

Physics

Special Topic: Active Matter

Physical principles at bio-nano interfaces with active matter

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Received 30 November 2023; Revised 14 January 2024; Accepted 23 January 2024; Published online 29 March 2024

Abstract: Active matter is characterized by out-of-equilibrium behaviors, offering an attractive, alternative route for revolutionizing disease diagnostics and therapy. A better understanding of how active matter interacts with cell membranes is critical to elucidating the underlying physical mechanisms and broadening the potential biomedical applications. This review provides a conceptual framework on the physiochemical mechanisms underlying active matter-biomembrane interactions. We briefly introduce the physical models of active matter and lipid membranes, and summarize the typical phenomena emerging from various active matter, including artificial active particles, cellular cytoskeletons, bacteria, and membrane proteins. Moreover, the remaining challenges and future perspectives of such non-equilibrium systems in living organisms are discussed. The findings and fundamental principles discussed in this review shed light on the rational design of activity-mediated cellular interaction, and could trigger better strategies to design and develop novel functional systems and materials toward advantageous biomedical applications.

Keywords: active matter, cell membrane, bio-nano interfaces, nonequilibrium physics, transmembrane transport

INTRODUCTION

Active matter is composed of particles, agents or constituents that consume energy and are thus out-of-equilibrium, which has demonstrated fascinating new patterns of self-organization not observed in equilibrium systems [1,2]. These constituent elements of active matter can induce directed motion, exert forces and induce shape deformations, or even to proliferate and annihilate. Active matter, ranging from molecular motors to groups of animals, exists at various spatial and temporal dimensions. Macroscopically, swarming insects, migrating animals, and human crowds can all be considered as active matter systems. Within living organisms, examples include the beating of the heart, the movement of muscles caused by non-equilibrium assemblages of cells. And at a more fundamental level, cell and bacterium represent active units. At the basic level, adenosine triphosphate (ATP) releases chemical energy to trigger protein conformational changes, facilitate intra-cellular cascades regulating signaling pathways, and manipulate the skeleton of the cell to control its shape and movement. Simultaneously, researchers have engineered self-propelled particles for delivering drugs into cells and studying the self-assembly of microscopic units.

Early studies primarily focused on the self-assembly behavior of bulk active matter. The self-assembly of

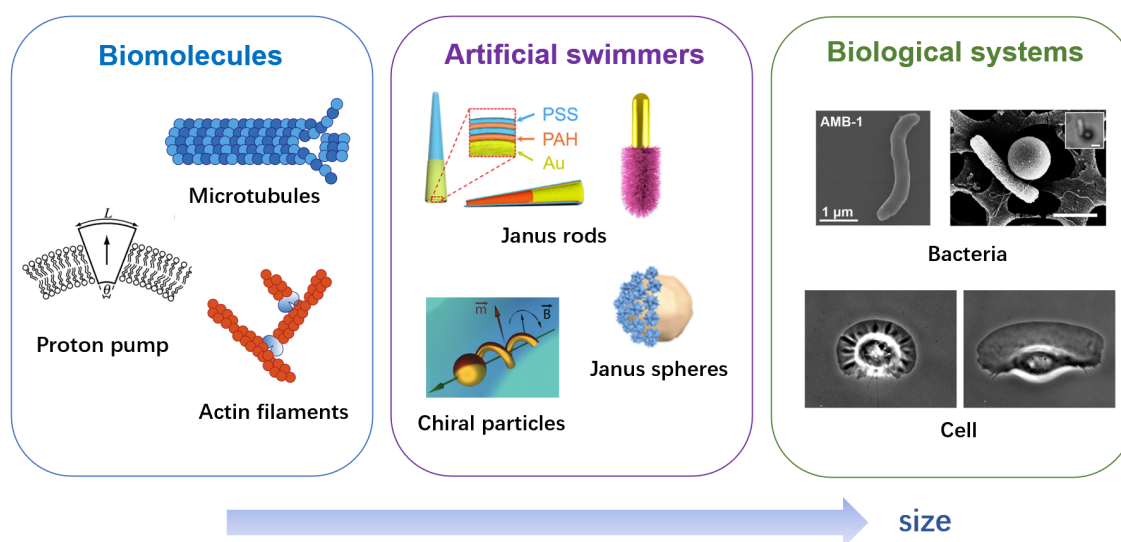


Figure 1 Active matter at different scales. Reprinted with permission from Refs. [22–24]. Copyright©2017, 2019, 2022 American Chemical Society. Reprinted with permission from Refs. [25,26]. Copyright©2018, 2020 WILEY-VCH. Reprinted with permission from Ref. [27]. Copyright©2020, The American Association for the Advancement of Science. Reprinted with permission from Ref. [28]. Copyright©2011 Barnhart *et al.* Reprinted with permission from Ref. [29]. Copyright©2005 American Physical Society.

passive spherical colloids with short-range isotropic interactions is commonly confined to close-packed hexagonal crystals. The introduction of activity has endowed particles with richer behaviors. The existence of the motility induces long-lived density fluctuations and phase separation without any attraction between particles [3–6]. The self-organization of non-spherical particles with multiple interactions also attracted extensive research. Colloidal hard rods form multilayered crystalline clusters at low concentrations, and as the concentration increases the crystallites get kinetically arrested [7]. For Dipolar Janus colloids, the non-uniform distribution of charges allows Dipolar Janus colloids to form active chains, swarms, and clusters [8,9]. Beyond colloidal particles, the cytoskeleton acts as nanomachines within cells, and understanding its spatial distribution and dynamic behavior is crucial. Unlike equilibrium networks, the input of energy in bundled active networks results in internally generated fluid flows and the motion of defects [10–13]. While the stresses produced by actin filaments and molecule motors are the reason for the contraction of actomyosin gels and the force generation in skeletal muscle [14–16]. However, the relationship between the motion of active matter and cellular membranes is far from established. The primary goal of this review is to clarify the physical mechanisms behind the deformation and fluctuations of cellular membranes induced by active force (Figure 1).

Conventional theories offer well-developed explanations for how cellular membranes deform in equilibrium, in which the bending and stretching energies of lipid membranes can be obtained via Helfrich energy expressions [17]. The total energy of cellular membranes under different conditions can be derived by performing variational methods and boundary conditions [18–21]. However, how active matter impacts this nonequilibrium phenomenon has been largely unexplored. There are significant challenges to investigating nonequilibrium systems in the framework of equilibrium physics. Therefore, the interaction between active matter and cell membranes is an urgent and critical issue to be addressed.

In this review, we provide a conceptual framework on the physiochemical mechanisms underlying active

matter-biomembrane interactions. Modeling methods of active matter and classical physical models of cell membranes are introduced. Then, we summarize the typical phenomena emerging from various active matter, including artificial active particles, cellular cytoskeletons, bacteria, and membrane proteins. The force-induced particle endocytosis and the motion coupling of multi-particle systems with membranes are described for artificial active particles. In the cytoskeleton section, we discuss how the cytoskeleton enhances membrane fluctuations, induces membrane deformation and motion, and reorganizes cell surface molecules. We then explore the effects of bacteria and membrane proteins on membrane fluctuations. Finally, the remaining challenges and future perspectives of non-equilibrium systems in living organisms are discussed. This review may appeal to those communities that are devoted to understanding and tuning activity-mediated cellular interaction in non-equilibrium systems.

PHYSICAL MODEL OF ACTIVE MATTER

Active Brownian particle model

While there is a wide variety of active matter, we discuss a general model to describe its microscopic motion. The motion of passive Brownian particles is due to the random fluctuations of the fluid molecules, which collide with the suspended particles and cause them to move erratically in all directions. For self-propelled Brownian particles, a dynamic interaction between random fluctuations and active swimming behavior propels them into a non-equilibrium state.

The translational diffusion coefficient of a spherical passive Brownian particle can be expressed as [30]

$$D_T = \frac{k_B T}{6\pi\eta R}, \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η denotes the fluid viscosity. The rotational diffusion coefficient of the particle is

$$D_R = \frac{k_B T}{8\pi\eta R^3}. \quad (2)$$

The characteristic time for particles moving in a straight line can be represented as $\tau_R = 1/D_R$.

To describe the motion of active particles, the active Brownian particle (ABP) model [30–33] becomes increasingly popular (Figure 2). Active colloids are conceptualized as Brownian particles subject to an additional propelling velocity of magnitude v applied along a fixed particle axis. The corresponding stochastic differential equations can be described as

$$\begin{cases} \dot{x} = v\cos\varphi + \sqrt{2D_T}\zeta_x, \\ \dot{y} = v\sin\varphi + \sqrt{2D_T}\zeta_y, \\ \dot{\varphi} = \sqrt{2D_R}\zeta_\varphi, \end{cases} \quad (3)$$

where $[x, y]$ denotes the particle position, φ represents its orientation, and ζ_x , ζ_y , and ζ_φ denote independent white noise stochastic processes with zero mean and a correlation function $\delta(t)$. For passive Brownian colloid, the average distance tends to vanish due to symmetry. While for an active colloid, the average trajectory follows a straight line along the x -direction $x(t) = \frac{v}{D_R}(1 - e^{-D_R t})$.



Figure 2 (A) Schematic of active Brownian particles in two dimensions. (B) Trajectories of the particle with different velocities. Reprinted with permission from Ref. [30]. Copyright©2016 American Physical Society.

Phenomenological model

For active matter with more complex structures, the ABP model proves inadequate for description. Indeed, ideal straight swimming only occurs when the left-right symmetry with respect to the self-propulsion direction remains unbroken. Helical swimming in three dimensions has been documented in various bacteria and sperm cells [34,35]. The introduction of an angular velocity ω in the rotational direction allows for describing particle rotation phenomenon [30].

$$\begin{cases} \dot{x} = v\cos\varphi + \sqrt{2D_T}\zeta_x, \\ \dot{y} = v\sin\varphi + \sqrt{2D_T}\zeta_y, \\ \dot{\varphi} = \omega + \sqrt{2D_R}\zeta_\varphi. \end{cases} \quad (4)$$

In addition to considering the rotational motion of active particles, the shape of particles can significantly influence their dynamics. For non-spherical particles, we can modify the particle motion equations as proposed in [36].

$$\mathbf{H} \cdot \mathbf{V} = \mathbf{K} + \boldsymbol{\chi}, \quad (5)$$

where $\mathbf{H}$ represents the grand resistance matrix. The generalized velocity, denoted as $\mathbf{V} = [\mathbf{v}, \boldsymbol{\omega}]$, encompasses the particle's translational and angular velocities, where $\mathbf{v}$ represents translational velocity and $\boldsymbol{\omega}$ denotes the angular velocity. Similarly, the generalized force, denoted as $\mathbf{K} = [\mathbf{F}, \mathbf{T}]$, includes the effective force $\mathbf{F}$ and torque $\mathbf{T}$ acting on the particle. The random vector $\boldsymbol{\chi}$ is a random vector characterized by a correlation of $2k_B T \mathbf{H} \delta(t)$.

In a real system, the active matter may experience additional forces such as gravity, forces originating from the interaction with other particles, and constraints such as motor proteins walking along microtubule directions. These forces can also be regarded as an “internal” force fixed on the particle's body coupled in Langevin equations.

THEORETICAL MODEL OF LIPID BILAYER

Energy representation of lipid bilayer

The cell membrane can be treated as a homogeneous elastic membrane, and its classical energy representation was proposed by Helfrich [17]. Helfrich characterized elastic strain caused by three ways: stretching, tilting, and bending. We initially focus on alterations in the area caused by stretching the mem-

brane. For small deformations, the elastic energy of stretching per unit area, denoted as w_s , is the square of the relative change in area $\Delta a / a$.

$$w_s = \frac{1}{2} k_s (\Delta a / a)^2, \quad (6)$$

where k_s represents the elastic modulus of stretching, expressed in energy per unit area. The corresponding stress, a force per length is

$$\sigma_s = k_s (\Delta a / a). \quad (7)$$

Next, we discuss the forces generated by tilt. For example, the application of an oblique magnetic field on magnetically anisotropic lipid molecules can tilt the membrane. The counter-balancing elastic torque per unit area, denoted as $\mathbf{m}_t$, should be provided by a normal force per unit length:

$$\sigma_t = \mathbf{m}_t \times \mathbf{e}, \quad (8)$$

where $\mathbf{e}$ is the unit vector parallel to the bilayer. The torque density is given by $\mathbf{m}_t = -k_t(\mathbf{n} \times \mathbf{d})$, $\mathbf{n}$ is the layer normal and the average $\mathbf{d}$ is orientation of the molecules. The elastic energy per unit area can be written as

$$w_t = \frac{1}{2} k_t (\mathbf{n} \times \mathbf{d})^2. \quad (9)$$

Finally, consider the curvature of the bilayer. The stress originated from a given point that the film is infinitely thin. The curvature-elastic energy per unit area can be expressed as

$$w_c = \frac{1}{2} k_c \left(\frac{\partial n_x}{\partial x} + \frac{\partial n_y}{\partial y} - c_0 \right)^2 + \bar{k}_c \left(\frac{\partial n_x}{\partial x} \frac{\partial n_y}{\partial y} - \frac{\partial n_x}{\partial y} \frac{\partial n_y}{\partial x} \right). \quad (10)$$

The curvature-elastic moduli, k_c and $\bar{k}_c$, have the dimension of energy. The linear term is accounted for by spontaneous curvature c_0 , which allows for the chemical compositions on each side of the cell membrane are different.

Deformations of bilayer vesicles

In most cases, bilayer vesicles exhibit rotational symmetry. Therefore, the shape of the vesicle can be described by a function $\psi(s)$, where s is the arclength of the vesicle, and ψ is the angle made by the layer normal to the polar axis. On any rotationally symmetric surface, two sets of orthogonal lines can be uniquely defined, corresponding to the meridians and parallels of a sphere. The principal curvatures of the surface along these lines are

$$c_m = \frac{d\psi}{ds}, c_p = \frac{\sin\psi}{r}, \quad (11)$$

where r is the distance between the vesicle and the polar axis. Limit considering a piece of bilayer between s_1 and s_2 . The total curvature-elastic energy of the vesicles can be expressed as

$$\int_{s_1}^{s_2} \left[\frac{1}{2} k_c (c_m + c_p - c_0)^2 + \bar{k}_c c_m c_p \right] ds. \quad (12)$$

We assume that the bilayer area remains constant, the auxiliary condition is incorporated:

$$\int_{s_1}^{s_2} ds = \text{const}. \quad (13)$$

We can establish an Euler-Lagrange equation for the shape of rotationally symmetric bilayers. By combining eqs. (12) and (13), we get the total energy expression for the vesicle, which can be solved by the

variational method.

$$f\left(\psi, \frac{d\psi}{ds}, r\right) = \frac{1}{2}k_c\left(\frac{d\psi}{ds} + \frac{\sin\psi}{r} - c_0\right)^2 + \bar{k}_c \frac{d\psi}{dr} \frac{\sin\psi}{r} + \lambda. \quad (14)$$

This method has been employed to solve various equilibrium state problems of cell membranes, such as the deformation induced by line tension in two-component lipid vesicles [19,37–39] and the energy change during particle endocytosis [20,21].

Membrane fluctuation

Biological membranes exhibit substantial structural fluctuations that arise from thermal excitation at physiological temperatures. For length scales larger than the average thickness of the membrane h_0 , a continuum model predicts the intensity of undulatory modes, characterized by amplitude $h(q)$, as a function of the wave vector q [40]

$$h^2(q) = \frac{k_B T}{A(k_c q^4 + \gamma q^2)}, \quad (15)$$

where k_c represents the bending modulus, γ is the surface tension, A is the system area, and $q = 2\pi/h_0$. The undulatory modes can be employed to gauge the bending stiffness of the cell membrane [41–43]. When active matters interact with the membrane, larger fluctuations ensue, disrupting the relationship $h^2 \propto q^4$. The emergence of new fluctuation patterns becomes a phenomenon worthy of investigation.

ACTIVE PARTICLES-BIOMEMBRANE INTERACTION

Applications of different types of active nanoparticle

Intracellular delivery of materials plays a crucial role in the field of biomedical sciences. In general, nucleic acids [44–47], proteins [48,49], small molecule drugs, as well as carriers like liposomes [50], nanoparticles [51–54], bacteria [26] and viruses, are all potential target materials. Nucleic acid delivery has shown remarkable progress, involving plasmid DNA and mRNA for gene expression, as well as small interfering RNA and microRNA for gene silencing. Additionally, delivery of protein biologics, including active inhibitory antibodies and stimulatory transcription factors, represents a potent yet underexplored approach for decoding and manipulating cellular functions [55]. Furthermore, in the realm of nanomedicine, a prototype nanoparticle known as semiconductor quantum dots (QDs) has demonstrated applications in imaging and treating tumor tissues [56,57]. Nanoparticles also enable the measurement of intracellular chemical and physical properties [58].

Despite the significant successes achieved by inert particles in various fields, challenges still remain. Such challenges stem from the restricted capacity to penetrate biological barriers, complex nanoparticle-cell interactions, unintended accumulation in body regions, and the lack of precise control over intracellular movement [59]. To address these issues, researchers have explored methods involving using chemical energy, acoustic fields and magnetic fields to render particles active. This enables nanoparticles to move more efficiently within biological tissues and overcome the energy barriers of cell membranes.

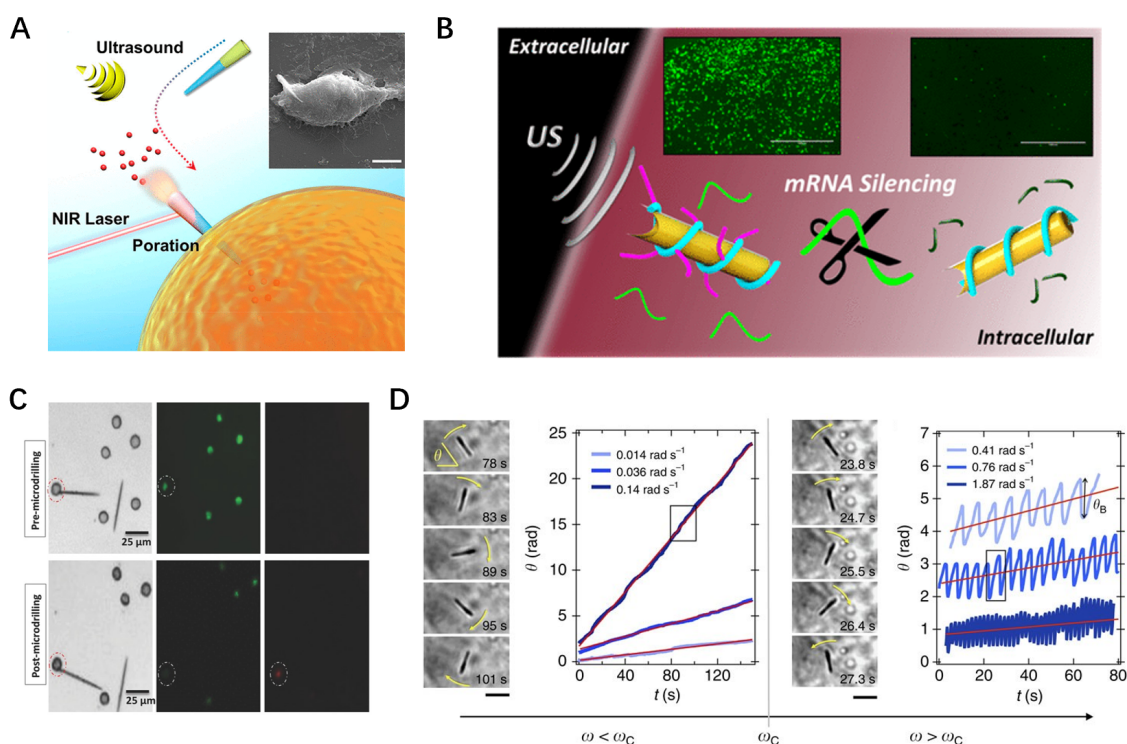


Figure 3 Acoustically and magnetically propelled nanomotors. (A) Schematic of the penetration of HeLa cells by AuNS-functionalized nanoswimmers. Reprinted with permission from Ref. [23]. Copyright©2019 American Chemical Society. (B) Intracellular siRNA delivery and gene-mRNA silencing using US-propelled siGFP/RCA-AuNWs. Reprinted with permission from Ref. [61]. Copyright©2016 American Chemical Society. (C) Brightfield and fluorescence images of HeLa cells before and after microdrilling. Reprinted with permission from Ref. [64]. Copyright©2015 Wiley-VCH. (D) Internalized wire subjected to a rotating field at varying frequencies. Reprinted with permission from Ref. [68]. Copyright©2016 Nature Research.

Acoustically driven forces enable precise control of the movement of nanoparticles, endowing them with the capability to overcome cellular barriers. Pioneering work by Wang *et al.* [60] demonstrated the internalization of gold rods in HeLa cells under an acoustic field. Considering the limited force generated by ultrasound, they further augmented ultrasound propulsion by introducing near-infrared (NIR) light in 2019 [23]. In this approach, an NIR laser was focused on the nanoswimmer, facilitating its rapid penetrating the cell membrane within 0.1 s (Figure 3A). The successful internalization of acoustically propelled nanomotors holds significant promise for various applications. One notable application involves utilizing gold nanowires (AuNWs) as carriers for nucleic acid transport, particularly for gene silencing [61,62]. AuNW, wrapped with a rolling circle amplification (RCA) DNA strand, demonstrated a 94% silencing efficiency within minutes when propelled by ultrasound-mediated delivery of siRNA-AuNWs (Figure 3B). This approach showed a substantial (~13-fold) improvement in silencing compared to statically modified nanowires [61].

Magnetic nanoparticles also serve as highly efficient carriers for drug and gene delivery [25,63]. Srivastava *et al.* [64] extracted calcified porous microneedles from the *Dracaena* sp. plant and coated these structures with a magnetic layer to facilitate cellular penetration through external magnetic actuation. These micro-drillers loaded with camptothecin have demonstrated effective tumor eradication. Carbon nanotubes contribute to the delivery of small molecules and peptides that are challenging to transport into cells [65,66]. Cai *et al.* [67] loaded nickel onto carbon nanotubes to enable the penetration of cells under a magnetic field drive

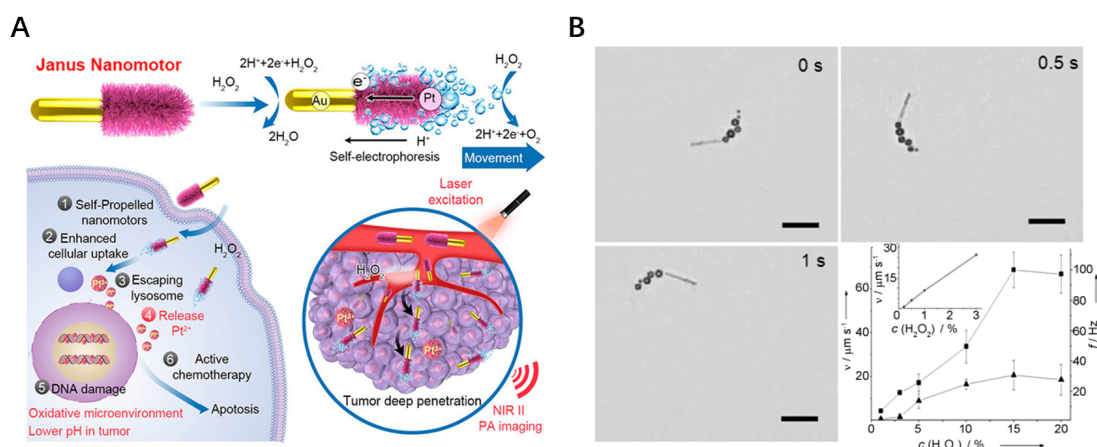


Figure 4 (A) Schematic representation of the application of JAuNR-Pt nanomotors for NIR-II PA imaging of deep tumor tissues and their antitumor efficacy. Reprinted with permission from Ref. [24]. Copyright©2022 American Chemical Society. (B) Time-lapse images of the motion of a nanorocket in 15% H₂O₂ solution. Reprinted with permission from Ref. [70]. Copyright©2013 Wiley-VCH.

(Figure 3C). Immobilizing the green fluorescent protein (EGFP) sequence onto the nanotubes achieved an unprecedentedly high transduction efficiency in Bal17 B-lymphoma, *ex vivo* B cells, and primary neurons, maintaining high viability after transduction. Furthermore, magnetically propelled micromotors are commonly employed for assessing mechanical intracellular environments [68,69]. Berret utilized rotational magnetic spectroscopy to measure the cytoplasm shear viscosity in living cells. Their findings indicate that the interior of living cells can be described as a viscoelastic liquid rather than an elastic gel [68] (Figure 3D).

In addition to acoustic and magnetic propulsion, chemical fuel-driven nanomotors hold the potential for diverse applications. Li *et al.* [24] synthesized a nanosized hydrogen peroxide (H₂O₂)-driven Janus gold nanorod-platinum (JAuNR-Pt) nanomotor designed to enhance second near-infrared region (NIR-II) photoacoustic (PA) imaging in deep tumor tissues and facilitate effective tumor treatment. The self-propulsion of the JAuNR-Pt nanomotor enables the continuous release of cytotoxic Pt²⁺ ions into the nucleus, inducing DNA damage and triggering cell apoptosis (Figure 4A). Wu *et al.* employed a nanoporous template for the synthesis of an H₂O₂-driven polymer multilayer tubular nanomotor (Figure 4B). The outer layers of the nanotube serve as a container, allowing for the loading of anticancer drugs and other therapeutic agents [70]. Biocompatible chemical fuels like urea are anticipated to enhance the feasibility of various applications [27,71]. Tang *et al.* [27] demonstrated a unique approach by modifying the platelet surface with a biotin-streptavidin-biotin binding complex to react with urease. This modification allows for the loading of doxorubicin onto nanoparticles, leading to the selective killing of MDA-MB-231 cells.

Dynamics of active particles interacting with membranes

Understanding the forces and energies related to the nanoparticle-cell interaction is crucial for the design of artificial nanoparticles, aiming to enhance their intracellular uptake efficiency. Upon particle contact with the membrane, a series of dynamic processes initiate. The process of uptake of nanoparticles involves curving the cell membrane and overcoming tension while stretching the membrane. Various forces, such as electrostatic, van der Waals, hydrophobic forces, ligand-receptor binding, are collectively lumped together as the

adhesion force, driving the endocytic process. Bending energy E_{bend} , tension energy E_{ten} , and adhesion energy E_{ad} can be defined to characterize the cellular uptake of particles [20]. The total adhesion energy required for complete wrapping nanoparticle is expressed as $E_{\text{ad}} = 4\pi R^2 w$, where w is the adhesion strength. Assuming a negligible spontaneous curvature ($\kappa_0 = 0$), the bending energy for full nanoparticle wrapping is given by $E_{\text{bend}} = 8\pi\kappa$, where κ is the bending rigidity. Additionally, the stretching energy is expressed as $E_{\text{ten}} = 4\pi R^2 \sigma$. Balancing the adhesion energy with membrane deformation energy establishes a lower limit for the nanoparticle radius, below which endocytosis becomes unattainable [72].

$$R_{\min} = \sqrt{\frac{2\kappa}{w-\sigma}}. \quad (16)$$

In instances where membrane wrapping is predominantly governed by specific interactions, the critical endocytic radius of particles is influenced by receptor density [73,74]. Simultaneously, particle properties such as shape [75,76], softness [21,77], and the cooperative effect of multiple particles [78,79] also impact their transmembrane transport processes.

The aforementioned studies primarily focus on passive particles, which endocytosis relies on adhesive energy. The dynamic processes of active particles interacting with membranes exhibit distinct differences from those of passive particles. Chen *et al.* [80] used Brownian dynamics simulations to investigate the process of endocytosis of active particles (Figure 5A) and provided theoretical analyses. By incorporating membrane bending stiffness, adhesive energy, and active forces, the system's total energy equation can be written as

$$E = \int 2\kappa H^2 dA_{\text{mem}} - \mu_A S + \Sigma \Delta A - Fz, \quad (17)$$

where H represents the mean curvature of the membrane, μ_A is the interaction energy between the particle and the membrane per unit area, ΔA is the excess membrane area resulting from its deformation, and z indicates the entry height of the particle during endocytosis. Taking the derivative of the total energy allows us to derive the uptake force, F_{up} . Additionally, a friction force can be expressed as $F_{\text{friction}} = 2\pi\eta R^2 \sin(\alpha)\dot{\alpha}$. By balancing the uptake force and the friction force, we can determine the critical Peclet number (Pe) governing the endocytic process:

$$Pe = \frac{8\pi\kappa - 4\pi\mu_A R^2}{k_B T \min_f [\mathcal{F}'(f)]}, \quad (18)$$

where $\mathcal{F}'(f)$ represents the relationship between the membrane wrapping z and the particle radius R . This equation overlaps with the simulation boundary of endocytosis (Figure 5B). In practical experiments, active particles may enter cells from various angles. The article also provides the optimal active force for maximum probability of cellular entry at different incident angles, indicating that active particles have an optimal force for entry at various angles. Beyond this optimal force, particles are prone to slipping from the membrane surface (Figure 5C).

The influence of multiple active particles colliding with a lipid vesicle has also been investigated [81]. A phase diagram is shown as a function of the self-propulsion speed v_0 and the vesicle-reduced volume $\hat{V}$ (Figure 6A and B). When the self-propulsion speed v_0 is zero, the system exhibits the thermodynamic behavior of the equilibrium state. As v_0 increases or reduced volume $\hat{V}$ decreases, the vesicle undergoes a sequence of shape transformations, transitioning from a spherical to prolate, oblate, and ultimately to a

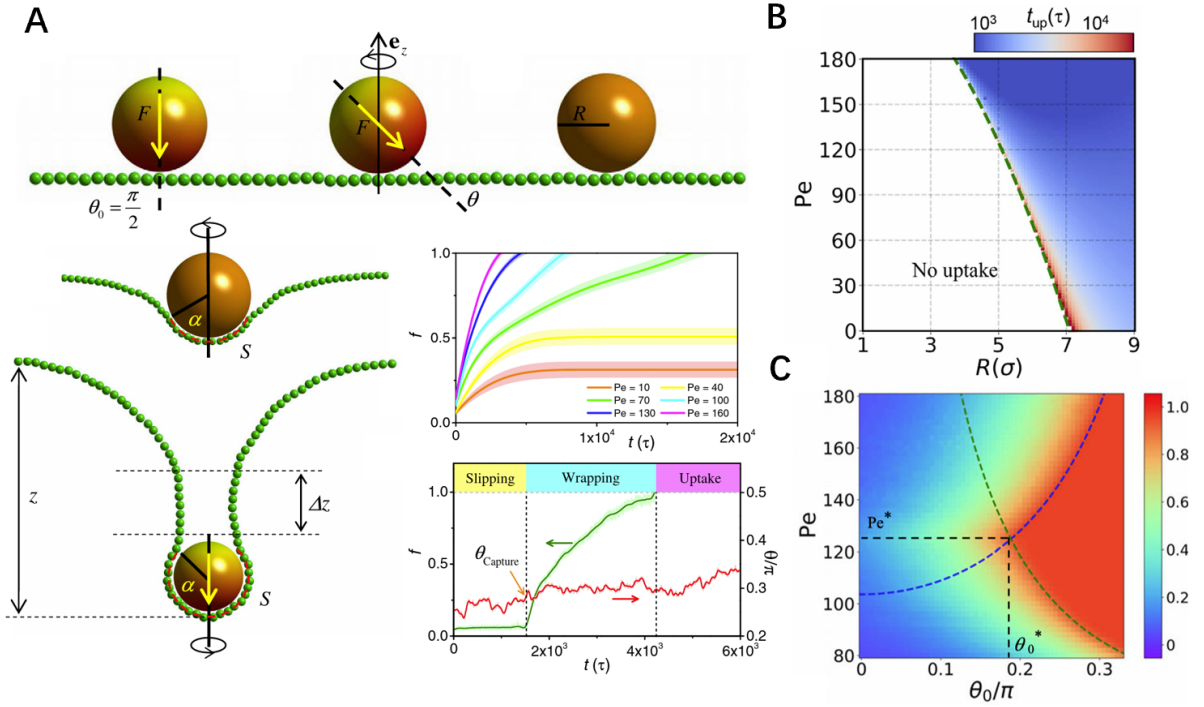


Figure 5 (A) Configurations of the active particle during the endocytosis process. (B) Uptake time plotted in the Pe - R space, with the boundary derived from the theoretical model. (C) Uptake probability P as a function of Pe and θ_0 . Reprinted with permission from Ref. [80]. Copyright©2020 American Physical Society.

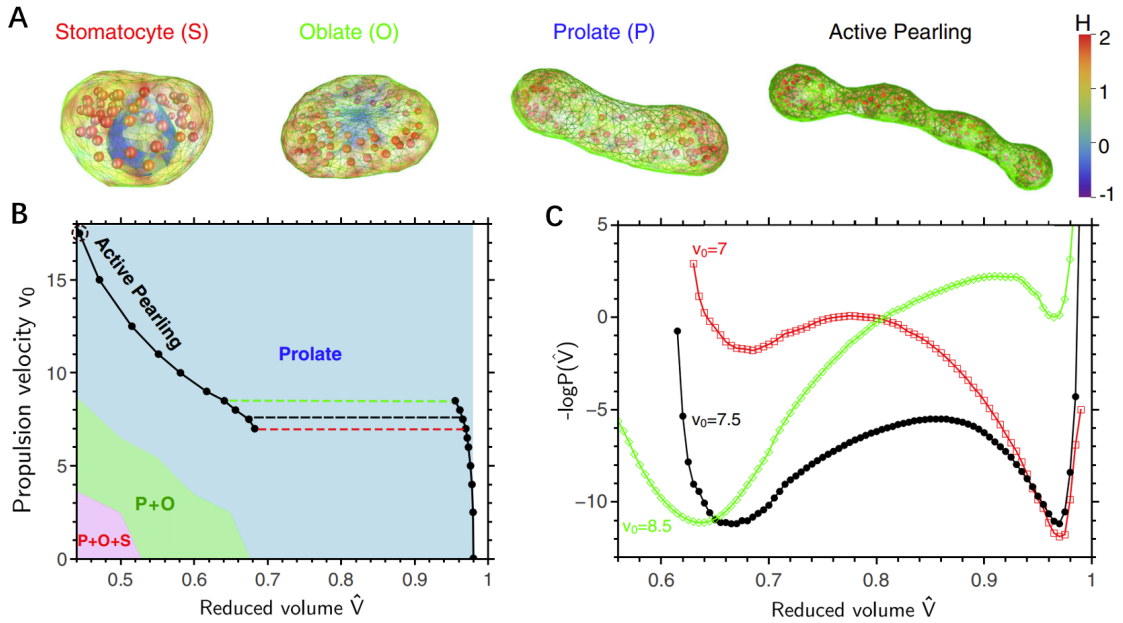


Figure 6 (A) Vesicle shapes change induced by the interplay between the elastic bending energy of the membrane and the swim pressure. Color bar represents the local membrane mean curvature H . (B) Phase diagram as a function of the self-propulsion speed v_0 and the reduced volume $\hat{V}$. (C) Free energy $-\log P(\hat{V})$ as a function of the reduced volume $\hat{V}$ for three values of v_0 . Reprinted with permission from Ref. [81]. Copyright©2019 American Physical Society.

stomatocyte. The researchers further employed forward flux sampling (FFS) to compute the stationary probability distribution of the reduced volume, $P(\bar{V})$ (Figure 5C). At lower activity, the membrane tends to stabilize in the stomatocyte state, with an increase in active forces causing a shift of the energy minimum towards the prolate state.

Vutukuri *et al.* [82] explored the shape transformation of giant unilamellar vesicles (GUVs) by varying the number of self-propelled particles (SPPs) and their activity. They categorized the membrane behavior into three distinct regimes. In the “tethering” regime (large Pe and low SPP volume fraction ϕ), long and thin tethers extend out from a mother vesicle. The “fluctuating” regime (small Pe) is characterized by quasi-spherical vesicle shapes and the absence of tethers. The “bola/prolate” regime (large Pe and ϕ) includes the formation of two or more satellite vesicles. By balancing the driving forces of active particle clusters, membrane bending rigidity, and both passive and active membrane tension, they theoretically determined transition points between these three states. Additionally, their study delved into the influence of active particles on the fluctuation spectrum. It was observed that in the small modes number (tension-dominated regime), activity significantly enhanced membrane fluctuations, while larger modes number (bending-dominated regime) remained essentially unaffected.

CYTOSKELETON BELOW CELL MEMBRANE

General introduction of cytoskeleton

The ability of cell to resist deformation, facilitate intracellular cargo transport, and change shape during movement all relies on the cytoskeleton. There are three main types of cytoskeletal: actin filaments, microtubules and intermediate filaments. All three can be organized into networks and possess the capability to restructure in reaction to external forces. They also play crucial roles in organizing and preserving the integrity of intracellular compartments [83].

Microtubules are the stiffest among these polymers. They are made of repeating α/β -tubulin heterodimers and are present in all eukaryotes [84]. Microtubules form radial arrays during interphase, serving as central hubs for intracellular traffic. In mitosis, they transform into the mitotic spindle, crucial for precise DNA segregation and chromosome alignment, driven by dynamic instability where individual microtubules rapidly switch between stable growth and rapid shrinkage [85].

Actin filaments are double-helical polymers composed of globular subunits arranged in a head-to-tail manner, resulting in molecular polarity within the filament [86]. These components have the ability to form highly organized and rigid structures, encompassing isotropic networks, bundled networks, and branched networks. Aligned filament bundles offer structural support for filopodial protrusions, pivotal in phenomena like chemotaxis (movement guided by a chemical gradient) and intercellular communication. In contrast, branched filament networks underpin the leading edge of most motile cells, facilitating the generation of forces essential for dynamic changes in cell morphology during phagocytosis.

Intermediate filaments are less stiffness than microtubules and actin filaments. They are crosslinked to each other and composed of various types of proteins, including keratins, vimentins, neurofilaments, and lamins. Intermediate filaments are particularly important in tissues subjected to mechanical stress, such as epithelial tissues, muscle cells, and neurons. Unlike microtubules and actin filaments, intermediate filaments lack

polarity, limiting their ability to facilitate the directional movement of molecular motors.

Active cytoskeletal breakdown thermodynamic equilibrium

In the nineteenth century, Browicz [87] observed that red blood cells exhibit flickering behavior under optical microscopy. And in subsequent studies, researchers concluded that the cellular cytoskeleton induces non-equilibrium fluctuations in the cell membrane. In such non-equilibrium systems, the fluctuation-dissipation (FD) theorem, which traditionally links thermal fluctuations of systems to their response to external forces, is no longer applicable [88–90]. Active microrheology (AMR) allows for the measurement of the mechanical properties of cell membranes containing dynamic fiber networks. While passive microrheology (PMR) is utilized to assess the activity of the network and deviations from the FD theorem.

A strong stiffening of the networks attributed to motor activity is found by AMR [91]. This method manipulates micrometer-sized embedded probe particles using a sinusoidally oscillated optical trap, generating a force F at frequency ω . The response function $\alpha(\omega)$ was derived from the measured probe particle displacement $u(\omega)$:

$$\alpha(\omega) = \frac{u(\omega)}{F}. \quad (19)$$

This response function is linked to the shear modulus G , via extending the Stokes relation [92] $\alpha = 1 / (6\pi Ga)$, where a represents the probe particle radius. The shear modulus G with a variety of concentrations of creatine phosphokinase (CPK) is shown in Figure 6C. The motors' contractile activity induces internal tensile stresses in the actin filaments, consequently enhancing the network's rigidity, as illustrated in Figure 6D.

In equilibrium system, where the membrane only affected by thermal forces, the power spectral density is

$$C(\omega) = \int u(t)u(0)\exp(i\omega t)dt. \quad (20)$$

According to the Fluctuation-Dissipation (FD) theorem, in equilibrium

$$\alpha''(\omega) = \frac{\omega}{2k_B T} C_{eq}(\omega). \quad (21)$$

The imaginary part of the response function $\alpha''(\omega)$ can be determined through active microrheology (AMR). And the right-hand side can be measured by passive microrheology (PMR). Active processes introduce additional fluctuations, so the right side is larger than the left side, thereby conflicting with the FD theorem. In the experiment by Mizuno *et al.* [91], no discernible difference between AMR and PMR results was observed for a duration of up to 5 h (Figure 7A). However, over longer durations, a noticeable distinction emerged, manifesting as significantly heightened fluctuations at frequencies below 10 Hz (Figure 7B). They attributed this phenomenon to a switching of the myosin minifilaments from a nonprocessive mode to a processive tension-generating mode.

Turlier *et al.* [93] employed optical microscopy to precisely measure the fluctuations of red blood cells and provided an analytical model and dissipative particle dynamics (DPD) simulations to give an explanation. They formulated spherical harmonic functions to characterize membrane shape. Then they employed the Langevin equation, incorporating dissipative forces, to describe the dynamics of membrane fluctuations:

$$\frac{d\mathbf{U}_{lm}(t)}{dt} = -W_{lm} \times \mathbf{U}_{lm}(t) + \mathbf{Z}_{lm}^{TH}(t) + \mathbf{Z}_{lm}^A(t), \quad (22)$$

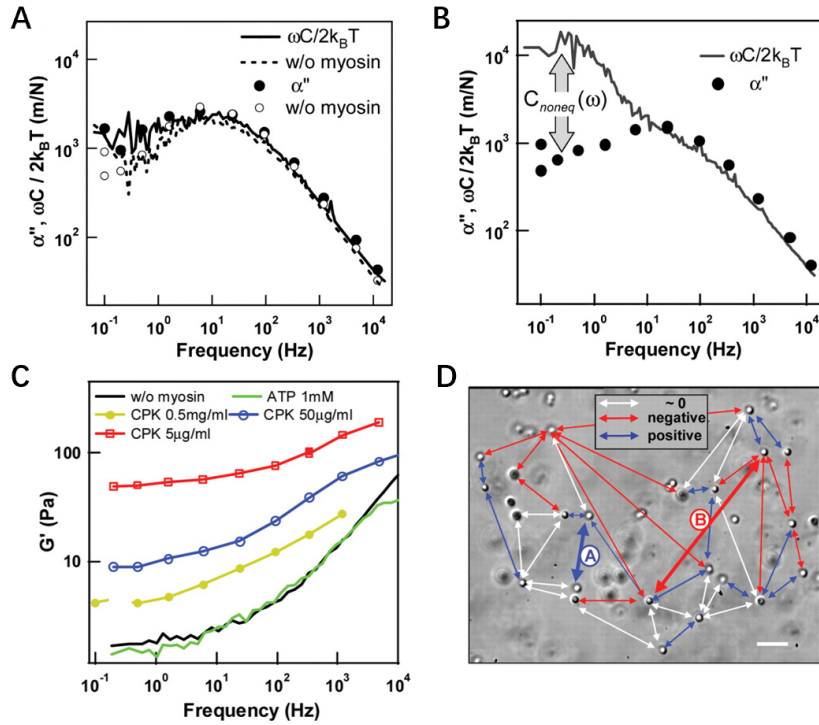


Figure 7 (A) The imaginary part of the response function α'' measured by AMR represented by circles. And the normalized power spectrum $\frac{\omega C}{2k_B T}$ measured by PMR represented by lines. Open circles and the dashed line denote cross-linked actin without myosin, while solid circles and the solid line represent networks with myosin 2.5 h post-sample preparation. (B) The same as (A) but 6.8 h after preparing sample with myosin. (C) Shear modulus $G'(\omega)$ at controlled [ATP] and creatine phosphokinase (CPK). (D) Correlated motion of particle pairs measured by video microscopy represents membrane contraction and relaxation. Reprinted with permission from Ref. [91]. Copyright©2007, The American Association for the Advancement of Science.

where W_{lm} is the relaxation frequency matrix for the membrane deformation U_{lm} . The thermal noise $Z_{lm}^{TH}(t)$ contributes to both bending and stretching fluctuations. An additional active noise, $Z_{lm}^A(t)$, arises from the spectrin network and affects only the stretching modes. The fluctuation spectrum of membrane shape for each spherical harmonic (l, m) can be computed in Fourier space as a sum of the dissipative part of the response function $\chi_{lm}''(f)$ and this active contribution

$$C_{lm}(f) = \frac{2k_B T}{2\pi f} \chi_{lm}''(f) + \frac{2n_a(1 - n_a \tau_a)}{1 + (2\pi f \tau_a)^2} |N_{lm}(f)|^2. \quad (23)$$

The authors assume that phosphorylation sites can transition between active and inactive states with rates k_a , and k_i . n_a is the mean activity defined as $n_a = k_a / (k_a + k_i)$. τ_a is an active timescale $\tau_a = (k_a + k_i)^{-1}$. $N_{lm}(f)$ describes the complex mode- and frequency-dependent propagation of tangential active noise on membrane fluctuations.

Cytoskeleton induce large scale deformations

Apart from inducing membrane fluctuations, the presence of sufficiently large active stresses can induce

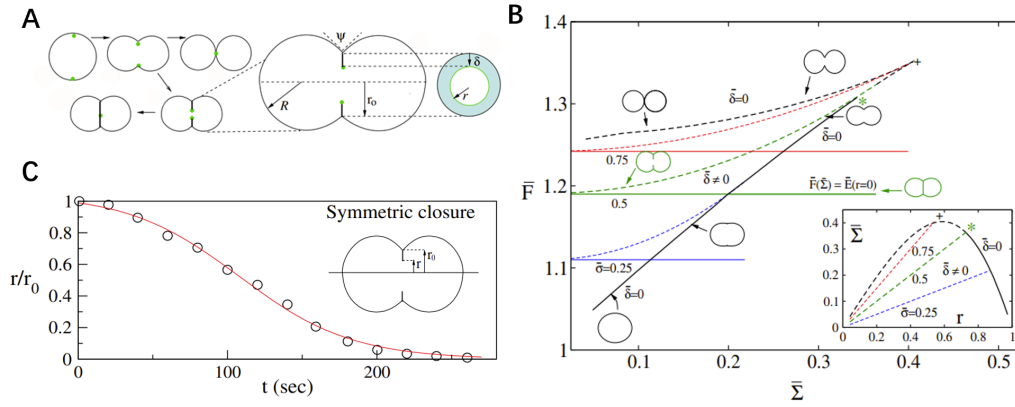


Figure 8 (A) Schematic of a dividing cell with axisymmetric contractile processes. (B) Phase diagram of normalized energy $\bar{F}$ as a function of normalized line tension $\bar{\Sigma}$. (C) Time evolution of the contractile ring's radius (r) during cytokinesis. Reprinted with permission from Ref. [97]. Copyright©2015 American Physical Society.

substantial deformations and flows, playing an important role in various cytoskeletal-mediated processes such as cell division and motility [15].

The occurrence of cytokinesis is constrained by the contractile ring, a structure composed of actin filaments, along with myosin motor proteins that interact to generate contractile stresses. As the contractile ring contracts, the radius of the furrow vanishes and the cell divides into two subvolumes. Cytokinesis is a complex process intricately regulated by multiple molecular factors [94–96]. However, a concise physical model can elucidate morphological changes in the cell membrane during the division process [97]. The driving force exerted by the contractile ring is conceptualized as equivalent to a line tension Σ . Incorporating it into the overall energy equation for the system, the total energy of the system can be written as

$$F(\psi, r) = R^2 \left[1 + \cos\left(\frac{\psi}{2}\right) \right] + \frac{\bar{\sigma}}{2} \left[R^2 \sin^2\left(\frac{\psi}{2}\right) - r^2 \right] + r\Sigma, \quad (24)$$

where $\frac{\psi}{2}$ is the angle between the furrow plane and the plasma membrane, R is the radius of the spherical cell, and $\bar{\sigma}$ is the normalized surface tension (Figure 8A). Minimization of the total energy yields the equilibrium shape of the membrane. Normalized energy $\bar{F}$ as a function of normalized line tension $\bar{\Sigma}$ is shown in Figure 7B. Accounting for viscous stress allows for the resolution of the dynamic process of the septum boundary r over time, as depicted in Figure 8C.

The biological details can be considered into the model of cytokinesis process [98]. The contractile force at the cell equator is proportional to myosin phosphorylation, which can be modeled through a Gaussian distribution. The impact of actin filament polymerization and depolymerization on cell volume can also be incorporated. By numerically solving the equilibrium equations for forces, membrane surface velocity fields, and determining membrane deformation at each time point, the evolution of membrane shape throughout the process can be deduced.

Cell movement is caused by the polarization of the actin network and the ‘walking’ of myosin motors along filaments. The deformation of cells during this motion can be described using the phase-field method [99,100]. By balancing bending force, surface tension force of the membrane, protrusion and retraction force caused by actin filaments, and friction force, the dynamic equation governing the evolution of the phase field ϕ can be derived as [99]

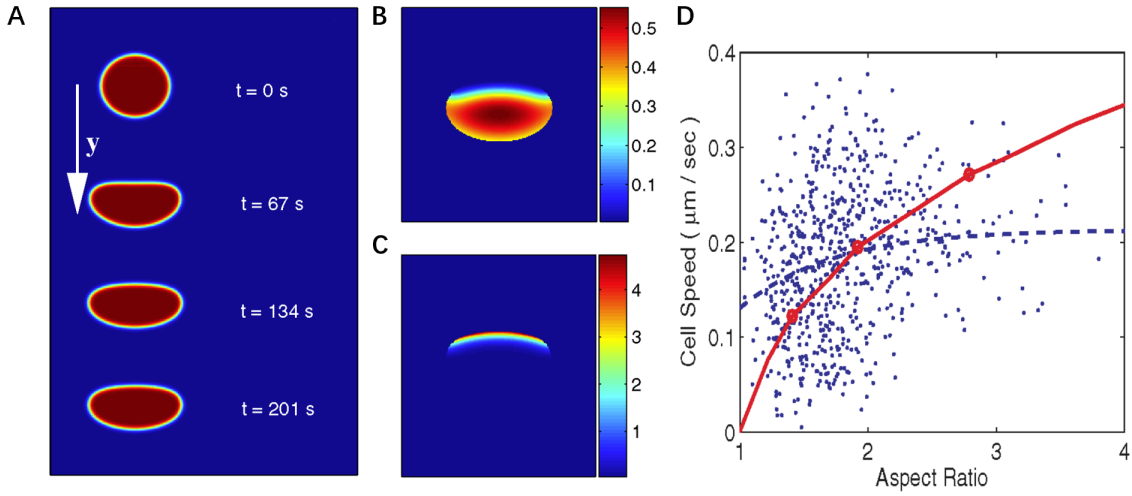


Figure 9 (A) Snapshot of a cell shape during motion. (B) Distributions of V in cell. (C) Distributions of W in cell. (D) Cell speed as a function of the aspect ratio. The solid line is simulation results and the dots are experimental results. Reprinted with permission from Ref. [99]. Copyright©2010 American Physical Society.

$$\tau \frac{\partial \phi}{\partial t} = -\kappa \left(\nabla^2 - \frac{G''}{\epsilon^2} \right) \left(\nabla^2 \phi - \frac{G'}{\epsilon^2} \right) + \gamma \left(\nabla^2 \phi - \frac{G'}{\epsilon^2} \right) - M_A \left(\int \phi d\mathbf{r} - A_0 \right) |\nabla \phi| + (\alpha V - \beta W) |\nabla \phi|, \quad (25)$$

where κ is the bending rigidity, γ is the surface tension, ϵ is a parameter controlling the width of the cell boundary. $G(\phi) = 18\phi^2(1-\phi)^2$ is a double-well potential with minima at $\phi = 0$ and $\phi = 1$, and M_A represents the cell area constraint factor. The force of protrusion and retraction correlates with the concentrations of cross-linked actin filaments (represented by V) and actin bundles (represented by W). The coefficients α and β determine the strength of these forces.

In Figure 9A, the simulation of the cell movement process is shown. Initially, the cell has an uneven distribution of W , causing it to retract from regions with higher W concentrations and initiate movement. Ultimately, the cell reaches a stationary state with a consistent speed and stable distributions of V and W (Figure 9B, C). The authors quantify different cell shapes using the aspect ratio S , defined as the ratio of cell width to length. They note that higher aspect ratios correlate with faster cell movement (Figure 9D).

Tjhung *et al.* [101] simulated the movement of three-dimensional cells. They varied the treadmilling parameter w_0 , the contractile activity ξ , and also the anchoring β , identifying four distinct cell motion states. Small β values result in a dominant finger-like structure (pseudopodium), while larger β values lead to fan-shaped geometries (lamellipodium) due to higher splay distortion at the cell's leading edge. Strong anchoring destabilizes the lamellipodium morphology, resulting in a non-motile, fried-egg-shaped cell. These simulations align with experimental studies investigating the impact of cell-substrate adhesion on cell motion [28,102].

Actin regulates cell surface molecules

Cell membrane is a highly dynamic and heterogeneous structure, which contains thousands of different phospholipids and proteins. The spatial organization of membrane surface molecules is not only determined by protein-protein interactions or lipid-phase segregation but is also driven by the ATP-fueled cortical actin

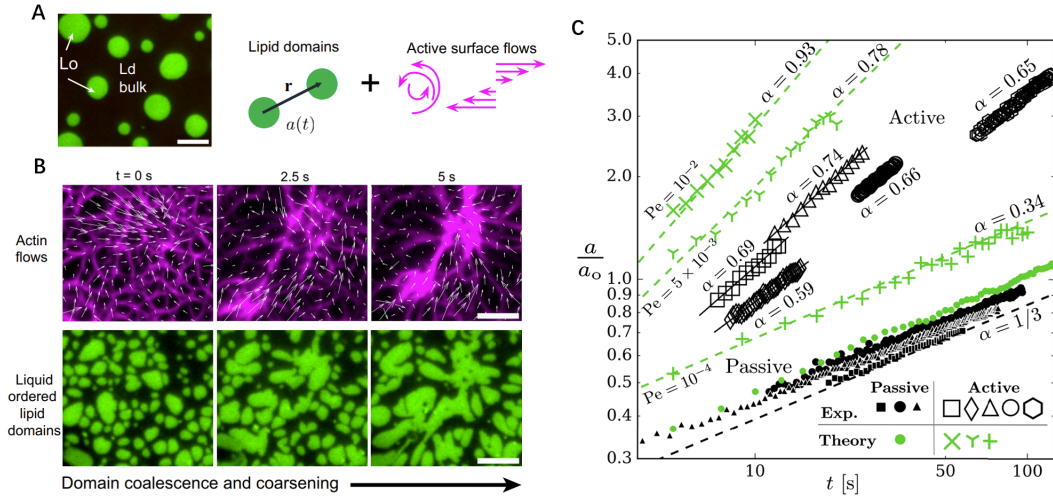


Figure 10 (A) Active flow drives lipid domain growth. (B) Timelapse images of actin (magenta) contraction and Lo lipid domains growing in time. (C) Lo lipid domain size $a(t)$ as a function of time t for experiments (black) and Cahn-Hilliard numerical calculations (green). Reprinted with permission from Ref. [105]. Copyright©2023 American Physical Society.

[103,104]. Investigating how nonequilibrium forces generated by the actin network impact the movement of phospholipids and proteins represents a compelling research question.

Arnold *et al.* [105] observed that activity-induced surface flows lead to the rapid coarsening of noncircular membrane domains that grow as $\sim t^{2/3}$ (Figure 10C). This results in a doubling of the growth exponent compared to passive coalescence. In their experimental setup, a lipid bilayer was deposited on a cushioned glass coverslip and decorated with filamentous actin (F-actin). Actin adheres to the bilayer through electrostatic attraction to positively charged DOTAP, which is abundant in the Ld phase. The Cahn-Hilliard model and Smoluchowski coagulation model were utilized to predict an α growth for domains' active convection.

Gowrishankar *et al.* [106,107] proposed that actin filaments can self-organize into asters, leading to cell surface molecules binding to dynamic actin form clusters. They assumed the presence of a static actin meshwork, with filaments dynamically combined with this framework. Subsequently, these filaments bound onto the inner leaflet of the plasma membrane through membrane-binding proteins. The authors described the active remodeling dynamics of polar actin filaments by their local concentration $c(\mathbf{r}, t)$, polar orientation $\mathbf{n}(\mathbf{r}, t)$, and apolar orientation tensor $\mathbf{Q}(\mathbf{r}, t)$. For simplicity, they only concentrated on polar filaments with polar ordering. They formulated the dynamical equations governing the motion of actin filaments:

$$\partial_t n = -\lambda(\mathbf{n} \cdot \nabla) \mathbf{n} + K_1 \nabla^2 \mathbf{n} + K_2 \nabla (\nabla \cdot \mathbf{n}) + \zeta \nabla c + \alpha \mathbf{n} - \beta |\mathbf{n}|^2 \mathbf{n} + \mathbf{f}_n, \quad (26)$$

$$\partial_t c = -\nabla \cdot \mathbf{J}_c = -\nabla \cdot (v_0 c \mathbf{n} - D_f \nabla c). \quad (27)$$

The terms on the right of the two equations represent contributions to the active forces/torques and current $\mathbf{J}_c$. Numerical solutions of the equations reveal that active actin filaments can be organized into stable aster-rich (and -poor) phases. This phenomenon arises naturally when there are adequate concentrations of treadmilling actin and active myosin. Active temperature is introduced to quantify the spatiotemporal stochasticity associated with the active dynamics. A decrease in the mean density of asters as the active temperature increased was observed.

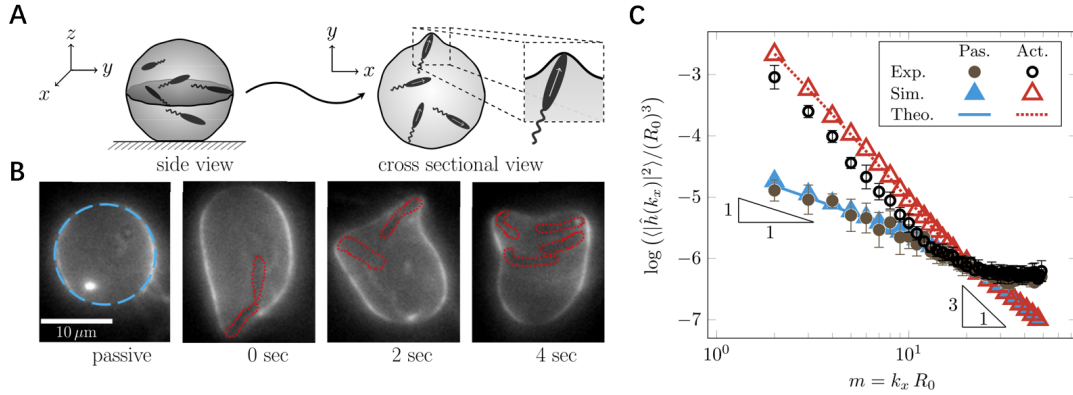


Figure 11 (A) Schematic of a GUV containing motile *Bacillus subtilis* PY79. (B) Snapshot of the experimental images. (C) Membrane height fluctuations spectrum for passive (brown, blue) and active (black, red) vesicles. Reprinted with permission from Ref. [112]. Copyright©2020 American Physical Society.

OTHER ACTIVE MATTER

Bacteria

Eukaryotic cells provide bacteria with a rich source of nutrients and a protected environment that promotes microbial replication. Bacteria enter into cells through specific receptor-ligand interactions, while host cells employ diverse strategies to destroy intracellular pathogens [108,109]. On the other hand, researchers have harnessed bacteria for therapeutic purposes, such as transforming them into drug-delivery vehicles. Din *et al.* [110] modified a bacterium with clinical significance to undergo synchronous lysis upon reaching a specific population density threshold. This process releases genetically encoded cargo for tumor treatment. Artificially designing and manipulating bacteria has broad application prospects [22,26,111].

Takatori and Sahu [112] investigated the impact of bacterial collisions with GUVs on membrane fluctuations, experimentally observing a significant increase in membrane fluctuation magnitude at low wave numbers. Analytical solutions for changes in cell membrane height in Fourier space can be obtained:

$$|\hat{h}(\mathbf{k})|^2 = \frac{k_B T}{\kappa k^4 + \lambda k^2} + \frac{N_p \tau_R}{\tau_T + \tau_R} \left(\frac{a^2 \bar{P} / R_0}{\kappa k^4 + \lambda k^2} \right)^2 e^{-a^2 k^2}, \quad (28)$$

where N_p represents the number of bacteria. It is assumed that a bacterium spends a reorientation time τ_R in contact with the membrane, followed by a travel time τ_T in the interior of the vesicle, and repeats this process. The theoretical predictions slightly overestimate experimental outcomes due to the neglect of bacteria-bacteria collisions, as illustrated in Figure 11C.

Protein pumps

Small molecules like oxygen, ethanol, and carbon dioxide can passively traverse the membrane through simple diffusion. However, for a substantial range of substances, cells must actively transport materials against electrical or concentration gradients. This process known as active transport, requires metabolic energy consumption, such as ATP or light absorption. It involves specialized membrane proteins that bind the transported substance on one side of the membrane, undergo a conformational change, releasing it on the

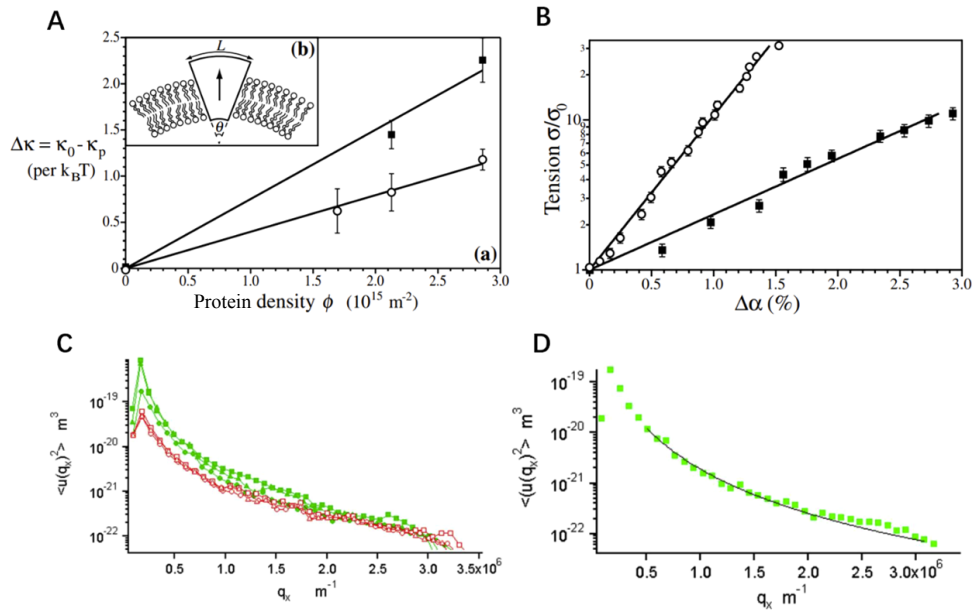


Figure 12 (A) Bending modulus difference between pure phospholipid membrane and protein-containing membrane. (B) The tension changes with relative area expansion without ATP (○) and with 1 mmol/L ATP (■). Reprinted with permission from Ref. [29]. Copyright©2005 American Physical Society. (C) Passive (red) and active (green) fluctuation spectrum of a single GUV containing BR. (D) Fit of active spectra by theory. Reprinted with permission from Ref. [115]. Copyright©2009 American Physical Society.

opposite side. The activity of these protein pumps induces a non-equilibrium state in the cell membrane, amplifying membrane fluctuations [113].

Girard *et al.* [29] selected the asymmetric sarcoplasmic reticulum Ca2-ATPase to investigate the effect of a transmembrane protein. They assessed the projected area/tension relationship through micropipette experiments [114]. Their findings revealed that the presence of protein decreases membrane bending stiffness (Figure 12A) and reduces membrane surface tension when excess area is held constant (Figure 12B). Faris *et al.* [115] used video-microscopy to analyze the fluctuation spectrum of giant vesicles containing bacteriorhodopsin (BR) pumps. Upon activation of the protein pumps, a notable increase in fluctuations in the low wavevector region was observed.

CONCLUSIONS AND PROSPECTS

A fundamental understanding of how active matter interacts with cell membranes is critical since active matter triggers a series of new phenomena beyond the framework of equilibrium physics. In this review, we provide a conceptual framework on the physiochemical mechanisms underlying active matter-biomembrane interactions. We first introduce the physical models of active matter and lipid membranes. Then, the typical phenomena emerging from various active matter are summarized, including artificial active particles, cellular cytoskeletons, bacteria, and membrane proteins. In particular, the relationship between particle activity and endocytosis efficiency, and the transformation of vesicle shape are described for artificial active particles. Moreover, we discuss how the cytoskeleton enhances membrane fluctuations, induces membrane de-

formation and motion, and reorganizes cell surface molecules. Membrane fluctuations caused by bacteria and protein pumps are also provided in analytical formula.

Compared with equilibrium physics, non-equilibrium systems are more challenging to obtain analytical solutions, and therefore often require the utilization and development of simulation or numerical techniques. Investigating the non-equilibrium phenomena generated by simplified and neat living systems will prompt us to understand life from the bottom up. The biological membrane system serves as a barrier that separates and protects the interior of a cell, which provides a valuable entrance for the investigation of these non-equilibrium systems. In addition, the cell membrane is composed of a variety of complex components, and changes in any component will produce a series of consequences and cause nontrivial changes in the dynamical behaviors. Furthermore, developing theoretical models to realize fundamental, physical analysis of such intriguing non-equilibrium systems points to an essential direction of this emerging and important field.

Funding

This work was supported by the National Science Foundation of China (22025302, 21873053 and 22202049). L.T.Y. acknowledges the financial support from the Ministry of Science and Technology of China (2022YFA1203203) and the State Key Laboratory of Chemical Engineering (SKL-ChE-23T01).

Author contributions

L.T.Y. conceived the project. X.J. and H.W. wrote the manuscript. All authors commented on the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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