNA of colloidal size ∼150 nm [59]) decreases significantly in starved *E. coli* due to a glasslike transition. (a) Two-dimensional trajectories of fluorescently labeled plasmids show evidence of cage trapping, hypothesized to result from crowding rather than network trapping. (b) Mean-square displacement from (a), plotted versus lag time, for fed and starved conditions (figure is from [59], with permission).

network found in eukaryotic cells. However, one glaring fact about glassy behavior in bacteria stands out above all others, from a colloidal hydrodynamics perspective: The volume fraction of the cytoplasm is typically reported to be in the range $0.2 \leqslant \phi \leqslant 0.4$, whereas in nonliving dispersions, the glass transition occurs in the range $0.57 \lesssim \phi \lesssim 0.59$, depending on the size polydispersity of the sample [66–69]. A number of factors could contribute to this apparent discrepancy (setting aside effects such as network trapping or measurement timescale). The authors propose that the large discrepancy in volume fraction where glassy dynamics occur could arise due to physical effects that change the effective particle size and thus volume fraction. Proposed factors include "bound water" or electrostatic repulsion hypothesized to increase effective particle size [59]. In addition, they propose that attractive forces that slow dynamics could play a role, as well as confinement effects. This is where computational modeling using biologically relevant size polydispersity, confinement, and hydrodynamic and nonhydrodynamic particle interactions can make fundamental contributions [70].

Glassy survival strategies are not confined to prokaryotes; indeed, similar starvation-induced glass transitions have been observed in budding yeast [71,72], associated with changes in cytosolic *p*H, where the glass transition was demonstrated to be essential for survival. The impact to survival is thought to result from both a reduction in energy consumption and an increase in protective mechanical stiffness [72]. While the evidence for glassy dynamics is convincing, how *p*H mechanistically changes physical conditions to trigger a glass transition has not been explored. Colloid science can contribute directly to such inquiry, for example, by noting that changes in *p*H can increase crowding by increasing effective particle size (via an electrostatic double layer) or reducing the volume of the overall cell (via osmotic-pressure-induced water exchange). Colloid science can also help by enabling detailed particle monitoring before, during, and after a glass transition [73]. More fundamentally, glassy dynamics change markedly with time, a hallmark of nonergodic behavior that will affect interpretation of results. Disentangling glassy aging from cell growth will in itself be fascinating work.

Such condition-dependent physical changes at the whole-cell scale demonstrate an essential role for colloidal-scale regulation in cell survival. To wit, aberrant liquid-glass transitions may play a crucial role in disease; for example, increased cytoskeletal fluidity in cancer cells is correlated with enhanced cellular motility and invasion [74]. This pervasive coupling of colloidal and biological function spurs entirely new questions to which the colloidal hydrodynamics community is uniquely poised to help answer. Colloidal glassy dynamics are evidently of critical importance in the operational mastery of cell quiescence (deeply related to aging) [75] or dormancy (key to bacterial survival) [76], and dysregulation of these states may play a role in hyperproliferation (driving

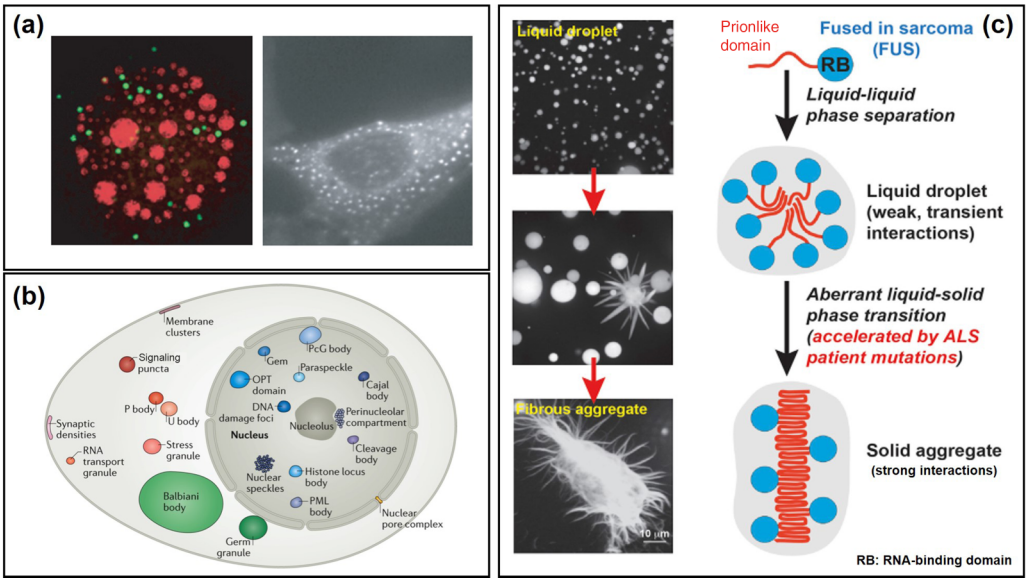


FIG. 4. Phase-separated biomolecular condensates are essential to healthy cell function. (a) Images of condensates in cells. On the left are nucleoli (red) and histone locus bodies (green) in the nucleus of an *X. laevis* oocyte, and on the right are purinosomes in a HeLa cell (from [92], with permission). (b) Diagram of known condensates in eukaryotic cells (from [89], with permission). (c) The ALS-associated protein FUS forms phase-separated condensates that convert to solid aggregates faster when the protein contains ALS patient-derived mutations (figure is from [91], with permission).

cancerous growth). Such states have been phenotypically observed (i.e., analogous to description via constitutive equations) across a wide range of cells, but have not been explained from *physical* first principles (i.e., conservation laws).

In summary, while experiments [66,69,77], theory [78,79], and dynamic simulation [73,80,81] have established extensive understanding of the colloidal glass transition in nonliving systems, detailed connection to biological parameters via modeling is nascent. The most obviously implicated physical factors are macromolecular crowding and size polydispersity; however, biologically relevant size polydispersity has been reported to be about $s = 0.52$ [82], which would shift the glass transition even higher and further away from the putative lowest value $\phi \approx 0.56$ in monodisperse hard-sphere systems, not closer to the reported maximum crowding in *E. coli*, $\phi \approx 0.40$. However, as noted above, other physical mechanisms such as changes in electrostatic potential [83] may drive the apparent volume fraction at the glass transition point lower, such as through changes in thermodynamic size or melting induced by colloidal-scale particle attractions [84–86].

### C. Phase-separated biomolecular condensates spatially control biological function

Phase separations in cells are an essential mechanism for intracellular regulation that enables control of biological functions by spatially concentrating some molecules and excluding others [Figs. 4(a) and 4(b)]. For example, the phase-separated nucleolus concentrates ribosome precursor molecules, facilitating localized ribosomal assembly within the nucleus in eukaryotes [87]. Without ribosomes (the factories that build peptides and proteins), construction of all matter in cells comes to a halt. Other biomolecular condensates play an important role in dynamic or transient environments where rapid local responses are needed [88,89]. For example, stress granules (phase-separated assemblies of proteins and translationally stalled mRNA) act as transient signaling hubs for enabling a quick response to unfavorable environmental conditions that would otherwise cause

cell death [90]. Breakdowns in phase separation also cause disease. For instance, dysregulation of phase-separated condensates is implicated in neurodegenerative diseases and aging, including Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS). In ALS, stable liquid compartments formed by the ALS-associated protein FUS in healthy cells are thought to undergo an accelerated aberrant liquid-solid phase transition in which liquid compartments become solid fibrous aggregates [91] [Fig. 4(c)]. Such biological phenomena are closely connected to arrested phase separation (e.g., the formation of networked gels) rather than the conventional phase separation between molecular liquids (i.e., liquids separated by a single interface).

The physical mechanisms underlying the formation and properties of arrested phase-separated colloidal condensates in cells have been successfully modeled using polymer theory as recently reviewed by Brangwynne *et al.* [92]. For example, Dignon *et al.* [93,94] investigated protein phase separation by first modeling protein (chain) dynamics in LAMMPS [19] and then phase separation in HOOMD [95]. The proteins were coarse grained as a chain of particles with biologically relevant bonding and interaction potentials. They successfully recovered temperature-dependent phase separation, for numerous protein types, which were qualitatively consistent with experimental results and Flory-Huggins theory for polymers [96]. Others have modeled proteins as colloids rather than polymers: Nguemaha and Zhou examined the role of RNA and protein attraction on liquid-liquid phase separation [97] by representing proteins and RNA as hard spheres with patchy surface attractions and modeling thermodynamically favorable configurations of mixtures using Monte Carlo simulations. Not surprisingly, they found that phase separation is acutely sensitive to the strength of attractive forces between proteins and RNA: Weak forces promote phase separation, medium forces suppress phase separation, and strong forces promote phase separation at high protein to RNA ratios while suppressing phase separation at low protein to RNA ratios. Overall, an explosion of interest in and study of phase separation in cells has produced impressive understanding of the biological role of changes in condensate structure, e.g., increasing reaction specificity, inhibiting reactions, and buffering, among others [89]. However, phase separation can also arrest and lead to formation of a networked gel, behavior well studied by soft-matter scientists. It would be interesting to identify whether such arrested phase separation also occurs in cells.

Beyond the localized processes a phase-separated domain controls, could the presence of condensates impact whole-cell function? Condensates can be expected to modify the flow and available volume for the remaining cytoplasm, potentially propagating long-range hydrodynamic or entropic effects that influence physical organization and dynamics, and thus biological behavior across the cell. Such cell-scale effects would be modulated at the colloidal level via, e.g., well-known protein-specific electrostatic profiles. Investigating such changes in whole-cell physics may suggest new therapeutic strategies for phase-separation dysregulation.

In summary, models aimed at investigating biological problems driven by colloidal-scale physics are emergent, and they highlight gaps in biological and physical modeling of particle interactions that are emblematic of broader opportunities to improve mechanistic understanding. Specifically, we see a need for direct connections of phenomena such as active motion, glass transitions, and phase separation to whole-cell functions like enhanced diffusion to support growth, dormancy for survival, and compartmentalization for cell propagation. One common feature of these knowledge gaps is relating microscopic forces as inputs to biological outputs at the whole-cell scale. We believe one solution can be found in the tools of suspension mechanics and low-Reynolds-number hydrodynamics, which complement existing methods in a way that permit their upscale to whole-cell physics.

## IV. FLUID MECHANICS: AN EXPANDING ROLE IN MODELING THE CELL

Expanding capabilities for modeling the fluid mechanics within cells offer a powerful approach for connecting microscopic physical processes to whole-cell biological functions. Historically, biochemical reactions and diffusion have been viewed as the primary drivers of cell function and thus are the focus of traditional models of cell processes. Numerous modeling methods have

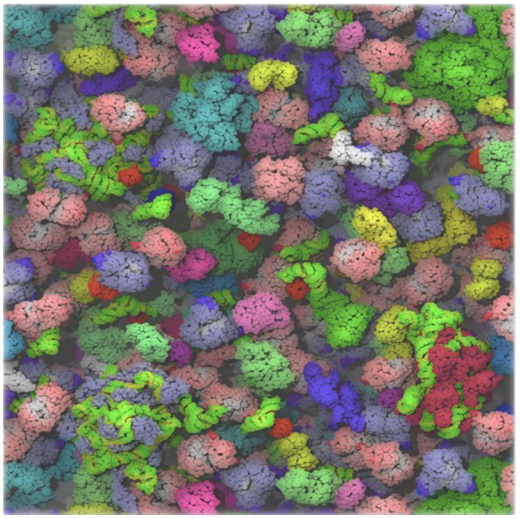

FIG. 5. The bacterial cytoplasm can be modeled via Brownian dynamics. Shown is an instantaneous snapshot of a Brownian dynamics simulation of 1008 macromolecules from McGuffee and Elcock. The 50 most abundant macromolecules in the *E. coli* cytoplasm are represented with experimentally determined shapes and relative abundances (figure is from [103], with permission).

addressed the connection between diffusion and reaction in cells, but abstraction of spatial context (viz., size and organization) results in extensive approximations that undermine their predictive value or value as discovery tools. For example, Bartol *et al.* [98] successfully reconstituted transient signals in neurons using the Monte Carlo tool MCell [99], kinetically modeling the reaction and diffusion of molecules represented as "ghost" particles, i.e., macromolecules without size, and with coarse-grained trajectories to manage computational cost. Doing so required insertion of an approximate diffusion rate and up to a dozen fitting parameters to obtain agreement between simulation and experiment. In an improvement to that approach, Khan *et al.* [100] resolved trajectories of point-particles using the computational tool Smoldyn [101] in order to model residence times of proteins (i.e., how long the protein remains in the correct position and orientation relative to a partner reaction molecule). Such binding dynamics are essential for signaling pathways in neurons. The study addressed a question that resides precisely at the regime of colloidal-scale modeling in cells: whether residence time is determined by binding affinity (durability of bonds competing with diffusion) or by the number of binding sites (distribution and quantity of attraction patches). Surprisingly, the study found that the distribution of binding sites exerted no influence on binding dynamics. However, the authors noted that this would likely change if the physical size of particles were modeled. Numerous other approaches are summarized in a review by Schöneberg *et al.* [102]. From a colloidal hydrodynamics perspective, incorporating particle size could improve diffusion-reaction models so that translational and rotational diffusion that affects number, orientation, and duration of particle encounters can be modeled in detail.

The emergent recognition that, beyond chemical reactions and diffusion, colloidal-scale physical processes orchestrate cell function is driving development of entirely new approaches for modeling cells. A familiar example of modeling Brownian motion in cells is the pioneering work by McGuffee and Elcock [103], who modeled a volume of cytoplasmic fluid crowded with macromolecules of finite size in a Newtonian solvent utilizing the Brownian dynamics algorithm originally developed by Ermak and McCammon (Fig. 5) [104]. In their mesoscale approach, particle positions are evolved from a displacement equation (the overdamped Langevin equation) and the solvent is implicitly modeled, dramatically reducing computational burden relative to molecular dynamics

(MD) approaches that model individual solvent molecules and inertia. A Lennard-Jones potential between the molecules provided detailed particle shape. Their landmark study was one of the first to demonstrate the power of Brownian dynamics in modeling the whole macromolecular composition of the cell interior: Processes that occur over timescales and length scales long compared to solvent motion then became computationally accessible. In this particular study, implementation and *ad hoc* adjustment of an attractive potential was required to recover experimentally measured diffusion rates for a model particle, green fluorescent protein (GFP), pointing to the key tradeoff made when using Brownian dynamics and other mesoscale models: The gain in computational speed and system size comes at the expense of the detailed molecular-scale interactions[3] available in MD simulations utilized extensively at the single-molecule scale in structural biology [106]. The Brownian dynamics algorithm was recently expanded by Sunol and Zia to implement confinement of a suspension by a spherical cavity as a simple model for a cell wall. This work further demonstrated the importance of physical resolution: Spherical confinement induces pronounced changes in osmotic pressure and viscosity, illustrating the key role that confinement and cell size may have on the liquid-glass transition in cells [107]. Overall, these studies demonstrate that Brownian forces and excluded volume can elucidate key cell functions and have potential to reveal insight into whole-cell phenomena. However, microscopic phenomena such as flow are also critical to whole-cell-scale function, as evidenced by the phenomenon of cytoplasmic streaming in cells first reported and characterized by Goldstein *et al.* [52,53].

Indeed, the past decade has seen a rapid expansion of modeling tools that combine biology with colloid and polymer science, incorporating microscopic forces that directly impact biological behavior. This union of biology and polymer science has resulted in identification of a slew of biological processes that can now be equivalently viewed as colloidal-scale physical processes. For example, the fluid dynamics community has shown that hydrodynamic or solvent-mediated interactions, which are neglected in the Brownian dynamics algorithm, are important in a number of intracellular processes. Grid-based methods, such as those implemented by Shelley and co-workers, have been used to study microtubule assemblies and have shown that hydrodynamic interactions in and membrane confinement of cells play a crucial role in the positioning of the mitotic spindle via different force transduction mechanisms that produce distinct intracellular flow profiles (Fig. 6) [61–64]. One of the most important contributions of this work is the claim that the flow structure can be a signature of a specific biological process: The authors showed that three different force transduction mechanisms for spindle positioning could all produce correct mitotic orientation and position, but each leads to a distinct flow profile. We propose the opportunity to systematically search for changes in whole-cell biological function that arise under the three distinct flows as a means to uncover mechanistic underpinnings, i.e., the microscopic physical processes that regulate whole-cell function. Using a similar approach, Saintillan *et al.* modeled chromatin (the DNA-protein bundle packed inside the nucleus of eukaryotes) as a long flexible Brownian polymer and showed that active extensions and resulting fluid disturbances lead to coherent motion of interphase chromatin (Fig. 7) [60]. Interrogation of the underlying polymeric forces and interactions have been shown to significantly affect these and other intracellular processes. For example, Spakowitz and co-workers have successfully integrated polymeric forces into their models to demonstrate the dynamic coupling between chromosomal loci [108], diffusion of DNA-binding proteins to target sites [109], and chromatin segregation due to epigenetic modifications [110], all of which are essential mechanisms of regulation in cells. Indeed, aberrant epigenetic modifications of DNA and consequent changes in DNA binding and expression may underlie a range of complex long-term

[3]These molecular-scale interaction potentials can be acquired via meticulous matching to experiment or companion MD simulations. The dissipative particle dynamics algorithm [105] straddles the two regimes of Brownian dynamics and molecular dynamics, modeling solvent particles using a frictional damping factor combined with a stochastic pairwise force, but its requirement for multiple adjustable parameters restricts its viability as a first-principles exploration tool.

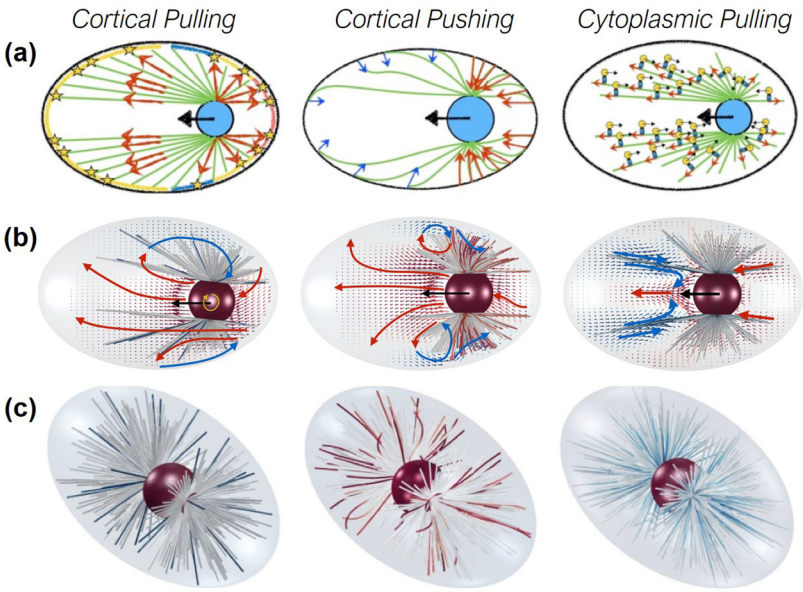


FIG. 6. Hydrodynamic flow plays an essential role in the positioning of the mitotic spindle. (a) Dynamic simulations of mitotic spindle positioning using three different putative force transduction mechanisms. (b) Each mechanism leads to distinct cytoplasmic flows within the model cell. (c) However, all three flows result in the same final positioning of the mitotic spindle, suggesting that these flows can be used to differentiate between underlying mechanisms. The simulations include hydrodynamic interactions but not Brownian motion (figure is from [64], with permission).

diseases including diabetes, hypertension, and coronary heart disease [111]. Overall, these studies demonstrate that key biological processes can be represented to a great extent via colloidal-scale physical forces that propagate to the whole-cell and even whole-organism scale.

However, an even bigger leap now seems plausible: that certain genes are responsible for purely physical processes as a means to provide and preserve essential aspects of life across a diversity of cell types. To interrogate this idea, we need to expand the toolkit for connecting physical forces to biological functions. For instance, polymer physics models often neglect many-body hydrodynamic effects while grid-based fluid mechanics methods have difficulty in modeling and

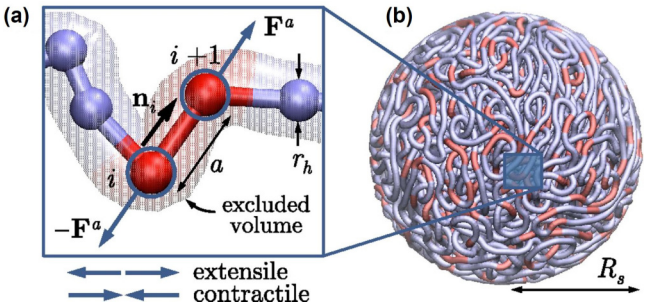


FIG. 7. (a) Active extensile forces within the nucleus can induce fluid disturbances that lead to (b) coherent motion of chromatin, represented here as a confined flexible chain. This work shows that long-ranged hydrodynamic coupling can lead to biologically relevant self-organization critical to gene expression (figure is from [60], with permission).

simulating Brownian motion and crowding. These shortcomings can be resolved by more extensive implementation of the concepts of colloidal suspension mechanics into models of subcellular problems in the biology and biophysics literature.

Expansions of capabilities already in progress include modeling membrane-confined suspensions with patchy attractions common on proteins in biology (where such attractions are well studied in nonliving colloidal self-assembly), active motion relevant to molecular motors (with similar motion well known in the low-Reynolds-number swimmer literature), and lubrication forces and many-body hydrodynamic interactions between macromolecules and the surrounding membrane, extensively studied in the suspension mechanics community but only recently appearing in models of the intracellular milieu [112,113]. These more detailed modeling methods can enhance the study of many biological processes including mitotic spindle positioning before cell division, where hydrodynamic interactions between the mitotic spindle and the background milieu may coordinate other biological processes relevant to the critical positioning of the mitotic spindle [64].

Integrated modeling of Brownian motion, crowding, size polydispersity, and fully coupled many-body hydrodynamic interactions within confining spherical enclosures has also recently become possible using the Confined Stokesian dynamics algorithm developed by Zia and co-workers [70,112–114]. The Confined Stokesian dynamics algorithm is a recent expansion of the Stokesian dynamics (SD) framework [115–120], which has long been recognized as the gold standard for modeling Brownian suspensions.[4] In SD, the suspending solvent itself is a Newtonian continuum that is implicitly modeled, vastly improving computational efficiency compared with molecular dynamics simulations that resolve each individual solvent molecule. Modeling the solvent implicitly frees computational power for representing macromolecules and other colloids. The regime of validity for modeling the suspending cytosol as a continuum fluid extends to particles that are at least five times the size of water molecules. Overall, this approach, where individual particles are suspended in a continuum fluid, makes possible the detailed representation of colloidal-scale interactions spanning contact to cell-length separation. In contrast to models that coarse grain the cytoplasm (fluid plus particles) as a fluid continuum (abstracting away individual particles), the confined Stokesian dynamics approach offers the advantage of direct representation of macromolecular crowding, measurement of particle dynamics such as self-diffusion and cooperative towing, detailed measurement of interaction times, and modeling of patchy attractions and repulsions that accurately represent biological conditions, among others.

Our recent expansion of SD to Confined SD incorporated a confining membrane [70,112–114]. This work demonstrated that many-body hydrodynamics must be included to accurately model colloidal motion in a model cell, viz., correct measurement of osmotic pressure, short- and long-time self-diffusion, viscosity, and cooperative motion. No fitting parameters are required; results emerge from solving conservation equations. The Confined Stokesian dynamics algorithm also demonstrated that interplay between particle-particle and particle-cavity interactions produces long-range cooperative motion, entrainment, and dynamical heterogeneity [113,114] that enable prediction of experimentally observed particle migration in, e.g., *Caenorhabditis elegans* early embryos during nuclear migration [122]. More generally, the work showed that a moving particle at the center of a cell drives nearby particles in the same direction but drives particles closer to the cell membrane in the opposite direction (Fig. 8) and provided a detailed position-dependent model for the transition between movement in the two directions. In more recent studies of polydisperse confined suspensions [70], entropic exclusion between a confining membrane and enclosed particles was shown to produce microstructural layering of particles that propagates inward from the cell membrane. This structure couples with hydrodynamics to produce dynamical heterogeneity, including regions of fast and slow dynamics (Fig. 9). This physical behavior may be implicated in

[4]Numerous other approaches for modeling particulate flows exist beyond those discussed in Sec. IV (each with its own strengths and weaknesses), summarized in Ref. [121]. Of these, Stokesian dynamics is recognized for efficiently [120] simulating Brownian suspensions without sacrificing accuracy.

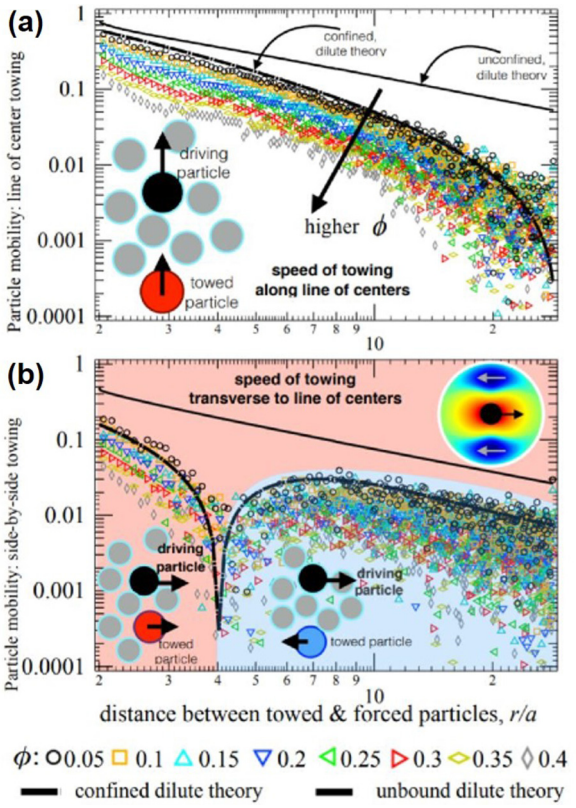


FIG. 8. Long-range hydrodynamic towing may regulate whole-cell physical dynamics. (a) If a particle (black) in a confined suspension is driven in a direction, any other particle in line (red) will be towed in the same direction. The strength of this towing is dependent on proximity between particles as well as volume fraction and confinement of the suspension. (b) Movement of particles that are not in line causes towing that changes direction depending on proximity to the wall: Particles near the center are towed along, but those near the wall are towed backward (figure is from [114], with permission).

biologically observed processes such as the colocalization of translation molecules and ribosomes near the cell membrane in *E. coli* during translation elongation [70,123]. The Confined Stokesian dynamics algorithm thus holds great promise to help unravel new physical mechanisms for cell regulation.

While such comprehensive tools are gaining traction, perhaps more ripe for development is cross-pollination of fluid mechanics tool developers, biologists, and biological modelers in thinking about how colloidal physics processes can alter cell function and behavior. Moving beyond the study of individual subcellular processes to the many remaining questions regarding whole-cell function would clearly benefit from integration of colloidal-scale physics into cell models.

## V. A UNIFIED BIOCOLLOIDAL FRONTIER

In this paper we have highlighted landmark achievements in the understanding and modeling of biological cells. The two disparate regimes of structural and systems biology have decoded how biology operates at the atomic and cellular scales, respectively, including how genetic information is stored in cells, how ribosomes chemically synthesize proteins, and how cells behave phenotypically across a wide range of healthy and diseased states. Spanning the gap

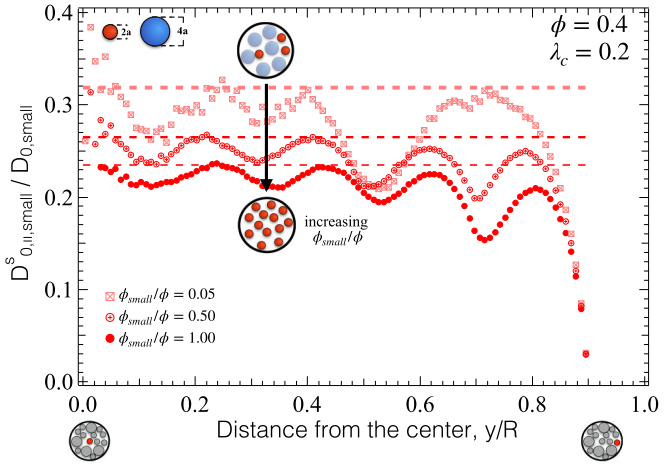


FIG. 9. Short-time self-diffusion of small particles ($D^s_{0,\text{small}}$) in spherically confined suspensions changes with different volume compositions of small and large particles and with distance from the center of confinement. The undulations are a consequence of structural heterogeneity and hydrodynamic interactions, viz., vanishing mobility at the wall [113]. The diffusivity of small particles is increasingly diminished in the direction parallel to the wall in suspensions with greater relative volume fraction of small particles. The horizontal lines are to guide the eye (figure is adapted from [70], with permission).

between these two regimes is a biocolloidal frontier, from which we suggest three primary takeaways.

(i) In Sec. III, study of the colloidal regime inside cells showed that microscale physics regulate whole-cell biological functions through active motion that speeds growth, glass transitions that trigger dormancy, and phase separations that regulate molecular interactions and store and manage genetic instructions. Overall, it revealed that there are whole-cell biological functions that need physics to explain them. However, whole-cell models and experiments need better representation of colloid physics, including new experimental approaches optimized for *in situ* interrogation of colloidal dynamics and microstructure.

(ii) In Sec. IV, study of subcellular processes revealed that colloidal-scale physics can represent their biological functions, viz., hydrodynamic flow and polymer dynamics that reposition the mitotic spindle during cell division and organize DNA to modify gene expression. These studies suggest that microscopic physics may even regulate subcellular biological functions. However, models in these studies need more biologically faithful representation of microscopic physical forces to connect to the physics of whole-cell function.

(iii) Overall, there is a disconnect between questions being revealed by colloidal-scale physics models focused on subcellular processes and questions that need to be asked to understand whole cell biological functions. Both modeling and questions hold a wealth of opportunity for contribution by the hydrodynamics community.

One might think of these opportunities as known unknowns. However, an even bigger frontier lies in systematically elucidating broad patterns in physical behavior that coordinate whole-cell biological functions, which may trigger questions we had not thought to ask before, such as why compartmentalization rather than reconstitution is the choice made by biology or whether genetic functions that are essential for life encode physical functions not yet known. Such expanded inquiry necessitates entirely new tools. One might view these opportunities as unknown unknowns.

### A. Known unknowns

Whole-cell models and experiments need better representation of colloidal physics, as well as new experimental approaches optimized for nondestructive observation of colloidal dynamics and microstructure. In Sec. III we reviewed three prominent examples of colloidal-scale physics that have been connected phenomenologically to whole-cell functions and pointed out that systematic links between first-principles physics (including physical laws such as the conservation of mass, momentum, and energy) and first-principles biology (including the central dogma, natural selection, and homeostasis) are nascent. The models incorporate substantial biological detail but often lack rigorous physical modeling needed to explain first-principles biology from first-principles physics. A plethora of opportunities thus exist. For example, future work in modeling active motion should include the self-motion of individual motor proteins localized to intracellular filaments via fluctuating bonds. Studies of the intracellular glass transition can benefit from recent advances in colloidal modeling including methods for executing jumps in volume fraction [73], tuning attractive forces [124], and modeling confinement [112,113,125,126] of suspensions of polydisperse size distribution. Finally, modeling of colloidal phase separation in cells must move beyond well-established methods of studying colloidal phase diagrams with constant size polydispersity, particle concentration, and interactive forces [127–130] in order to capture the nonequilibrium dynamics in cells; current efforts include methods for modulating interparticle colloidal forces with temperature fluctuations and temporal dependence [124] as well as methods for studying the transient formation of gels due to varying interparticle attractions and repulsions [131–135]. Altogether, the combined detailed modeling of active motion, glassy dynamics, and phase separations in cells may reveal deeper connections between the transition of uncompartmentalized abiotic molecules into life [136] and is essential for cell-scale operational mastery. Alongside such refinements in modeling, new experimental inputs will also be required. A colloidal hydrodynamicist might wish for detailed measurement of the spatial locations of every macromolecule in a cell, as they evolve over time, as well as measurement of physical interactions between macromolecules in the form of tables of force or potential versus relative orientation or separation between particle pairs.

Overall, models aimed at understanding biological problems driven by colloidal-scale physics are gaining traction (exemplified by models of chromatin dynamics [60], the positioning of the mitotic spindle [63,64], and chromatin segregation due to epigenetic modifications [110]). However, one gap they share is relating physical microscopic forces to whole-cell-scale biological functions. Doing so requires advancement to models that simultaneously include Brownian motion, macromolecular crowding, confinement, many-body hydrodynamic interactions, complex attractive and repulsive potentials responsive to *p*H and salinity, deformability, and many more, where interactive physical forces (inputs) and biological functions (outputs) are derived from detailed measurements in biology experiments, which itself will require new physics-based methods.

Nascent models of whole-cell intracellular physics are working toward this goal, for example, the Confined Stokesian dynamics algorithm developed by Zia and co-workers [70,112,113]. A hallmark of this algorithm is the accurate modeling of the hydrodynamic coupling between colloidal particles and the cell membrane at virtually any level of crowding. Even at such a level of hydrodynamic fidelity, our models are still biologically simple compared to real cells filled with viscoelastic networks, lined with porous media, and bound by deformable permeable membranes [137–142]. Future expansions of this model must thus include substantial algorithmic changes to make these biologically relevant conditions more tractable. For example, viscoelastic networks found in cells are made up of polymers that have been extensively and successfully modeled via the bead-spring model. An implementation of a suspension with bead-spring macromolecules can be envisioned as an expansion of the Stokesian dynamics algorithm [143]. Additionally, large dynamic porous media inside cells such as the nucleoid can be coarse grained as a Brinkmann medium and implemented in Confined Stokesian dynamics. More sophisticated representations of deformable or even viscoelastic membranes will require developments in theory to obtain the correct Green's functions for such an enclosure and mathematical relationships to describe resulting particle-wall

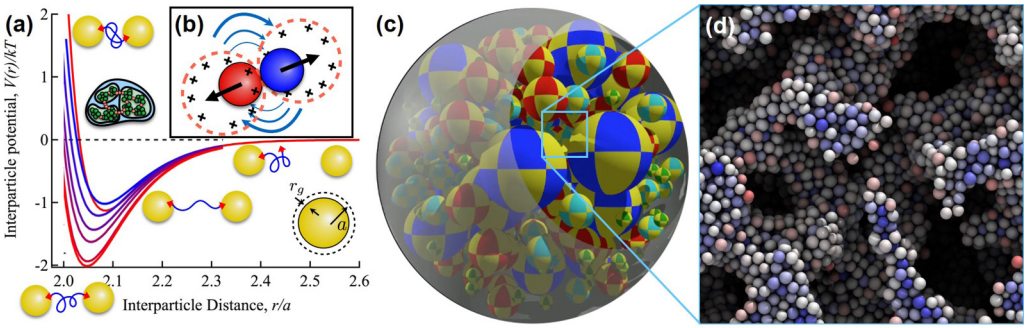


FIG. 10. The Cellular Stokesian dynamics algorithm [70,112,113] can be used to model whole-cell processes. As one example, the algorithm can be used to study (a) phase separation at the colloidal scale resulting from interparticle attractions, characterized by (b) an interaction potential, which may comprise variable-distance repulsions and attractions that are uniformly distributed over molecular surfaces or patchy. In Cellular Stokesian dynamics models, (c) hydrodynamic interactions and size polydispersity in a cell coupled with (d) attractive forces can thwart phase separation, instead inducing gelation and formation of fibrous or ropy networks reminiscent of those implicated in ALS and other age-related diseases [figures (a) and (b) are from [124], (c) is from [70], and (d) is from [131], with permission].

interactions. This effort to leap forward toward representing whole-cell biological functions will lead to what we call Cellular Stokesian dynamics (Fig. 10).

### B. Unknown unknowns

Are there unknown physics-based processes that are essential for life or otherwise regulate whole-cell functions across multiple length scales and timescales? The examples discussed in this paper suggest that the answer is yes, that as-yet discovered physical processes at the biocolloidal frontier regulate cell function. Such a conclusion can additionally be inferred from efforts in synthetic biology to construct artificial cells from a minimal gene set [144,145]. For example, over a 25-year arc that started by identifying a minimal model cell (*Mycoplasma genitalium*), we have both gained and lost ground in understanding how genes instantiate life. In 1995, the concept of a minimal gene set required for life was set [146] and four years later [147] gene disruption technology established that 23% of the 480 genes essential to life in the model Mycoplasma served an unknown function. Nearly two decades later, further study in the closely related *Mycoplasma mycoides*, which should have a very similar essential gene set [145], revealed that 32% of life essential genes serve an unknown function. Thus, despite 25 years of effort, the greatest success thus far in constructing a living cell from its constituent molecules required "booting up" a nonliving cell with a genome transplant [145,146,148,149]. While this is a stunning achievement, the mechanistic role of 149 genes in just *Mycoplasma mycoides* alone remains a mystery.

How do we know what genes do in the first place? For context, a simplified explanation is as follows. Historically, genes were screened for their functionality using random mutagenesis (such as via radiation or mutagenic chemicals) so as to disrupt genes within natural genomes. Subsequently lost functionality would be assigned to the disrupted genes. However, these tests are typically limited to identifying chemical or biological functions. For example, in bacteriophage T7, 19 genes were discovered to have roles in DNA synthesis, virus particle maturation, and lysis by mapping disrupted functions to disrupted genes in randomly generated mutants [150]. More generally, there are many biochemical processes that convert reactants (A) to products (B); one can sequentially knock out every gene in the genome and measure the amount of B via, e.g., fluorescence microscopy or mass spectrometry, and determine how each gene affects the conversion of A to B. This idea can be applied to many whole-cell behaviors to determine the biochemical functions of genes.

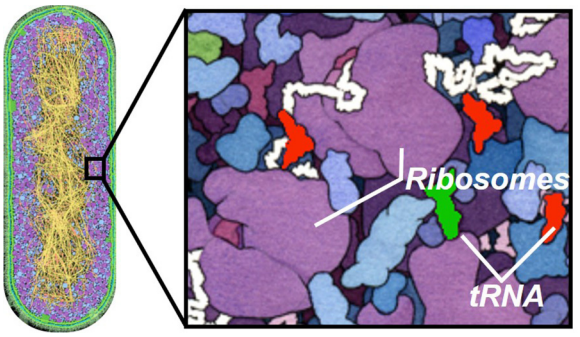


FIG. 11. Translation elongation in *E. coli* is an ideal model problem for elucidating the colloidal physics underpinning whole-cell behavior. While the nucleoid is confined to the center (gold region), translation occurs in the nucleoid-excluded perimeter, where tRNA complexes (red or green) search for matching ribosomes (purple). The search is a complex combinatoric and transport process that spans both regions and involves cell-scale changes in spatial organization as growth rates change [123] (figure is adapted from [3], with permission).

However, what if the function of a gene is physical? What tests should we be doing? How can we use new tests to systematically search for physics-controlled processes inside cells across multiple length scales and timescales, for functions we cannot now see or do not yet know are there? The first step is the judicious selection of model problems.

### *Choosing a model problem*

We propose that when searching for physical functions, especially those that span colloidal physics and cell biology, an ideal model problem is one that is essential to life regardless of cell type, condition, age, or species; is constrained by colloidal-scale transport; and operates across subcellular to whole-cell scales. For example, in our research we have selected one such model problem: translation elongation in a simple model bacterium, *E. coli* [123]. Translation elongation is essential to life: An mRNA chain containing genetic instructions is decoded by a ribosome, which uses the information to orchestrate assembly of amino acids into a growing polypeptide chain, which ultimately forms a protein. Proteins are the building blocks for the majority of all biomolecules inside bacteria; without proteins, bacteria do not exist. While the chemical process of protein polymerization executed by ribosomes is well understood [151], translation elongation also requires the physical delivery of a specific sequence of amino acids. Each amino acid must be transported from elsewhere in the cytoplasm by a carrier molecule, a transfer-RNA (tRNA) complex. The colloidal-scale physics underlying the searching, transport, and matching dynamics involved in this physical process are a green field.

Spatial heterogeneity and cell-spanning transport clearly play a role in translation elongation. The protein polymerization process itself takes place in localized volumes within the cytoplasm, since actively translating ribosomes are bound to large mRNA complexes that remain relatively immobile. Despite this localization, ribosomes somehow recruit translation molecules like tRNA complexes from the entire cytoplasmic volume. Thus, while tRNA complexes can access the entire cytoplasm, ribosomes are mostly excluded between the cell wall and a centrally located nucleoid (DNA) [152–154] as illustrated in Fig. 11. The two key molecules involved also have different sizes, indicating that polydispersity plays a role: Approximated as spheres, tRNA complexes have a radius of 5.9 nm [155] and ribosomes have a radius of 13 nm [156]. These tRNA complexes and ribosomes must successfully execute a combinatorial search process for one another across space and time, whereby any particular ribosome must correctly acquire one of 42 unique types of tRNA during a search-and-check process that rejects mismatched combinations. Overall, the process of translation

elongation integrates chemical reactions and physical transport across molecular, colloidal, and whole-cell length scales and is essential to all life.

Of note, the essentiality of translation elongation suggests that the physics involved have been highly optimized via evolutionary selective pressures over the past $3 \times 10^9$ years. That is, if there is interesting physics to be found, it is likely to be found here. The complex interplay of these processes at the colloidal scale suggests new mysteries in whole-cell physics that we are exploring via physical modeling [123]. Our work suggests that by including even the simplest spatial resolution, i.e., entropic exclusion and Brownian motion, we can partly explain long-standing discrepancies between measured elongation rates [157] and kinetics-centered models [158]. Applying colloidal behaviors discovered from models of spherically confined polydisperse colloidal dispersions with coupled many-body hydrodynamic interactions (cf. Figs. 8 and 9) suggests several new mechanisms that power this process and may lead to connections with life-essential genes of unknown function [70,107,112,113,123].

### C. Prospects

To realize the goal of operational mastery of biological cells, future research should have as an ultimate goal a whole-cell model, where underlying physical laws enable prediction of biological behavior. One promising route to this goal is the sequential assembly of spatially resolved, physically modeled, biologically accurate, experimentally validated subcellular processes into a whole-cell model, much the way a battery, steering assembly, suspension system, electronics, and tires are assembled separately and then sequentially inserted into an automobile chassis. However, we do not yet have all the parts or the instructions for how they work or go together: There may be undiscovered physical functions essential to building the whole-cell model. The leap forward seems to require inferring the unseen from the seen, much as Einstein inferred the existence of the atom from particle diffusion. If the trajectory of scientific discovery is any example (e.g., Mendel's pea flowers, Darwin's finches, or Brown's diffusion), the mere presence of life-essential genes of unknown function is a clear signal that genes may hold these physical instructions. Here, instead of physical features implying biological function, biological features may imply physical function. The vision for physics-based models should thus focus on development of model problems that reveal as-yet undiscovered physical functions in cells. From a practical standpoint, new tools are needed: We will need extensive algorithmic advancement, more compute power, more high-fidelity biological data relevant to colloidal interactions and motion, new ways to visualize dynamics and mesoscale structure in cells, and new ways of collaborating between biology, physics, and engineering.

## ACKNOWLEDGMENTS

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