



Title	Study of cellular adaptability and mechanical homeostasis
Author(s)	上田, 唯花
Citation	大阪大学, 2026, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/105510
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

The University of Osaka

Study of cellular adaptability and mechanical homeostasis

YUIKA UEDA

MARCH 2026

Study of cellular adaptability and mechanical homeostasis

A dissertation submitted to
THE GRADUATE SCHOOL OF ENGINEERING SCIENCE
THE UNIVERSITY OF OSAKA
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY IN ENGINEERING

BY

YUIKA UEDA

MARCH 2026

Abstract

Living systems possess a remarkable capacity to maintain internal stability in the face of environmental perturbations, a phenomenon known as homeostasis. Although this adaptive capability is well established at the level of whole organisms, mechanical homeostasis at the cellular scale is equally fundamental and guides the remodeling of intracellular architecture in response to physical cues. The molecular constituents that participate in these processes, including genes and proteins, have been extensively catalogued. However, the emergent physical principles that coordinate these components across hierarchical scales and give rise to adaptive behaviors, which do not appear in nonliving matter, remain poorly understood. In this study, we present multi-scale mechanistic frameworks that combine theoretical modeling, computational simulations, and quantitative experiments to clarify how adaptability emerges from interactions among three hierarchical levels: intracellular structural proteins, cytoskeletal networks, and entire cellular systems. We first identify statistical laws that determine how cells regulate the lengths of structural proteins in order to maintain mechanical homeostasis, which serves as a foundational requirement for cellular adaptability. Building on this foundation, we then demonstrate through phase transition theory and numerical simulations that the remodeling of cytoskeletal networks is driven by critical transitions that depend on the stiffness of the environment. To understand how cells preserve robust adaptability under noise, we introduce a quantitative framework based on nonequilibrium physics that separates intrinsic functional fluctuations from thermal noise. Contrary to the classical view that emphasizes microtubule-based motors, our results show that actomyosin activity is the predominant source of these functional fluctuations. We further demonstrate that cellular senescence profoundly reorganizes this fluctuation landscape. Senescent cells display an increase in the magnitude of fluctuations but a marked reduction in their diversity. This loss of heterogeneity indicates a decline in regulatory entropy, which provides a mechanistic link between altered intracellular dynamics and the diminished adaptive capacity that characterizes aging. Taken together, these findings establish a comprehensive physical mechanism that connects molecular statistics with cellular physiology and highlights the active regulation of fluctuations as an essential feature of living matter.

Contents

I Introduction	1
I -1. Cellular adaptability	1
I -2. Hierarchical organization of the actomyosin cytoskeleton	2
I -3. Tensional homeostasis	3
I -4. Equilibrium and nonequilibrium descriptions of biological systems	5
I -5. Mechanochemical regulatory mechanisms	6
I -6. Our motivation	8
II What is the requirement for cellular tensional homeostasis? – Theoretical analysis	
II -1. Summary	11
II -2. Introduction	12
II -3. Methods	12
II -3.1 Constraint function	12
II -3.2 Objective function	14
II -3.3 Lagrange multipliers	17
II -3.4 Parameters	17
II -4. Results	18
II -4.1 The relative effect of the two entropies	18
II -4.2 The effect of force in AFs	19
II -4.3 The individual effect of formation entropy	20
II -4.4 The individual effect of dispersion entropy	22
II -5. Discussion	23
II -6. Supporting Material	25
III Emergence of multiple set-points of cellular homeostatic tension	
III -1. Summary	42
III -2. Introduction	43
III -3. Methods	44
III -3.1 Model description	44
III -3.2 Parameters	46
III -4. Results	48
III -5. Discussion	49
IV Asymmetric response emerges between creation and disintegration of force-bearing subcellular structures as revealed by percolation analysis	
IV -1. Summary	51
IV -2. Introduction	52
IV -3. Model Analysis	54

IV-3.1 Distribution of AFs	55
IV-3.2 Analysis of SFs, FAs, and actin meshwork	55
IV-3.3 Binding and unbinding of AFs	55
IV-3.4 Monte-Carlo simulation	56
IV-3.5 Determination of the number of AFs	56
IV-3.6 Minimal model analysis	57
IV-4. Results	58
IV-4.1 The effect of cellular tension generation potential on percolation	58
IV-4.2 The effect of AF length distribution on percolation	59
IV-4.3 Adaptive response to change in cellular tension generation potential	62
IV-5. Discussion	63
IV-6. Appendix	65
IV-7. Supporting Material	68
V Adaptive flexibility of cells through nonequilibrium entropy production	
V-1. Summary	69
V-2. Introduction	70
V-3. Theory and Methods	71
V-3.1 Entropy production	71
V-3.2 Maximum binding energy	74
V-3.3 Probability distribution of contractile unit structures	74
V-3.4 Quantification of contractile unit length	76
V-4. Results	76
V-4.1 Contractile unit distributions and their biophysical implications	76
V-4.2 Contractile unit organization and remodeling under energetic and environmental constraints	78
V-5. Discussion	80
VI Hierarchical phase transitions as mechanical checkpoints of intracellular organization	
VI-1. Summary	84
VI-2. Introduction	85
VI-3. Model overview	87
VI-3.1 Phase transition 1 – Formation of stress fibers as a physical phase transition	87
VI-3.2 Phase Transition II – Directional alignment of stress fibers as a physical phase transition	93
VI-3.3 Phase Transition III – Lateral aggregation of aligned stress fibers as a physical phase transition	96
VI-4. Experimental verification of cellular phase transition induced by the stiffness	100

VI-5. Discussion	102
VI-6. Supporting Information	106
VII Revealing cellular functional fluctuations through nonequilibrium physics	
VII-1. Summary	110
VII-2. Introduction	111
VII-3. Material and methods	112
VII-3.1 Cell culture and treatments	112
VII-3.2 Objective function	113
VII-3.3 Fluorescence correlation spectroscopy (FCS)	113
VII-3.4 Nonequilibrium model for analyzing nonthermal forces	114
VII-3.5 Analysis of diffusion heterogeneity	116
VII-4. Results	118
VII-4.1 Modulation of myosin activity directly impacts cellular active fluctuations	118
VII-4.2 Kinesin and dynein are not responsible for the fluctuations	119
VII-4.3 Kinesin and dynein are not responsible for the fluctuations	120
VII-4.4 Combined inhibition of myosin activity and actin polymerization	120
VII-4.5 Cellular senescence leads to significantly increase active fluctuation	121
VII-4.6 Smaller particles experience stronger fluctuations and respond more sensitively to actomyosin modulation	121
VII-4.7 Cellular senescence simplifies diffusion mode	123
VII-4.8 Impact of myosin activities on diffusion modes	124
VII-5. Discussion	126
VIII Conclusion	128
References	130

I Introduction

I -1. Cellular adaptability

Cellular adaptability is a key fundamental and defining characteristic of living systems (I .1,2). Unlike static objects or simple reactive systems, biological entities exist in a state of continuous flux, maintaining their systems through the active sensing and processing of a vast array of internal and external environmental cues. This capacity to adaptively modify internal architecture, morphology, and biological functions is not merely a passive response to external forces, but a highly coordinated and sophisticated regulatory process. It allows cells to remain robust and functional across diverse physiological regimes, ensuring survival in the face of fluctuating conditions that characterize the microscopic world. This biological flexibility stands in stark contrast to the design principles of artificial machines. In the field of engineering, systems such as automobiles or robotic devices are typically built upon a pre-programmed logic, where responses to environmental changes are limited to specific parameters and scenarios anticipated during the design and manufacturing process. When such an artificial system encounters a situation outside its predefined specifications, it often fails because its structural and functional rules are rigid and lack the capacity for self-reorganization. In contrast, living cells exhibit a versatile flexibility that transcends the limitations of fixed instructions. Biological systems handle novel situations and stochastic noise not by adhering to a rigid blueprint, but by utilizing a dynamic remodeling of their subcellular constituents. This adaptability enables cells to navigate unpredictable environments and maintain operational stability.

The implications of cellular adaptability extend to the most essential processes of life, governing the spatiotemporal transitions required for tissue development and maintenance (I .3). For instance, during cell proliferation, the stepwise maturation of intracellular structures acts as a series of mechanical checkpoints that align the physical state of the cytoplasm with the timing required for proper cell cycle progression (I .4,5). Furthermore, during differentiation, mechanical cues from the extracellular matrix guide distinct intracellular orders that influence cell fate and lineage specification (I .6,7). The biological importance of studying these adaptive mechanisms is further highlighted by the severe consequences of their failure. A decline in adaptive capacity or a malfunction in homeostatic regulation is frequently the root cause of various pathological states. When cells fail to maintain stable mechanical phases or properly reorganize their cytoskeleton under stress, they may enter pro-inflammatory states that lead to the progression of diseases such as atherosclerosis(I .8,9). In the context of cancer, the loss of normal adaptive constraints allows for aberrant mechanical behaviors and invasive transitions (I .10). Additionally, the

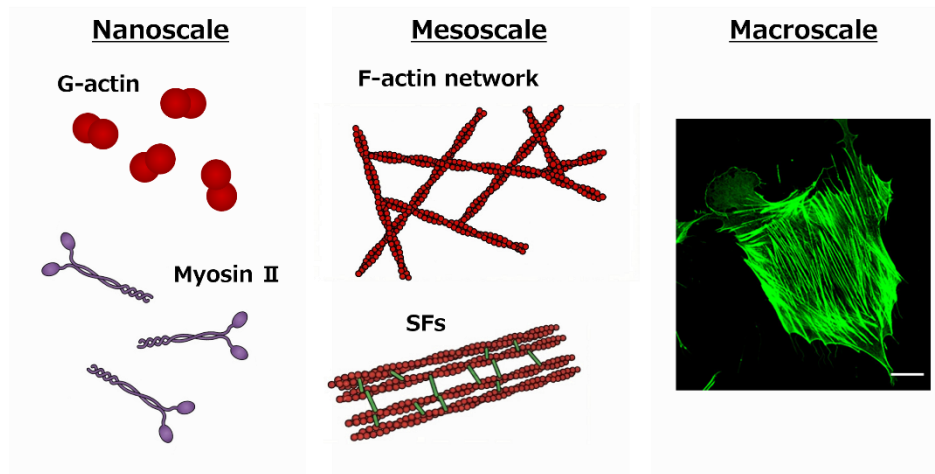


Fig. I -1 Hierarchical organization of the actomyosin cytoskeleton.

The cellular mechanical architecture spans multiple length scales, emerging from the self-organization of nanometer-sized molecular building blocks (G-actin and myosin II) into mesoscopic structures (F-actin networks and stress fibers). These local interactions integrate to determine the global mechanical behavior at the macroscopic cellular level.

process of cellular senescence has been shown to reshape the fluctuation landscape of the cytoplasm, leading to a reduction in regulatory flexibility and a diminished ability to respond to environmental demands (I .11). Therefore, understanding the principles, both biochemical and physical, that govern how cells execute precise adaptive responses amidst stochastic noise remains one of the most compelling challenges.

I -2. Hierarchical organization of the actomyosin cytoskeleton

The structural integrity and adaptive capacity of living cells arise from a sophisticated hierarchical organization that spans multiple length scales, from nanometer-sized molecules to macroscopic cellular structures (I .12). In classical engineering, artificial machines are constructed as assemblies of discrete, pre-designed parts. The functionality of these systems is typically determined by a rigid blueprint, where each component is manufactured to meet specific tolerances and then integrated into a fixed architecture. In such systems, the relationship between individual parts and the whole is static, and any deviation from the original design often leads to a loss of performance or mechanical failure. In contrast, cellular architecture is not a product of external assembly but emerges through a process of spontaneous self-organization. This self-organizing nature allows for an integrated hierarchy where macroscopic order is continuously maintained through the collective behavior of its microscopic constituents. Unlike engineered structures, where the properties of the whole

are a simple summation of its parts, the cell functions as a multi-scale system where the interactions between different levels are dynamic and reciprocal. This ensures that the cell can maintain structural coherence while remaining flexible enough to reorganize its entire framework in response to environmental changes.

At the core of this cellular mechanical hierarchy is the cytoskeleton, a dynamic network of biopolymers that provides both mechanical stability and the machinery for force generation (I .13,14). The actomyosin system, in particular, exemplifies how local molecular interactions propagate across scales to determine global cellular behavior. At the nanometer scale, G-actin monomers polymerize into filamentous F-actin (I .15,16). These filaments then interact with various actin-binding proteins, such as cross-linkers and molecular motors like myosin II, to form diverse architectures including branched networks, isotropic meshes, and highly aligned bundles known as stress fibers. Unlike the static joints in man-made trusses, the connections within the cytoskeletal network are transient and governed by specific binding energies, such as “turnover”, allowing the structure to continuously remodel itself while maintaining mechanical continuity.

The interaction between these hierarchical levels is characterized by a high degree of mechanical coupling. For instance, the contractile forces generated by myosin motors at the molecular level are integrated across the actin network to produce macroscopic tension at the cellular scale (Fig. I -1). This cross-scale communication is not unidirectional; the macroscopic boundary conditions, such as the stiffness of the extracellular matrix, feed back to influence the arrangement and stability of the microscopic filaments. In stress fibers, this is evidenced by the periodic organization of α -actinin and myosin, forming sarcomere-like contractile units that allow the cell to behave as a coherent mechanical entity.

I -3. Tensional homeostasis

Homeostasis is one of the most essential functions unique to living systems, enabling them to maintain a stable internal state despite continuous fluctuations in the external environment. This concept, originally described as the maintenance of the "milieu intérieur (internal environment)" allows biological organisms to preserve physiological variables, such as temperature, pH, and nutrient concentrations, within narrow functional ranges (I .17). At the cellular level, this principle of stability extends beyond biochemical parameters to include the mechanical state of the cell, a phenomenon known as tensional homeostasis (I .18–20). Tensional homeostasis refers to the active process by which nonmuscle mesenchymal cells, such as fibroblasts and endothelial cells, maintain a constant level of intracellular tension and

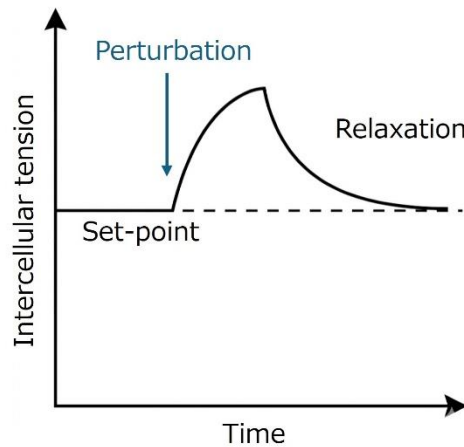


Fig. I -2 Schematic representation of cellular tensional homeostasis.

Upon mechanical perturbation, intracellular tension transiently deviates from its baseline but is actively regulated to return to a specific homeostatic set-point. This relaxation process is mediated by cytoskeletal remodeling, ensuring mechanical stability and consistent sensitivity to environmental cues.

associated signaling activities (Fig. I -2). While passive materials simply remain deformed or fail under external loads, living cells sense mechanical perturbations, such as substrate stretching or fluid shear, and trigger structural remodeling to relax these stresses back to a basal set-point. This mechanical stability provides a consistent baseline, or "mechanical quietness," which is necessary for cells to accurately detect and respond to new environmental cues without being overwhelmed by persistent noise.

The primary machinery responsible for this homeostatic control is the actomyosin cytoskeleton. The internal tension is sustained through a delicate balance between highly organized contractile bundles, known as stress fibers (SFs), and a more stochastic, directionless network of actin filaments (AFs) that pervades the cytoplasm. SFs serve as the major force-bearing elements, particularly in well-spread cells, and can undergo maturation to reach higher tension levels. For instance, the recruitment of α -smooth muscle actin (α -SMA) to preexisting bundles allows SFs to reinforce themselves, leading to the emergence of secondary, "superature" homeostatic set-points (I .21,22). This transition demonstrates that tensional homeostasis is not a rigid state but an adaptive one that can shift depending on the mechanical demands of the environment, such as increased substrate stiffness.

The physiological importance of maintaining tensional homeostasis is underscored by its role in regulating biochemical signaling. Persistent mechanical stress is a known trigger for pro-inflammatory and growth-related pathways, such as the MAPK cascade (I .23). By actively relaxing internal tension, cells prevent the chronic activation of these signals. Consequently, a failure in this homeostatic regulation is a hallmark of various diseases. For example, the loss of tensional set-points is closely linked to the progression of atherosclerosis, chronic inflammation, and the invasive transformation of cancer cells (I .8,9). Thus, understanding the principles that govern the stability and transitions of these homeostatic states is vital for both fundamental biophysics and the development of new clinical perspectives.

I -4. Equilibrium and nonequilibrium descriptions of biological systems

Living systems are fundamentally characterized by their departure from thermodynamic equilibrium. Unlike isolated systems that inevitably evolve toward a state of maximum entropy, cells are open systems that continuously exchange energy and matter with their surroundings. This nonequilibrium nature is primarily driven by the constant flux of chemical energy, most notably through the hydrolysis of adenosine triphosphate (ATP) (I .24). This energy consumption powers molecular motors, maintains ion gradients, and drives the active reorganization of the cytoskeleton, generating active stresses that cannot be explained by thermal fluctuations alone.

Despite this inherent nonequilibrium character, the framework of equilibrium statistical mechanics remains an exceptionally powerful tool for deciphering the complexity of living system (I .25). The application of concepts such as the Boltzmann distribution and free energy minimization has enabled the successful modeling of a wide array of biological phenomena across multiple scales. A prominent example is the quantitative description of gene regulatory networks and cell fate transitions. "Waddington landscape" can be quantified using statistical mechanical principles to map the stochastic paths of development and differentiation (I .24). In these contexts, treating complex interactions between DNA, proteins, and RNA as being in "effective equilibrium" allows for the characterization of quasi-stable states that define cellular identity (I .26–28).

Simultaneously, the limitations of equilibrium descriptions have necessitated the

development of nonequilibrium physical frameworks, such as active matter theory and stochastic thermodynamics. These approaches are essential for understanding phenomena that explicitly break time-reversal symmetry and violate the fluctuation-dissipation theorem. For instance, research on the mesoscopic fluctuations within the cytoplasm has revealed that biological systems operate far from equilibrium to enhance sensitivity and speed in cellular responses (1.29). These nonequilibrium features are not merely biological noise but are functional elements that allow cells to execute precise mechanical tasks amidst stochasticity.

In unraveling complex cellular phenomena, we argue that it is insufficient to simply conclude that life is a nonequilibrium system. Rather, a critical challenge lies in identifying the extent to which a biological process can be understood through the lens of equilibrium physics. By clarifying where equilibrium descriptions fail, we can precisely isolate and define the specific roles played by nonequilibrium dynamics. We contend that integrating both equilibrium and nonequilibrium perspectives is essential for decoding the hierarchy of physical principles that govern living systems. This dual approach not only provides a more profound understanding of cellular adaptability but also contributes to expanding the boundaries between biology and physics, offering a unified framework to explore the origins of biological order and its breakdown in disease or senescence.

I -5. Mechanochemical regulatory mechanisms

Biochemical signaling constitutes the internal control circuitry of the cell, functioning as a sophisticated information-processing system that interprets environmental cues to coordinate appropriate biological responses. While these pathways can be likened to engineered electronic circuits in their ability to relay and amplify signals, they possess a unique complexity characterized by an inherent balance between precision and stochasticity. Unlike the rigid, deterministic logic of man-made devices, cellular signaling involves significant fluctuations and noise, which are not merely errors but essential features that enable diverse and flexible outputs. This interplay between accuracy and inaccuracy allows the cell to navigate unpredictable environments, providing the regulatory plasticity required for versatile adaptation across various scales.

Within this complex circuitry, the Rho family of small GTPases serves as a master

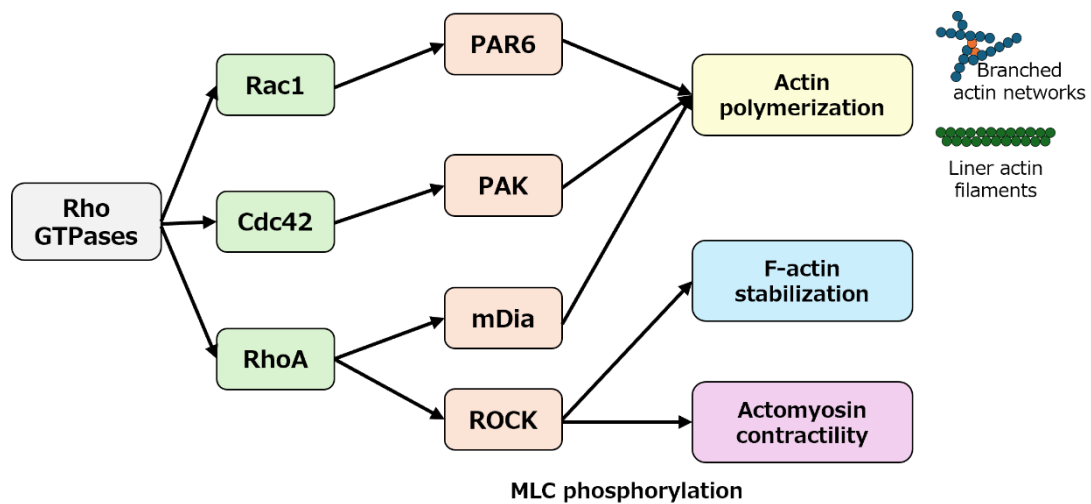


Fig. I -3 Mechanochemical regulatory circuits governed by Rho GTPases.

The Rho family of GTPases acts as a master regulatory hub coordinating cytoskeletal dynamics. Rac1 and Cdc42 drive actin nucleation and polymerization through the Arp2/3 complex and formins (structural supply), while the RhoA/ROCK pathway governs actomyosin contractility via MLC phosphorylation (force generation).

regulatory hub that integrates diverse upstream signals (Fig. I). These proteins act as molecular switches that cycle between an inactive GDP-bound state and an active GTP-bound state, a process strictly regulated by guanine nucleotide exchange factors (GEFs). Specifically, GEFs facilitate the activation of RhoA by promoting the exchange of GDP for GTP in response to mechanical or chemical stimuli. Once activated, RhoA-GTP interacts with multiple downstream effectors to trigger a cascade of events that govern both the structural supply and the contractile state of the actin cytoskeleton (I .30,31).

Signaling through Rac1 and Cdc42 activates the Arp2/3 complex via WAVE and WASP, respectively, promoting the formation of branched actin networks. In contrast, RhoA signaling activates formins such as mDia, which nucleate long, linear actin filaments. These filaments serve as the structural backbone for SFs. The activity of mDia downstream of RhoA ensures a steady supply of linear actin polymers that can be bundled into higher-order contractile structures (I .32,33). Furthermore, the maintenance of a flexible and responsive cytoskeleton requires the continuous turnover of actin filaments, a process mediated by actin-binding proteins such as cofilin. By preferentially binding to ADP-actin and severing older filaments, cofilin accelerates filament disassembly and promotes depolymerization, thereby enabling the efficient recycling of actin monomers into the cytoplasmic pool. This dynamic

turnover allows the cell to rapidly redeploy its cytoskeletal resources and prevents the actin network from becoming a static, rigid lattice (I .34,35).

The generation of mechanical force within the cell is a tightly regulated process that depends on the activation of nonmuscle myosin II, primarily orchestrated by Rho-associated coiled-coil-containing protein kinase (ROCK). As a serine/threonine kinase and a major downstream effector of RhoA, ROCK functions as a molecular integrator that translates biochemical signals into mechanical tension. This regulation is achieved through a dual mechanism that ensures robust and sustained contractility. First, ROCK directly phosphorylates the regulatory myosin light chain (MLC) at serine 19, enhancing the actin-activated ATPase activity of myosin II and promoting the assembly of myosin into bipolar filaments. Second, ROCK phosphorylates the myosin-binding subunit of myosin light chain phosphatase (MYPT1), thereby inhibiting phosphatase activity. This inhibition prevents the dephosphorylation of MLC, a process referred to as calcium sensitization, which allows cells to maintain high levels of contractile tension even under basal intracellular calcium concentrations. Through this multifaceted regulation, ROCK determines the magnitude of active stress within the actomyosin network, effectively bridging transient biochemical signaling events and persistent mechanical states of the cytoskeleton (I .36–38).

However, while these biochemical signaling pathways define the accessible molecular states and the turnover rates of cytoskeletal components, they alone cannot fully account for the emergence of complex spatial orders. The resulting structural organization is fundamentally governed by physical principles and emergent properties. Once upstream regulators, including guanine nucleotide exchange factors (GEFs), RhoA, and ROCK, establish key physical parameters such as active stress, binding affinities, and actin monomer concentration, the system spontaneously evolves toward a mechanically constrained steady state dictated by fundamental physical principles. In this unified framework, biochemical signaling determines the timing and permissibility of cytoskeletal reorganization by tuning the control parameters, whereas physical principles ensure the robustness and reproducibility of the resulting structures.

I -6. Our motivation

The primary motivation of our work is to elucidate the fundamental mechanisms that define

livingness, the quintessential essence of biological systems. This essence is most strikingly manifested in the cellular remarkable adaptive capacity and its ability to maintain tensional homeostasis. As explored in the preceding sections, cells sense their ever-changing environments and flexibly reorganize their internal architecture to preserve stability. However, the sheer complexity of these processes, where microscopic fluctuations give rise to macroscopic order, presents a significant challenge to our understanding.

Contemporary research has largely focused on identifying specific molecular components and signaling pathways. With the advancement of measurement technologies, it has become possible to acquire large-scale datasets that map these constituents with high precision. However, the mere identification of elements is insufficient to explain how a cell, which is far more than a simple assembly of its parts, generates sophisticated emergent functions. Life remains teeming with unexplained phenomena, and even at this moment, discoveries that defy our current imagination may be occurring. For such a dynamic subject, the underlying physical rules may not be fully explainable by established physical theories alone. By bridging the gap between biochemistry and physics, this work endeavors to confront these challenging questions, aiming to deepen our understanding of biological systems and contribute to the pioneering of new academic frontiers.

This dissertation is organized as follows to provide a multifaceted exploration of cellular adaptability and homeostasis. In chapter II, the theoretical requirements for maintaining cellular tensional homeostasis are investigated. By focusing on the length distribution of actin filaments, a constrained optimization problem is formulated to determine the optimal architectural configuration required for sustained tension by the framework of thermodynamics and statistical mechanics. In chapter III, the emergence of multiple homeostatic set-points is explored. The recruitment of α -SMA and the resulting reinforcement of stress fibers are modeled to demonstrate how different stable tension states can arise depending on the mechanical environment. In chapter IV, percolation theory is employed to analyze the dynamics of cytoskeletal remodeling. The physical origins of the observed asymmetry between the creation and disintegration of force-bearing structures are elucidated through the lens of network connectivity. In chapter V, the adaptive flexibility of cells is considered from the perspective of nonequilibrium entropy production. By quantifying structural randomness, it is shown how the inherent variability in sarcomere-like assemblies lowers energy barriers for remodeling, enabling flexible responses to environmental demands. In chapter VI, the process of intracellular organization depends on the stiffness of

surrounding environment is treated as a series of hierarchical phase transitions. A statistical-mechanical framework is developed to describe how mechanical cues guide distinct structural orders through threshold-dependent transitions. In chapter VII, functional fluctuations within the cell are revealed through nonequilibrium physics. By distinguishing nonthermal fluctuations from thermal noise, this study demonstrates how cellular senescence reshapes the fluctuation landscape and diminishes regulatory flexibility.

Finally, while the approaches presented here explain only a fraction of the vast phenomena of cellular adaptability and homeostasis, we offer a new lens through which to view the cell. By reinterpreting biological events through the perspective of physical inevitability, this work seeks to provide a principled foundation for understanding the dynamic nature of life.

II What is the requirement for cellular tensional homeostasis? – Theoretical analysis

II -1. Summary

Tensional homeostasis is a cellular process whereby nonmuscle cells such as fibroblasts keep a constant level of intracellular tension and signaling activities. Cells are allowed thanks to tensional homeostasis to adapt to mechanical stress, but the detailed mechanism remains unclear. Here we address from a theoretical point of view what is required for maintaining cellular tensional homeostasis. A constrained optimization problem is formulated to analytically determine the probability function of the length of individual actin filaments (AFs) responsible for sustaining cellular tension. An objective function composed of two entropic quantities measuring the extent of formation and dispersion of AFs within cells is optimized under two constraint functions dictating a constant amount of actin molecules and tension that are arguably the two most salient features of tensional homeostasis. We then derive a specific probability function of AFs that is qualitatively consistent with previous experimental observations, in which short AF populations preferably appear. Regarding the underlying mechanism, our analyses suggest that the constraint for keeping the constant tension level makes long AF populations smaller in number because long AFs have a higher chance to be involved in bearing larger forces. The specific length distribution of AFs is thus required for achieving the constrained objectives, by which individual cells are endowed with the ability to stably maintain a homeostatic tension throughout the cell, thereby potentially allowing cells to locally detect deviation in the tension, keep resulting biological functions, and hence enable subsequent adaptation to mechanical stress. Although minimal essential factors are included given the actual complexity of cells, our approach would provide a theoretical basis for understanding complicated homeostatic and adaptive behavior of the cell.

II -2. Introduction

Homeostasis is responsible for the maintenance of living systems, in which their internal and external elements exhibit complex interactions. In addition to the well-known presence at the organismal level, homeostasis has actually been recognized to be of importance at the individual cellular level. Tensional homeostasis is one of the known homeostatic mechanisms, by which nonmuscle cells such as fibroblasts, endothelial cells, and cancer cells are allowed to sustain a constant level of tension through turnover of their constituent proteins (II . 1–4). Cellular tension is thus relaxed to the basal level over time even upon mechanical stress that disturbs intracellular tensional balance (II . 5, 6). Mechanical stresses such as cell stretch and fluid shear stress are known to trigger signaling pathways, and importantly such signals, typically associated with cell growth and inflammation, are suppressed concomitantly with the recovery of cellular tension. Thus, tensional homeostasis plays crucial roles in functional adaptation of nonmuscle cells to mechanical environment and consequently has been implicated in many diseases such as atherosclerosis (II . 7–12); however, the detailed mechanism remains largely unknown.

Given that living systems are comprised of many structural/functional “layers” that are highly complicated but closely associated with each other, deciphering the homeostasis at the cell layer level will contribute to deeper understanding of the whole homeostasis and resulting adaptive behavior at the organismal level. With this motivation, we must be in a position to tackle this challenging but exciting topic, namely the mechanism underlying cellular tensional homeostasis. Here we discuss physical aspects of the mechanism from a theoretical point of view bridging subcellular and cellular layers. Specifically, we address what is the requirement for actin filaments (AFs), a major cytoskeletal component that undergoes turnover, to allow for sustaining a constant level of tension over the cytoplasm. A constrained optimization problem is then formulated to describe the probability function of the length of individual AFs. Our result yielded a probability function with no local maxima unlike the Gaussian distribution but consistent with experimental observations, describing how the homeostasis at the cellular level is achieved on the underlying basis of the molecular level.

II -3. Methods

II -3.1 Constraint function

We aim to derive the length distribution of the individual AFs in cells according to the method of Lagrange multipliers. The number of actin monomers that constitute an AF with a length of l_i is n_i where i is a positive integer. We normalize the length (effective diameter) of individual actin monomers to be unity, and AF length is proportional to the number of constituent actin monomers; accordingly, $l_{i+1} = l_i + l_1 = l_i + 1$. We consider two equality

constraints. First, we assume that the total number of actin monomers within the cell, N , remains constant in accordance with the fact that actin is commonly used as a "housekeeping" protein in Western blotting analysis, and is thus described as

$$N = \sum n_i. \quad (\text{II} - 1)$$

The probability that actin monomers belong to a population of AFs with a length of l_i is described by

$$p_i = \frac{n_i}{N} \quad (\text{II} - 2)$$

where the sum of the probabilities is unity given Eq. (II-1).

We impose another equality constraint to capture the essence of tensional homeostasis. Cells are known to sustain preexisting tension of a similar magnitude over the cytoplasm, which is borne by the meshwork of AFs undergoing interactions with myosin II (II. 12–16). These observations suggest that the expected value of the tension in individual AFs of the meshwork is constant. The expected value F is then described as an equality constraint by

$$F = \sum p_i f_i \quad (\text{II} - 3)$$

where f_i is a tension borne by an AF with a length of l_i . The tension in the AF meshwork, which finally gives rise to tensional homeostasis, is generated by the dynamic interaction with myosin II. To bear the tension, individual AFs must be long in length enough to make contact with other surrounding AFs to form a meshwork (II. 17–20). Otherwise, the tension is not transmitted across the cytoplasm even with the presence of myosin II activity. In other words, longer AFs would more frequently bear a level of tension compared to shorter ones. Besides, because there is an upper limit of the myosin II activity, individual AFs would sustain up to a certain level of forces. To capture these features, the relationship between f_i and l_i is described by a sigmoid function

$$f_i = \frac{f_0}{1 + \exp(l_0 - l_i)} \quad (\text{II} - 4)$$

where f_0 and l_0 represent the maximum force and the length with the maximum slope of

force, respectively (Supporting Material Fig. II-S1).

II -3.2 Objective function

What is appropriate for the objective function of the constrained problem that characterizes tensional homeostasis? AFs, responsible for bearing cellular tension, are subject to turnover, by which the constituent monomers are polymerized and/or depolymerized. This continuous turnover allows intracellular AFs to fluctuate in length, and from an entropic perspective they are supposed to take as many states as possible at equilibrium. Thus, the probability distribution of AF length would be partly determined to increase a specific entropy that dictates formation of AFs with extensive fluctuations and is here described by

$$S_f = k_b \ln W \quad (\text{II} - 5)$$

where k_b and W denote the Boltzmann constant and the number of states, respectively. Under the principle of equal a priori probabilities of each AF length, the number of states of actin molecules is described by

$$W = \frac{N!}{n_1! n_2! \cdots n_m!} \quad (\text{II} - 6)$$

where m is a group of AFs with the longest length. Using Stirling's approximation and Eqs. (II-1) and (II-2), Eq. (II-5) is rewritten as

$$S_f = -k_b N \sum p_i \ln p_i \quad (\text{II} - 7)$$

(see Supporting Material for derivation). We adopt this formation-associated entropy as one of the objective functions.

As already discussed above, AFs must be distributed throughout the cytoplasm to form a meshwork where preexisting tension is sustained. As another objective function, we then consider an entropy related to the spatial distribution of AFs given that extensive dispersion

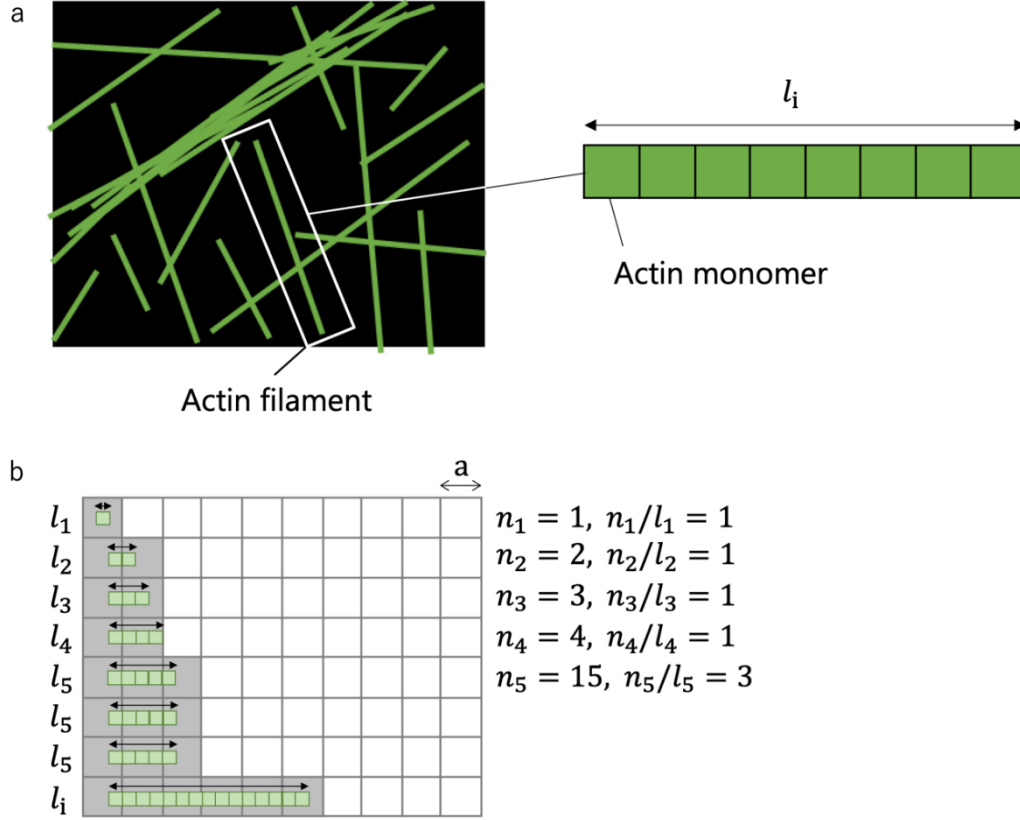


Figure II-1 Schematic to explain S_d . (a) Schematic of the cytoplasm (black) where AFs (green) assumed to be formed by one-dimensional association of actin monomers to have a length of l_i are spatially distributed. (b) The cytoplasm is divided by compartments with a length of a . AFs (green) occupy some of the compartments (gray). The arrows in the cell show the length of AFs. An example of l_i comprising of 15 actin monomers is shown. See Supporting Material for detailed explanation.

of the AFs would allow the cells to be stabilized. Actin-based structures include directional architectures such as stress fibers that align in a specific direction, but the cytoplasmic “directionless” AF meshwork is more commonly present in nonmuscle cells (II. 17–20). The presence of the random meshwork is supposed to allow the cells to stabilize the whole cell architecture (II. 13, 14) as well as to sense and respond to mechanical cues arising from arbitrary directions (II. 11, 21). To evaluate this dispersion-associated entropy, we analyze the extent of intracellular regions where AFs are dispersed depending on their length; here, if all actin monomers form a single long AF, the region of the cell to be occupied, mechanically supported, and communicated by the AF will be limited in space; meanwhile, if actin monomers instead form many short AFs, they overall would be able to cover a large region

upon dispersion.

For the analysis, the cytoplasm is modeled to consist of compartments, the number of which is sufficiently larger than that of AFs (Fig. II -1). For each AF, the degree of intracellular dispersion is evaluated by counting the maximum number of compartments to which the AF can be distributed. The number of the compartments that an AF can occupy is partly determined by its length l_i and the compartment size a . We do not consider deformation of AFs for simplicity, and thus only one-dimensional arrangement is analyzed. The maximum number of compartments that can be covered by all the existing AFs is expressed by the following equation (see Supporting Material for detailed explanation):

$$G = \frac{n_1}{l_1} + 2 \sum_{i=2}^{1+a} \frac{n_i}{l_i} + 3 \sum_{j=2+a}^{1+2a} \frac{n_j}{l_j} + \dots = \frac{n_1}{l_1} + \sum_{j=1}^{\frac{m-2}{a}+1} \sum_{i=2+a(j-1)}^{1+aj} (j+1) \frac{n_i}{l_i}. \quad (\text{II} - 8)$$

As n_i/l_i denotes the number of individual AFs of length l_i , we can compute using Eq. (II - 8) the contribution of each length population to G , which we denote as A_i . In other words, Eq. (II -8) is alternatively described as

$$G = \sum A_i \frac{n_i}{l_i}. \quad (\text{II} - 9)$$

G measures the extent of intracellular dispersion of AFs, which is identical in concept to entropy. To evaluate its effect on the stabilization of the cell, we introduce a constant k'_b having the same unit as that of the Boltzmann constant k_b to define dispersion entropy

$$S_d = k'_b \sum N \frac{A_i}{l_i} p_i. \quad (\text{II} - 10)$$

Given that formation entropy S_f and dispersion entropy S_d are independent of each other, we describe the overall objective function or the overall entropy of the system S as the sum of them, i.e.,

$$S = -k_b N \sum p_i \ln p_i + k'_b \sum N \frac{A_i}{l_i} p_i. \quad (\text{II} - 11)$$

Note that to be exact the overall entropy characterizing highly complex systems like cells would contain numerous entropy-associated factors with a form of the product/sum of them.

For example, the product of two hierarchical entropies may be needed to appropriately characterize the overall system if we look at the details of the larger factor. The entropy in such a complex system will thus be practically determined by the final coarseness of the model. The sum, on the other hand, describes independent factors; but, if the effect is small enough, its contribution would be ignored. In the present case, we have considered the two independent entropies, i.e., how AFs are created (S_f) and how AFs are dispersed (S_d), both of which are supposed to be the two most essential factors in the AF-based cellular tensional homeostasis; and, their relative contributions are evaluated by changing the ratio of effective Boltzmann constants k_b and k'_b , each of which relates the number of their respective possible microstates to the macroscopic cell state.

II -3.3 Lagrange multipliers

Introducing Lagrange multipliers α and β for the constraint function Eq. (II -1) (divided by N and substituted to Eq. (II -2)) and Eq. (II -3), respectively, the partial derivative of the Lagrangian function

$$\mathcal{L} = S - \alpha \left(\sum p_i f_i - F \right) - \beta \left(\sum p_i - 1 \right) \quad (\text{II} - 12)$$

is taken with respect to p_i to find a specific distribution of AFs that maximizes the objective function of Eq. (II -12) under the constraints. Thus, the probability function of AFs that stabilizes the cell system at equilibrium is obtained by the method of Lagrange multipliers together with thermodynamic considerations to be

$$p_i = \frac{\exp \left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i \right) / k_b \right]}{\sum \exp \left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i \right) / k_b \right]} \quad (\text{II} - 13)$$

where V , λ , and T denote the volume, preexisting strain, and temperature of the cell, respectively (see Supporting Material for derivation).

II -3.4 Parameters

The following parameters are used unless otherwise stated: $N = 1,000$, $k_b = 1.38 \times 10^{-23}$ J/K, $T = 309.5$ K, $V = 6 \times 10^{-16}$ m³, $\lambda = 0.2$, $l_1 = 1$, $a = 10$, $f_0 = 10^{-12}$ N, and $l_0 = 80$. Here, the values for T and V were chosen from a typical temperature and cell volume, respectively;

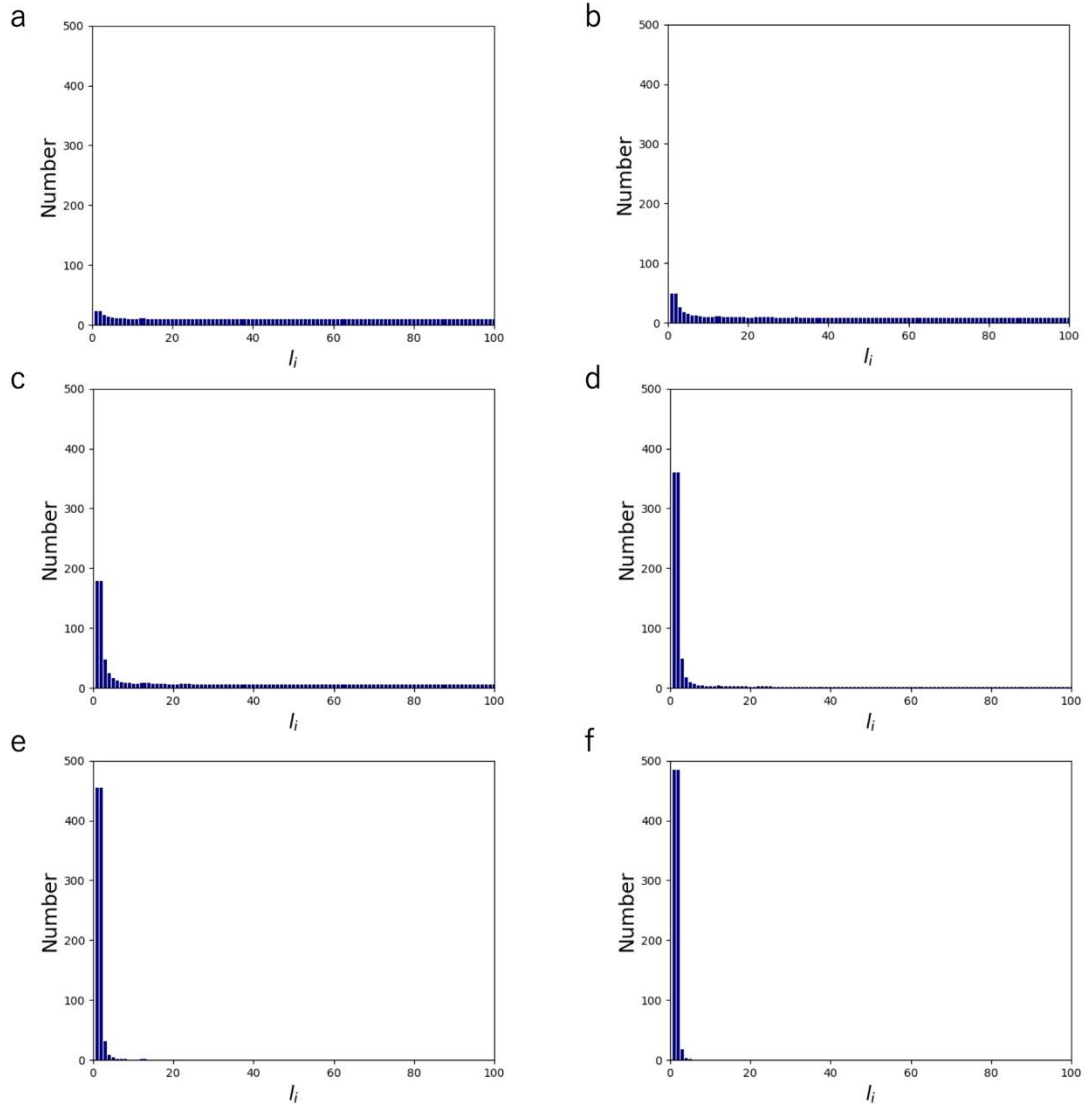


Figure II-2 Length distribution of AFs comprising of $N = 1,000$ monomers at $k_b'/k_b = 1$ (a), $k_b'/k_b = 2$ (b), $k_b'/k_b = 4$ (c), $k_b'/k_b = 6$ (d), $k_b'/k_b = 8$ (e), and $k_b'/k_b = 10$ (f). With a larger k_b'/k_b , populations of AFs with shorter lengths increase in the cell.

the value for N was chosen arbitrarily; the values for the spatial parameters a and l_0 are arbitrarily chosen and are normalized by l_1 ; the value for k_b is taken from the Boltzmann constant; and, the values for λ and f_0 are chosen from in vivo (II.12–15) and in vitro data (II.22), respectively.

II-4. Results

II-4.1 The relative effect of the two entropies

To capture the basic feature of the appearance probability of actin molecules p_i derived as

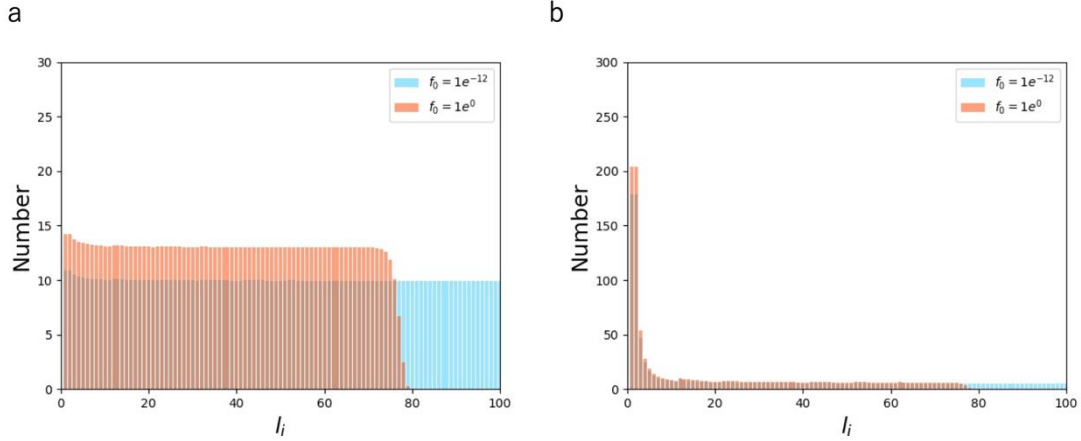


Figure II-3 Length distribution of AFs comprising of $N = 1,000$ monomers with $f_0 = 10^{-12}$ (blue) and $f_0 = 1$ (orange) at $k'_b/k_b = 0.1$ (a) and $k'_b/k_b = 4$ (b).

a function of length l_i , the effect of changing the ratio of k'_b to the constant k_b was analyzed. At $k'_b/k_b = 1$, the distribution was approximately comparable in number for all AF lengths (Fig. II-2a). As k'_b/k_b is raised up to 10, the distribution is skewed toward short AFs (Fig. II-2b-f). Further smaller and larger k'_b/k_b resulted in a distribution similar to that at $k'_b/k_b = 1$ and 10, respectively (Supporting Material Fig. II-S2). Thus, lowering the relative contribution of k'_b , or namely weighting the effect of S_f , gives rise to a uniform distribution of AF length. Meanwhile, increasing the relative contribution of k'_b , or weighting the effect of S_d , gives rise to a distribution skewed to short AFs. Their log-log plots suggest that, with the raised k'_b/k_b , the distribution tends to approach an exponential distribution (Supporting Material Fig. II-S3). Thus, the overall distribution does not display a peak at a specific length as in a normal distribution and is not preferentially dominated by long AFs.

II-4.2 The effect of force in AFs

The effect of increasing the maximum force f_0 that individual AFs sustain was analyzed at $k'_b/k_b = 0.1$ (Fig. II-3a) and 4 (Fig. II-3b), in which the distribution is uniform and skewed to short AFs, respectively, as already described above. In both cases, the increase in f_0 up to 10^0 N resulted in suppression of AF populations with a length longer than l_0 (set to be 80 here). To compensate for the suppressed population, the appearance probability was uniformly raised in the other populations. This behavior is observed because of the constraint of the expected value of force given by Eqs. (II-3) and (II-4). More specifically, long AFs have a higher chance to be involved in bearing larger forces, and thus the constraint makes long AF populations smaller in number. We also analyzed the effect of changing the threshold length l_0 in Eq. (II-4) while keeping f_0 unchanged at 10^{-12} N, but almost no change was

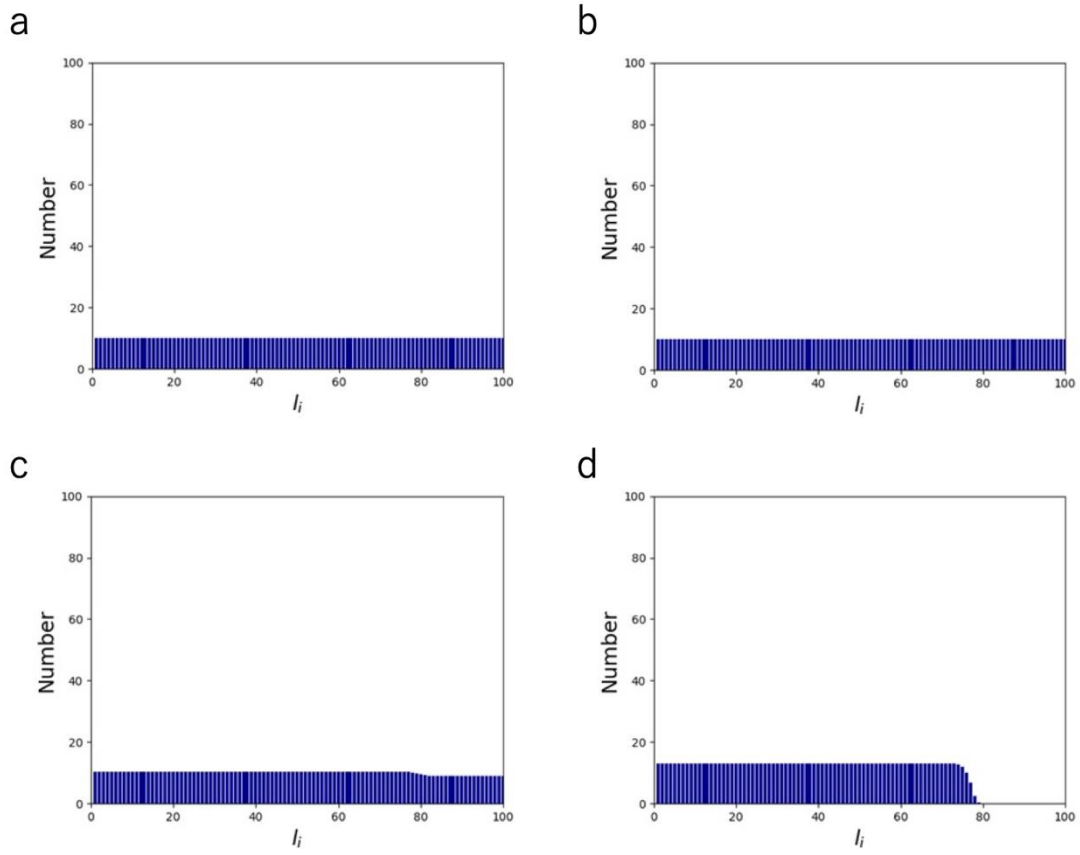


Figure II-4 Length distribution of AFs comprising of $N = 1,000$ monomers at $f_0 = 10^{-6}$ (a), $f_0 = 10^{-4}$ (b), $f_0 = 10^{-2}$ (c), and $f_0 = 10^0$ (d).

observed with $l_0 = 20$ and 80 both at $k'_b/k_b = 0.1$ and 4 (Supporting Material Fig. II-S4). In relation to investigating the role of force, the effect of changing the strain λ , which was introduced in the process of defining the internal energy of the cell from a thermodynamic perspective (see Supporting Material), was also analyzed. The distribution was almost insensitive to the value of λ (0.2 or 0.8) as analyzed at $k'_b/k_b = 4$ (Supporting Material Fig. II-S5). The absence of response to these parameters is touched on again in the next section.

II -4.3 The individual effect of formation entropy

We have so far considered both S_f and S_d for characterizing the overall objective function Eq. (II-11). To evaluate the sole effect of S_f , here we put $k'_b = 0$ in the probability function of Eq. (II-13) where the effect of S_d is omitted. Unlike the case with the two entropies where short AFs appear more frequently (Fig. II-2), the distribution becomes uniform all along the length at a maximum force f_0 of more than 10^{-4} N (Fig. II-4a, b). As f_0 is increased to 10^{-2} N, a stepwise increase toward shorter AFs appears at the position of l_0 set here to be 80

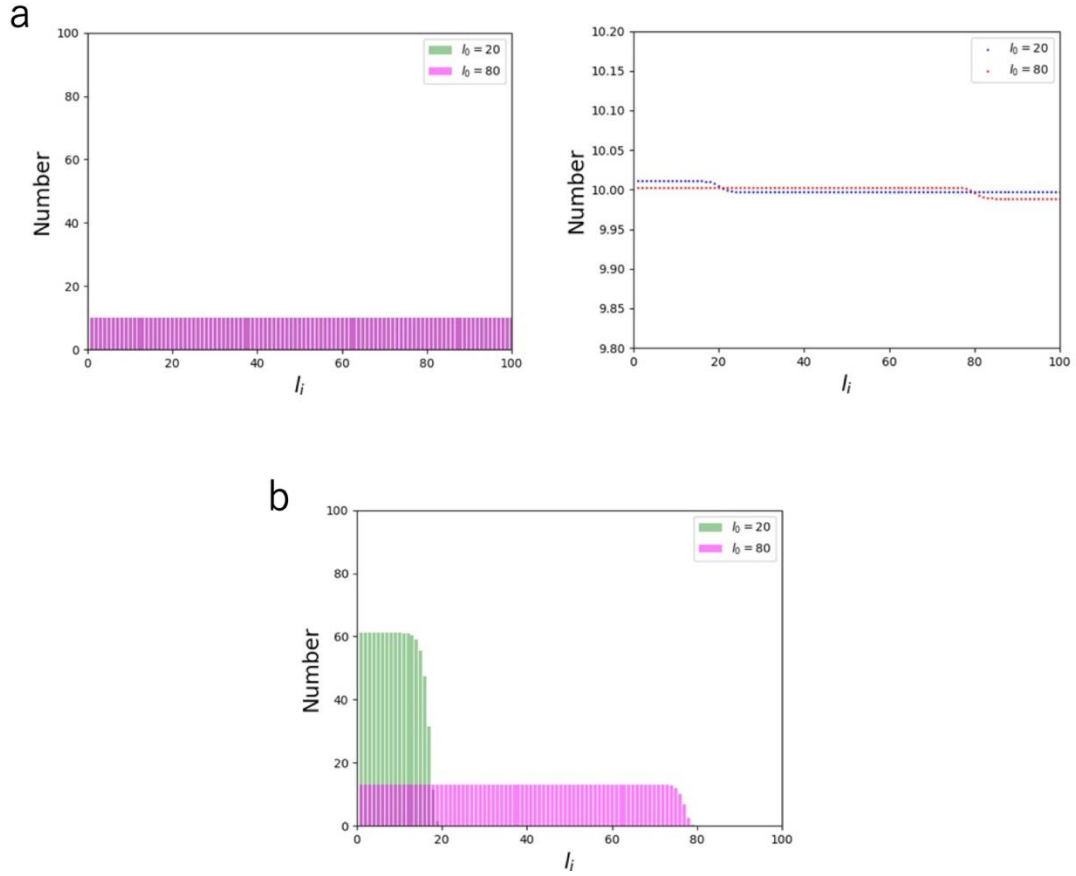


Figure II-5 Length distribution of AFs comprising of $N = 1,000$ monomers with $l_0 = 20$ (green) and $l_0 = 80$ (pink) at $f_0 = 10^{-4}$ (a) and $f_0 = 10^0$ (b). For visual clarity, point plots are also shown on the right for the case of a with magnified vertical axis.

(Fig. II-4c). With a further increase in f_0 to 10^0 N, the AF population with a length longer than l_0 completely disappears, and accordingly the short population is lifted upward (Fig. II-4d). This behavior creating a threshold for appearance is consistent with the case involving the two entropies (Fig. II-3) and can thus be explained in the same way as described above; namely, the increase in the maximum force lowers the appearance probability of long AF populations.

We next analyzed the effect of changing the threshold length l_0 , which had little effect in the presence of S_d in the objective function (Supporting Material Fig. II-S4). We found that, in the absence of S_d , only a subtle change is observed at a low f_0 of 10^{-4} N regardless of $l_0 = 20$ or 80 (Fig. II-5a), while the extent of the change becomes profound at an f_0 of 10^0 N where a steep stepwise change occurs at the position of l_0 (Fig. II-5b). Nevertheless, the shape of the uniform distribution does not change except for the position of the threshold for appearance.

In relation to investigating the role of force, we analyzed the effect of λ as it partly determines the sensitivity of entropy to force, in which an increased λ is supposed to increase the sensitivity (Supporting Material Eq. II-S28). Unlike the case with the two entropies where λ has only little effect (Supporting Material Fig. II-S5), increasing λ from 0.2 to 0.8 does provide a uniform increase in the probability for AF populations with a length shorter than l_0 (Supporting Material Fig. II-S6). As the increase in λ strengthens the sensitivity to force, it also causes an effect similar to that caused by the increase in f_0 , i.e., the uniform lift in probability below l_0 due to the constraint of constant expected value of force (Figs. II-3, 4, and 5). The lift becomes particularly detectable in the absence of S_d as the probability is low and flat over lengths less than l_0 , while it is hard to detect the subtle change in the presence of S_d where the probability is high at short lengths (Fig. II-S5).

II -4.4 The individual effect of dispersion entropy

Unlike the above case that S_d is eliminated with $k'_b = 0$, p_i is not obtained for the case eliminating S_f as k_b is included in the denominator of Eq. (II-13). As S_d takes a larger value for shorter AF lengths in a monotonically varying manner (Eq. (II-10)), the absence of local maxima in the case considering S_f as the only objective function leads to that the probability is not analytically derived by the method of Lagrange multipliers. The probability that maximizes S_f under the two constraints dictating the constant amount of actin monomers and force would be one in which shorter AFs more frequently appear. Indeed, increasing the ratio of k'_b/k_b (i.e., decreasing the relative impact of k_b on k'_b) approaches such a distribution (Fig. II-2).

In relation to S_d , the effect of A_i denoting the coefficient of n_i/l_i in Eq. (II-9) and measuring how each length population is spatially dispersed was analyzed. The relationship between A_i and l_i as a function of the compartment size a shows that a smaller value of a , which more finely divides the cell, increases more steeply with l_i (Supporting Material Fig. II-S7a). At a small a , the number of compartments occupied by AFs increases relatively sensitively with increase in AF length; meanwhile, it does not necessarily increase at a large a until the AF length exceeds a certain level to finally make incremental changes. The length required for the stepwise increase is determined by the number of summation operations for i in Eq. (II-8), specifically $(1 + aj) - \{2 + a(j - 1)\} + 1 = a$; thus, each increment occurs upon a length of a . To evaluate the contribution of each actin molecule to the dispersion with different AF lengths, the number of occupied compartments per unit length (per bound actin molecule), i.e., A_i/l_i , was analyzed (Supporting Material Fig. II-S7b). A_i/l_i is large for short AFs and decreases with increased AF length to converge to the reciprocal of a , indicating that the efficiency for intracellular dispersion of individual actin monomers is

lowered by being associated with long AFs, but there is a limitation value determined by the compartment size a or equivalently the monomer size. We also analyzed how different values of a affect the length distribution of AFs at different k'_b/k_b (Figs. II-S8 and S9). The results show that increasing a as well as k'_b/k_b both shifts the distribution toward shorter AF length populations because the increase in a and k'_b/k_b both decreases the efficiency for dispersion (Supporting Material Fig. II-S7) and the weight on S_f that leads to a uniform distribution (Fig. II-2), respectively. It is important to note that our aim here is to capture the qualitative features of the probability distribution rather than the specific number; thus, as the trend is consistent regardless of the value of a as examined here, we kept $a = 10$ for the other parts of this study.

II -5. Discussion

Nonmuscle cells such as endothelial cells are known to adapt to mechanical stress applied from arbitrary directions (II.8, 11, 21). What makes this mechanical adaptation possible is the presence of tensional homeostasis, which allows the cells to sustain a constant level of tension over the cytoplasm through turnover of their constituent proteins (II.23–25). To address the physical requirement for tensional homeostasis, here we considered what type of actin molecule populations is required to provide a specific mean tension throughout the cytoplasm. The nature of having various AF lengths in nonmuscle cells – which are supposed to behave adaptively in accordance with time-varying intracellular and extracellular milieu – is distinct from that of muscle cells – which are in contrast specialized to exhibit unidirectional contractile activity. Accordingly, for example, nebulin is expressed specifically within muscle cells to stabilize AFs to possess a fixed length (II.26).

Physicochemical mechanisms determining the length of AFs have been analyzed mostly on their in vitro dynamic behavior where typically the rate of polymerization/depolymerization, viscosity, and diffusion as a function of the length were considered (II.27–30). AFs in vitro were then predicted to have an exponential distribution, which is qualitatively consistent with experimental observations (II.31). The effect of some actin-binding proteins was also analyzed, yielding in some cases a distribution with a local maximum (II.32–37). Regarding the AF distribution in living cells, on the other hand, its involvement in constructing filopodia/lamellipodia was investigated (II.38). However, previous studies have not explicitly focused on actin meshwork, namely the basic intracellular architecture (II.17–20). Thus, to our knowledge, there is no study that investigated how individual cells are endowed with tensional homeostasis thanks to their AF resources. Unlike in vitro studies, there are only limited experimental data available on the AF length distribution within animal cells, apart from that in *Dictyostelium discoideum* (II.39); but, observations of cell breakage after

phalloidin-mediated AF stabilization suggest that the probability is clearly higher for shorter AFs within cells (II.40), thus consistent with our prediction.

To extract the physical features of tensional homeostasis, we considered the two independent factors: S_f and S_d that are related to the formation of the force-bearing elements and to the spatial distribution of the elements, respectively. S_f indicates in what manner each AF is likely to be formed. For example, if all the molecules have the same length, the number of states is then unity, meaning that they can only be in a fixed state and are thus unstable as a whole since stochastic fluctuations are not allowed. If actin molecules instead take various lengths, they would be more stabilized by having a larger number of states. S_d , on the other hand, indicates in what manner AFs are likely to be dispersed in the cell. Because these two quantities are both identical in concept to entropy, we introduced k'_b to modulate the weight of S_d versus S_f , enabling the comparison of the relative impact to the overall system. With regards to the importance of S_d , recent studies have demonstrated that mechanosensitive actin-binding proteins such as filamin are distributed over the cytoplasm (II.21, 41, 42). These cytoplasmic sensors play a role in maintaining cell functions by converting mechanical information such as intracellular tension into biochemical information such as change in binding affinity, presumably locally detecting deviation in the homeostatic tension and hence keeping the functional integrity of the cell (II.14). The intracellular dispersion of AFs may thus not only be induced according to the natural law of physics, but also it may be serving for such biological functions.

Studies aimed at characterizing the properties of individual proteins have been extensively conducted as technical advances have made it possible to perform sophisticated measurements at the molecular level, while their collective behaviors are not necessarily taken into sufficient account. However, living organisms are not merely a collection of elements with separate functions but exist through complex interactions among structurally and functionally hierarchical “layers”, thereby allowing the whole system to acquire universal features of life such as flexibility, adaptivity, and resulting maintenance of biological activities. It is necessary, given the actual complexity of cells, to limit the scope of our research to arguably the most essential factor in tensional homeostasis, i.e., the involvement of AFs; but, by describing a simple model, we captured the salient features at the cellular and molecular levels. Specifically, we derived the length distribution of AFs not by considering isolated individuals but by analyzing the population, in which the cellular constraints of having a constant level of actin monomers as well as tension turn out that the probability decreases as the length increases. Our prediction is in agreement with experimental observations made on cells (II.40). Exploring the involvement of other actin-regulating proteins (II.43) will be the subject of future challenging but exciting investigations, for which our approach would

provide a basis. To this end, as we discussed just after the introduction of the overall entropy of Eq. (II -12), further elaborate formulations of entropies and appropriate constraints are key to describing the characteristics and roles of the newly added elements.

II -6. Supporting Material

II -S1: Figure II -S1

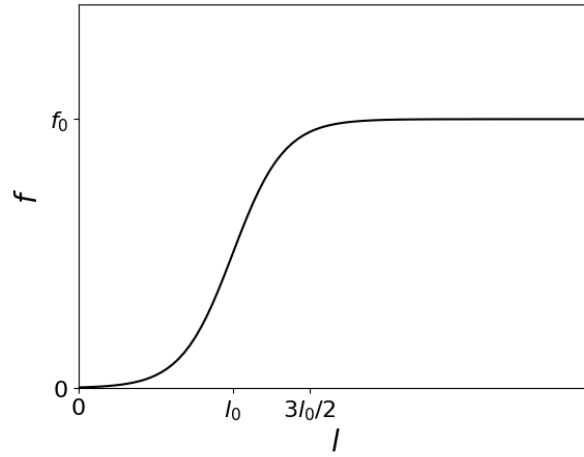


Fig. II -S1 The relationship between force f_i and AF length l_i . The force increases up to f_0 , where l_0 characterizes the position of the transition.

II-S2: Derivation of Eq. (II-7)

From Eqs. (II-5) and (II-6), the formation-associated entropy is described by

$$S_f = k_b \ln \frac{N!}{n_1! n_2! \cdots n_m!} \quad (\text{II} - \text{S1})$$

Stirling's approximation on Eq. (II-S1) together with Eq. (II-1) yields that

$$\begin{aligned} S_f &= k_b (\ln N! - (\ln n_1! + \ln n_2! + \cdots)) \\ &= k_b (\ln N! - \sum \ln n_i!) \\ &= k_b (N(\ln N - 1) - \sum n_i (\ln n_i - 1)) \\ &= k_b (N \ln N - \sum n_i \ln n_i). \end{aligned} \quad (\text{II} - \text{S2})$$

With Eq. (II-2),

$$\begin{aligned} S_f &= k_b (N \ln N - \sum N p_i \ln(N p_i)) \\ &= k_b (N \ln N - \sum N p_i (\ln N + \ln p_i)) \\ &= -k_b N \sum p_i \ln p_i. \end{aligned} \quad (\text{II} - 7)$$

II -S3: Explanation of Eq. (II -8)

$$G = \frac{n_1}{l_1} + 2 \sum_{i=2}^{1+a} \frac{n_i}{l_i} + 3 \sum_{j=2+a}^{1+2a} \frac{n_j}{l_j} + \dots = \frac{n_1}{l_1} + \sum_{j=1}^{\frac{m-2}{a}+1} \sum_{i=2+a(j-1)}^{1+aj} (j+1) \frac{n_i}{l_i} \quad (\text{II} - 8)$$

As described in the main text, n_i is the number of actin monomers in the cell involved in constructing AFs of length l_i . Thus, n_i/l_i represents the number of individual AFs of length l_i . The diameter of each molecule is smaller than a that is the compartment size, indicating that the number of compartments that can be occupied by a single actin monomer (or an AF of length l_1) is always unity as the monomer is the smallest countable unit, which determines the first term in the middle expression of Eq. (II -8). Thus, $i = 1$ represents a group of AFs with the shortest length. As described in the main text, the diameter and filament lengths of actin molecules are normalized by the unit length $l_1 = 1$. The compartment size of $a = 1$ means that the diameter of actin molecules is equal to the length of each compartment; thus, the number of compartments occupied by a single AF with a length of, e.g., $l_i = 10$ is also 10. Let us consider how the occupied compartments are counted in general with $a > 1$ based on some examples shown in Fig. II -1b where a case with $a = 3l_1 = 3$ is drawn. Here, the maximum number of compartments that can be occupied by two (l_2), three (l_3), and four (l_4) bound actin molecules is all 2 as indicated by the gray-shaded area in the figure. If actin polymerizes to have a length of l_5 composed of five actin monomers, the maximum number of compartments occupied by this AF is 3 as again indicated by the gray-shaded area. The count for these 2 and 3 consecutive compartments corresponds to the second and third terms in the middle expression of Eq. (II -8), respectively. Here, the number of the AFs is also considered; namely, in the case of the population of $i = 5$, the number of the AFs is $n_5/l_5 = 15/5 = 3$. The general form, in which the maximum number of the compartments occupied by AFs is counted in the same way, corresponds to the right expression of Eq. (II -8), where m is, again, a group of AFs with the longest length.

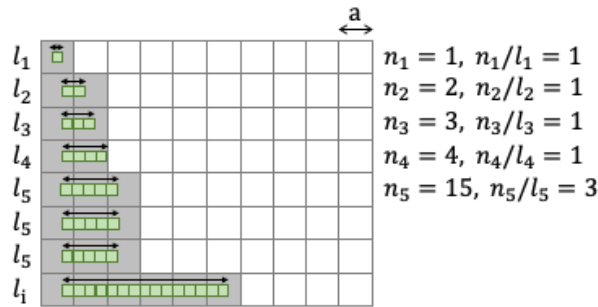


Fig. II -1b

II-S4: Derivation of Eq. (II-14)

The partial derivative of Eq. (II-13) with respect to p_i is

$$\frac{\partial \mathcal{L}}{\partial p_i} = \sum \left[-k_b N \ln p_i - k_b N + k'_b N \frac{A_i}{l_i} - \alpha f_i - \beta \right]. \quad (\text{II} - \text{S3})$$

The objective function $\mathcal{L}$ takes an extreme value at

$$\frac{\partial \mathcal{L}}{\partial p_i} = 0, \quad (\text{II} - \text{S4})$$

which provides the existence probability for the present purpose

$$p_i = \exp \left[\left(k'_b \frac{A_i}{l_i} - \frac{\alpha}{N} f_i - \frac{\beta}{N} \right) / k_b - 1 \right]. \quad (\text{II} - \text{S5})$$

Next, to determine the Lagrange multipliers α and β , Eq. (II-S5) is substituted partially into Eq. (II-12) for the overall entropy, yielding

$$\begin{aligned} S &= -k_b N \sum p_i \left[\left(k'_b \frac{A_i}{l_i} - \frac{\alpha}{N} f_i - \frac{\beta}{N} \right) / k_b - 1 \right] + k'_b \sum N \frac{A_i}{l_i} p_i \\ &= \alpha \sum p_i f_i + \beta \sum p_i - k_b N. \end{aligned} \quad (\text{II} - \text{S6})$$

With Eqs. II-(1)–(3), Eq. (S6) is expressed as

$$S = \alpha F + \beta - k_b N. \quad (\text{II} - \text{S7})$$

The partial derivative of Eq. (II-S7) with respect to the expected value of force F is

$$\frac{\partial S}{\partial F} = \alpha + F \frac{\partial \alpha}{\partial F} + \frac{\partial \beta}{\partial F}. \quad (\text{II} - \text{S8})$$

With Eqs. (II-1) and (II-2), the summation of p_i shown by Eq. (II-S5) must be unity:

$$\sum \exp \left[\left(k'_b \frac{A_i}{l_i} - \frac{\alpha}{N} f_i - \frac{\beta}{N} \right) / k_b - 1 \right] = 1. \quad (\text{II} - \text{S9})$$

The partial derivative of Eq. (II-S9) with respect to F is

$$\sum \frac{1}{k_b} \left(-\frac{f_i}{N} \frac{\partial \alpha}{\partial F} - \frac{1}{N} \frac{\partial \beta}{\partial F} \right) \exp \left[\left(k'_b \frac{A_i}{l_i} - \frac{\alpha}{N} f_i - \frac{\beta}{N} \right) / k_b - 1 \right] = 0. \quad (\text{II} - \text{S10})$$

With Eqs. (II-1), (II-2), and (II-S5), Eq. (II-S10) is reduced to

$$-\frac{1}{k_b} \left\{ \sum \left(\frac{f_i}{N} \frac{\partial \alpha}{\partial F} + \frac{1}{N} \frac{\partial \beta}{\partial F} \right) p_i \right\} = 0, \quad (\text{II} - \text{S11})$$

and therefore

$$F \frac{\partial \alpha}{\partial F} + \frac{\partial \beta}{\partial F} = 0. \quad (\text{II} - \text{S12})$$

With Eq. (II-S8), the partial derivative of the overall entropy S with respect to F is

$$\frac{\partial S}{\partial F} = \alpha, \quad (\text{II} - \text{S13})$$

suggesting that the Lagrange multiplier introduced to find the optimal distribution, α , can be expressed using the overall entropy and force expectation value.

Next, we discuss the specific form of α given that the entropy derived from a microscopic point of view based on statistical mechanics, maximizing the objective function at equilibrium, coincides with that derived from a macroscopic point of view based on thermodynamics. For the thermodynamic consideration, we consider a cell with a volume of V and a preexisting strain of λ . As we already discussed, the presence of the preexisting tension (or concomitant mechanical strain) is aimed at capturing the feature of tensional homeostasis. The strain varies spatially and temporarily at the microscopic view, while at the macroscopic view we consider an average level of strain distributed throughout the cytoplasm. The expected value of force F introduced in Eq. (II-3) is regarded as stress in the following thermodynamic model.

The first law of thermodynamics gives

$$dU = d'Q + d'W \quad (\text{II} - \text{S14})$$

where U , Q , and W denote the internal energy, the heat given to the system, and the work done to the system, respectively. Let us assume an elastic relationship between stress F and strain λ , i.e.,

$$F = E\lambda \quad (\text{II} - \text{S15})$$

where E denotes the elastic modulus of the cell, and it turns out that the strain energy is

$$k = \int F d\lambda = \frac{1}{2} E \lambda^2 = \frac{1}{2} F \lambda, \quad (\text{II} - \text{S16})$$

and accordingly the whole strain energy is described using volume V to be

$$K = V k = \frac{1}{2} V F \lambda. \quad (\text{II} - \text{S17})$$

The work caused by change in stress, which in other words is the change in strain energy, is therefore

$$d'W_{\Delta\text{stress}} = \frac{1}{2} V \lambda dF, \quad (\text{II} - \text{S18})$$

in which cell volume is assumed to be constant (i.e., Poisson's ratio = 0.5).

The strain or intracellular deformation is generated within cells at tensional homeostasis upon the actin–myosin II interaction using the energy obtained from the ATP-mediated chemical reaction. Therefore, the strain energy discussed here is not the result of work done "by external forces" as in the case of conventional spring elasticity, but is the result of work done "by the system itself." In other words, as the cell system we discuss here includes subcellular parts that do work (namely, generate force and deform), the work done to the system must be opposite in sign to that done "by the system itself." It also turns out that, in the present system, the work done "by the system itself" will increase in amount as the strain/deformation-associated intracellular stress F increases. Therefore, change in work of the entire system is

$$d'W = -d'W_{\text{stress}} = -\frac{1}{2}V\lambda dF. \quad (\text{II} - \text{S19})$$

With this, Eq. (II-S14) turns out

$$dU = TdS - \frac{1}{2}V\lambda dF \quad (\text{II} - \text{S20})$$

where T is the thermodynamic temperature. The free energy G is defined as

$$G = U - TS, \quad (\text{II} - \text{S21})$$

and its change is

$$dG = dU - TdS - SdT, \quad (\text{II} - \text{S22})$$

or with Eq. (II-S20)

$$dG = -\frac{1}{2}V\lambda dF - SdT. \quad (\text{II} - \text{S23})$$

Describing the total differential form of $G(F, T)$,

$$dG(F, T) = \left(\frac{\partial G}{\partial F}\right)_T dF + \left(\frac{\partial G}{\partial T}\right)_F dT. \quad (\text{II} - \text{S24})$$

From Eqs. (II-S23) and (II-S24),

$$\left(\frac{\partial G}{\partial F}\right)_T = -\frac{1}{2}V\lambda. \quad (\text{II} - \text{S25})$$

The partial derivative of the free energy with respect to F at constant temperature yields

$$\left(\frac{\partial G}{\partial F}\right)_T = \left(\frac{\partial U}{\partial F}\right)_T - T \left(\frac{\partial S}{\partial F}\right)_T. \quad (\text{II} - \text{S26})$$

From Eqs. (II –S25) and (II –S26),

$$-\frac{1}{2}V\lambda = \left(\frac{\partial U}{\partial F}\right)_T - T\left(\frac{\partial S}{\partial F}\right)_T. \quad (\text{II} - \text{S27})$$

At constant temperature where there is no change in internal energy, Eq. (II –S27) is reduced to

$$\left(\frac{\partial S}{\partial F}\right)_T = \frac{V\lambda}{2T}, \quad (\text{II} - \text{S28})$$

describing the effect of intracellular stress on entropy derived from a macroscopic thermodynamic point of view. This positive relationship – distinct from the negative one for a conventional spring where stretch reduces the extent of fluctuations, the number of possible microstates of the constituents, and thereby the entropy as well – is reasonable because the stress of current interest F is, as already mentioned, originated from the intracellular actin–myosin II interaction. More specifically, F is interpreted to be an indicator of the activity of the intracellular components rather than a factor compelling their movement. Thus, an increased F and resulting activation of the actin–myosin II interaction stabilize the cell structure as well as increase the entropy. Given that Eqs. (II –S13) and (II –S28) are equal at equilibrium,

$$\alpha = \frac{V\lambda}{2T}. \quad (\text{II} - \text{S29})$$

Substituting Eq. (II –S29) into (II –S5) yields

$$p_i = \exp \left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i - \frac{\beta}{N} \right) / k_b - 1 \right]. \quad (\text{II} - \text{S30})$$

To determine β , the constant part of Eq. (II –S30), which is independent of i , was expressed as B , and then

$$p_i = B \exp \left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i \right) / k_b \right] \quad (\text{II} - \text{S31})$$

Where

$$B = \frac{1}{\exp\left[\frac{\beta}{k_b N} + 1\right]}. \quad (\text{II} - \text{S32})$$

With Eqs. (II-1) and (II-2), the summation of p_i must be unity so that

$$\sum B \exp\left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i\right)/k_b\right] = 1, \quad (\text{II} - \text{S33})$$

indicating that

$$B = 1/\sum \exp\left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i\right)/k_b\right], \quad (\text{II} - \text{S34})$$

and

$$\beta = -k_b N(\ln B + 1). \quad (\text{II} - \text{S35})$$

Consequently,

$$p_i = \frac{\exp\left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i\right)/k_b\right]}{\sum \exp\left[\left(k'_b \frac{A_i}{l_i} - \frac{V\lambda}{2TN} f_i\right)/k_b\right]}. \quad (\text{II} - 14)$$

II-S5: Figure II-S2

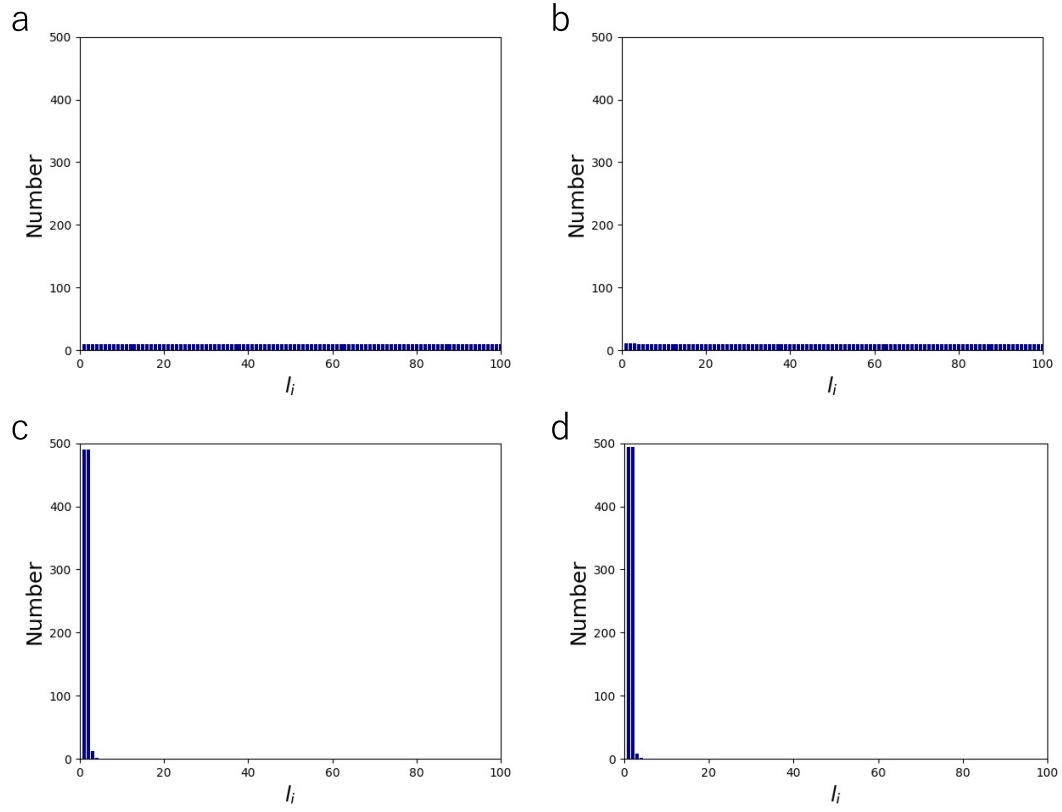


Fig. II-S2 Length distribution of AFs comprising of $N = 1,000$ monomers at $k_b'/k_b = 10^{-2}$ (a), $k_b'/k_b = 10^{-1}$ (b), $k_b'/k_b = 11$ (c), and $k_b'/k_b = 12$ (d).

II-S6: Figure II-S3

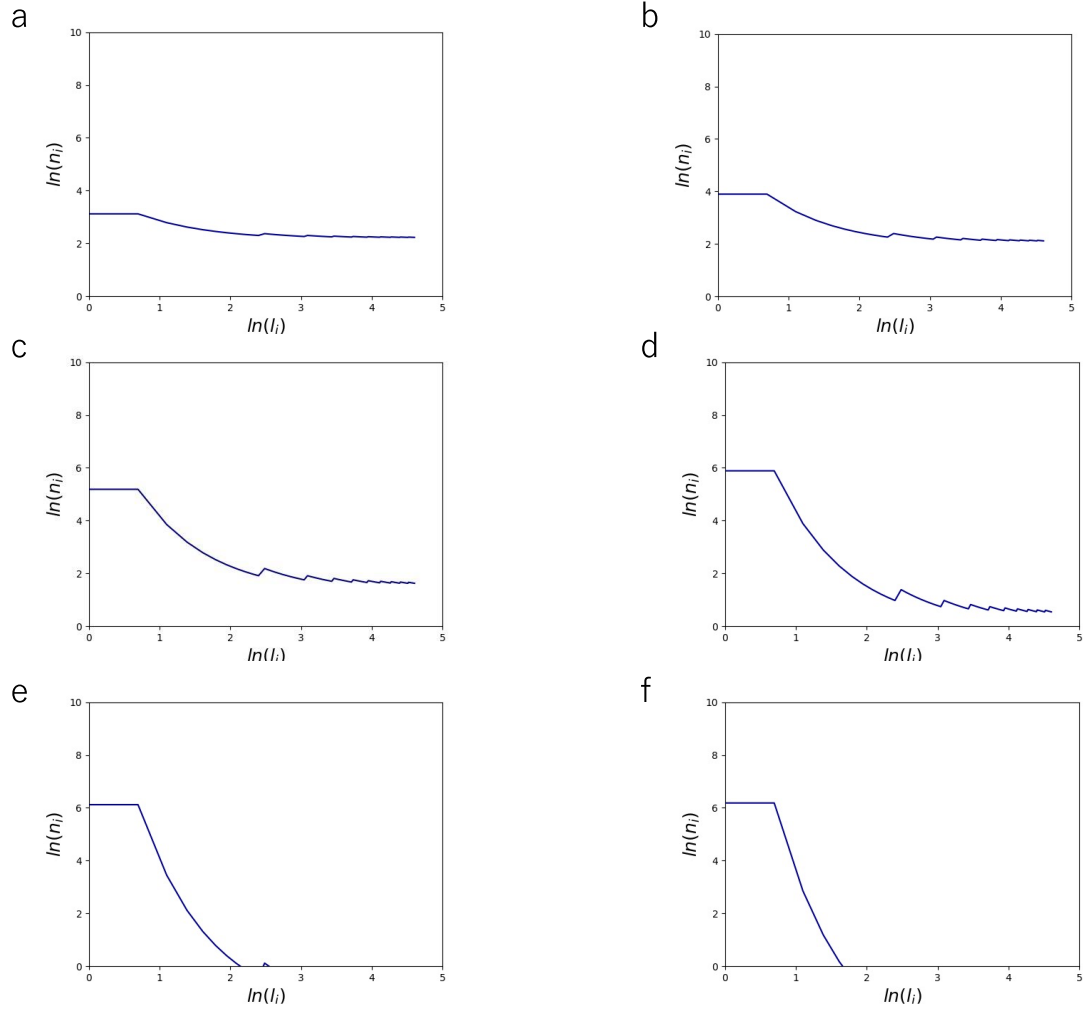


Fig. II-S3 Log-log graphs of Fig. II-2, showing the length distribution of AFs comprising of $N = 1,000$ monomers at $k_b'/k_b = 1$ (a), $k_b'/k_b = 2$ (b), $k_b'/k_b = 4$ (c), $k_b'/k_b = 6$ (d), $k_b'/k_b = 8$ (e), and $k_b'/k_b = 10$ (f).

II-S7: Figure II-S4

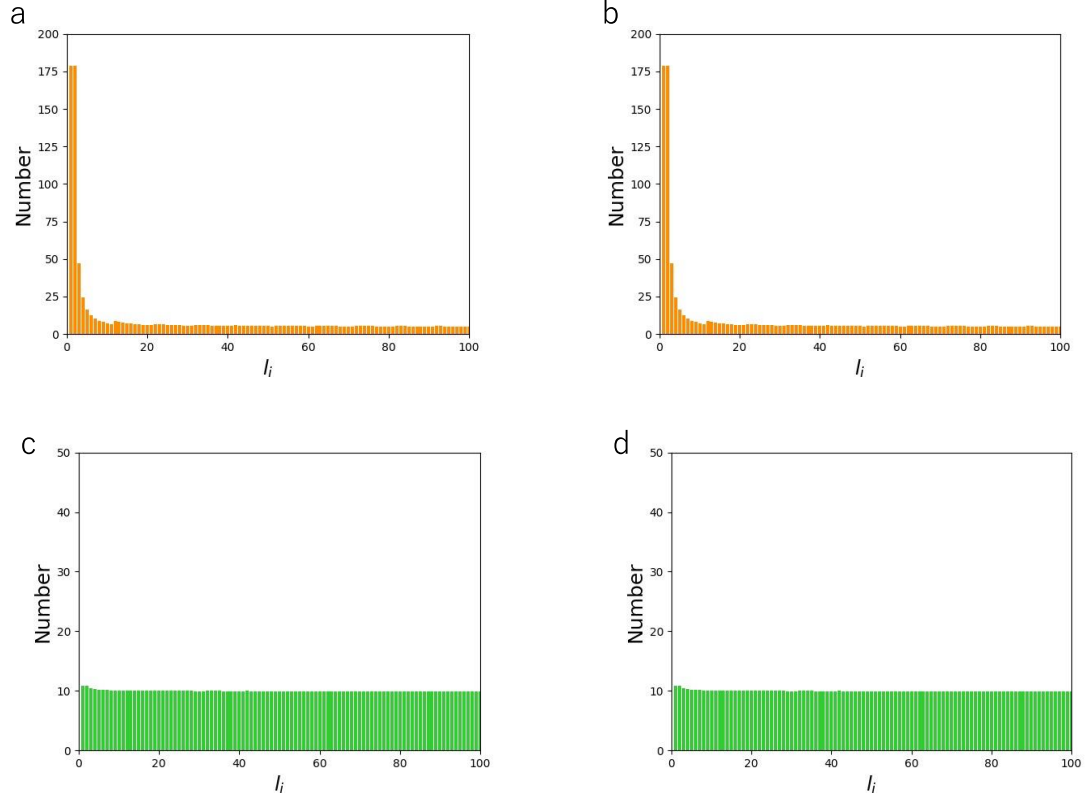


Fig. II-S4 Length distribution of AFs comprising of $N = 1,000$ monomers at $k_b'/k_b = 4$ and $l_0 = 20$ (a), $k_b'/k_b = 4$ and $l_0 = 80$ (b), $k_b'/k_b = 0.1$ and $l_0 = 20$ (c), and $k_b'/k_b = 0.1$ and $l_0 = 80$ (d), showing that the distribution does not depend on l_0 .

II-S8: Figure II-S5

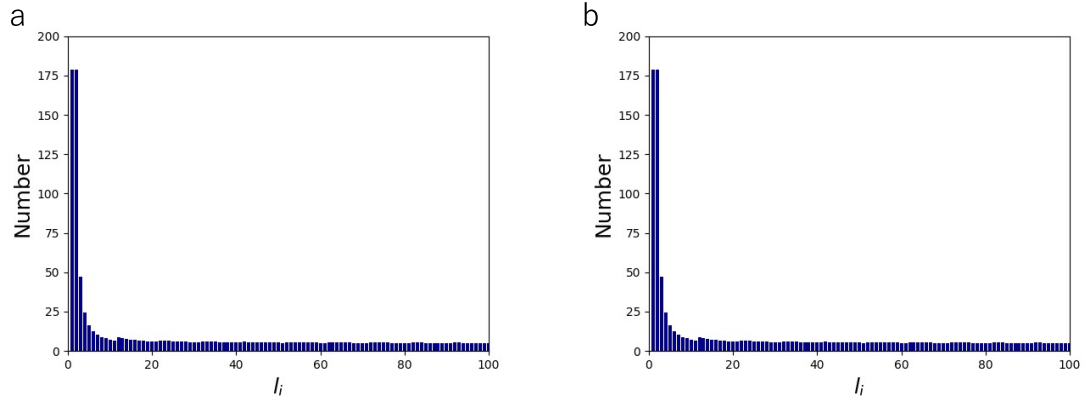


Fig. II-S5 Length distribution of AFs comprising of $N = 1,000$ monomers at preexisting cell strain (prestretch) of $\lambda = 0.2$ (a) and $\lambda = 0.8$ (b).

II-S9: Figure II-S6

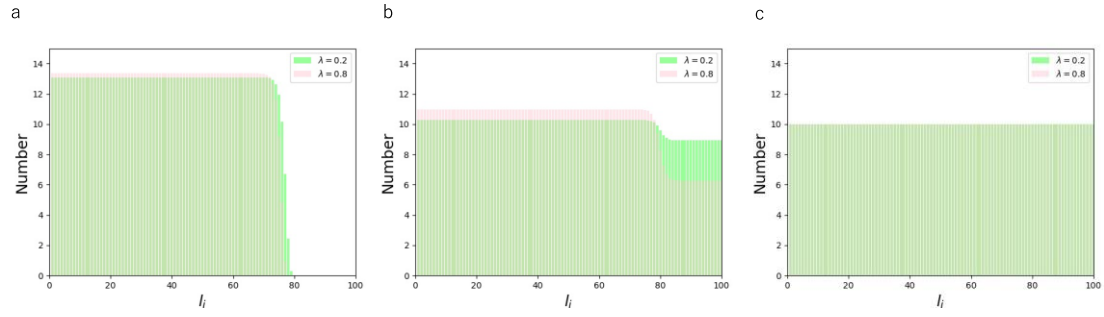


Fig. II-S6 Length distribution of AFs comprising of $N = 1,000$ monomers for preexisting cell strain of $\lambda = 0.2$ (green) and $\lambda = 0.8$ (pink) at $f_0 = 10^0$ (a), $f_0 = 10^{-2}$ (b), and $f_0 = 10^{-4}$ (c).

II-S10: Figure II-S7

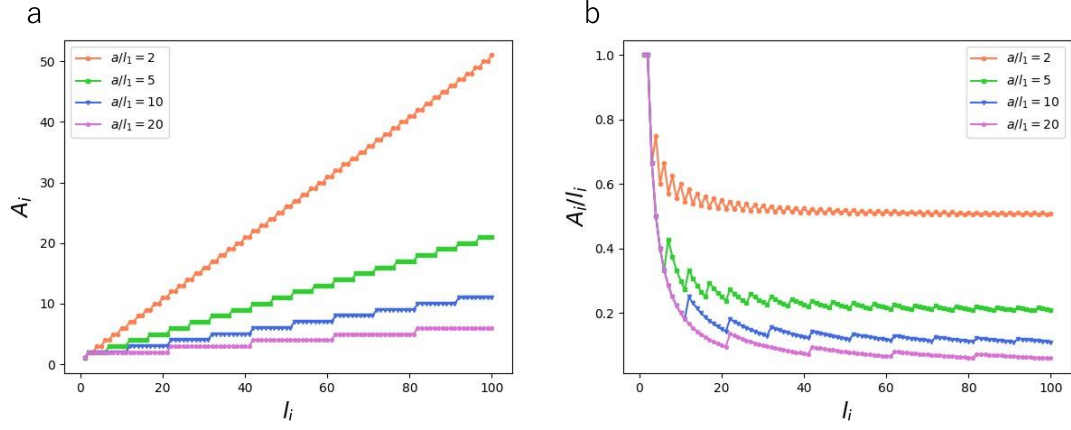


Fig. II-S7 The effect of changing the ratio of compartment size a to actin monomer size l_1 . In the analysis, the size is normalized by l_1 as described in Methods section. (a) A_i , which determines S_d , as a function of l_1 at different a ; $a/l_1 = 2$ (orange), $a/l_1 = 5$ (green), $a/l_1 = 10$ (blue), and $a/l_1 = 20$ (purple). (b) A_i/l_i as a function of l_1 at different a ; $a/l_1 = 2$ (orange), $a/l_1 = 5$ (green), $a/l_1 = 10$ (blue), and $a/l_1 = 20$ (purple). Note that $l_1 = 1$ for the rest of the analyses in the present study.

II-S11: Figure II-S8

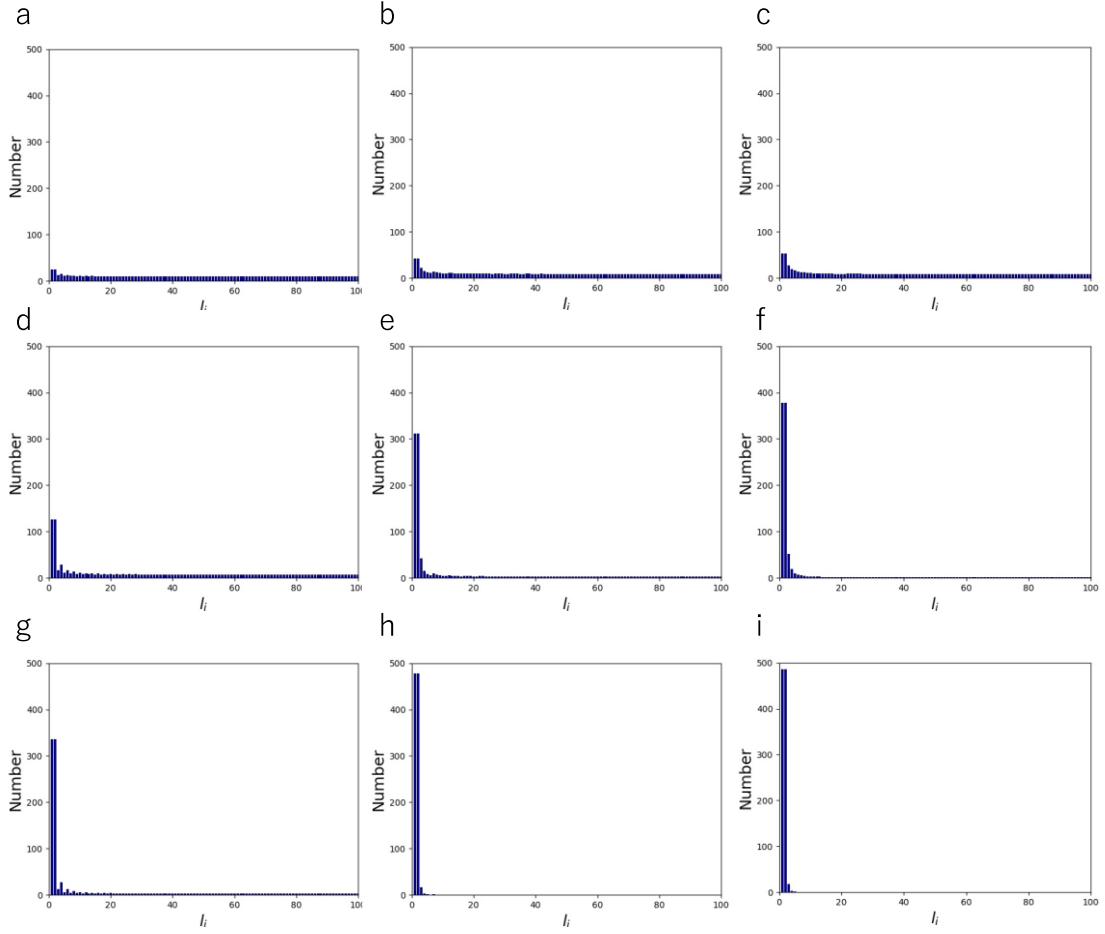


Fig. II-S8 Length distribution of AFs comprising of $N = 1,000$ monomers at $k_b'/k_b = 2$ and $a/l_1 = 2$ (a), $k_b'/k_b = 2$ and $a/l_1 = 5$ (b), $k_b'/k_b = 2$ and $a/l_1 = 20$ (c), $k_b'/k_b = 6$ and $a/l_1 = 2$ (d), $k_b'/k_b = 6$ and $a/l_1 = 5$ (e), $k_b'/k_b = 6$ and $a/l_1 = 20$ (f), $k_b'/k_b = 10$ and $a/l_1 = 2$ (g), $k_b'/k_b = 10$ and $a/l_1 = 5$ (h), and $k_b'/k_b = 10$ and $a/l_1 = 20$ (i). Cases at $a/l_1 = 10$ are shown in Fig. II-2.

II-S12: Figure II-S9

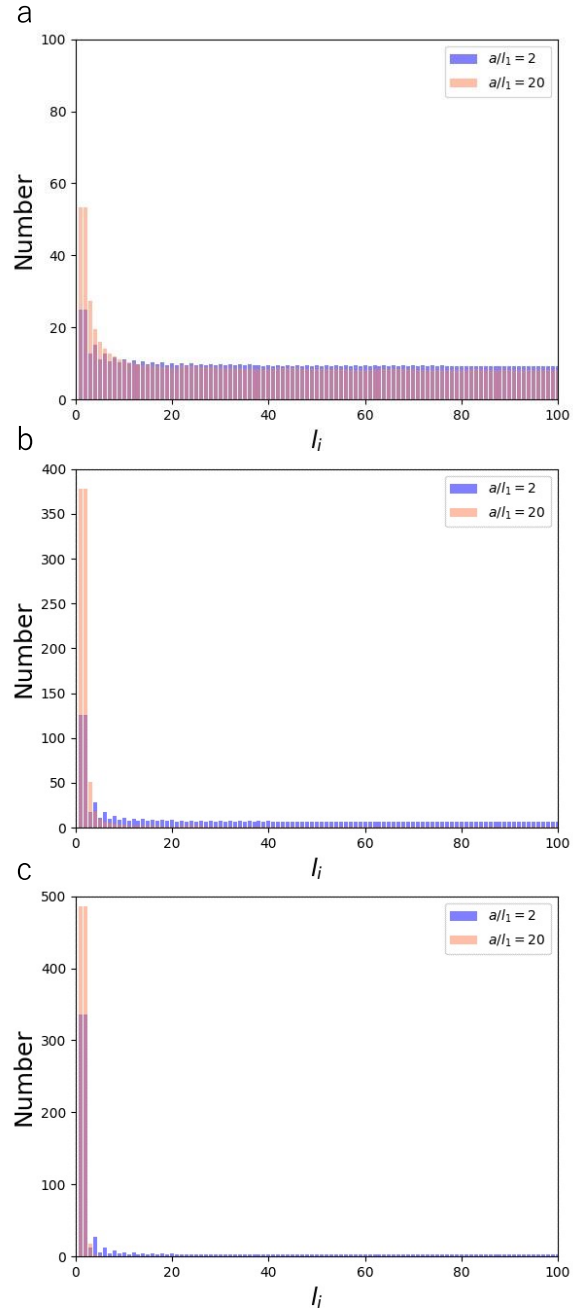


Fig. II-S9 Length distribution of AFs comprising of $N = 1,000$ monomers for $a/l_1 = 2$ (blue) and $a/l_1 = 20$ (orange) at $k_b'/k_b = 2$ (a), $k_b'/k_b = 6$ (b), and $k_b'/k_b = 10$ (c) taken partly from Fig. II-S8.

III Emergence of multiple set-points of cellular homeostatic tension

III-1. Summary

Stress fibers (SFs), a contractile actin bundle in nonmuscle mesenchymal cells, are known to intrinsically sustain a constant level of tension or tensional stress, a process called cellular tensional homeostasis. Malfunction in this homeostatic process has been implicated in many diseases such as atherosclerosis, but its mechanisms remain incompletely understood. Interestingly, the homeostatic stress in individual SFs is altered upon recruitment of α -smooth muscle actin in particular cellular contexts to reinforce the preexisting SFs. While this transition of the set-point stress is somewhat a universal process observed across different cell types, no clear explanation has been provided as to why cells end up possessing different stable stresses. To address the underlying physics, here we describe that imposing a realistic assumption on the nature of SFs yields the presence of multiple set-points of the homeostatic stress, which transition among them depending on the magnitude of the cellular tension. We derive non-dimensional parameters that characterize the extent of the transition and predict that SFs tend to acquire secondary stable stresses if they are subject to as large a change in stiffness as possible or to as immediate a transition as possible upon increasing the tension. This is a minimal and simple explanation, but given the frequent emergence of force-dependent transformation of various subcellular structures in addition to that of SFs, the theoretical concept presented here would offer an essential guide to addressing potential common mechanisms governing complicated cellular mechanobiological responses.

III-2. Introduction

Stress fibers (SFs), a contractile actin bundle, are known to intrinsically sustain a constant level of tension in nonmuscle mesenchymal cells (III. 18). Specifically, the force sustained in individual SFs ranges from approximately one to several tens of nano-Newtons, while their cross-sectional area is several square micrometers, giving rise to a tension or tensional “stress” (i.e., the force divided by the cross-sectional area) of several $\text{nN}/\mu\text{m}^2$ (III. 1, 32). Under this cellular regulation called tensional homeostasis, perturbations applied to SFs upon mechanical stresses such as cyclic stretch or fluid shear stress onto the cells are relaxed over time through structural remodeling, allowing the stress in SFs to remain at the intrinsic set-point value (III. 17). Malfunction in this homeostatic control or adaptive response has been implicated in chronic pro-inflammatory states that potentially lead to diseases such as atherosclerosis and cancer (III. 3, 10).

Interestingly, the set-point stress in SFs is altered upon recruitment of α -smooth muscle actin (α -SMA) (III. 11). Specifically, Hinz and colleagues found in myofibroblasts that a homeostatic stress of $\sim 3.8 \text{ nN}/\mu\text{m}^2$ is sustained at individual focal adhesions (FAs), to which SFs are serially connected at their termini, with an area of $\leq 7.5 \mu\text{m}^2$; but, at other FAs with a larger area of $> 7.5 \mu\text{m}^2$ to which they refer as “supermature” FAs, a greater magnitude of stress of $\sim 12 \text{ nN}/\mu\text{m}^2$ is instead sustained (III. 9, 12). This jump in the set-point stress value is induced by modulating mechanical cues to increase the magnitude of the force in the connected SFs, for example by plating cells on a stiff substrate, and is accompanied by new association of α -SMA to the preexisting SFs, with which originally β -cytoplasmic actin isoforms are associated. Supermature adhesions are induced in other cell types such as osteoblasts and hepatic stellate cells (III. 2, 23). Similar force-dependent transformation of small adhesions into large ones called fibrillar adhesions have also been reported in endothelial cells and smooth muscle cells (III. 7, 28, 36). Collectively, the force-driven transition of the homeostatic cellular stress that individual SFs sustain is thus a somewhat universal process.

Despite these observations, no clear explanation has been provided as to why cells end up possessing different stable stresses. In the present theoretical work, we describe that a specific constitutive model depicting the nature of SFs yields the presence of multiple set-points of the homeostatic stress, which transition among them depending on the magnitude of the sustained force. This is a simple explanation, but given the frequent emergence of force-dependent transformation of various subcellular structures in addition to that of the SF–FA complex, the concept presented here may provide a fundamental insight into the underlying physics of complicated cellular mechanobiological responses.

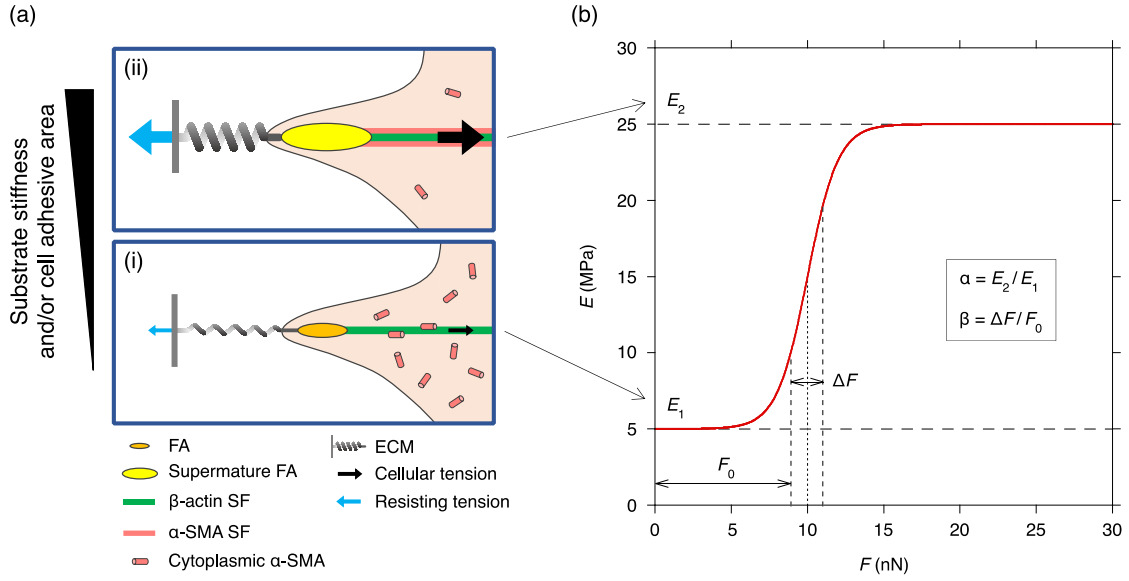


Fig. III-1 Force-dependent increase in elastic modulus of SFs. (a) α -SMA incorporation to a large tension-bearing SF depicted according to (III. 9). Tension is relatively low (i) and high (ii) in magnitude at a soft and stiff extracellular milieu, respectively, as represented by the elongation of the extracellular matrix (ECM) spring. (b) The constitutive relationship for the elastic modulus of SFs E as a function of the tension F described by Eq. (III-1).

III-3. Methods

III-3.1 Model description

α -SMA is incorporated to and reinforces preexisting SFs when they are allowed to exhibit a contractile force that is high in magnitude enough to trigger the incorporation (III. 9, 11, 12) (Fig. 1a). It has also been reported that cellular elastic modulus is elevated upon increased localization of α -SMA along the length of SFs (III. 20, 21, 29, 31, 35). Given these observations, we assume that the elastic modulus of individual SFs E is altered as a function of the contractile force F , produced and sustained in the SFs, in a sigmoid manner described by

$$E(F) = \frac{E_2 - E_1}{2} \tanh \rho + \frac{E_1 + E_2}{2} = \frac{E_1(\alpha e^\rho + e^{-\rho})}{e^\rho + e^{-\rho}} \quad (\text{III} - 1)$$

Where

$$\rho = \frac{F - F_0}{\Delta F}, \quad (\text{III} - 2)$$

$$\alpha = E_2/E_1 (\geq 1), \quad (\text{III} - 3)$$

and E_1 , E_2 , F_0 , and ΔF denote the lower and upper limits of the elastic modulus, the force that provides the average elastic modulus, and the characteristic range of force where this transition takes place, respectively (Fig. III-1b). More specifically, E_1 and E_2 represent the elastic modulus of SFs without (low-force) and with (high-force) α -SMA incorporation, respectively. The rationale for using the sigmoid function comes from the fact that the elastic modulus is not infinitely increased but instead has a limit value even on high-stiffness substrates (III. 21, 26, 33).

The strain increment in individual SFs measured from the primary set-point state, ε , is specifically described by substituting the force-dependent elastic modulus $E(F)$ to, for simplicity to capture the main physics, the one-dimensional Hooke's law

$$\varepsilon = \frac{\sigma}{E(F)} = \frac{\sigma(e^\rho + e^{-\rho})}{E_1(\alpha e^\rho + e^{-\rho})} \quad (\text{III} - 4)$$

where σ is the corresponding stress increment. Differentiating ε by σ yields

$$\frac{\partial \varepsilon}{\partial \sigma} = \frac{(e^\rho + e^{-\rho})(\alpha e^\rho + e^{-\rho}) - 2F \frac{\alpha - 1}{\Delta F}}{E_1(\alpha e^\rho + e^{-\rho})^2}. \quad (\text{III} - 5)$$

The second derivative of ε with respect to σ is

$$\frac{\partial^2 \varepsilon}{\partial \sigma^2} = -\frac{4A(\alpha - 1)}{E_1 \Delta F (\alpha e^\rho + e^{-\rho})^3} \left\{ (\alpha e^\rho + e^{-\rho}) - \frac{F}{\Delta F} (\alpha e^\rho - e^{-\rho}) \right\} \quad (\text{III} - 6)$$

where A denotes the cross-sectional area of individual SFs, and hence $F = \sigma A$. A may also be modelled using a sigmoid function. However, there is a report that cellular elastic modulus is increased by a factor of several tens (III. 20) or at least by 3–4 folds (III. 21) upon increased α -SMA expression, while it is unlikely that the cross-sectional area of individual SFs is increased by that amount given that the transition can occur with no substantial change in the lateral width of SF-associated FAs (III. 9). Besides, the critical behavior of this system is determined by a non-dimensional parameter T_s defined below

regardless of the complexity introduced by the second sigmoid function. Thus, we assume A is a constant to focus our discussion on the most noticeable observations as well as to make the analysis mathematically more tractable. At $\frac{\partial \varepsilon}{\partial \sigma} = 0$ and $\frac{\partial^2 \varepsilon}{\partial \sigma^2} = 0$ where the slope of the ε - σ curve is zero at its inflection point, $\rho = 0$, and hence $F = F_0$; furthermore, in this condition, the following non-dimensional parameter T_s turns out to be equal to the unity:

$$T_s = \frac{1}{(\Delta F/F_0)} \frac{\alpha - 1}{\alpha + 1} = \frac{\alpha - 1}{\beta(\alpha + 1)} \quad (\text{III} - 7)$$

where

$$\beta = \Delta F/F_0. \quad (\text{III} - 8)$$

The inflection point of the ε - σ curve also satisfies

$$\varepsilon = \frac{2\sigma}{E_1(\alpha + 1)}. \quad (\text{III} - 9)$$

Analysis of the sign of $\frac{\partial \varepsilon}{\partial \sigma}$ and $\frac{\partial^2 \varepsilon}{\partial \sigma^2}$ indicates that ε always increases with σ at $T_s < 1$, while ε has both a local maximum and a local minimum at $T_s > 1$. As described above, this tendency for T_s to determine the sign of $\frac{\partial \varepsilon}{\partial \sigma}$ and $\frac{\partial^2 \varepsilon}{\partial \sigma^2}$ holds also true in a situation where the cross-sectional area A is represented as a function of F using a sigmoid function similar to that of $E(F)$. It turns out that strain energy

$$S = \frac{1}{2} \sigma \varepsilon = \frac{E(F)\varepsilon^2}{2} = \frac{\sigma^2}{2E(F)} \quad (\text{III} - 10)$$

has, as is the case with conventional materials, a single local minimum for stress at $T_s \leq 1$; meanwhile, S has two local minima for stress at $T_s > 1$.

III-3.2 Parameters

To visualize the results where ε and S are evaluated as a function of the variable σ , E_1 is

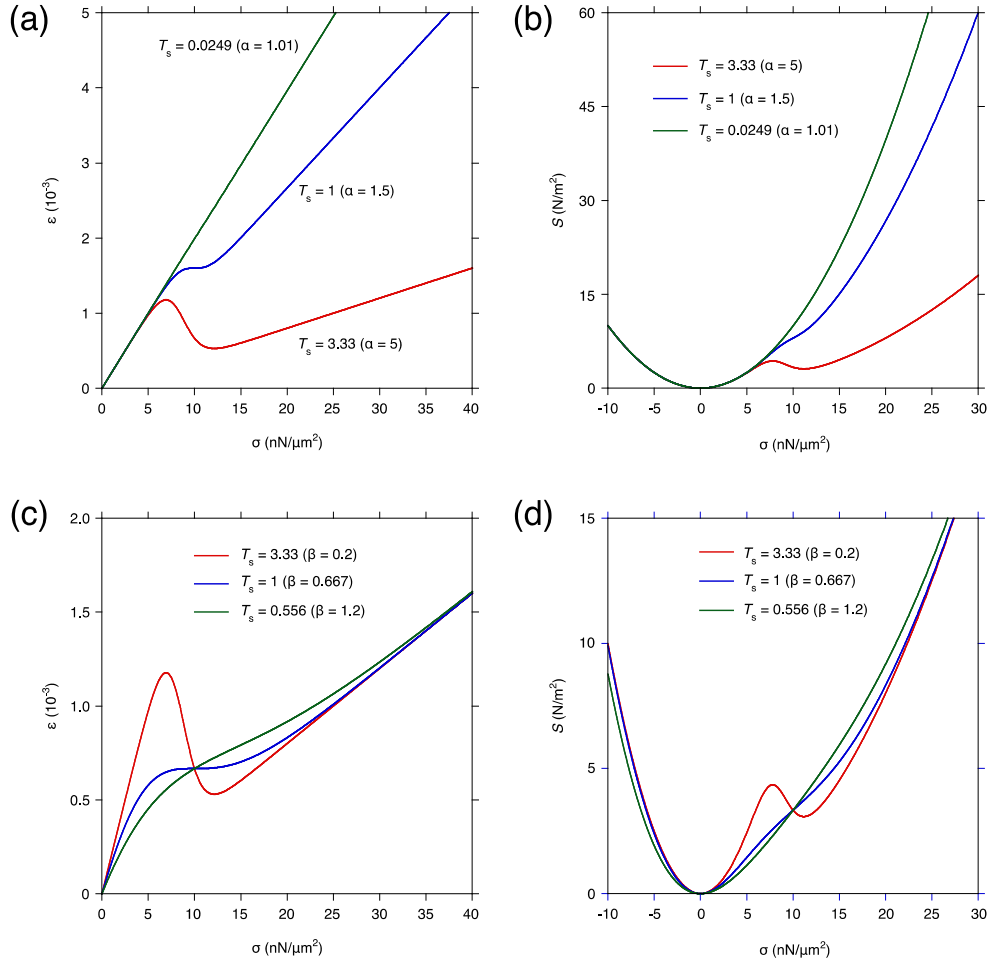


Fig. III-2 The effect of changing the non-dimensional parameters T_s , α , and β on the strain ε and strain energy S as a function of stress σ , the variables of which are all measured from the primary set-point state. (a, b) ε (a) and S (b) with different values of T_s and α at $\beta = 0.2$. (c, d) ε (c) and S (d) with different values of T_s and β at $\alpha = 5$.

assumed to be 5 MPa (III. 4). F_0 and A are assumed to be 10 nN and $1 \mu\text{m}^2$ in accordance with the magnitude of tension in and the cross-sectional area of single SFs, respectively (III. 4, 25). Regarding the rationale for the value of α , which measures the ratio of E_2 to E_1 and is defined to be equal to or more than the unity (Fig. III-1b), a 3–4 times increase in cellular elastic modulus was detected upon increased α -SMA expression along the length of SFs in valve interstitial cells (III. 21). In a separate study, the elastic modulus was increased ~52-fold (from 0.85 kPa to 44 kPa) for normal human lamina cribrosa cells and ~30-fold (from 1.1 kPa to 33 kPa) for glaucoma lamina cribrosa cells upon increased α -SMA expression along SFs (III. 20). To cover these ranges, an α of 1–50 is analyzed. Regarding the value of ΔF ,

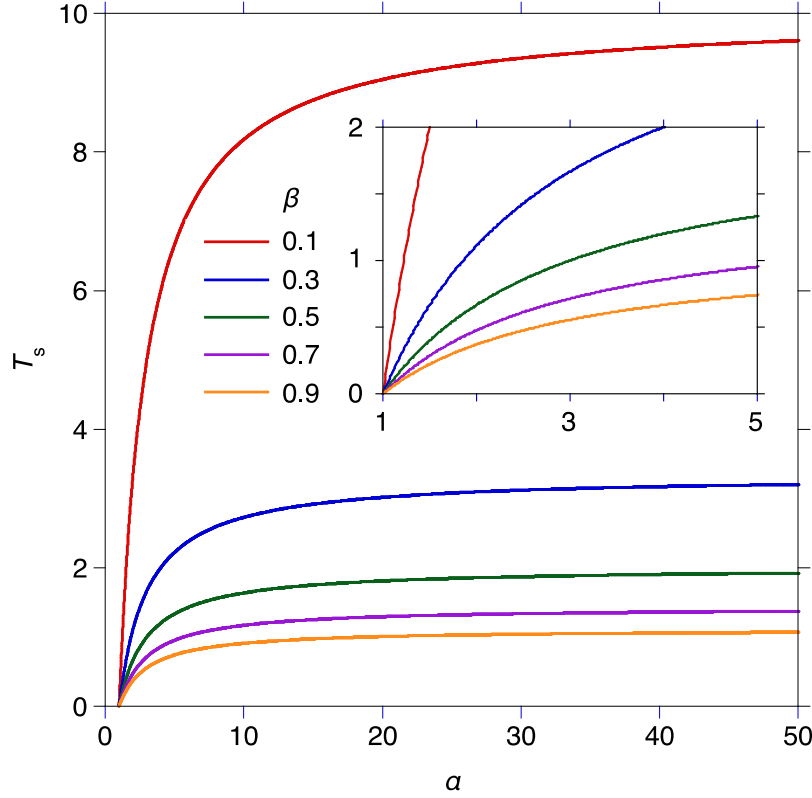


Fig. III-3 The relationship among the non-dimensional parameters T_s , α , and β . The inset shows an enlarged view of the same relationship. $T_s = 1$ represents the threshold for displaying a secondary stable stress.

Eq. (7) suggests that the relative value of $\Delta F/F_0$ ($= \beta$) essentially matters for the critical parameter T_s rather than their respective absolute values; thus, β is varied under the fixed F_0 value of 10 nN, which then uniquely determines the value of ΔF .

III-4. Results

The effect of changing α on the relationship between the strain ε and stress σ , both measured from the primary set-point state, was investigated at a fixed β value of 0.2 (Fig. 2a). As mathematically shown above, specific examples illustrate that ε increases monotonically with σ at $T_s < 1$ (specifically, $T_s = 0.0249$ with $\alpha = 1.01$). In contrast, ε has both a local maximum and a local minimum at $T_s > 1$ (with $\alpha = 5$) with an inflection point at $\rho = 0$, i.e., $\sigma = F_0/A = 10 \text{ nN}/\mu\text{m}^2$. At the threshold value of $T_s = 1$ (with $\alpha = 1.5$), ε increases with σ except for $\sigma = 10 \text{ nN}/\mu\text{m}^2$ (i.e., $\rho = 0$) where its slope is zero. Accordingly, the strain energy S has a single local minimum at $T_s \leq 1$, whereas two local minima appear at $T_s > 1$ (Fig. III-2b).

The effect of changing β on the relationship between ε and σ was likewise investigated at

a fixed α value of 5. Specific examples demonstrate that ε increases monotonically with σ at $T_S < 1$ (specifically, $T_S = 0.556$ with $\beta = 1.2$). At the threshold value of $T_S = 1$ (with $\beta = 0.667$), ε increases with σ while having a zero-slope at $\sigma = 10 \text{ nN}/\mu\text{m}^2$ (i.e., $\rho = 0$) (Fig. III-2c). As a consequence, S has a single local minimum at $T_S \leq 1$, whereas two local minima appear at $T_S > 1$ (Fig. III-2d). Thus, the emergence of the two stable stress values, corresponding to the two local minima, is observed in a range of $T_S > 1$. The inflection point of the ε - σ curve is independent of β as described by Eq. (III-9), and thus all the curves pass through it (Fig. III-2c). Meanwhile, ε value at the inflection point is decreased with increasing α as is clear from Eq. (III-9) (Fig. III-2a).

Visualization of the comprehensive parametric change in α and β shows that $T_S > 1$, in which two stable stresses are emerged, is achieved at a high and a low value of α and β , respectively (Fig. III-3). In other words, SFs tend to acquire secondary stable stresses if they are subject to as large a change in elastic modulus as possible or to as immediate a transition as possible upon increasing the tension according to Eq. (III-1).

III-5. Discussion

The size of individual FAs reversibly increases or decreases according to the tension borne in the connected SFs, eventually allowing the intracellular stress to remain constant over time (III. 8, 18). Meanwhile, if the substrate is stiff enough to bear a large tension in the FA-SF complex, a phase transition occurs to upgrade the homeostatic stress by modulating the molecular composition of the SFs (III. 9). Upon this transition, α -SMA is recruited to such SFs bearing a large tension, leading to an increase in the elastic modulus (III. 20, 21, 29, 31, 35). The possession of these homeostatic tensional stresses, known as “tensional homeostasis,” is widely observed across different mesenchymal cell types such as fibroblasts and endothelial cells (III. 5, 14, 24, 27, 30). Owing to this homeostatic control, cellular tension is relaxed to the basal level even under mechanical stresses such as cell stretch and fluid shear stress (III. 3, 6, 13, 17). These stresses are known to trigger signaling pathways associated with cell growth and pro-inflammation, while importantly such signals are suppressed over time concomitantly with the recovery of cellular tension. Tensional homeostasis thus allows cells to circumvent chronic activation of pro-inflammatory signals, and its malfunction has been implicated in various diseases that include atherosclerosis and cancer (III. 10, 18).

Despite the tremendous significance in biology and medicine, mechanisms of tensional homeostasis remain largely unknown probably because of the necessity of requiring interdisciplinary knowledge related to physics as well as molecular biology. At least, no clear explanation has been put forward regarding the physical origin of the environmental cue-

dependent induction of a secondary homeostatic tensional stress. In this regard, our model described here is simple and minimal but capable for the first time of explaining all the key observations in a physically plausible manner. Specifically, it is known that the actual SFs undergo an increased tension upon increasing cellular adhesive area and/or stiffness of the substrate as these two mechanical cues enable efficient tension transmission between the substrate and SFs (III. 12). Such an increased tension renders the existing SFs less stable, resulting in a phase transition from a β -cytoplasmic actin-dominant phase to an α -SMA-dominant one followed by the stiffening of the updated SFs (III. 21, 26). The constitutive relation given by Eq. (III-1) captures this feature. We thereby show that it gives rise to the transition to a state where another homeostatic stress is stably sustained, revealing the physical essence of why multiple set-points exist in cellular tensional homeostasis. Rigorous experimental validation of our theoretical prediction will be the subject of future investigation, but at least it is consistent with existing observations.

Context-dependent protein recruitment to existing SFs is observed not only for α -SMA but also for the septin cytoskeleton (III. 15, 16) and eEF2 elongation factor (III. 22) elicited during the epithelial–mesenchymal transition and the cellular senescence, respectively. Enhancement of cellular contractility is followed in these transitions, implying concomitant increase in their elastic properties as well. Given the similarity to the behavior analyzed here, our explanation of why cells end up experiencing different stable stresses may govern such transitions of SFs in general. The present study may also provide insights into why different higher-order forms are present in force-bearing actin-based structures such as podosomes and invadosomes, which are known to take different architectures in a context-dependent manner (III. 19, 34). Our theoretical work may thus offer a promising route to addressing such unsolved challenges of highly complicated cellular mechanobiological processes.

IV Asymmetric response emerges between creation and disintegration of force-bearing subcellular structures as revealed by percolation analysis

IV-1. Summary

Cells dynamically remodel their internal structures by modulating the arrangement of actin filaments (AFs). In this process, individual AFs exhibit stochastic behavior without knowing the macroscopic higher-order structures they are meant to create or disintegrate, but the mechanism allowing for such stochastic process-driven remodeling of subcellular structures remains incompletely understood. Here we employ percolation theory to explore how AFs interacting only with neighboring ones without recognizing the overall configuration can nonetheless create a substantial structure referred to as stress fibers (SFs) at particular locations. We determined the interaction probabilities of AFs undergoing cellular tensional homeostasis, a fundamental property maintaining intracellular tension. We then showed that the duration required for the creation of SFs is shortened by the increased amount of preexisting actin meshwork, while the disintegration occurs independently of the presence of actin meshwork, suggesting that the coexistence of tension-bearing and non-bearing elements allows cells to promptly transition to new states in accordance with transient environmental changes. The origin of this asymmetry between creation and disintegration, consistently observed in actual cells, is elucidated through a minimal model analysis by examining the intrinsic nature of mechano-signal transmission. Specifically, unlike the symmetric case involving biochemical communication, physical communication to sense environmental changes is facilitated via AFs under tension, while other free AFs dissociated from tension-bearing structures exhibit stochastic behavior and binding to existing structures. Thus, both the numerical and minimal models demonstrate the essence of intracellular percolation, in which macroscopic asymmetry observed at the cellular level emerges not from microscopic asymmetry in the interaction probabilities of individual molecules, but rather only as a consequence of the manner of the mechano-signal transmission. These results provide novel insights into the role of the mutual interplay between distinct subcellular structures with and without tension-bearing capability.

IV-2. Introduction

One of the salient features of the cell structure is its adaptive capability to remodel the architecture according to the roles required in a given environment. In eliciting this capability, individual actin filaments (AFs) serving as the primary structural component exhibit stochastic behavior without “knowledge” of the macroscopic structure they are meant to create (IV.23, 29, 40). AFs instead merely interact with neighboring elements, culminating in the collective creation of a cell structure comprised of specific actin-based structures (IV.4, 10). This feature further implies the potential of cells to update the internal structure by modulating the probability of the interaction as well as the quantity or characteristics of the components. Currently, the mechanism behind this flexible creation and disintegration of the cell structure utilizing the stochastic behavior of individual components remains only partially understood.

The above perspective, focusing on the cellular hierarchy comprised of structural components interacting with each other, evokes a concept in physics known as percolation theory (IV.2). Percolation theory describes how stochastic phenomena at a microscopic level yield an order at a macroscopic level by examining the interconnection among nodes within the system. Numerous probabilistic processes have been explored in diverse fields of science with the framework of percolation, which include invasion percolation, oriented percolation, and bond percolation (IV.1, 15). The percolation theory has drawn the interest of physicists because of its simple setup as well as non-trivial phenomena it exhibits in bridging different hierarchies. On the other hand, while some aspects of the cell structure have been scrutinized through the approach of percolation theory (IV.1, 7, 24, 27, 32, 39), its adaptive nature driven by transient environmental changes remains largely unknown.

Among actin structures in mesenchymal non-muscle cells such as fibroblasts, actin meshwork exists as a fundamental cytoplasmic element (IV.5, 33, 37). In addition, stress fibers (SFs) are expressed particularly on a stiff substrate while physically connecting separate focal adhesions (FAs) at their termini. SFs are comprised of bundles of AFs cross-linked by non-muscle myosin II and α -actinin to exert contractile tension onto the FAs (IV.18, 25). In cells residing on less stiff substrates, less amounts of SFs appear in the cytoplasm together with highly developed disordered actin meshwork. Thus, there is an exchange of the constituent AFs between the coexisting structures, actin meshwork and SFs, allowing the cells to adapt to the given environment. The interplay among actin molecules has been analyzed from a conventional percolation perspective (IV.7, 39). Meanwhile, significant ambiguity persists regarding the biological role of the concurrent presence of actin meshwork bearing less tension and SFs bearing more pronounced tension in cellular adaptive response to environmental stimuli.

Here we perform Monte Carlo simulations based on a percolation model to investigate

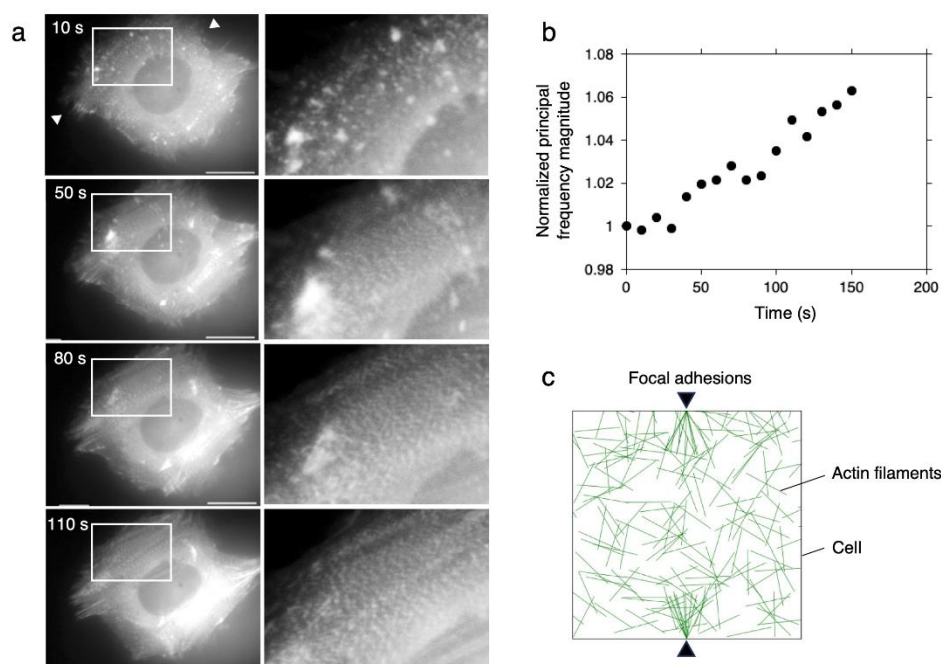


Figure IV-1 Percolation nature of intracellular structures. (a) Live cell imaging to observe the formation of SFs in A7r5 cell lines expressing α -actinin-1 prepared by a standard protocol (see Supporting Material). Cells are initially treated with (-)-blebbistatin to disrupt SFs, followed by washout at $t = 0$ to observe subsequent recovery. Because of the typically slower turnover of FAs compared to that of SFs, some cell-substrate adhesions are still remained (triangles), acting as an anchor to the substrate. As contractility recovered over time, SFs visualized by the periodic patterns of α -actinin-1 appear to link the partly remaining FAs. The right panels display a magnified view of the white rectangles. Scale, 20 μm . (b) Analysis of the time-series images with fast Fourier transformation, extracting the principal frequency component at each time and normalizing it against the value at $t = 0$. The observed increase over time suggests a transition, after the washout, in α -actinin-1 as well as coexisting AFs from a random meshwork having a low level of frequency magnitude to directional stress fibers (SFs) having a high level of frequency magnitude. (c) Cell model with two dimensional square lattices. AFs (green) are initially randomly distributed in the cell. We evaluate the formation of SFs by calculating the connection between the two FAs (triangles) located at the top and bottom edges of the cell.

how the stochastic behavior of AFs can lead to the distinction between directional tension-bearing SFs and random non-tension-bearing meshwork while also accommodating with environmental changes. The model incorporates the binding and unbinding probabilities of

AFs considering the influence of cellular tensional homeostasis, a fundamental property of proliferative non-muscle cells (IV.3, 39). Tensional homeostasis allows for keeping a constant level of intracellular tension through turnover, and hence it is also referred to as mechanical homeostasis. With this function, mechanotransduction-mediated biochemical signals activated upon the tension are also maintained constant, circumventing chronic activation of pro-inflammatory signaling and hence facilitating the ability of cells to adapt to applied mechanical stress (IV.12). Our percolation model encompasses actin meshwork and SFs without explicitly considering numerous other proteins and structures that actually exist in real cells such as non-muscle myosin II and filopodia/lamellipodia (IV.18). Regardless of this simplicity inherent to percolation theory focusing on the most essential factors (i.e., tension-bearing and non-bearing elements), our primary goal here is not to mimic such actual complex phenomena but rather to extract the role of the coexistence of the distinct structures in cellular adaptation. We also consider a minimal analytical model to delineate the distinction between the responses to elevation and reduction in homeostatic tension observed in the percolation analysis.

IV-3. Model Analysis

IV-3.1 Distribution of AFs

The actual cells are comprised of a variety of molecules and are thus complicated; but, to focus on the indispensable factor associated with intracellular force transmission, we explicitly consider only the involvement of AFs. To extract the percolation nature observed in actual cells (Fig. IV-1a, b), individual AFs are modeled to be a line segment of a length L and are distributed randomly in a two-dimensional $x - y$ plane that represents a cell with a square shape (Fig. IV-1c). The position of one end of AFs (x_1, y_1) is given randomly within the cell plane at each time step, and the other end (x_2, y_2) is expressed by

$$x_2 = x_1 + L \sin(b) \quad (\text{IV} - 1)$$

$$y_2 = y_1 + L \cos(b) \quad (\text{IV} - 2)$$

$$b = \pi(-1 + 2 \text{ rand}) \quad (\text{IV} - 3)$$

where b is the radian angle of the line segment, and rand is provided with a value between 0 and 1 according to a uniform random number generator. Unless otherwise stated, the length of individual AFs L is 0.2, the number of individual AFs is 250, and each cell edge length is 1.0.

IV-3.2 Analysis of SFs, FAs, and actin meshwork

FAs are represented by two different points, each of which is located at the center of the facing edges of the square cell plane (Fig. IV-1c). SFs are defined as a state of AFs that align within a specific angle ($-\pi/3 \leq \theta \leq \pi/3$) and collectively link the two separate FAs, in which θ is the radian angle measured from the line connecting the two FAs. The rationale for the directional alignment of AFs is that the actual SFs are comprised of bundled AFs bearing tension, distinct from the entangled actin meshwork bearing little tension compared to SFs. Thus, to prevent unintentional inclusion as SFs when large numbers of AFs lack specific alignment but linking the two FAs, it was necessary to define SFs based on the consistent alignment. We assumed that $n = 10$ AFs are linked at its one end to the FAs all the time, while the direction of the individual AFs can change randomly over time. These specific AFs are considered to constitute a part of the FAs and are thus not counted as being within the population of AFs. The actin meshwork is defined here to be a state composed of AFs that are not associated with SFs.

IV-3.3 Binding and unbinding of AFs

AFs that constitute SFs undergo continuous turnover, namely molecular exchange with the surrounding actin meshwork, and thus the binding and unbinding with the existing SFs occur simultaneously (IV.30). To mimic this feature, the binding and unbinding probabilities are defined as follows:

$$K_{\text{on}} = K_{\text{on}}^0 \exp\left(\frac{N_s - N_c[t]}{N_0}\right) \quad (\text{IV} - 4)$$

$$K_{\text{off}} = K_{\text{off}}^0 \exp\left(\frac{N_c[t] - N_s}{N_0}\right) \quad (\text{IV} - 5)$$

where N_s and $N_c[t]$ represent the number of AFs that is determined by the steady state tension in a SF undergoing tensional homeostasis and the actual number of AFs that constitute a SF at time t , respectively; N_0 is a normalizing coefficient set to be 100; and, K_{on}^0 and K_{off}^0 are the binding and unbinding probabilities at the initial state and set to be 0.35 and 0.20 (IV.13, 38), respectively. These equations suggest that K_{on} is exponentially increased if the actual number of AFs constituting a SF is lower than that under steady state tensional homeostasis, while K_{off} is rather increased if the actual number is, on the contrary, larger than that at tensional homeostasis. Note that there is no deterministic situation in intracellular activities so that both of the probabilities will never be zero. The rationale behind this formula is that SFs are thickened in response to increased tension (IV.4, 9), which is

allowed by the mechanoresponsive properties (IV.11, 13); specifically, the lifetime of non-muscle myosin on AFs is extended upon tension due to the catch bond mechanism, eventually increasing their number associated with SFs. Thus, $N_c[t]$ is supposed to increase over time with tension development. Meanwhile, N_s refers to a specific number of AFs that is more intrinsic to the cell state. Specifically, the phosphorylation of myosin regulatory light chain (MRLC) dictates the potential allowing for the non-muscle myosin–AF interaction, and the phosphorylation level varies depending on the intracellular and extracellular milieu (IV.11, 22). For example, cells on softer substrate exhibit lower levels of MRLC phosphorylation, and it ends up in lower levels of tension and hence thinner SFs or lower numbers of associated AFs. Given this intrinsic property of cells, N_s is given as a parameter interpreted as a function of the MRLC phosphorylation reflecting the intrinsic cell state. On the other hand, AFs forming the meshwork assumed to lack tension-bearing capacity and hence remain unaffected by K_{on} and K_{off} , which are mechano-signals transmitted through tension.

IV-3.4 Monte-Carlo simulation

The distribution of meshwork-associated AFs is first determined based on Eqs. IV-(1)–(3), which is followed by the determination of the specific AFs forming SFs. The values of K_{on} and K_{off} are then determined by counting the number of SF-associated AFs $N_c[t = 0]$ based on Eqs. IV-(4)–(5), and the state of individual AFs at the following time steps is likewise determined. In this process, AFs stay at their same positions with K_{on} , while they change their positions again randomly with K_{off} . The subsequent evolution of the position of AFs is analyzed by arbitrary numbers of steps regarded to be time t . At each time step t , the presence and absence of the AF-mediated link between the FAs yields 1 and 0, respectively (Fig. IV-S1). As the time evolution is dependent on the initial position of AFs and the stochastic event, the same Monte-Carlo simulation was repeated by 10^4 to obtain the Percolation Probability (PP) that represents the mean of the probability to span between the FAs, or the probability to form a SF, at each time t . PP for forming actin meshwork, which links facing edges of the cell plane as described above, is also obtained.

IV-3.5 Determination of the number of AFs

To determine $N_c[t]$, i.e., the number of AFs involved in the SF at time t , a line segment intersection detection algorithm using vector cross product is employed. The condition that the AFs cross with each other is described by using the vector cross product as

$$sd < 0 \quad (\text{IV} - 6)$$

$$s = \overrightarrow{P_1P_2} \times \overrightarrow{P_1P_3} = (x_2 - x_1)(y_3 - y_1) - (x_3 - x_1)(y_2 - y_1) \quad (\text{IV} - 7)$$

$$d = \overrightarrow{P_1P_2} \times \overrightarrow{P_1P_4} = (x_2 - x_1)(y_4 - y_1) - (x_4 - x_1)(y_2 - y_1) \quad (\text{IV} - 8)$$

where $P_1(x_1, y_1)$ is one endpoint of an AF forming a line segment, while $P_2(x_2, y_2)$ is the other endpoint; likewise, $P_3(x_3, y_3)$ and $P_4(x_4, y_4)$ describe the different endpoints of another AF. The presence of intersection is thus analyzed for all the combinations of individual AFs to count the total number associated with the FAs. AFs orienting within an angle of $-\pi/3 \leq \theta \leq \pi/3$ are picked up in this process as described above to finally obtain $N_c[t]$ as the number constituting a straight SF.

IV-3.6 Minimal model analysis

Minimal model analysis was carried out to investigate the mechanism underlying the discernible response between the creation and disintegration of SFs observed in the percolation analysis. Model assumptions, parameters, and predictions are described in Appendix.

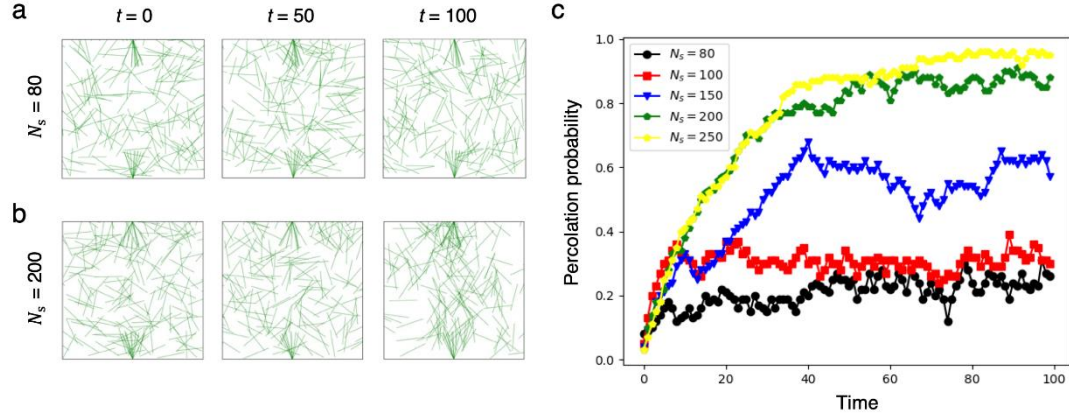


Figure IV-2 Percolation analysis. (a, b) Distribution states of AFs at $t=0$, $t=50$, and $t=100$ for $N_s=80$ (a) and $N_s=200$ (b). (c) Time evolution of the percolation probability for $N_s=80$ (black), $N_s=100$ (red), $N_s=150$ (blue), $N_s=200$ (green), and $N_s=250$ (yellow). With a larger N_s , the probability for forming SFs converges to a larger value.

IV-4. Results

IV-4.1 The effect of cellular tension generation potential on percolation

First, the effect of changing N_s on $N_c[t]$ was investigated. N_s denotes a specific number of AFs that reflects the inherent potential of cells to generate tension as already described, while $N_c[t]$ is the number of AFs involved in SFs. The length of individual AFs and each cell edge were set to be 0.2 and 1.0, respectively. Most AFs are involved in actin meshwork at $t=0$ because they are initially distributed randomly in the cell (Fig. IV-2a, b). To analyze how the Percolation Probability (PP) changes over time, where PP measures the probability of whether AFs form a SF or not, we repeated the simulation 10^4 times for each of $N_s = 80, 100, 150, 200$, and 250. The average behavior shows that PP gradually increases over time because the lifetime of AFs on a SF is extended upon binding due to the K_{on} -mediated catch bond behavior to allow the SF to be stabilized (Fig. IV-2c). The SF finally reaches a steady state where the association and dissociation are approximately balanced while subject to constant turnover, in which meshwork and SFs coexist. A greater value of PP was obtained at the steady state with a higher value of N_s , which raises K_{on} and lowers K_{off} to stabilize the existing SF. Thus, the majority of AFs are involved in meshwork without building SFs at a low N_s , while the association of AFs with SFs is increased with N_s , finally allowing the dominant population of AFs to reverse from that for meshwork to that for SFs. The sum of the different populations for meshwork and SFs remains constant at 250 during this interaction.

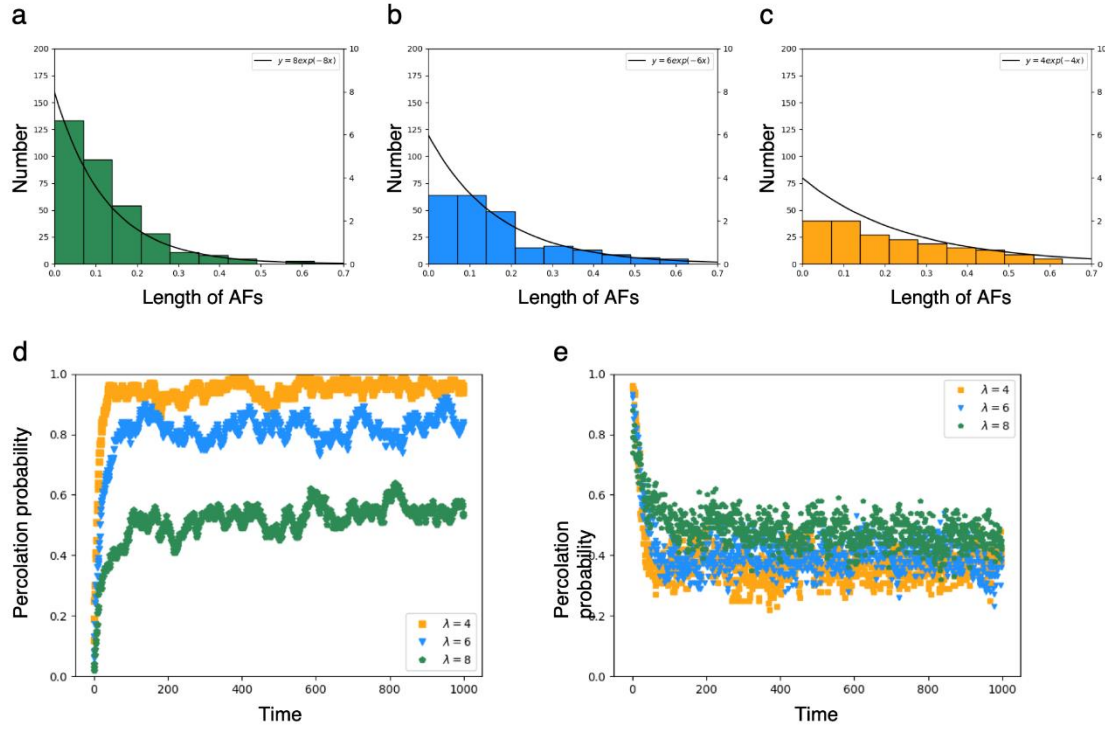


Figure IV-3 The effect of AF length distribution on percolation probability. (a, b, c) Probability density function and number of AFs at different length distributions. $y = 8e^{-8x}$ (a), $y = 6e^{-6x}$ (b), and $y = 4e^{-4x}$ (c). (d) Time evolution of the percolation probability of SFs. (e) Time evolution of the percolation probability of actin meshwork.

IV-4.2 The effect of AF length distribution on percolation

The need for having specific AF lengths is often focused on at the level of single subcellular structures such as primary cilia and muscle sarcomeres (IV.17, 19). For example, sarcomeres are optimized for efficient contraction by expressing nebulin protein to keep a fixed length of individual AFs (IV.14). In contrast, only little discussion has been made on the necessary length of AFs for non-muscle cells. We previously investigated based on statistical mechanics and thermodynamics the physical requirement for allowing non-muscle cells to maintain tensional homeostasis (IV.35). Specifically, we assumed that AFs as a tension-bearing component must have as diverse lengths as possible and occupy as much space as possible in the cytoplasm in accordance with the requirement for maximizing entropy. The theoretical consequence was that AFs need to have more short subpopulations than long ones, and in fact the length distributions obtained were in qualitative agreement with those that have been observed in experiments. Other potential biological benefits for possessing

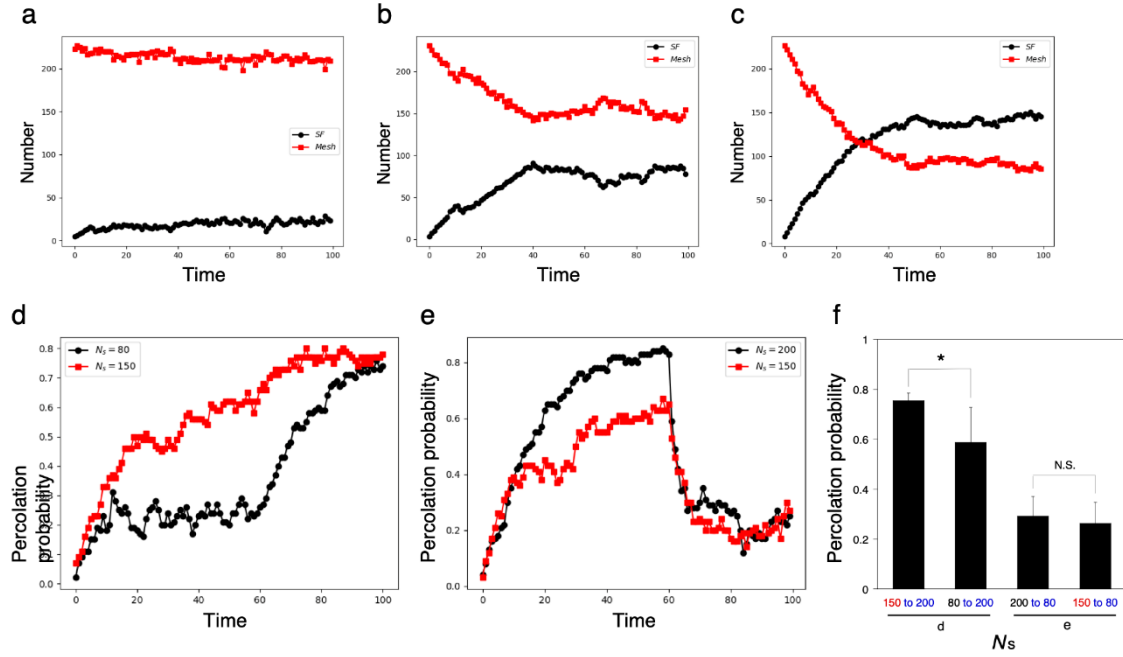


Figure IV-4 Adaptive response of the tension-mediated percolation nature. (a, b, c) Time evolution of the number of AFs, which build SFs (black) and meshwork (red) at $N_s = 80$ (a), $N_s = 150$ (b), and $N_s = 200$ (c). (d) Response to a stepwise change in N_s given at $t = 60$ from $N_s = 80$ (black) and $N_s = 150$ (red) to $N_s = 200$. (e) Response to a stepwise change in N_s at $t = 60$ from $N_s = 200$ (black) and $N_s = 150$ (red) to $N_s = 80$. (f) Evaluation of the difference in d and e. All the PP values were collected in the time period between $t = 60$ and 100 in each case, and the variation in the value was analyzed with student t -test. Asterisk indicates a statistical difference ($p < 0.01$), suggesting that there is a delay in the adaptation period between the two curves in d, whereas there is no delay in e.

such specific AF length distributions have not been thoroughly addressed in prior studies. With the present numerical approach to analyze the intracellular percolation, we then tackled this question.

The effect of providing a variety of AF lengths on PP was then investigated, while keeping the same total length of AFs. We determined the length distribution of individual AFs by employing an exponential function,

$$y(x; \lambda) = \lambda e^{-\lambda x} \quad (\text{IV} - 9)$$

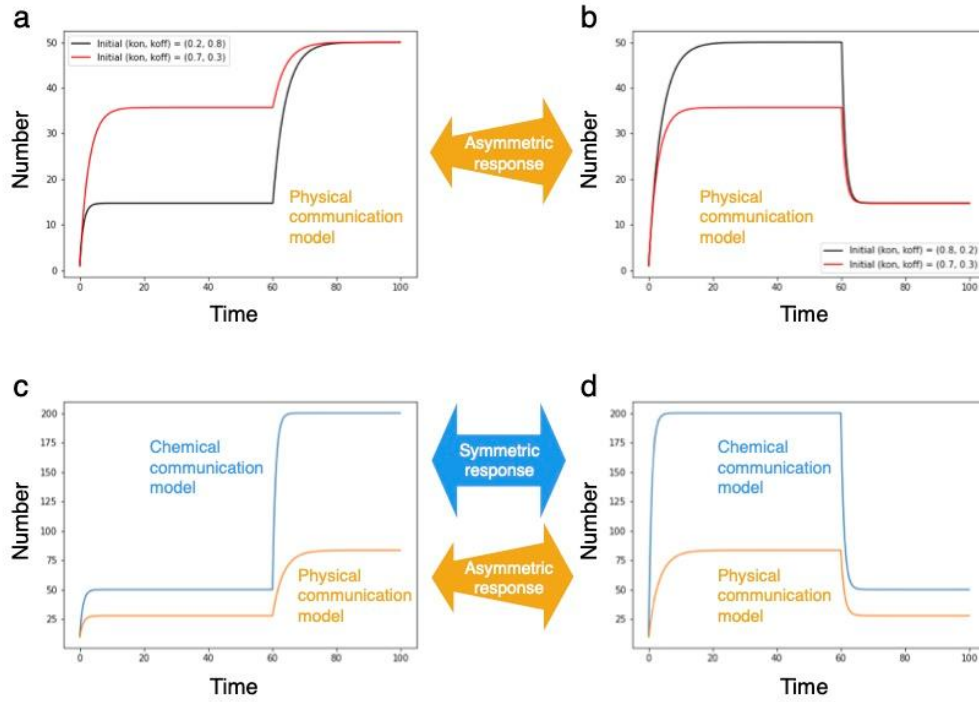


Figure IV-5 Minimal model analysis to decipher the mechanism of the asymmetric response. (a, b) Time-series change in N_b (Number) of the physical communication model. (c, d) Time-series change in N_b (Number) of the chemical communication model.

where x is the length, and λ is a parameter that characterizes the distribution. The rationale for introducing this exponential distribution is that previous studies including ours have indicated that the length of AFs inside of living cells has an exponential distribution-like probability function (IV.21, 35). We gave λ a value of 8, 6, or 4 to have a distribution inclined to short length regions to a highest (Fig. IV-3a), middle (Fig. IV-3b), or weakest degree (Fig. IV-3c), respectively. The time course of PP shows that the most uniform length distribution ($\lambda = 4$) and the one most inclined to shorter length regions ($\lambda = 8$) allow SFs and actin meshwork to be relatively easily formed, respectively (Fig. IV-3d, e). Meanwhile, the middle one ($\lambda = 6$) exhibits a tendency in between them. Thus, the length distribution affects the preferred choice of cross-linking style in the cytoplasm, in which meshwork requires tension transmission throughout the cytoplasm so that short AF populations suitably contribute to the formation, whereas SFs bear a tension in particular directions so that the presence of longer AFs more efficiently enables such unidirectional communication.

IV-4.3 Adaptive response to change in cellular tension generation potential

Next, the effect of changing the value of N_s in the process of percolation development was investigated, which aims at analyzing the adaptive response of the cell model to environmental change. As N_s reflects the cellular capability of generating tension, which varies in accordance with the surrounding milieu, the temporal change in N_s simulates a process in which cells are exposed to pharmacological, signaling, or biophysical stimulation; e.g., cells increase and decrease the capability of generating tension on stiff and soft substrates as well as with increased and decreased Rho-kinase activity as the kinase for MRLC, respectively (IV.22).

First, we confirmed that the system approximately reaches the steady state by $t = 60$ after percolation formation initiated at $t = 0$ at $N_s = 80, 150$, and 200 (Fig. IV-4a, b, c). We then analyzed the effect of increasing the value of N_s from 80 or 150 to 200 in a stepwise manner at $t = 60$ (Fig. IV-4d). The states of $N_s = 80$ and 150 represent the cell where actin meshwork exists predominantly over SFs (Fig. IV-4a) and certain levels of actin meshwork and SFs coexist (Fig. IV-4b), respectively, and then the response to a stepwise increase to 200 where SFs should be more dominant (Fig. IV-4c) was analyzed. The analysis showed that the increase in N_s from 80 to 200 takes longer time compared to that from 150 to 200 , which seems evident given the smaller quantities of SFs in the former case at $t = 60$. Next, we decreased the value of N_s , on the contrary, from 150 or 200 to 80 at $t = 60$ (Fig. IV-4e). Interestingly, unlike the above case that PP reaches the same level of a steady state upon the increased N_s at a different timepoint, the present PP sharply decreases to a steady state upon the change in N_s in both cases.

To evaluate the difference in the rate of the response, all the PP values were collected during an adaptive period of between $t = 60$ and 100 for each condition and were analyzed with student t -test. The statistical analysis shows that the PP values were significantly higher for the change of N_s from 150 to 200 compared to that from 80 to 200 . Meanwhile, there is no significant difference in the PP values during the adaptive period between the change of N_s from 200 to 80 and that from 150 to 80 . These results suggest that the extent of the coexistence of actin meshwork and SFs does impact the duration of percolation formation, in which advanced coexistence (with an initial N_s value of 150) is advantageous for rapid adaptation to complete percolation than less developed conditions (with an initial N_s value of 80), whereas interestingly it has no delay effect for the percolation collapse.

Finally, we considered a minimal model (Appendix) to interpret the mechanism behind the difference in response to the time-varying N_s found in the percolation analysis. We demonstrate that the asymmetry between creation and disintegration in response to

environmental changes is intrinsic to mechano-signal transmission mediated by physical tension (Appendix, Fig. IV-5).

IV-5. Discussion

What is intriguing and essential in general percolation analysis is the consideration of a hierarchical system comprising at least two layers, in which the individual elements of the lower layer interact with neighboring elements at a local level without recognizing the overarching structure, and then only modulating the stochastic rate of these interactions at the microscopic level can lead to an emergence of a new structure at the macroscopic level. These hierarchical systems can be observed in individual adherent cells, in which tension plays a pivotal role in mechanosensitive regulation in the actin meshwork-based cytoplasm to form SFs at suitable locations. Cells thus flexibly reconfigure their internal structures in accordance with the surrounding milieu, exhibiting an inherent percolation nature in their adaptive behavior.

More specifically, directional SFs are created from and maintained within the random actin meshwork (IV.37), acting as a physical connector between separate FAs in a process reminiscent of general percolation. Our percolation analysis simulated the stochastic interaction among AFs as a microscopic component and the potential emergence of SFs as a macroscopic structure spanning the entire cell. The binding/unbinding probability of the tension-bearing element AFs is modulated by N_s that represents the cellular capacity to generate homeostatic tension, which is basically determined in actual cells by each cell type and environment, thus serving as a global parameter. There is another factor that locally modulates the probability, namely $N_c[t]$, which denotes the number of AFs associated with an existing SF. $N_c[t]$ is transmitted as a mechano-signal through the tension-bearing AFs without recognizing the overarching cell structure, but nonetheless can contribute to the formation of SFs at particular locations. Thus, the states of microscopic elements and their collective entity are coordinately interrelated within the hierarchical cell system, and a change in one layer impacts the others by leveraging the inherent nature or percolation to flexibly cope with continuously generating intracellular/extracellular perturbations. This percolation-based perspective is essential in comprehending the environment-dependent self-assembly of living systems composed of multiple interacting components, allowing proliferative cells to alleviate the stress inevitably provided from time-varying intracellular and extracellular milieu (IV.8, 12).

Our percolation analysis showed that a high and low level of N_s leads to a different cell state where SFs and actin meshwork are more preferably expressed, respectively. These results agree well with experimental observations given that N_s is associated with the level

of MRLC phosphorylation (IV.22). N_s in reality reflects more general capability of the cell to generate homeostatic tension so that this parameter is actually affected by various environmental cues. For example, cells more preferably express actin meshwork and SFs on a softer and stiffer substrate and at a smaller and larger adhesive area, respectively (IV.26), and this selective architectural style can also be interpreted to correspond in our percolation analysis to a case with a low and high value of N_s . Furthermore, our results showed that the length distribution of AFs modulates the intracellular structure even if the value of N_s is the same. Specifically, the distribution inclined toward shorter length populations more stabilizes actin meshwork, suggesting that cells also achieve adaptation by modulating the statistical nature of AF length.

We found that the coexistence of SFs with larger amounts of meshwork induces a greater delay in reaching a steady state following an increase in N_s , while it causes no delay in relation to reduction in N_s regardless of the meshwork amount. In other words, SFs are created at a rate contingent on the quantity of preexisting actin meshwork, while interestingly their disassembly always occurs rapidly. Actual cells indeed bring about disassembly of SFs slightly earlier than their assembly, in which disassembly is observed within several minutes, while assembly typically takes several tens of minutes in response to mechanical stress (IV.9, 11, 20, 21, 31). This tendency can be explained in our model by focusing on that AFs associated with SFs are the elements responsible for transmission of physical tension as a change in K_{on} . Meanwhile, AFs belonging to meshwork merely continue to move randomly. Thus, SFs must first be created, if cells are initially comprised only of meshwork, for the transmission of physical signals and then be increased in number to finally reach a steady state. This asymmetric behavior may also be related to the common response of cells under transient mechanical stress, which shows prompt fluidization followed by gradual resolidification (IV.3, 34).

We then attempted to explain the origin of the disparity between assembly and disassembly based on a minimal model (Appendix). Tendencies akin to those in the percolation analysis were obtained, suggesting that physical communication via tension transmission intrinsically induces an asymmetric response to environmental changes. From a biological perspective, it may be reasonable to propagate signals at a consistent rate irrespective of assembly and disassembly if both exert an equally significant impact on the entire system. Conversely, if the impacts differ between them, possessing such asymmetric rates can present potential benefits. There are always cases that provide cells with an impactful change upon assembling subcellular structures. Focusing on metabolic processes, for example, anabolism to create structures involves net energy consumption, while catabolism to disintegrate does not. In addition, creating subcellular structures lower the

degree of freedom at the whole cell level rather than disintegrating them, thereby leading to decrease in structural flexibility by increased constraint. Therefore, it seems advantageous to create structures as meticulously as possible in harmony with dynamically changing environments or otherwise to shift to a rapid collapse once deemed unnecessary. Overall, our analysis highlights the asymmetric rate regulation between the creation and disintegration of subcellular structures, providing new insights to the mechano-signal communication in cells.

IV-6. Appendix

A minimal analytical model is described to contemplate the difference in the adaptive response observed in the percolation analysis (Fig. IV-4). Let the total number of AFs N remain constant over time and comprised of the AFs in the binding state N_b and those in the unbinding state N_u , namely,

$$N = N_b + N_u. \quad (\text{IV} - \text{A1})$$

We examine the time course of the bound population N_b by distinguishing the means of communications with the surrounding environment between physical and chemical ways within cells.

Physical communication via tension transmission occurs exclusively via specific AFs under binding with other AFs. Bound AFs can take two states at the subsequent time step: they either remain bound with a probability of k_{on} or unbind with a probability of k_{off} , in which k_{on} and k_{off} can vary in accordance with the magnitude/direction of the tension reflecting the instantaneous environmental conditions (as simulated in the percolation analysis by the stepwise change in N_s). Unbound AFs, in contrast, exhibit only stochastic behavior following a probability distinct from k_{on} and k_{off} . More in detail, unbound AFs undergo two steps to restore binding: random diffusion toward SFs and bind with them. The diffusion may be affected in real cells by environmental changes such as alterations in intracellular concentration, but these changes are not suitable for direct comparison with the changes physically transmitted by tension. The time course change of N_b is then described by

$$\frac{\partial N_b}{\partial t} = -\frac{k_{\text{off}}}{k_{\text{on}} + k_{\text{off}}} N_b + A N_u \quad (\text{IV} - \text{A2})$$

where A is a binding probability of unbound AFs, which is not related to the environment-associated tension. Thus, only bound AF populations, and not unbound ones, are directly subjected to k_{on} and k_{off} reflecting mechano-signals.

Time course change of the physical communication model using Eq. (IV-A2) actually shows a tendency similar to that of the percolation model (Fig. IV-5a, b). Specifically, a concomitant increase in k_{on} from 0.7 to 0.8 and decrease in k_{off} from 0.3 to 0.2 (Fig. IV-5a, orange), with a constant total value of $k_{\text{on}} + k_{\text{off}} = 1$ and a constant value of $A = 0.05$, reaches a steady state earlier than another concomitant increase in k_{on} from 0.2 to 0.8 and decrease in k_{off} from 0.8 to 0.2 (Fig. IV-5a, blue). Meanwhile, a concomitant decrease in k_{on} from 0.8 to 0.2 and increase in k_{off} from 0.2 to 0.8 (Fig. IV-5b, blue) reaches a steady state simultaneously with another concomitant decrease in k_{on} from 0.7 to 0.2 and increase in k_{off} from 0.3 to 0.8 (Fig. IV-5b, orange). Thus, an asymmetric behavior between creation and disintegration, driven by an increase in N_b and a decrease in N_b , respectively, consistent with the percolation simulation result (Fig. IV-4), is obtained even in the minimal model.

On the other hand, chemical communication independent of physical binding is described by

$$\frac{\partial N_b}{\partial t} = -\frac{k'_{\text{off}}}{k'_{\text{on}} + k'_{\text{off}}} N_b + \frac{k'_{\text{on}}}{k'_{\text{on}} + k'_{\text{off}}} N_u \quad (\text{IV} - \text{A3})$$

where the binding probability k'_{on} and unbinding probability k'_{off} may vary depending on intracellular and extracellular environmental conditions. The transition of AFs from binding to unbinding can reflect respective environmental changes in both physical and chemical communications. Note however that the transition from unbinding to binding in physical communication occurs in a manner unrelated to the environmental changes, but that in chemical communication can reflect them. Thus, an asymmetry is inherently present between physical and chemical communications: only bound AFs communicate with the environment in the former through k_{on} and k_{off} , whereas both bound and unbound AFs do that in the latter through k'_{on} and k'_{off} .

The mode of creation and disintegration is compared between the physical and chemical communication models (Fig. IV-5c, d). Specifically, a concomitant increase in k_{on} from 0.2 to 0.8 and decrease in k_{off} from 0.8 to 0.2 are applied to both the physical (Fig. IV-5c, orange dashed; $A = 1.0$) and chemical (Fig. IV-5c, blue solid) communication models. Separately, a concomitant decrease in k_{on} from 0.8 to 0.2 and increase in k_{off} from 0.2 to 0.8 are applied to both the physical (Fig. IV-5d, orange dashed) and chemical (Fig. IV-5d,

blue solid) communication models. These results suggest that, while the creation is delayed compared to disintegration in the physical communication as already observed (Fig. IV-5a, b), both creation and disintegration occur rapidly in the chemical communication. Thus, unlike the asymmetric behavior intrinsic to the tension transmission or mechano-signals, the chemical communication yields a symmetric response between creation and disintegration, suggesting that the minimal model captures the essence of the phenomena of interest.

IV-7. Supporting Material

Figure IV-S1

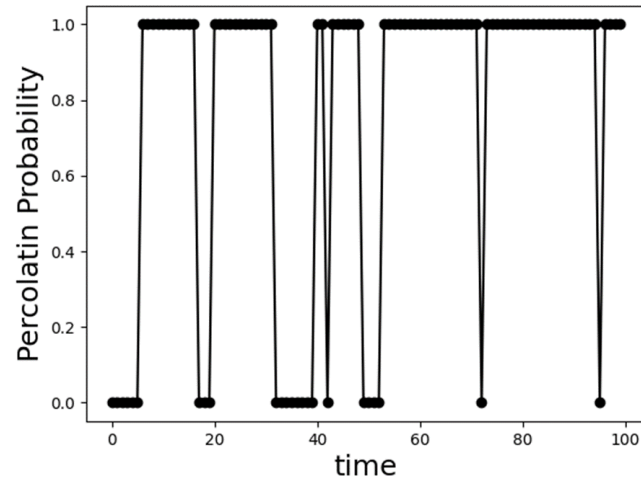


Fig. IV-S1 One case of the percolation simulation, in which 1 is provided if the separate FAs are connected, while 0 is provided if they are not connected.

Methods for cell preparation in Figure IV-1

Rat aortic smooth muscle cell lines (A75r, ATCC) were cultured with low-glucose (1.0 g/L) Dulbecco's Modified Eagle Medium (Wako) containing 10% (v/v) heat-inactivated fetal bovine serum (SAFC Biosciences) and 1% penicillin-streptomycin (Wako) in a humidified 5% CO₂ incubator at 37°C. An expression plasmid encoding non-muscle α -actinin-1 was transfected to cells using Lipofectamine LTX and Plus Reagent (Thermo Fischer Science) according to the manufacturer's instruction. To initially disrupt SFs, (-)-blebbistatin (10 μ M, Wako) was applied to cells for 20 minutes.

V Adaptive flexibility of cells through nonequilibrium entropy production

V-1. Summary

Cellular adaptation to environmental changes relies on the dynamic remodeling of subcellular structures. Among these, periodic actomyosin assemblies are fundamental to the organization and function of the cytoskeletal architecture. In muscle-type cells, sarcomeres exhibit ordered structures of consistent lengths, optimized for stable force generation. By contrast, nonmuscle-type cells exhibit greater structural variability, with sarcomere-like periodic units of varying lengths that contribute not only to force generation but also to adaptive remodeling upon environmental cues. These structural differences have traditionally been attributed to the specific protein compositions unique to each cell type. However, the functional significance of such periodic unit variability remains poorly understood within a unified framework. Here, we propose a conceptual model grounded in nonequilibrium physics to provide a unified perspective on structural variability in cytoskeletal adaptation. Specifically, we demonstrate that the effective binding strength of these contractile units can be evaluated by quantifying structural randomness through Shannon entropy. The increased entropy associated with the inherent randomness of sarcomere-like assemblies in nonmuscle-type cells lowers the energy barrier for cytoskeletal remodeling, enabling flexible adaptation to environmental demands. In contrast, the ordered sarcomere arrangements in muscle-type cells correspond to higher binding energies, stabilizing cytoskeletal configurations for sustained force generation. While structural disorder is often regarded as a source of instability, our analysis reveals that it can serve as a driver of cytoskeletal remodeling and a foundation for adaptive cellular behavior. Thus, our study provides a unified theoretical foundation for understanding cytoskeletal adaptability across diverse cell types by integrating structural randomness into a nonequilibrium framework.

V-2. Introduction

Living cells adaptively reorganize their internal structures in response to changes in both their internal and external environments (V. 1,2). This structural adaptability is fundamental to individual cellular processes such as differentiation, proliferation, and apoptosis, as well as to higher-order processes such as tissue development and wound healing (V. 3,4). The cytoskeleton is central to the physical structure and force generation of cells, maintaining its integrity through organized protein complexes. Among these, periodic actomyosin structures, primarily composed of actin, myosin II, and α -actinin, function as fundamental units of cellular contractile architecture.

In striated muscles, these periodic assemblies are referred to as sarcomeres and constitute the core of myofibrillar organization (V. 5–9). Meanwhile, similar sarcomere-like periodic structures are also present in ventral stress fibers of nonmuscle cells (V. 10–18). Compared to the uniform length and alignment of muscle sarcomeres, these nonmuscle counterparts exhibit greater structural variability; and even within muscle cells, interestingly, younger ones display broader distributions of sarcomere lengths, indicating greater structural randomness than mature ones (V. 19,20). Furthermore, this variability is more pronounced in motile cells than in nonmotile cells (V. 11,12). Muscle sarcomeres primarily support coordinated contractility within tissues (V. 5), while nonmuscle sarcomere-like structures are involved in diverse dynamic cellular processes including migration, division, and structural remodeling upon environmental changes (V. 21–24).

In biological research, traditionally, these differences have been attributed to the unique properties of specific proteins expressed in each cell type, often within a reductionist framework. While this molecular perspective is indispensable for identifying components and enabling targeted manipulation, it can be complemented by a comparative physical approach that seeks a unified interpretation of how architectural variability is intrinsically linked to distinct cellular functions. Prior studies from a physical perspective have aimed to elucidate the mechanisms for the self-organization of actomyosin periodicity (V. 20–22,25,26), with some attempts in exploring how their contractions generate cellular tension and in turn maintain cytoskeletal organization (V. 5,27,28). However, how the variability of such periodic structures contributes to cellular functions remains an open question, as no unified theoretical framework has been established.

Here, we describe a conceptual model based on a nonequilibrium physics framework to provide a unified interpretation of the relationship between subcellular structural variability and adaptability. We analytically derive constraints on the probability distribution of these structures and the effective binding energy among their constituent units to evaluate how

structural variability enhances adaptability. To incorporate nonequilibrium effects, we formulate a Fokker–Planck equation describing the probability distribution of actomyosin-based periodic configurations and consider entropy production as an indicator of the energetic cost required for maintaining these nonequilibrium structures, specifically referring to the cytoskeletal system composed of contractile units. We also analyze the length distribution of actomyosin contractile units from experimental results on different cell types and interpret its functional implications within our theoretical framework. Finally, we explore the parameter space of our model to investigate how variations in structural properties influence adaptability. Our results suggest a fundamental physical principle, indicating that actomyosin organization should be understood not merely at the scale of individual contractile units, but as an emergent system operating at the cellular level to enhance adaptive flexibility. Interestingly, while structural randomness often impairs system function in engineered systems, it instead serves as a key driver of adaptability in living organisms.

V -3. Theory and Methods

V -3.1 Entropy production

In striated muscle, the sarcomere is a well-defined periodic structure composed mainly of actin, myosin, and α -actinin, among other proteins. In contrast, non-muscle cells often exhibit actomyosin structures with irregular, sarcomere-like periodicity. In this study, we collectively refer to the canonical sarcomeres in muscle cells and the sarcomere-like structures in non-muscle cells as contractile units, defined by the periodic spacing between α -actinin-rich regions along actomyosin bundles (Fig. V -1). This operational definition serves as a marker of intracellular structural organization. For the purposes of our modeling framework, we consider the system to be the cytoskeletal structure composed of contractile units, while the surrounding intracellular environment, assumed to be sufficiently large, serves as a heat bath that remains close to thermal equilibrium. While many actomyosin-binding proteins contribute to remodeling in specific contexts, their roles differ substantially between muscle and nonmuscle cells. By contrast, actin, myosin II, and α -actinin represent the only structural components whose isoform differences may be directly compared across both systems, and thus these can be assumed as elements comprising the cytoskeletal system, without excluding possible contributions from other proteins.

We then express the nonequilibrium entropy production during the growth process of the cytoskeletal structure while nearby contractile units bind together or unbind. Given the

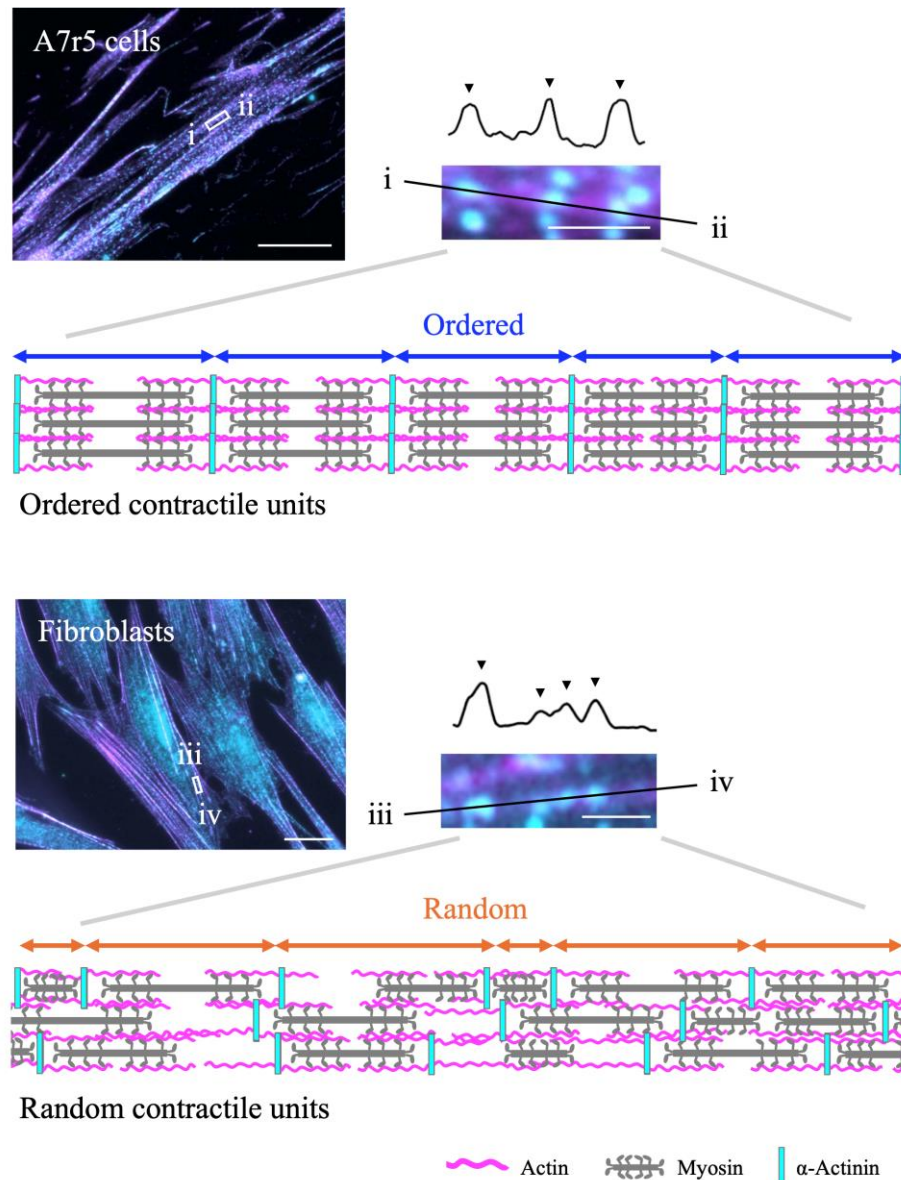


Fig. V -1 Schematic of contractile unit structure. The upper diagram (blue) shows ordered sarcomeres with specific lengths, while the lower diagram (orange) shows their disordered counterparts with varying lengths. The upper image corresponds to A7r5 cells, and the lower to fibroblasts, illustrating representative examples. White rectangles are magnified to evaluate the intensity of α -actinin (cyan) along the indicated F-actin bundles (magenta). Arrowheads indicate peaks in the intensity curves. Scale, 20 μm (left) and 2 μm (magnified).

inherent complexity of cytoskeletal systems, including the involvement of over a hundred distinct proteins in actin contractile bundles (V . 29), modeling at the individual protein level would inevitably require selective assumptions, potentially obscuring the broader functional roles underlying contractile unit fluctuations. To circumvent this, we adopt a coarse-grained approach in which the combined action of these proteins is treated as equivalent to contractile unit-level binding and unbinding, using the contractile unit as the fundamental unit of analysis.

The total entropy production, considered for both the system and the environment, is defined as

$$\sigma = \Delta S_{sys} + \Delta S_{bath} \geq 0 \quad (V - 1)$$

where ΔS_{sys} and ΔS_{bath} are the change in entropy of the system and of the surrounding environment, respectively (V . 30). We consider the probability distribution of the contractile unit length in one dimension, describing its randomness, defining the probability of its length state x as $P(x)$. In nonequilibrium systems, ΔS_{sys} can be expressed using Shannon entropy as follows (V . 26):

$$\Delta S_{sys} = - \sum P(x) \ln P(x). \quad (V - 2)$$

For ΔS_{bath} , we assume that the environment can freely exchange constituent contractile unit elements and energy with the preexisting cytoskeletal structure and is therefore described by a grand canonical distribution under this theoretical assumption. The change in entropy of the environment is then given by

$$\Delta S_{bath} = -\beta(\Delta E - \Delta\mu) \quad (V - 3)$$

where ΔE is the energy change in the cytoskeletal structure due to contractile unit binding, $\Delta\mu$ is the change in chemical potential as contractile units transition from the particle bath to within the cytoskeletal structure, and $\beta = 1/k_b T$ representing the inverse thermal energy,

with k_b being the Boltzmann constant ($1.38 \times 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$) and T the absolute temperature (310.15 K). Using Eqs. (V –2) and (V –3), the total entropy production is expressed by

$$\sigma = - \sum P(x) \ln P(x) - \beta(\Delta E - \Delta\mu) \geq 0, \quad (\text{V} - 4)$$

showing that the entropy production during the forming process of the cytoskeletal structure is always positive.

V -3.2 Maximum binding energy

We define ΔE in Eq. (V –3) as the energy change due to contractile unit binding to the cytoskeletal structure, corresponding to the binding energy, or $\Delta E = \Delta E^b$. From Eq. (V –4), the maximum binding energy is described by

$$\Delta E^b \leq \Delta E_{max}^b = \Delta\mu - \frac{1}{\beta} \sum P(x) \ln P(x). \quad (\text{V} - 5)$$

Based on the second law of thermodynamics, the nonequilibrium formation of the cytoskeletal structure driven by contractile unit binding is accompanied by a positive entropy change. This constraint imposes a limit on the binding energy, which represents the strength of the interaction between contractile units, with a more negative binding energy indicating a stronger binding. While unbinding can also be described by reversing the signs of ΔE and $\Delta\mu$, we focus on the binding process as a representative case that captures the essential thermodynamic behavior.

V -3.3 Probability distribution of contractile unit structures

We also define $P(x)$ based on a simple conceptual model that captures the variations in contractile unit length, while using experimental data to examine its quantitative trends across different cell types. Given that the contractile unit exhibits a biophysical response

characterized by a potential energy landscape that is inherently convex downward with respect to its stable length x_0 , we express the potential energy $U(x)$ at contractile unit length x as

$$U(x) = \frac{1}{2}k(x - x_0)^2 \quad (\text{V} - 6)$$

where k represents the restoring contribution of the contractile unit. The convexity of this function follows from the inherent properties of the actomyosin structure, which we justify in Supporting Note 1, and extend to asymmetric configurations in Supporting Note 2. Using the Fokker-Planck equation, the time evolution of $P(x, t)$ is described by

$$\frac{\partial P(x, t)}{\partial t} = -\frac{\partial}{\partial x}\{-k(x - x_0)P(x, t)\} + \frac{\partial^2}{\partial x^2}\{DP(x, t)\} \quad (\text{V} - 7)$$

where D is the diffusion coefficient, representing the probabilistic spread of contractile unit configurations in relation to energy stability; in this conceptual model, D captures the effective diffusive contribution of thermal fluctuations and actomyosin-driven dynamics as a coarse-grained representation without explicitly modeling these mechanisms. By solving Eq. (V -7) under the steady-state condition, we obtain the stationary probability distribution as

$$P(x) = \frac{\exp\left(-\frac{k(x - x_0)^2}{2D}\right)}{\sum \exp\left(-\frac{k(x - x_0)^2}{2D}\right)} \quad (\text{V} - 8)$$

where $P(x)$ satisfies the normalization condition $\sum P(x) = 1$. We quantitatively analyzed the maximum binding energy ΔE_{max}^b in Eq. (V-5) by considering specific $P(x)$. For this analysis, D was set to $10 \mu\text{m}^2/\text{s}$ based on typical molecular diffusion coefficients (V. 31–34), and $\Delta\mu$ was estimated to be on the order of 20 kJ/mol from experimental measurements of the energy associated with ATP hydrolysis (V. 35,36). While $\Delta\mu$ does not correspond to a specific reaction, it reflects the average energetic input required to organize disordered components

into more ordered structures. Thus, $\Delta\mu$ serves as a coarse-grained chemical potential that integrates diverse chemophysical contributions involved in contractile unit formation.

V-3.4 Quantification of contractile unit length

The actual contractile unit length was estimated as a representative value using previously published experimental images from multiple sources, including myofibrils isolated from mouse skeletal muscles (V . 37), primary mice myotubes (V . 38), and neonatal rat cardiomyocytes (V . 39), which predominantly express striated muscle-type actin and myosin molecules; and human fibroblasts (V . 40), PtK2 long-nosed potoroo epithelial kidney cells (V . 40), gerbil fibroma cells (V . 40), and A7r5 rat aortic smooth muscle cells (V . 41), which express nonstriated muscle-type actin and myosin molecules. Among them, A7r5 cells also express α -SMA, a smooth muscle-specific actin isoform commonly used as a marker for smooth muscle cells and myofibroblasts; these cells adopt a synthetic phenotype and therefore do not form the side-polar myosin filaments typical of fully differentiated smooth muscle cells (V . 42,43). Images of endogenous α -actinin immunostaining (V . 38–41) were analyzed by using ImageJ/Fiji software (NIH). Fluorescence intensity profiles were analyzed along the longitudinal axis of multiple contractile units to measure the distance between adjacent α -actinin–positive regions, with their centers defined by intensity maxima (Fig. V - 1). The measured inter-peak distance was thus used to define the contractile unit length. We analyzed $n = 40$ – 48 contractile units for each of the three striated muscle types (myofibrils, myotubes, and cardiomyocytes), and $n = 53$ – 56 contractile units for each of the four nonstriated muscle types (fibroblasts, PtK2, gerbil fibroma, and A7r5 cells) to obtain the respective distributions. To illustrate representative sarcomere distributions, we also hereby performed immunostaining for α -actinin and actin filaments on A7r5 cells following method (V . 41) and on human foreskin fibroblasts following method (V . 29) (Fig. V - 1). Rather than performing statistical testing, we focused on extracting characteristic length scales from documented samples to provide a comparison across different conditions.

V-4. Results

V-4.1 Contractile unit distributions and their biophysical implications

Contractile structures in both muscle and nonmuscle cells exhibit a common periodic organization, yet differ markedly in structural variability. Muscle-type sarcomeres form highly ordered, three-dimensional lattices with uniform lengths, whereas nonmuscle contractile units lack such precise organization and appear more heterogeneous (V . 41). This observed

variability, however, may not merely be a visual distinction but rather reflect differences in the effective binding strength among contractile unit components. To examine this possibility, we analyzed contractile unit length distributions across different cell types and quantified their structural variability.

Specifically, in myofibrils isolated from mouse skeletal muscles (hereafter referred to as Skeletal myofibrils), primary mouse myotubes (Myotubes), neonatal rat cardiomyocytes (Cardiomyocytes), which all predominantly express striated muscle-type actin and myosin molecules and are characterized by relatively large contractile forces, sarcomeres were consistently observed to maintain a specific length (Fig. V-2a). To quantify the consistency of sarcomere length, we calculated Shannon entropy for each distribution (Fig. V-2b), confirming that sarcomeres in muscle-type cell types exhibit lower levels of entropy, indicative of a more ordered structural arrangement. In contrast, contractile units in human fibroblasts (Fibroblasts), PtK2 long-nosed potoroo epithelial kidney cells (PtK2 cells), and gerbil fibroma cells (Fibroma cells), which express nonstriated muscle-type actin and myosin molecules and are characterized by less contractile force, exhibited greater length variability and higher entropy. Thus, while nonmuscle contractile units retain their periodic structure (V. 15,44), they show a relatively random organization compared to muscle sarcomeres. A7r5 rat aortic smooth muscle cells (A7r5 cells), which coexpress both α -SMA and muscle-type α -actin, displayed an intermediate level of structural randomness among the analyzed cell types.

The effective binding energy for different actomyosin structures was estimated using Eq. (V-5) and the measured distributions, showing its proportionality to the change in system entropy at a fixed chemical potential of -20 kJ/mol (Fig. V-2c). Nonmuscle contractile units exhibit lower binding energies than muscle sarcomeres, suggesting a greater capacity for structural remodeling. This trend is consistent with experimental observations (V. 5,10–17,45), showing that nonmuscle contractile units undergo more dynamic reorganization than their muscle counterparts. Here, we define adaptability as the structural plasticity that enables contractile units to reorganize in response to environmental cues. From this perspective, a lower binding energy corresponds to a more flexible state, as it allows easier structural rearrangement in response to external perturbations. This interpretation supports our notion that the ordered arrangement of contractile units is not merely a property of individual elements, but an emergent system property that enables structural adaptability at the cellular scale.

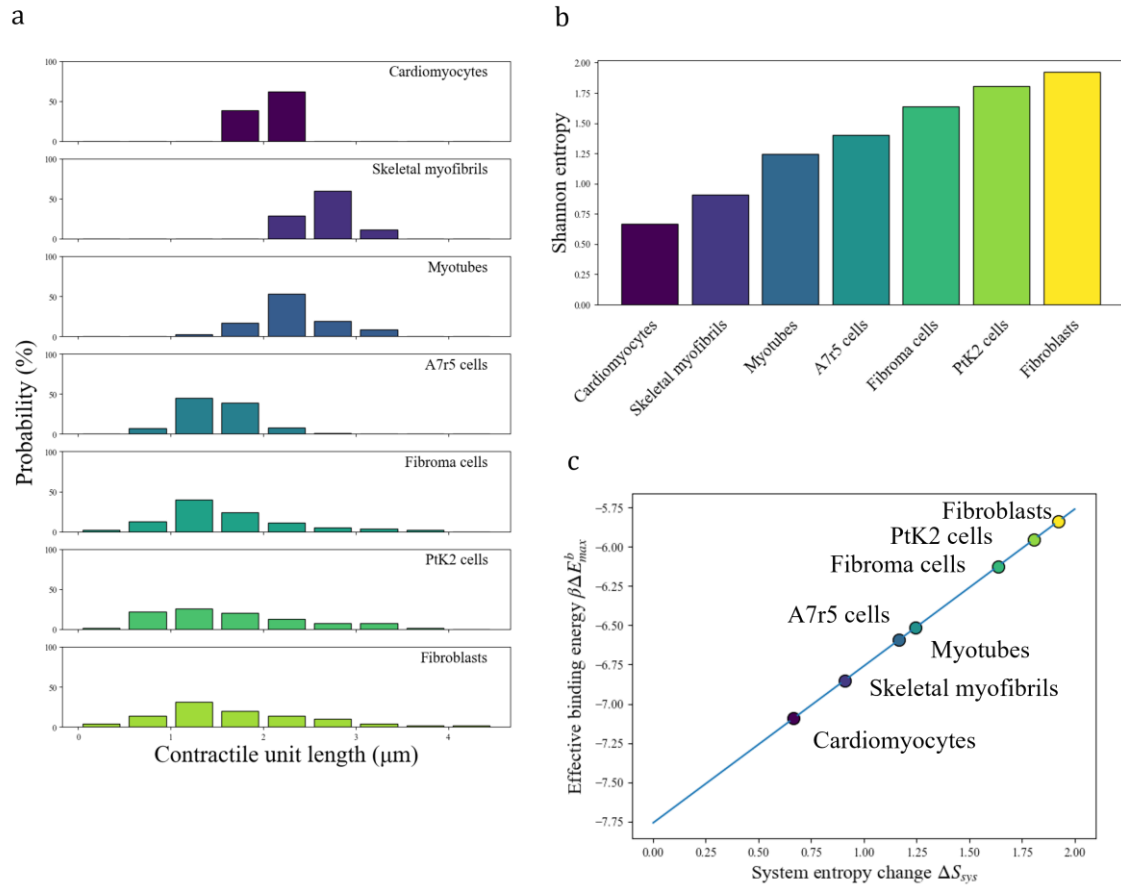


Fig. V-2 Quantification of contractile unit length variability and estimation of binding energy. (a) The actual contractile unit length was quantified using previously published experimental images from multiple sources, including neonatal rat cardiomyocytes (V.39), mouse skeletal myofibrils (V.37), primary mouse myotubes (V.38), A7r5 smooth muscle cells (V.41), gerbil fibroma cells (V.40), PtK2 epithelial cells (V.40), and human fibroblasts (V.40). (b) Shannon entropy was calculated for each distribution. (c) The binding energy for different types was estimated using Eq. (V-8) and the distributions shown in Fig. V-3 at a fixed chemical potential of -20 kJ/mol.

V -4.2 Contractile unit organization and remodeling under energetic and environmental constraints

Next, we extend our investigation using our framework to systematically examine how variations in key parameters influence structural stability and adaptability. The probability distribution for contractile unit lengths was analyzed using the model described by Eq. (V-

8). These distributions follow a Gaussian distribution with variance $\sqrt{D/k}$, which arises from the balance between stochastic fluctuations and restoring contribution of contractile units (Fig. V-3a). As k increases, the variance in contractile unit length decreases, resulting in more stable localization around a specific length. Conversely, as k decreases, the variance increases, leading to a broader length distribution and a more random actomyosin structure.

As the restoring contribution k increases, structural order becomes higher and entropy decreases (Fig. V-3a, blue), reducing the thermodynamic drive for progressive assembly. Consequently, the maximum achievable binding energy becomes more negative, limiting the elongation of cytoskeletal structures as described in Eq. (V-5). On the other hand, when the restoring contribution is small, the resulting disordered configuration increases entropy (Fig. 3a, red), diminishing the relative contribution of binding energy and facilitating cytoskeletal assembly and hence flexible remodeling. Although the model defines k as a parameter that determines structural order, the one-to-one correspondence between k and the distribution $P(x)$ allows reciprocal interpretation, in which ordering may also reflect stronger restoring effect. While our model focuses on steady-state organization, such reciprocal interpretation also suggests that structural order may dynamically fluctuate in response to temporal changes in the effective restoring-to-fluctuation ratio (k/D), as may occur under varying environmental conditions. For example, increased fluctuations could elevate D and thereby reduce structural order, whereas suppressed fluctuations may effectively enhance k and restore order. These results suggest that cytoskeletal structures with random organization, such as those found in nonmuscle cells, are more adaptable to dynamic growth and remodeling. In contrast, ordered structures, like those in muscle cells, require larger changes in binding energy for structural reorganization, indicating lower flexibility for dynamic remodeling. Interestingly, the value of x_0 does not affect the limits of the binding energy because entropy is determined not by the absolute stable length but by the probability of the structure being randomly distributed (Fig. V-3b).

The relationship with the chemical potential reveals a positive correlation with the maximum binding energy as explicitly described in Eq. (V-5) (Fig. V-3c). A small difference in chemical potential (Fig. V-3c, red regions) corresponds to a reduced impact of the binding energy, allowing for easier remodeling of the structures. While our discussion has focused on the extent of the contractile unit randomness, it should be noted that chemical potential depends on component concentration, which varies with intracellular environmental conditions. Therefore, even with the same randomness in the cytoskeletal system, the behavior is actually influenced by environmental factors including concentration.

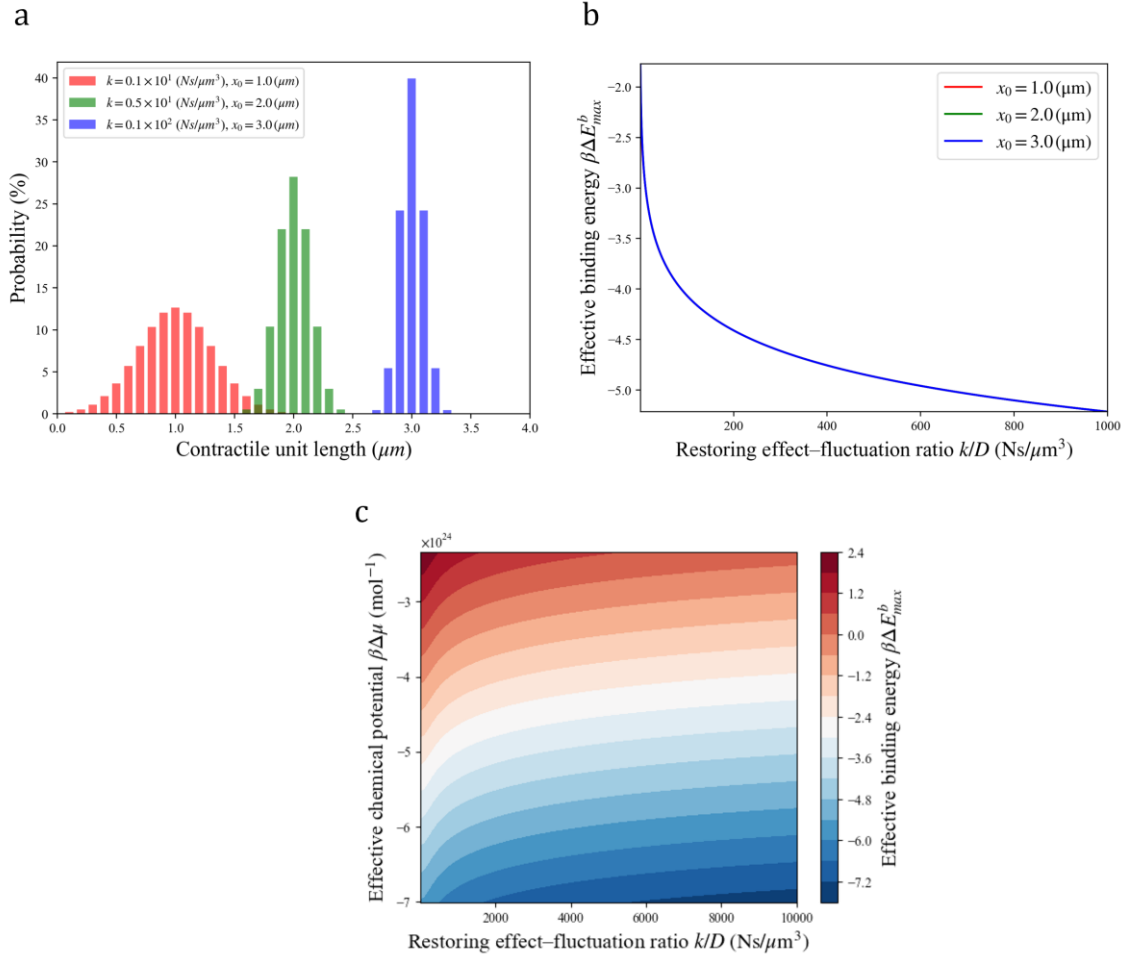


Fig. V-3 Quantification of maximum binding energy and its dependence on structural and energetic factors. (a) Length distribution of contractile units at $k = 10^2$ [N/ μm] (red), 10^3 [N/ μm] (green), and 10^4 [N/ μm] (blue). With a smaller k , the distribution of contractile unit lengths exhibits greater variability, indicating increased structural diversity. (b) Relation between restoring contribution and maximum binding energy. A higher restoring contribution corresponds to a lower maximum binding energy, indicating stronger binding between constituent elements. (c) Dependence of maximum binding energy on restoring contribution and chemical potential. A lower chemical potential results in a lower maximum binding energy.

V-5. Discussion

In this work, we developed a nonequilibrium physical model as a unified framework to reinterpret the relationship between subcellular structural variability and cellular adaptability across hierarchical scales. Note that this study does not aim to theoretically explain the origin of sarcomere randomness; rather, our conceptual model is designed to describe how the intrinsic properties of contractile units are linked to the observed structural variability, which

reflects a state of lower energetic barriers and, in turn, allows for more flexible remodeling. Although the model is intentionally minimal, while inevitably leaving system-specific molecular details outside its scope, this simplification enables clearer insights into the relationship between structural variability and adaptability.

Previous studies have largely focused on self-organizing mechanisms of sarcomere patterning (V.21,22,25,26), but these mechanisms were often examined in isolation, without a unifying framework linking them to cellular adaptive responses. In particular, it remained unclear how both muscle sarcomeres and nonmuscle sarcomere-like structures collectively contribute to the ability of cells to respond to environmental changes. Our model integrates essential physical factors such as restoring effects and fluctuations into an energy-based description of actomyosin contractile unit length distributions. Using the Fokker-Planck equation, we derived the probability distribution of contractile unit lengths (Eq. (V-8)), revealing that higher restoring effects induce less random structural variations around a specific length. This finding is consistent with experimental observations, in which muscle cells exhibit ordered sarcomere structures, whereas nonmuscle cells display more dispersed configurations (V.5,10–17,45,46).

To bridge the structural variations with energetic constraints, we analyzed entropy production associated with cytoskeletal remodeling caused by contractile unit binding. In our model, the cytoskeletal structure, consisting of serially connected contractile units, is treated as a system in grand canonical contact with its surrounding environment, resulting in a nonequilibrium state characterized by continuous energy exchange and persistent entropy production. By determining Shannon entropy (V.47) from the probability distribution of contractile unit lengths, we derived a limit on binding energy (Eq. (V-5)), constrained by the requirement of nonnegative entropy production in nonequilibrium systems (V.30). Our analysis thus provides a framework for understanding how structural randomness governs the energy landscape of cytoskeletal remodeling. In muscle-type cells with high restoring effect and ordered sarcomere lengths, the requirement for entropy production necessitates substantial binding energy for cytoskeletal elongation. In contrast, in nonmuscle-type cells with lower restoring effect and more random structures, the entropy resulting from structural disorder allows for more flexible cytoskeletal remodeling with minimal binding energy. Thus, these cytoskeletal structures achieve flexible responsiveness to environmental cues facilitated by the randomness-driven entropy. Interestingly, young muscle cells display greater randomness in sarcomeres than mature cells (V.19), potentially suggesting that early-stage cells may be more adaptable and then gradually form specialized structures optimized for stable contractile function as they mature, as supported by recent experimental

observations (V.48). Note that binding energy is also modulated by the chemical potential, which depends on intracellular component concentrations. For example, in environments rich in contractility-promoting molecules, stable cytoskeletal structures with strong binding energies can form even in the presence of random structures. Thus, cytoskeletal structures can remodel in response to conditions within the system as well as those in the environment. Our theoretical descriptions capture this cellular behavior, aligning with experimentally observed relationships across the different hierarchies, namely between actomyosin structures and cells (V.5,10–17,45).

While we have primarily discussed the qualitative features, we also estimated the effective binding energy for different types of cytoskeletal structures (Fig. V-2). We found that the estimated trend is consistent with experimental observations. To interpret this trend, we focus here on two major actin cross-linkers, α -actinin and myosin. Nonmuscle α -actinin isoforms have been reported to exhibit higher dissociation constants K_d for actin binding, ranging from 2.96 to 3.96 μM (V.49), compared to muscle α -actinin isoforms, which display lower K_d values of 0.4 μM (50) or 0.59 μM (V.51). This difference suggests that muscle α -actinin binds actin more stably, stabilizing the sarcomere more effectively than nonmuscle α -actinin. Regarding myosin, the K_d value of muscle myosin isoforms (28.2 nM) is higher than that of nonmuscle myosin ones (4.6 nM) (V.52), suggesting a higher affinity of nonmuscle myosin for actin. Functionally, muscle myosin molecules undergo rapid cross-bridging cycles to facilitate muscle contraction, whereas nonmuscle myosin molecules remain attached to actin to sustain cellular tension along stress fibers. These distinct roles suggest that α -actinin, rather than myosin, facilitates structural reorganization through its more flexible interactions with actin, thereby supporting dynamic cellular adaptability.

Such entropy-associated structural variability is also evident in other biological systems. For example, the extracellular matrix exhibits tissue-specific differences in collagen organization, where highly aligned fibers confer mechanical stability (e.g., tendons), whereas disordered networks enhance adaptability and remodeling capacity (e.g., skin, wound healing) (V.53). A comparable principle is reflected in the evolutionary divergence; for example, within the myosin II family, the nonmuscle isoform contributes to heterogeneous and dynamic cytoskeletal networks, supporting versatile functions, while the striated muscle isoform forms highly ordered sarcomere structures optimized for stable contraction (V.54). These examples illustrate a common trade-off between structural order and functional adaptability, consistent with the core mechanism of our model, in which increased structural entropy facilitates flexible responses to environmental demands.

In conclusion, we developed a physical framework for the unified evaluation of

contractile unit variability across different cell types, linking subcellular structural randomness to cellular adaptability. We suggest that structural disorder, often perceived as disadvantageous, actually enhances cellular adaptability by increasing entropy. This randomness is not limited to actomyosin contractile units but also appears inherently in other cellular components, indicating that extending our model could reveal additional mechanisms working across other biological hierarchies. Thus, our framework provides a basis for exploring the complex relationships between cellular physical structures and functions.

VI Hierarchical phase transitions as mechanical checkpoints of intracellular organization

VI-1. Summary

Living cells inherently reorganize their intracellular structures in response to mechanical cues from their environment. Among these responses, the formation of actin-based stress fibers exhibits a series of structural transitions depending on substrate stiffness: from disordered states on soft substrates, to partial alignment, and eventually to bundled formations as stiffness increases. While these transformations have been well documented, the physical principles underlying their emergence remain elusive. Here we develop a statistical-mechanical framework demonstrating that these structural changes arise through a hierarchy of threshold-dependent phase transitions dictated by energy–entropy competition. This formulation provides a thermodynamic basis for understanding how distinct cytoskeletal orders become favored under different mechanical regimes. We propose that these transitions serve as mechanical checkpoints that coordinate intracellular organization during G1-phase spreading. These findings reveal how mechanical cues guide distinct intracellular orders through a physically constrained hierarchy of transitions.

VI-2. Introduction

Living cells inherently exhibit the ability to adaptively modify their internal architecture in response to changes in both internal and external conditions. One striking manifestation of this adaptive capacity is the reorganization of actin stress fibers (SFs) in response to the mechanical stiffness of the extracellular environment. Numerous experimental studies have demonstrated that both the extent and organization of SFs strongly depend on substrate stiffness (VI.1). On highly compliant substrates, SFs are rarely observed; once the stiffness exceeds a certain threshold, SFs begin to appear, though they remain randomly oriented. As the stiffness increases further, SFs progressively align in a common direction, and eventually, at an even higher threshold, these aligned fibers bundle together laterally to form thick actin cables (VI.2–5). These stepwise transformations in actin organization are also observed in temporal succession when cells spread on sufficiently stiff substrates (VI.6–8).

These structural transitions occur spontaneously in response to environmental conditions, without changes to the molecular identity of the system. This behavior is reminiscent of phase transitions in physical systems, in which macroscopic order emerges through changes in external parameters, even when microscopic constituents remain unchanged. Phase transitions have long been a central topic in statistical mechanics and condensed matter physics (VI.9). In recent years, similar concepts have been introduced to biology; for example, in the study of liquid–liquid phase separation within cells where biomolecular condensates form and dissolve in response to physiological cues (VI.10). However, the stiffness-dependent, stepwise transitions in cytoskeletal organization have not yet been examined through this physical framework despite their apparent resemblance to phase-transition-like behavior. This gap is notable given that substrate stiffness is known to regulate essential cellular processes including proliferation, differentiation, and apoptosis, i.e., topics that lie at the core of mechanobiology (VI.11,12). A deeper understanding of how intracellular structure responds to gradual changes in mechanical stiffness could therefore provide fundamental insights into the environment-dependent behavior of living systems.

Here we develop a theoretical framework based on statistical mechanics to explain the stiffness-induced transitions in intracellular actin organization. Using a simplified lattice model of actin filament arrangement, we characterize distinct states that emerge from different spatial configurations of filaments, and we identify the essential conditions under which transitions between these states occur. Although other biological factors such as myosin activity or molecular signaling pathways are not explicitly modeled, they are implicitly embedded in the physical parameters of the system. This approach provides a minimal but physically grounded explanation for the spontaneous reorganization of intracellular structures in response to mechanical cues. These findings suggest that stepwise structural

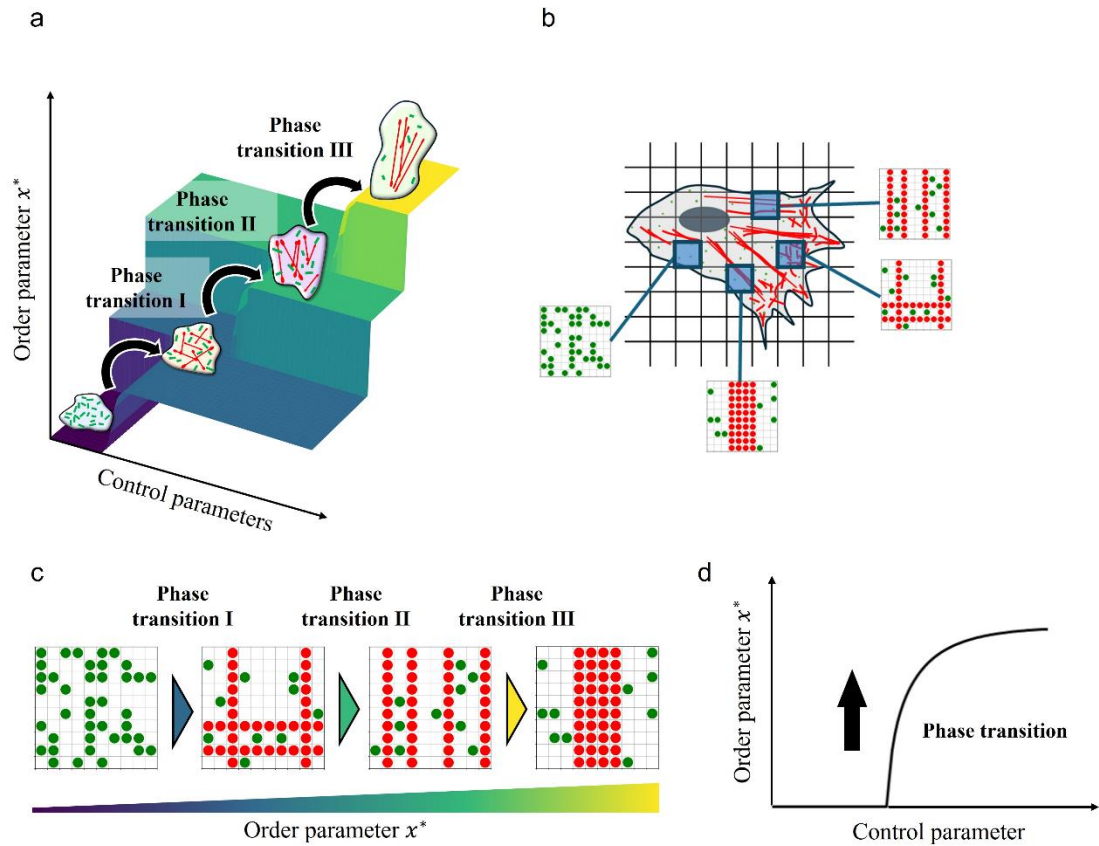


Figure VI-1 Overview of cellular stepwise transitions. (a) Intracellular actin structures undergo sequential phase transitions in response to control parameters such as substrate stiffness. (b) Conceptual illustration of sub-systems within a cell: different cytoplasmic regions may occupy distinct structural states, each represented by a lattice-based local domain used in the model. (c) Lattice-based cell models to analyze each transition: initially, randomly distributed actin particles (green) create long filaments (red) (I), then align along a common direction (II), and eventually undergo interfiber coupling (III) with each transition characterized by a distinct order parameter. (d) Schematic depiction of a discontinuous change in the order parameter at a critical value of the control parameter, illustrating a phase transition.

transitions may act as successive mechanical checkpoints that coordinate intracellular organization with the cell cycle.

VI-3. Model overview

Cells exhibit a sequence of structural transitions in actin organization as a function of substrate stiffness (Fig. VI-1) (VI.3). In response to increasing stiffness, cells progress through four qualitatively distinct states: i) a disordered phase in which actin particles are randomly distributed; ii) a bundled phase in which these particles aggregate into SFs with random orientations; iii) an aligned phase in which SFs acquire a common direction; and iv) an aggregated phase in which aligned SFs coalesce laterally. This sequence of state changes represents a hierarchy of physical phase transitions that govern how cells adapt to mechanical cues. To explore the fundamental principles governing these transitions, we construct a theoretical framework based on statistical mechanics. Unlike conventional phase transition theories based on a single order parameter, the present framework describes a hierarchy of distinct orders, each emerging at a different stiffness threshold and inherited by subsequent phases. To analyze this hierarchy, we develop distinct theoretical models for each of the three phase transitions, SF formation (Phase Transition I, PT1), directional alignment (Phase Transition II, PT2), and fiber aggregation (Phase Transition III, PT3), each capturing a different level of structural organization. These models share a common physical perspective rooted in statistical mechanics but differ in their formulation according to the specific dominant order in each phase. Because actual cells may exhibit different structural states in different regions, the current system can be interpreted as a sub-system of a larger cellular domain. Each model therefore represents a coarse-grained local portion rather than the entire cell.

VI-3.1 Phase transition 1 – Formation of stress fibers as a physical phase transition

We begin by considering the phase transition process, in which randomly distributed actin particles form SFs, characteristic of PT1. Here, actin particles denote coarse-grained actin-based structural units that represent material progressing toward the next structural state, ranging from monomeric actin that polymerizes into filaments to filament segments that assemble into SFs. The cell is represented as a two-dimensional square with a side length of a , on which actin particles are distributed (Fig. VI-2a), where a denotes the side length measured in lattice units, with the lattice spacing set to unity; numerically, a also corresponds to the number of lattice sites per side. We focus on spontaneous phenomena that emerge independently of macroscopic changes in cell shape or volume, which are not included in the present model. We also assume that the temperature of the cell remains

constant. The Helmholtz free energy of the cell is defined as follows:

$$A = E - TS \quad (\text{VI} - 1 - 1)$$

where E , T , and S represent energy, temperature, and entropy, respectively. We derive the specific form of Eq. (VI-1-1) by considering the distribution of actin particles. The square cell is subdivided into smaller units referred to as a lattice. The total number of lattice sites is equal to the area of the cell V :

$$V = a^2. \quad (\text{VI} - 1 - 2)$$

Let N_a represent the total number of actin particles within the cell, which remains constant as actin works as a "housekeeping" protein. The cell is sufficiently large relative to the area occupied by all actin particles to ensure $N_a < V$, and each lattice site can be occupied by at most one actin particle.

We define SFs as the arrangement of actin particles that align in a straight line from one edge of the cell to the other (Fig. VI-2a, red). Any other configurations are considered to be randomly distributed actin particles (Fig. VI-2a, green). Although SFs in actual cells may not always form perfect straight lines, we define SFs in this way to enable a strict count of the possible microscopic states, or microstates, of this macroscopic cell system. Here note that, in statistical mechanics systems such as those applied to biological contexts (VI.13–17), the distinction between macroscopic and microscopic variables is not simply determined by physical size. In the case of a protein statistical mechanics, for example, countable degrees of freedom typically include the length of the protein chain, while uncountable degrees of freedom would refer to the relative coordinates of individual atoms within the protein structure. In addition, while SFs in real cells can vary in length and do not necessarily span the entire cell, we consider the length a in our lattice model as the minimum length required to count as an SF. For more complex cases involving SFs of varying lengths or shapes, the current system may be interpreted as a sub-system of a larger cellular domain, but such analyses are beyond the scope of this study, which focuses on the essential aspects of the phenomena.

Let L_s represent the number of SFs formed by actin particles. To characterize the phase state, we define an order parameter, which is a macroscopic variable that determines the degree of order within the cell, based on the extent to which SFs are formed. Specifically, the order parameter x_1 is defined as the ratio of actin particles involved in SF formation to the

total number:

$$x_1 = \frac{L_s a}{N_a} \quad (\text{VI} - 1 - 3)$$

When $x_1 = 0$, no SFs are formed, indicating a completely disordered state. Meanwhile, when $x_1 = 1$, all actin particles form SFs, representing a fully ordered state within the cell. We determine the specific form of the entropy by counting the possible microstates. Actin particles can exist in two distinct states: as part of non-SFs or as part of SFs. First, the number of possible microstates of actin particles or non-SFs (Fig. VI-2a, green) is given by:

$$W_p = \frac{(V - L_s a)!}{(N_a - L_s a)! (V - N_a)!} \quad (\text{VI} - 1 - 4)$$

where the available lattice sites are those not involved in SF formation (i.e., $V - L_s a$), and the number of available actin particles and empty lattice sites are $N_a - L_s a$ and $V - N_a$, respectively. Similarly, the number of possible microstates of SFs (Fig. VI-2a, red) is given by

$$W_s = \frac{2a!}{L_s! (2a - L_s)!}, \quad (\text{VI} - 1 - 5)$$

in which the number of possible configurations is calculated for arranging a total of L_s SFs. We do not distinguish between SF configurations that are symmetrical with respect to the starting position, either vertically or horizontally. The number of possible microstates is thus calculated within the range of $a + a = 2a$. Taken together, the system entropy is described, with the Stirling approximation, by

$$\begin{aligned} S_1 &= k_b \ln W_p W_s \\ &= k_b \left\{ (V - N_a x_1) \ln(V - N_a x_1) - N_a (1 - x_1) \ln N_a (1 - x_1) - (V - N_a) \ln(V - N_a) \right. \\ &\quad \left. + 4a \ln a - 2a \ln 2 - \frac{N_a}{a} x_1 \ln N_a x_1 - \left(2a - \frac{N_a}{a} x_1 \right) \ln(2V - N_a x_1) \right\} \quad (\text{VI} - 1 - 6) \end{aligned}$$

where k_b is the Boltzmann constant (Fig. VI-S1).

Next, to describe the energy E_1 , we define the single-particle energy of an actin particle as μ_1^0 and the interaction energy due to binding as μ_1^s , respectively; μ_1^0 is an intrinsic energy that

reflects the inherent properties of the actin particle and is considered a constant, while μ_1^s represents the energy associated with the stability of the binding and is generally a function that can vary depending on chemical and physical influences from the surrounding environment. A stiffer substrate allows cells to generate larger traction forces due to the law of action and reaction (VI.12), and given that actin–myosin complexes including SFs become more stable under such increased tension, a property known as a catch bond (VI.18–22), the interaction energy is expressed as a function of tension F_1 :

$$\mu_1^s(F_1) = B \exp(-CF_1) \quad (\text{VI} - 1 - 7)$$

where B is the interaction energy due to binding in the absence of tension, and C is the constant that determines the effect of F_1 . The total energy of the cell is expressed using the number of non-SF actin particles, $N_a - L_s a$, and the number of particles forming SFs, L_s (Fig. VI-S2):

$$E_1 = \mu_1^0(N_a - L_s a) + \mu_1^s L_s a \quad (\text{VI} - 1 - 8)$$

From Eqs. (VI-1-6) and (VI-1-8), the free energy of Eq. (VI-1-1) is expressed as

$$A_1 = \mu_1^0(N_a - x_1 N_a) + B N_a x_1 \exp(-CF_1) - T k_b \left\{ (V - N_a x_1) \ln(V - N_a x_1) - N_a(1 - x_1) \ln N_a(1 - x_1) - (V - N_a) \ln(V - N_a) + 4a \ln a - 2a \ln 2 - \frac{N_a}{a} x_1 \ln N_a x_1 - \left(2a - \frac{N_a}{a} x_1\right) \ln(2V - N_a x_1) \right\} \quad (\text{VI} - 1 - 9)$$

To derive the conditions for PT1, we take the partial derivative of Eq. (VI-1-9) with respect to x_1 :

$$\frac{\partial A_1}{\partial x_1} = N_a \left[(\mu_1^s - \mu_1^0) - k_b T \left\{ -\ln(V - N_a x_1) + \ln N_a(1 - x_1) - \frac{1}{a} \ln N_a x_1 + \frac{1}{a} \ln(2V - N_a x_1) \right\} \right] \quad (\text{VI} - 1 - 10)$$

The order parameter x_1^* that satisfies $\partial A_1 / \partial x_1 = 0$ is

$$x_1^* \approx 1 - \frac{V}{N_a} \exp \frac{\mu_1^s - \mu_1^0}{k_b T}, \quad (\text{VI} - 1 - 11)$$

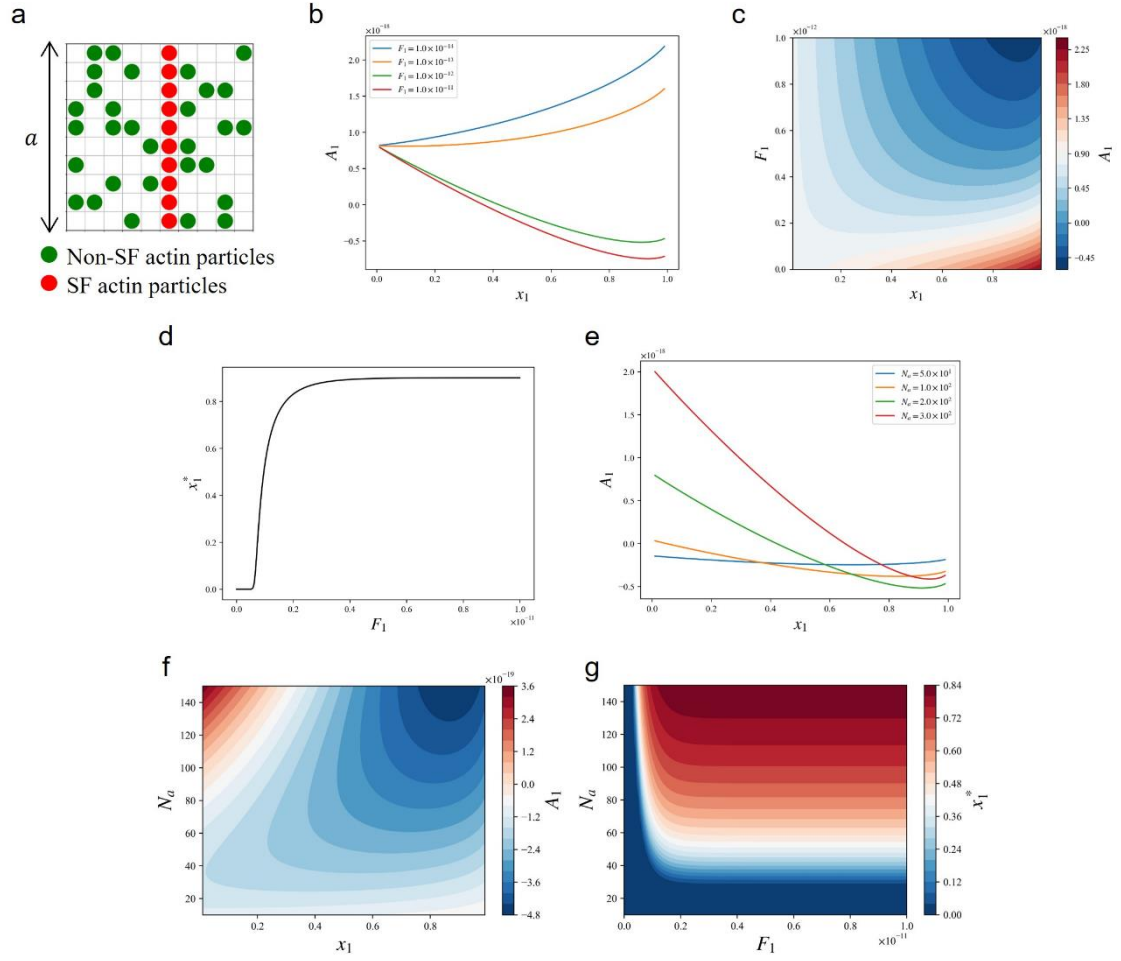


Figure VI-2 Analysis of Phase Transition I (PT1). (a) Schematic of a cell lattice model. Actin particles are classified as non-SF (green) or SF (red). (b) Free energy as a function of the order parameter and sustained tension. (c) Map of free energy as a function of the order parameter and sustained tension. (d) Order parameter that minimizes free energy as a function of sustained tension. (e) Free energy as a function of the order parameter and actin concentration. (f) Map of free energy as a function of the order parameter and actin concentration. (g) Critical order parameter as a function of the effective actin concentration and sustained tension.

in which the approximation $V \gg N_a$ is used. The free energy has an extremum in $0 < x_1^* < 1$, indicating that a symmetry-breaking phase transition is induced at $x_1^* \neq 0$. Thus, the criterion for PT1 is given by

$$\mu_1^s - \mu_1^0 < k_b T \ln \frac{N_a}{V}, \quad (\text{VI} - 1 - 12)$$

suggesting that if the interaction energy μ_1^s is low, the cell system becomes stable by forming SFs and establishing order. In other words, the system spontaneously moves towards an ordered state characterized by SF formation when substrate stiffness is high. Conversely, if μ_1^s is high, the cell is more stable when actin particles remain randomly distributed without forming SFs. While this model assumes constant cell volume and actin particle number, the result suggests that a higher effective concentration of actin particles, N_a/V , facilitates SF formation. Interestingly, even though this model does not explicitly consider the effect of concentration, its impact on the cell stability is revealed within the framework of statistical mechanics.

Using the more rigorous Eq. (VI-1-10), we numerically show the relationship between the order parameter x_1 and the free energy A_1 (Figs. VI-2b-2g). Hereafter, unless otherwise stated, these parameters are used: $N_a = 200$ (corresponding to 20 actin particles per filament in the calculation), $T = 309.5$ K, $k_b = 1.38 \times 10^{-23}$ J/K, $F_1 = 1 \times 10^{-12}$, $a = 20$, $B = 1.5 \times 10^{-20}$, and $C = 2.5 \times 10^{12}$. B and C , which affect the magnitude of the energy, are determined given that the binding free energy of proteins generally falls within the range of 1–10 kcal/mol (VI.23). These parameter magnitudes were chosen within physically reasonable orders consistent with intracellular conditions, such as typical levels of actomyosin-generated tension. Because the model is necessarily conceptual and not intended to reproduce absolute experimental values, the use of biologically plausible orders ensures that the resulting phase transitions reflect realistic cellular regimes rather than numerically arbitrary choices. Under conditions where the tension F_1 is small, the free energy A_1 reaches its minimum at $x_1 = 0$ (Fig. VI-2b, blue and orange), indicating that the cell system is stable in a disordered state where actin particles are randomly distributed without forming SFs. Once F_1 exceeds a certain threshold described by Eqs. (VI-1-7) and (VI-1-11), A_1 shifts to its minimum at higher values of x_1 (Fig. VI-2b, green and red), suggesting that in environments with larger forces, such as those with greater substrate stiffness, the cell system is more stable as it adopts an ordered phase characterized by the presence of one or more SFs. In environments where the total number of effective actin particles N_a is small, the free energy A_1 reaches its minimum at $x_1 = 0$ (Fig. VI-2e, blue), suggesting that the cell system is stable in a state where actin particles are randomly distributed. As N_a increases, in contrast, the free energy minimum shifts to a larger value of x_1 (Fig. VI-2e, green and red), indicating that cells with higher actin concentrations are more stable in an ordered phase where SFs are formed (Figs. VI-2f and 2g). The analytical results

for the order parameter x_1^* , at which the free energy is minimized, also demonstrate a similar phase transition as in the numerical analysis (data not shown).

VI-3.2 Phase Transition II – Directional alignment of stress fibers as a physical phase transition

We explore the mechanism of the phase transition from a cell structure with SFs formed in random directions to one where SFs acquire a specific orientation, characteristic of Phase Transition II (PT2). In the previous phase, the presence of SFs alone was regarded as an ordered state. In contrast, PT2 is interpreted as the emergence of a new order, where SFs align in a particular direction. In this new phase, SFs align either vertically or horizontally (Fig. VI-3a). Although in actual cells not all actin particles form SFs, we consider that the cell is entirely in an SF-forming phase to focus on the essential aspects of this specific phase transition. In addition, while actual SFs can align in various directions, we count only those aligning vertically or horizontally. While our current model focuses on the actin cytoskeleton within a simplified two-dimensional lattice, it can be extended to include additional cellular components, higher-dimensional lattices, or curved SF arrangements. These extensions would increase the complexity of energetic and entropic terms while retaining the core theoretical structure. In particular, cases involving SFs of varying lengths, shapes, or orientations can be addressed by interpreting the current model as a sub-system within a larger cellular domain.

Within the square lattice framework as used for PT1, let us consider the case where the total number of SFs is n_2 . We denote the number of SFs aligning along the x -axis as n_2^x (Fig. VI-3a, green) and those aligning along the y -axis as n_2^y (red), and thus

$$n_2 = n_2^x + n_2^y \quad (\text{VI} - 2 - 1)$$

The new order parameter that determines the phase state here is defined as follows to evaluate the extent to which SFs are aligned in the same specific directions.

$$x_2 = \frac{n_2^x - n_2^y}{n_2} \quad (\text{VI} - 2 - 2)$$

When $x_2 = 0$, SFs are evenly distributed between the two axes, indicating a disordered state; meanwhile, when $x_2 = 1$ or $x_2 = -1$, SFs are aligned only along either the x -axis or y -axis, reflecting a state with directional order. The number of possible microstates

determined from the distribution of SFs is described by

$$W = \frac{n_2!}{n_2^x! n_2^y!}, \quad (\text{VI} - 2 - 3)$$

and then the entropy is described, with the Stirling approximation, by

$$\begin{aligned} S_2 &= k_b \ln W \\ &= k_b \left(n_2 \ln n_2 - \frac{1+x_2}{2} \ln \left(\frac{1+x_2}{2} n_2 \right) - \frac{1-x_2}{2} \ln \left(\frac{1-x_2}{2} n_2 \right) \right) \end{aligned} \quad (\text{VI} - 2 - 4)$$

(Fig. VI-S3). We define the energy of a single SF bearing a tension of F_2 based on the catch-bond properties:

$$\mu_2(F_2) = \mu_2^0 \exp \left(-\frac{F_2}{F_2^0} \right), \quad (\text{VI} - 2 - 5)$$

in which F_2 ($= f_2 a$) is the maximum tension that fully formed single SFs can sustain; f_2 is the unit tension, F_2^0 is a normalizing factor, and a is the cell edge length as in PT1. Tension in proliferative cells is known to be produced by the interaction between ubiquitous nonmuscle actin and myosin II filaments, which occurs when these proteins align in a straight configuration (VI.24,25). Based on this, we assume that when one SF crosses another perpendicularly, the intersection point lacks the linear structure effective for tension generation and maintenance. If an SF does not experience such interference from others, the tension it can generate reaches its maximum value of F_2 . The tensions in the x -axis and y -axis directions are then given as follows:

$$\begin{aligned} F_2^y &= f_2(a - n_2^x) \\ F_2^x &= f_2(a - n_2^y) \end{aligned} \quad (\text{VI} - 2 - 6)$$

From Eqs. (VI-2-5) and (VI-2-6), the energy E_2 and free energy A_2 are given by:

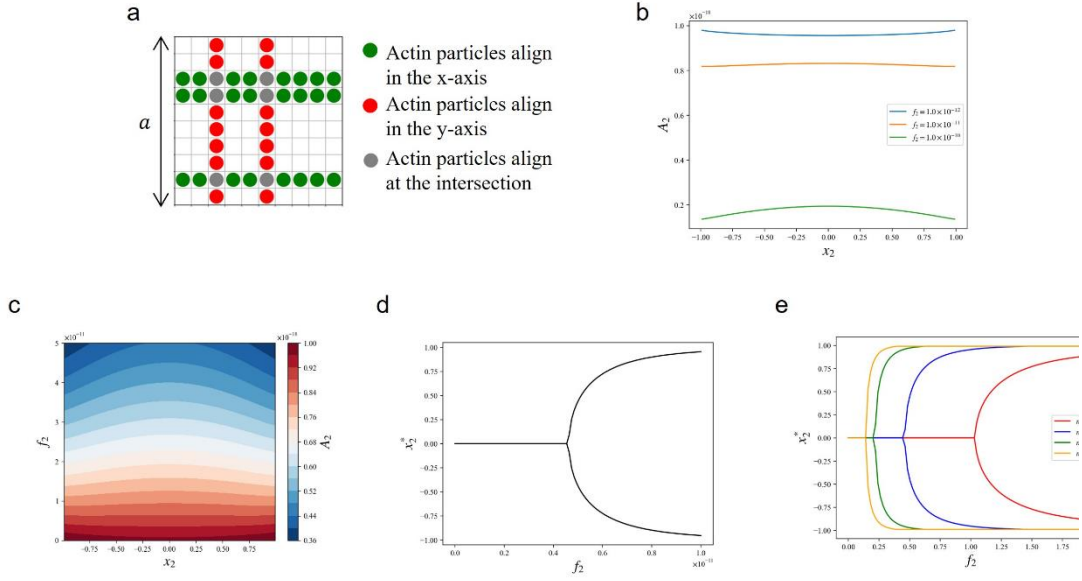


Figure VI-3 Analysis of Phase Transition II (PT2). (a) Schematic of a cell lattice model for PT2. Actin particles align in the x -axis (green) or y -axis (red) to form SFs, in which the tension generation is abrogated at the intersection (gray). (b) Free energy as a function of the order parameter and sustained tension. (c) Map of free energy as a function of the order parameter and sustained tension. (d) Order parameter corresponding to the minimum free energy as a function of the sustained tension. (e) Order parameter corresponding to the minimum free energy as a function of the total number of actin particles and sustained tension.

$$E_2 = n_2^y \mu_2(F_2^y) + n_2^x \mu_2(F_2^x) \quad (\text{VI} - 2 - 7)$$

(Fig. VI-S4) and

$$A_2 = \mu_2^0 \left\{ \frac{1-x_2}{2} n_2 \mu_2^0 \exp \left(-\frac{f_2 \left(a - \frac{(1+x_2)n_2}{2} \right)}{F_2^0} \right) + \frac{1+x_2}{2} n_2 \mu_2^0 \exp \left(-\frac{f_2 \left(a - \frac{(1-x_2)n_2}{2} \right)}{F_2^0} \right) \right\} \\ - T k_b \left(n_2 \ln n_2 - \frac{1+x_2}{2} \ln \left(\frac{1+x_2}{2} n_2 \right) - \frac{1-x_2}{2} \ln \left(\frac{1-x_2}{2} n_2 \right) \right), \quad (\text{VI} - 2 - 8)$$

respectively. To analyze the critical condition, taking the second derivative of Eq. (VI-2-8) with respect to x_2 and substituting $x_2 = 0$ yield

$$\frac{\partial^2 A_2(0)}{\partial x_2^2} = \frac{n_2^2 f_2 \mu_2^0}{4F_2^0} \left(-4 + \frac{f_2 n_2}{F_2^0} \right) \exp \left(-\frac{f_2 \left(a - \frac{n_2}{2} \right)}{F_2^0} \right) + n_2 T k_b. \quad (\text{VI} - 2 - 9)$$

With the approximation $f_2 \left(a - \frac{n_2}{2} \right) / F_2^0 \ll 1$ and solving $\partial^2 A_2(0) / \partial x_2^2 = 0$,

$$f_2^* = \frac{2 \left(F_1^0 \mu_2^0 n_2 - F_2^0 \sqrt{\mu_2^{0^2} n_2^2 - k_b \mu_2^0 n_2^2 T} \right)}{\mu_0 n_2^2}, \quad (\text{VI} - 2 - 10)$$

and thus the cell system stabilizes at $x_2 = 0$ when $f_2 < f_2^*$, and at $x_2 \neq 0$ when $f_2 > f_2^*$.

Using the rigorous Eq. (2-8) with $\mu_2^0 = 10^{-19}$, $F_2^0 = 10^{-9}$, and $n_2 = 10$ (i.e., 10 fiber units), we visualized the relationship between the free energy A_2 and the order parameter x_2 (Fig. VI-3b, blue and orange), showing that A_2 reaches its minimum at $x_2 = 0$ when the force f_2 is small. Meanwhile, the free energy has a convex shape and reaches its minimum at $x_2 \neq 0$ when the force is large (Fig. VI-3b, green). Thus, SFs sustaining low tension remain stable with random orientation, but once the tension exceeds a certain threshold the influence of energy becomes more dominant than entropy, leading to a phase where SFs align in a consistent direction (Fig. VI-3c, d). When the total number of distinct SFs, n_2 , is small, the order parameter corresponding to the minimum free energy begins to change at higher force levels (Fig. VI-3e). In contrast, when n_2 is large, the change in the order parameter starts at lower force levels, suggesting that PT2 occurs more easily with more SFs under the same force conditions than with fewer SFs.

VI-3.3 Phase Transition III – Lateral aggregation of aligned stress fibers as a physical phase transition

Next, we focus on the mechanism of Phase Transition III (PT3) in which SFs, after acquiring directional alignment in PT2, undergo further transformation to undergo interfiber coupling. As in the previous sections, we consider a simple model where directionally aligned SFs are distributed within a square cell lattice of side length a with constant volume and temperature (Fig. VI-4a). In actual cells, not all SFs align in a single direction, but we focus on the key aspects of how directionally aligned SFs cluster after PT2. Let the total number of SFs be n_3 , and let n_3^{SF} and n_3^{ass} represent the number of independent and assembled ones, respectively:

$$n_3 = n_3^{SF} + n_3^{ass} \quad (\text{VI} - 3 - 1)$$

We consider the case where a single aggregate is present. Although multiple aggregates form in actual cells, this complexity can be addressed by adjusting entropy calculations. Here, however, the focus is on establishing a framework for analyzing the fundamental principles and significance of PT3. The order parameter is defined as follows to evaluate the extent to which SFs form aggregates:

$$x_3 = \frac{n_3^{ass}}{n_3} \quad (\text{VI} - 3 - 2)$$

SFs are independently distributed at $x_3 = 0$, reflecting a disordered state, while they undergo interfiber coalescence at $x_3 = 1$. The number of microstates is expressed as follows, focusing on the distribution patterns:

$$W = \frac{(a + 1 - n_3^{ass})!}{(n_3 - n_3^{ass})! (a - n_3^{ass})!} \quad (\text{VI} - 3 - 3)$$

The entropy is described, with the Stirling approximation, by

$$\begin{aligned} S_3 &= k_b \ln W \\ &= k_b \{ (a + 1 - n_3 x_3) \ln(a + 1 - n_3 x_3) - n_3 (1 - x_3) \ln n_3 (1 - x_3) - (a - n_3) \ln(a - n_3) \} \end{aligned} \quad (\text{S3} - 4)$$

(Fig. VI-S5). The energy of individual SFs is given by Eq. (VI-2-5) as used in PT2,

$$\mu_3 = \mu_3^0 \exp\left(-\frac{c f_3}{F_3^0}\right), \quad (\text{VI} - 3 - 5)$$

but the interpretation here is that c is a concentration-dependent parameter of binding proteins such as α -actinin that stabilize the connections between SFs. The energy and free energy are then be expressed by

$$E_3 = n_3 \left\{ (1 - x_3) \mu_3^0 \exp\left(-\frac{c_{SF} f_3}{F_3^0}\right) + x_3 \mu_3^0 \exp\left(-\frac{c_{ass} f_3}{F_3^0}\right) \right\} \quad (\text{VI} - 3 - 6)$$

(Fig. VI-S6), and

$$A_3 = n_3 \left\{ (1 - x_3) \mu_3^0 \exp\left(-\frac{c_{SF} f_3}{F_3^0}\right) + x_3 \mu_3^0 \exp\left(-\frac{c_{ass} f_3}{F_3^0}\right) \right\} - T k_b \{ (a + 1 - n_3 x_3) \ln(a + 1 - n_3 x_3) - n_3 (1 - x_3) \ln n_3 (1 - x_3) - (a - n_3) \ln(a - n_3) \}, \quad (\text{VI} - 3 - 7)$$

respectively, where c_{SF} and c_{ass} are c for independently distributed SFs and assembled SFs, respectively. To Differentiating Eq. (VI-3-7) with respect to x_3 is

$$\frac{\partial A_3}{\partial x_3} = n_3 \left(-\mu_3^0 \exp\left(-\frac{c_{ass} f_3}{F_3^0}\right) + \mu_3^0 \exp\left(-\frac{c_{SF} f_3}{F_3^0}\right) \right) - T k_b (\ln(a + 1 - n_3 x_3) + \ln(n_3 - n_3 x_3)), \quad (\text{VI} - 3 - 8)$$

and the order parameter at $\partial A_3 / \partial x_3 = 0$ is expressed as

$$x_3^* = \frac{(a + 1) \exp\left(\frac{-\mu_3^0 \exp\left(-\frac{c_{ass} f_3}{F_3^0}\right) + \mu_3^0 \exp\left(-\frac{c_{SF} f_3}{F_3^0}\right)}{T k_b}\right) - n_3}{n_3 \left(\exp\left(\frac{-\mu_3^0 \exp\left(-\frac{c_{ass} f_3}{F_3^0}\right) + \mu_3^0 \exp\left(-\frac{c_{SF} f_3}{F_3^0}\right)}{T k_b}\right) - 1 \right)}. \quad (\text{VI} - 3 - 9)$$

The free energy reaches an extremum and stabilizes at this specific $0 < x_3^* < 1$, as long as x_3^* is not equal to 0. The condition for PT3 is now described by

$$-\mu_3^0 \exp\left(-\frac{c_{ass} f_3}{F_3^0}\right) + \mu_3^0 \exp\left(-\frac{c_{SF} f_3}{F_3^0}\right) < T k_b \ln \frac{n_3}{a + 1}, \quad (\text{VI} - 3 - 10)$$

indicating that even when the energy difference on the left side remains unchanged, PT3 can still occur if the total number of SFs is high.

Visualization of PT3 with $n_3 = 10$ (i.e., 10 fiber units) and $F_3^0 = 1 \times 10^{-9}$ shows that entropy dominates when the tension is weak with a low binding protein concentration, leading to a state where the free energy is minimized at $x_3 = 0$ (Fig. VI-4b, blue and orange). On the other hand, a high binding protein concentration leads to stabilization of the cell system with SF aggregates (Fig. VI-4b, green). One straightforward interpretation of the parameter

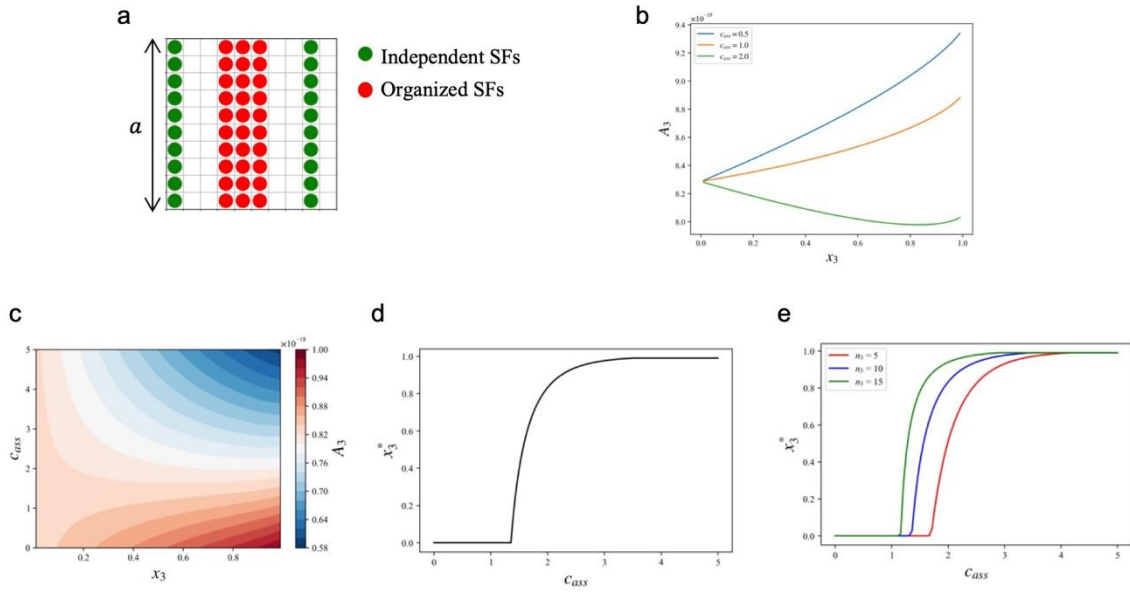


Figure VI-4 Analysis of Phase Transition III (PT3). (a) Schematic of a cell lattice model for PT3. Directionally aligned SFs are either distributed independently (green) or organized into assembled structures (red). (b) Free energy as a function of the order parameter and effective binding protein concentration. (c) Map of free energy as a function of the order parameter and binding protein concentration. (d) Order parameter corresponding to the minimum free energy as a function of binding protein concentration. (e) Order parameter corresponding to the minimum free energy as a function of the total number of SFs and binding protein concentration.

c_{ass} is the concentration of actin binding proteins that allow SFs to engage in interfiber coupling, but another is that c_{ass} also represents the internal and external factors that drive changes in the size of SFs through the enlargement of focal adhesions and consequently increase the sustained tension, which is also enhanced by substrate stiffness (Fig. VI-4c, d) (VI.26–30). The threshold concentration decreases as the total number of SFs increases (Fig. VI-4e). This observation is consistent with the theoretically derived phase transition conditions (Eqs. (VI-3-9) and (VI-3-10)).

VI-4. Experimental verification of cellular phase transition induced by the stiffness

The hierarchical structural reorganization of the actin cytoskeleton was experimentally investigated across varying substrate stiffnesses to validate the theoretical framework (Fig. VI-5). We revealed that HFF-1 cells on soft 7 kPa substrates exhibit a disordered actin phase (Fig. VI-5a, top). As the substrate stiffness increases to 20 kPa and 41 kPa, a distinct transition to a filamentous phase becomes evident, with glass substrates supporting the development of prominent stress fiber bundles (Fig. VI-5b–d, top). The corresponding orientation maps further illustrate this progression, showing a transition from random local orientations on soft gels to high directional alignment on rigid substrates (Fig. VI-5a–d, bottom).

To quantitatively evaluate the emergence of structural order, spatial autocorrelation analysis was performed on local cytoplasmic domains (Fig. VI-5e). To eliminate variations in absolute intensity between different imaging settings, each autocorrelation function $g(r)$ was normalized by its maximum value at $r = 0$. The resulting decay profiles demonstrate that structural correlation persists over significantly longer distances on stiffer substrates. The correlation length ξ , calculated as the distance at which the correlation intensity, shows a significant and monotonic increase from 7 kPa to glass (Fig. VI-5f). Furthermore, the anisotropy values, which evaluate the degree of global fiber alignment, exhibit a corresponding rise with increasing substrate stiffness (Fig. VI-5g).

These experimental observations provide direct evidence for the hierarchical phase transitions predicted by our thermodynamic model. The extension of the correlation length ξ corresponds to PT1, representing the spontaneous transition from a disordered particulate phase to a bundled filamentous phase, which validates the behavior of the order parameter x_1 . Simultaneously, the increase in anisotropy marks the onset of PT2, reflecting the directional alignment of the formed fibers as represented by the order parameter x_2 . Collectively, these results demonstrate that substrate stiffness acts as a key control parameter that sequentially triggers cytoskeletal ordering through the competition between thermodynamic energy and entropy.

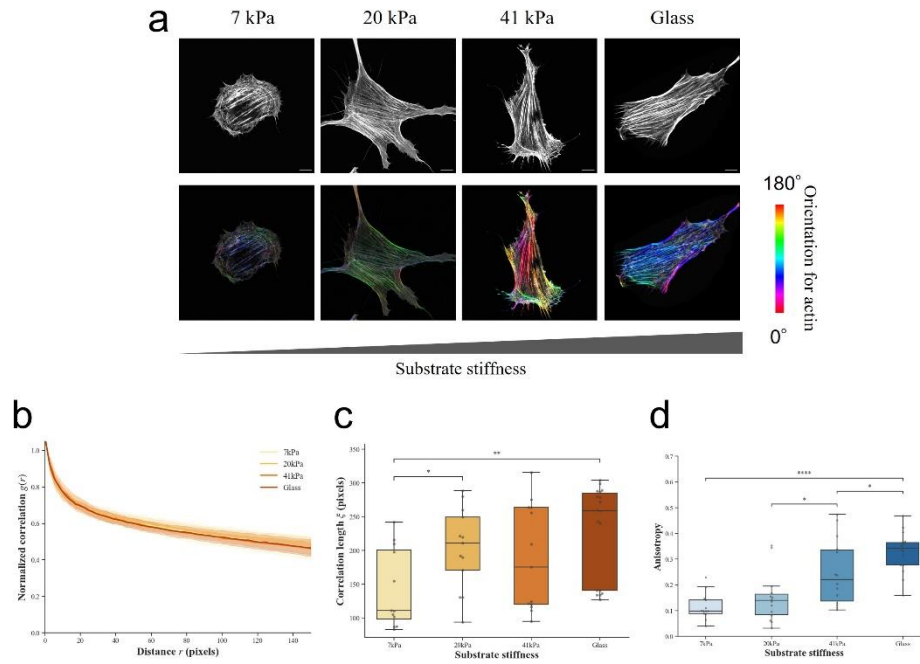


Figure VI-5 Experimental validation of stiffness-dependent hierarchical structural transitions. (a-d) Top panels: HFF-1 cells stained for F-actin on polyacrylamide gel substrates with stiffnesses of 7 kPa, 20 kPa, and 41 kPa, and glass substrate. Bottom panels: Corresponding local orientation maps for actin staining, where the different colors indicate the local orientation of actin filaments as defined by the provided color map. (e) The normalized spatial autocorrelation function. To eliminate absolute intensity variations between different imaging settings, each autocorrelation curve was normalized by its maximum value at $r = 0$. The curves represent the mean values for each condition ($n=10\sim15$ cells per group), with the shaded areas indicating the standard deviation (SD). (f) Box plot showing the correlation length ξ across the different substrate stiffnesses. ξ was determined by fitting the decay curves in (e) with an exponential function $g(r) \propto \exp(-r/\xi)$, serving as an experimental proxy for the order parameter ξ in PT1. A significant increase in ξ on stiffer substrates indicates the emergence of structural order (fiber formation) through energy–entropy competition. (g) Comparison of anisotropy values calculated. Higher anisotropy values on rigid substrates reflect the transition from a randomly oriented bundled phase to a directionally ordered aligned phase.

VI-5. Discussion

Many studies have described how living cells reorganize their internal structures in response to mechanical cues in terms of signaling molecular pathways (VI.31–35), but such approaches often leave open the question of what makes those responses necessary rather than merely possible. The present study addresses this gap by developing a statistical-mechanical framework that accounts for intracellular reorganization as a physically inevitable response to environmental stiffness. Statistical-mechanical perspectives have previously been applied mainly at higher biological scales, for example by modeling collections of cells as interacting ensembles (VI.36). In contrast, the present formulation operates at a more microscopic level by treating a single cell as an ensemble of interacting molecular units. Within this perspective, intracellular reorganization can be understood as a physically inevitable consequence of the trade-off between energy and entropy governed by extrinsic or intrinsic stiffness. This viewpoint moves beyond descriptive accounts and provides a principle-based explanation for the stepwise formation and organization of actin structures, highlighting the physical logic that underlies cytoskeletal adaptation.

A key contribution of this study is the identification of hierarchical phase transitions that progressively govern the organization of actin structures in response to increasing substrate stiffness. Unlike previous approaches that rely on the fine-tuning of molecular signaling parameters, these transitions arise from threshold-dependent shifts in free energy landscapes driven by substrate stiffness-dependent actin interactions (Fig. 6). Our theoretical framework captures how distinct intracellular orders emerge sequentially, each inheriting structural features from the preceding state. Much like minimal models in statistical physics that extract essential behavior from complex systems, our approach isolates the fundamental energetic relationships that make such transitions physically favored under specific mechanical conditions.

These phase transitions have implications beyond structural stability. When cells fail to maintain a stable phase, especially when SFs cannot properly form or align under mechanical stress, they may enter a pro-inflammatory state, potentially leading to chronic inflammatory diseases like atherosclerosis (VI.37–39). Focal adhesions at the termini of SFs convert mechanical tension into biochemical signals, with insufficient tension leading to sustained activation of pathways such as MAPK and subsequent disease progression (VI.40,41). While previous studies have elucidated these molecular mechanisms in detail (VI.42,43), our model offers a complementary, systems-level perspective that explains how such states may arise from failures in physically governed structural organization.

The sequence of structural transitions observed as cells spread on stiff substrates can be interpreted as a gradual process of internal mechanical maturation. This progression is

driven by intracellular percolation (VI.27), in which actin–myosin interactions increasingly form SFs that support tension generation, leading to a rise in intracellular stiffness. As cells thus also stiffen internally, they become more capable of proceeding to subsequent structural transitions, with each state providing a foundation for the next. While cell cycle progression is canonically regulated by biochemical checkpoints such as those involving cyclins and cyclin-dependent kinases, it is during the G1 phase when cells must attach and spread that essential cytoskeletal reorganization (VI.3–5) occurs to support entry into later phases (VI.44). This stepwise maturation of intracellular structures, for which we have described the underlying physical conditions, may serve as a series of “mechanical” checkpoints that align cytoskeletal organization with the timing required for proper cell cycle progression.

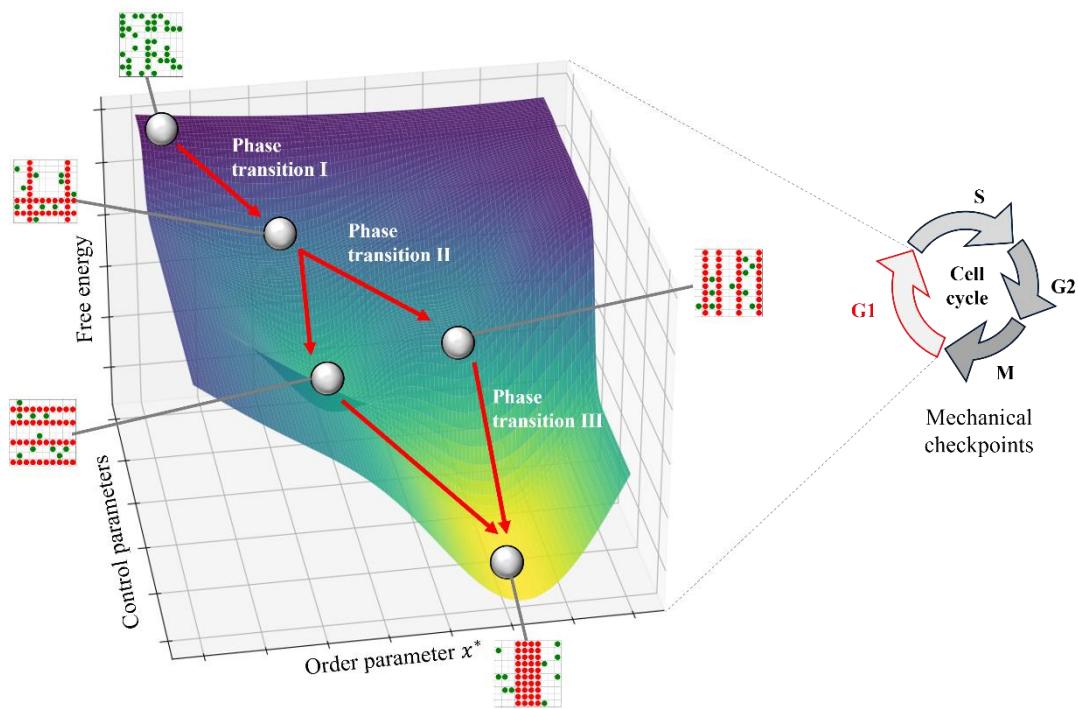


Figure VI -6 Hierarchical free-energy landscape of mechanical cue-dependent intracellular transitions. A schematic free-energy surface illustrating three discrete cytoskeletal transitions (PT I–III) driven by increasing control parameters such as substrate stiffness and the extent of cell spreading. Representative configurations highlight the structural regimes associated with each transition. The right panel relates this hierarchy to G1-phase mechanical checkpoints that coordinate cytoskeletal maturation with cell-cycle progression.

While the notion of mechanical checkpoints has appeared in previous studies, typically referring to threshold-dependent behaviors in cell polarization (VI.45), focal adhesion dynamics (VI.46,47), or mechanosensitive differentiation processes (VI.48–50), these descriptions have remained largely phenomenological. In contrast, our framework reveals that such transitions are not merely empirically observed switching behaviors but are thermodynamically inevitable, governed by the competition between energy and entropy. We reinterpret mechanical checkpoints as emergent outcomes of physical constraints, offering a more principled understanding of how intracellular structure and potentially cell fate become mechanically coordinated. Another area of research that may be relevant to our theory is the study of topological defects in aligning multicellular structures (VI.51). Within this thermodynamic viewpoint, the resulting free-energy landscapes can be viewed as a conceptual molecular-scale analogue of the classical Waddington landscape, illustrating how mechanical inputs reshape the structural fate of intracellular assemblies and highlighting the hierarchical nature of the experimentally observed transitions (Fig. 6).

Given that SFs comprise more than one hundred associated proteins as revealed in our recent work (VI.52), their full molecular diversity lies far beyond the scope of any model. In addition, because cytoskeletal architecture varies across regions of a cell, individual parts of the cytoplasm can be at distinct stiffening stages during spreading. From this combined perspective, each phase transition in our framework is naturally interpreted as the dominant structural behavior within a local sub-system rather than the entire cytoplasm. The present model focuses on the essential physical elements governing actin organization, while remaining extensible because additional molecular influences can be represented by modifying the statistical weights or constraints that determine which filament configurations contribute most to the ensemble. For example, regulation by actin-destabilizing molecules such as cofilin (VI.24) can be modeled, without introducing explicit dynamical descriptions, by biasing the weighting toward shorter filament states within the same statistical-mechanical framework.

In recent years, the concept of liquid–liquid phase separation has provided a valuable framework for understanding the dynamic organization of membraneless compartments in cells (VI.53). Equilibrium thermodynamics and statistical physics have proven powerful for explaining how complex molecular behaviors can emerge through physically interpretable transitions without invoking specific signaling pathways. Our study shares a similar spirit but extends it to a distinct class, namely cytoskeletal systems shaped adaptively by mechanical cues. By incorporating the catch-bond behavior, an essential property of actin–myosin mechanobiology (VI.54), into our statistical mechanical model, we demonstrate that cytoskeletal structures undergo a hierarchy of phase transitions, extending the framework

beyond chemical condensation.

While living cells are fundamentally nonequilibrium systems, structural changes such as cytoskeletal organization often occur under conditions where equilibrium-based approaches remain highly effective. This perspective is consistent with successful equilibrium-based analyses in biological physics, including those applied to protein folding (VI.55), the mechanosensitivity of focal adhesions (VI.56,57), and lipid and biomolecular phase separations (VI.53,58), where equilibrium thermodynamics and statistical mechanics have yielded valuable insights despite the underlying systems being intrinsically open. By showing how much of the observed behavior can be explained within this equilibrium framework, our study can help clarify where nonequilibrium effects such as temporally varying mechanical inputs or spatially heterogeneous energy landscapes may begin to play a defining role, suggesting that cells likely integrate both equilibrium and nonequilibrium strategies for structural regulation.

Our statistical mechanical analysis establishes a framework for understanding how stepwise phase transitions in SF organization arise as a physically inevitable response to environmental stiffness. While our current formulation focuses on the actin cytoskeleton within a simplified two-dimensional lattice, it can be extended to include additional cellular components, higher-dimensional lattices, or curved SF arrangements, allowing for more accurate representations of actual cellular architecture. These extensions would increase the complexity of energetic and entropic terms but retain the core theoretical structure. In particular, cases involving SFs of varying lengths or shapes can be accommodated by interpreting the current model as a sub-system within a larger cellular domain. Although such detailed modeling is beyond the scope of this study, our work provides a foundation upon which future research can build and may contribute to a unifying understanding of how living systems reorganize their internal structures across diverse biological contexts.

VI-6. Supporting Information

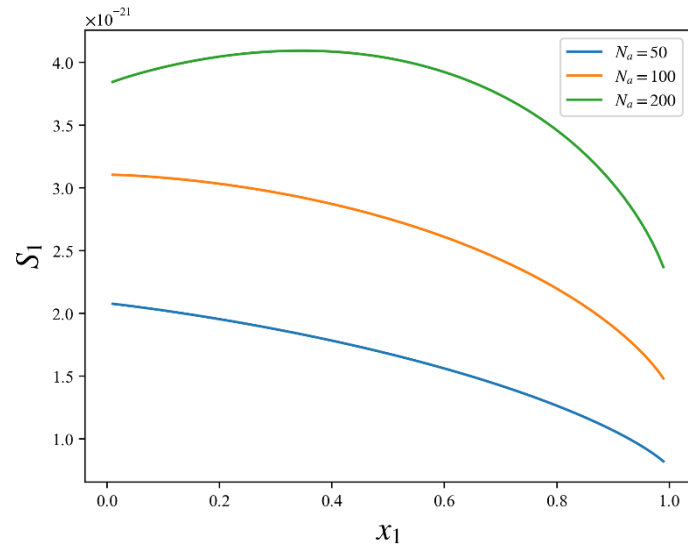


Fig. VI-S1 Entropy vs. order parameter for PT1.

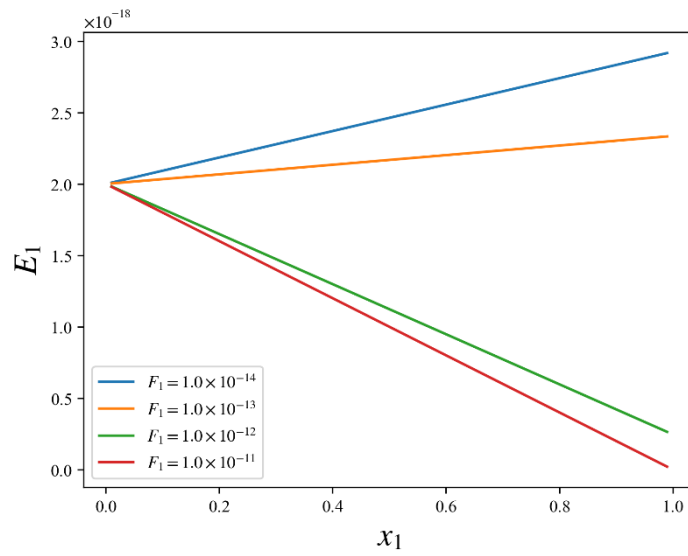


Fig. VI-S2 Energy vs. order parameter for PT1.

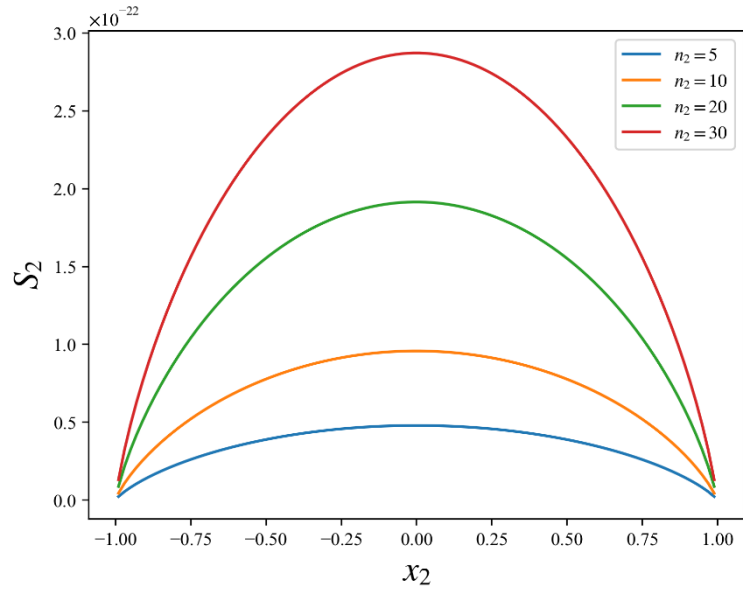


Fig. VI-S3 Entropy vs. order parameter for PT2.

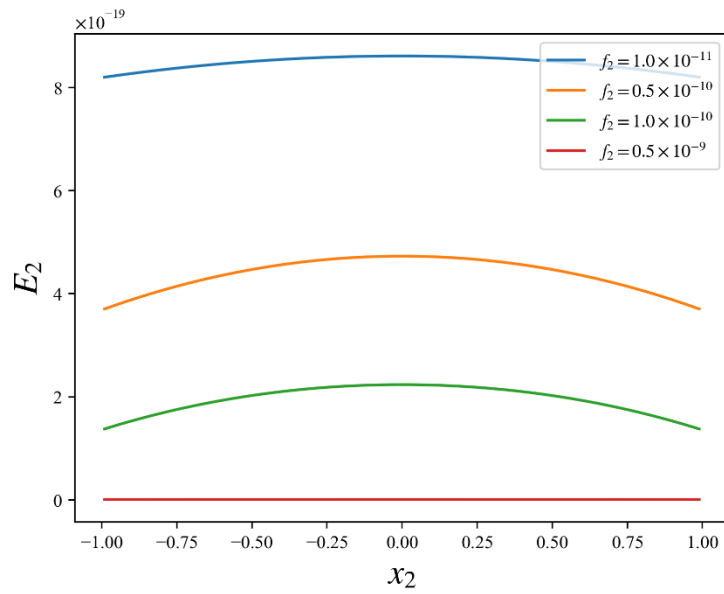


Fig. VI-S4 Energy vs. order parameter for PT1.

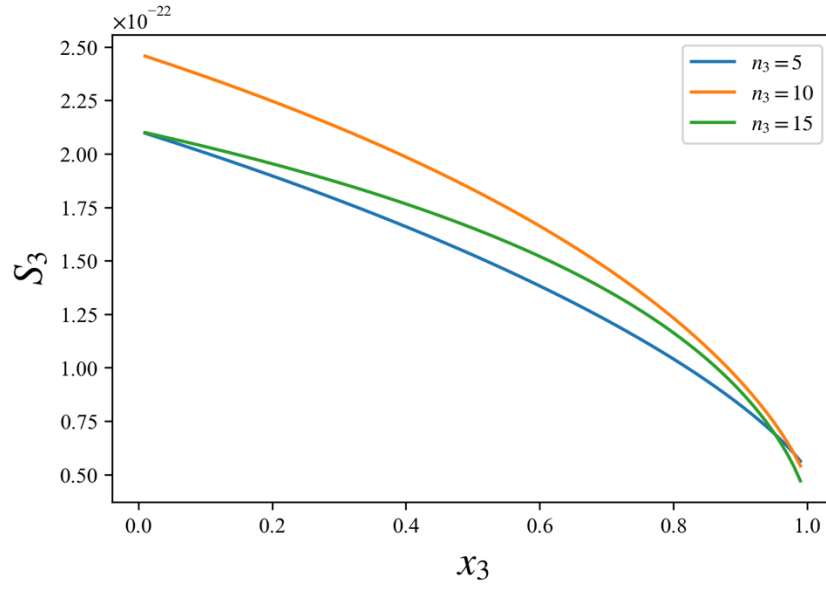


Fig. VI-S5 Entropy vs. order parameter for PT3.

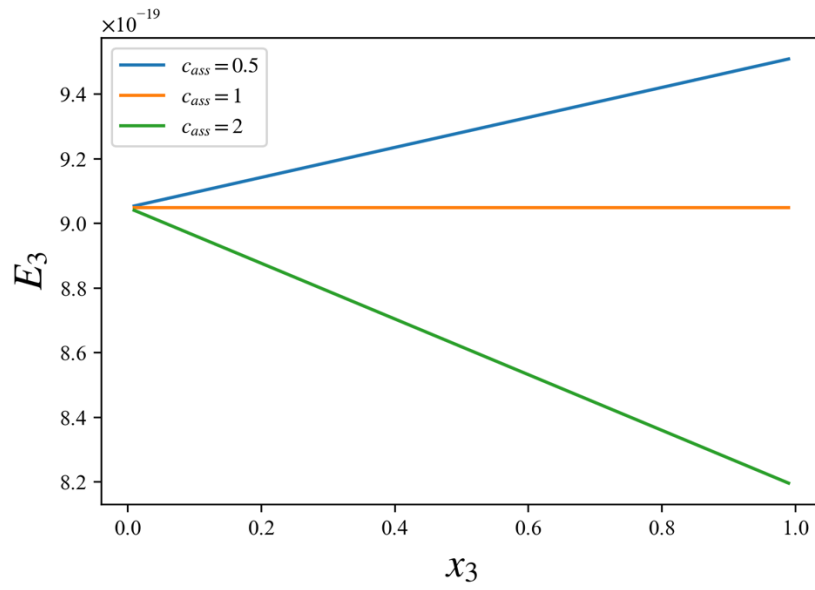


Fig. VI-S6 Energy vs. order parameter for PT3.

Methods for cell and hydrogel preparation in Figure VI-5

Primary human foreskin fibroblasts (HFF-1, ATCC) were cultured as a monolayer in high-glucose Dulbecco's Modified Eagle's Medium (DMEM, Wako) supplemented with 15% fetal bovine serum (Sigma-Aldrich) and 1% penicillin-streptomycin solution (Wako). Cells were kept at 37°C in a humidified incubator with 5% CO₂. The medium was changed once every 3 days, and cells were passed at a split ratio of 1:3 when 80% confluence was reached.

Polyacrylamide (PAA) gels were synthesized on activated glass coverslips to provide substrates with defined elastic moduli. The coverslips were initially cleaned with 70% ethanol for 30 minutes, functionalized with 2% (3-aminopropyl) APTES in acetone for 10 minutes, and subsequently treated with 2.5% glutaraldehyde in phosphate-buffered saline (PBS) for 30 minutes to ensure covalent gel adhesion. The mechanical properties of the PAA gels were controlled by varying the weight ratios of acrylamide to bis-acrylamide (8:0.48, 8:0.264, 5:0.3, and 5:0.1 % w/v), with polymerization initiated by ammonium persulfate (APS) and TEMED.

VII Revealing cellular functional fluctuations through nonequilibrium physics

VII-1. Summary

Living cells have a remarkable ability to sense environmental changes and adapt their internal structure, morphology and motility with high accuracy. However, at the microscopic scale, all cellular components, molecules, structures and even entire cells, are continuously exposed to stochastic fluctuations. Although the molecular pathways and signaling mechanisms underlying adaptive responses have been extensively studied, how cells preserve such sophisticated adaptability in a noise-dominated environment remains poorly understood. Here, we propose a quantitative framework based on nonequilibrium physics to distinguish intrinsic cellular fluctuations from thermal noise, thereby revealing functional fluctuations actively regulated by cells. By integrating fluorescence correlation spectroscopy with theoretical analyses of nonequilibrium dynamics, we identified nonthermal fluctuations that reflect active regulation within living cells. Our results reveal that, surprisingly, intracellular fluctuations are not predominantly governed by microtubule-based motors such as kinesin or dynein, but are instead influenced by actomyosin activity. Furthermore, we demonstrate that cellular senescence reshapes the fluctuation landscape, with increased magnitude while decreased diversity of fluctuations. This reduction in fluctuation heterogeneity suggests a decline in regulatory flexibility and provides a mechanistic link between intracellular fluctuations and the reduced adaptive capacity observed in senescent cells. Together, these findings suggest intracellular fluctuations as a functional readout of cellular adaptability and highlight their active regulation as a key determinant of the physiological state of cells. Our work elucidates the physical basis of how cells maintain adaptability among pervasive fluctuations and introduces fluctuation dynamics as a means to explore cellular senescence.

VII-2. Introduction

Living cells possess a remarkable capacity to sense environmental cues and adapt their biological processes such as their structure and motility (VII.1,2). This adaptive behavior is fundamental to physiological processes such as proliferation and differentiation, and its impairment is often linked to pathological states (VII.3,4). While biochemical signaling pathways have been extensively mapped, cells must execute these precise responses in a microscopic environment dominated by stochastic noise. Understanding how cells maintain robust adaptability amidst such fluctuations requires integrating physical principles with molecular biology.

The mechanical landscape of the cytoplasm is largely shaped by the activity of motor proteins like the interaction between nonmuscle myosin II and actin filaments, ubiquitous cytoskeletal components, kinesin, and dynein (VII.5). By consuming ATP, motor proteins generate nonthermal forces that are transmitted through the cytoskeleton network, driving structural rearrangements necessary for cellular adaptation (VII.1,6,7). Through mechanotransduction, cells convert these physical signals into biochemical responses (VII.8,9). While the forces of individual motors have been characterized in vitro (VII.10,11), quantifying the collective nonthermal forces that drive adaptive responses within the complex environment of a living cell remains a significant challenge.

These intracellular forces drive the system far from thermodynamic equilibrium, resulting in persistent molecular fluctuations (VII.7,12–15). The molecular fluctuations observed in cells thus include not only thermal fluctuations due to Brownian motion but also nonthermal ones driven by the universal forces intrinsic to proliferative cells. Analysis of these fluctuations can provide insights into the mechanical properties of cells arising from nonequilibrium dynamics. In this regard, Einstein demonstrated the connection between microscopic thermal fluctuations and macroscopic quantities (VII.16), laying the groundwork for the Fluctuation-Dissipation Theorem (FDT), a cornerstone of statistical physics that links thermal fluctuations to the response of a system to external stimuli. Extensive research has focused on analyzing cellular mechanical properties by examining the violation of the FDT in molecular dynamics, allowing the nonthermal forces that drive fluctuations, elastic moduli, and energy dissipation in cells to be quantified (VII.7,12,13,17–24). However, these studies primarily require the measurement of both active fluctuations and passive responses to external stimuli in single particle measurements. This approach using specialized equipment is not easily accessible to most cell biologists, which limits detailed understanding of the intracellular collective

fluctuations and their functional roles.

Here, we propose a quantitative framework based on nonequilibrium physics to distinguish intrinsic cellular fluctuations from thermal noise. By integrating fluorescence correlation spectroscopy (FCS) with an analytical model of nonequilibrium dynamics, we identify nonthermal fluctuations that reflect active cellular regulation. Our results demonstrate that intracellular fluctuations are primarily governed by actomyosin activity rather than microtubule-based motors. Furthermore, we show that cellular senescence reshapes this fluctuation landscape, where a reduction in fluctuation heterogeneity correlates with a decline in adaptive capacity. This work establishes intracellular fluctuations as a functional readout of cellular adaptability and provides a physical basis for exploring the physiological shifts associated with aging.

VII-3. Material and methods

VII-3.1 Cell culture and treatments

Primary human foreskin fibroblasts (HFF-1, ATCC) were cultured as a monolayer in high-glucose Dulbecco's Modified Eagle's Medium (DMEM, Wako) supplemented with 15 % fetal bovine serum (Sigma-Aldrich) and 1 % penicillin-streptomycin solution (Wako). Cells were kept at 37 °C in a humidified incubator with 5 % CO₂. The medium was changed once every 3 days, and cells were passaged at a split ratio of 1:3 when 80 % confluence was reached. For cellular senescence experiments, HFF-1 cells were passaged until they reached passage 26 (p26), considered as senescent cells, which were compared with younger cells at passage 9 (p9).

For pharmacological treatments, cells were incubated with various inhibitors prior to and during the measurements. To inhibit myosin II activity, cells were treated with (-)-blebbistatin at concentrations of 10 μ M or 20 μ M. calyculin A (5 nM) was used to enhance myosin II activity. To investigate the role of other motor proteins, cells were treated with the dynein inhibitor ciliobrevin D (10 μ M and 20 μ M) or the kinesin inhibitor monastrol (10 μ M and 20 μ M). The role of the actin cytoskeleton was assessed using the actin polymerization inhibitor cytochalasin D (10 μ M and 20 μ M) and the actin filament stabilizer jasplakinolide (100 nM). Microtubule polymerization was inhibited using nocodazole (10 μ M and 20 μ M). Combined

inhibition of actomyosin was performed using a mixture of (-)-blebbistatin and cytochalasin D.

VII-3.2 Plasmid construction and transfection

The fluorescent protein mClover2 was used as a tracer for intracellular fluctuation measurements. The primary plasmid used in this study was mClover2-C1 (Add gene plasmid #54577, a gift from Michael Davidson). For experiments investigating the effect of tracer particle size, tandem constructs of mClover2 were created. The plasmids of 2-mClover2 and 3-mClover2, containing two and three mClover2 units respectively, were constructed by Gibson assembly. Fragments were obtained by PCR amplification of the mClover2-C1 (VII.25). Cells were transfected with the plasmids 8 hours after seeding using Lipofectamine LTX and Plus Reagent (Thermo Fischer Scientific) according to the manufacturer's instructions.

VII-3.3 Fluorescence correlation spectroscopy (FCS)

To probe intracellular dynamics, we utilized fluorescence correlation spectroscopy (FCS), a technique that analyzes the spontaneous fluctuations of fluorescence intensity, $I(t)$, from diffusing molecules in a microscopic observation volume. The statistical properties of these fluctuations are quantified by the fluorescence intensity time-autocorrelation function (ACF), $G(\tau)$, defined as:

$$G(\tau) = \frac{\langle \delta I(t) \delta I(t + \tau) \rangle}{\langle I(t) \rangle^2} \quad (\text{VII} - 1)$$

where $\delta I(t)$ represents the fluctuation from the mean intensity, and τ is the lag time.

FCS measurements were performed on a confocal laser scanning microscope (Olympus FV3000) equipped with a 60× oil-immersion objective (NA 1.42). Live cells, cultured on glass-bottom dishes for 24 hours post-transfection, were maintained at 37°C and 5% CO₂ using a stage-top incubator. The pinhole was set to 200 μm, which defined a 3D Gaussian observation volume with a radial dimension of $\omega_{xy} = 0.211 \mu\text{m}$ and a structural parameter of $\omega_z/\omega_{xy} = 2.67$.

The autocorrelation function can be expressed in terms of the diffusion coefficient D and the average particle number $\bar{N}$ as follows

$$G(\tau) = \frac{1}{\bar{N}} \left(1 + \frac{4D\tau}{W_{xy}^2} \right)^{-1} \left(1 + \frac{4D\tau}{W_y^2} \right)^{-1}. \quad (\text{VII} - 2)$$

VII-3.4 Nonequilibrium model for analyzing nonthermal forces

To quantify the nonthermal forces within cells, we develop a nonequilibrium physical framework derived from the violation of the Einstein relation. The time evolution for the intracellular particle density ρ at position x is described by

$$\frac{\partial \rho}{\partial t} = u \frac{\partial \rho}{\partial x} + D \frac{\partial^2 \rho}{\partial x^2} \quad (\text{VII} - 3)$$

where u is the average particle velocity, t is time, and D is the diffusion coefficient, under a natural assumption that the intracellular space in question is sufficiently large relative to the size of the individual particles. At a steady state with negligible macroscopic particle flow, ρ remains constant over time, allowing the steady state density $\rho_{st}(x)$ to be described as

$$\rho_{st}(x) = \rho_0 \exp\left(-\frac{u}{D}x\right) \quad (\text{VII} - 4)$$

where ρ_0 is the particle density at $x = 0$. In the presence of a constant external force f , the potential energy of a particle at x is fx . The statistical average of the potential energy per particle is described by

$$\frac{\int_0^\infty dx f x \rho_{st}(x)}{\int_0^\infty dx \rho_{st}(x)} = \frac{\int_0^\infty dx f x \rho_0 \exp\left(-\frac{u}{D}x\right)}{\int_0^\infty dx \rho_0 \exp\left(-\frac{u}{D}x\right)} = f \frac{D}{u}. \quad (\text{VII} - 5)$$

Within living cells, there exist both the random thermal force f_T due to molecular collisions and the nonthermal force f_A generated by the interaction between ubiquitous motor proteins (Fig. VII-1). The total force f per particle is then

$$f = f_T + f_A. \quad (\text{VII} - 6)$$

Only the potential energy associated with f_T follows the law of equipartition of energy, in which it equals the energy received from the surroundings as seen in Brownian motion. From Eqs. (VII-5) and (VII-6),

$$(f - f_A) \frac{D}{u} = k_B T \quad (\text{VII} - 7)$$

where k_B is the Boltzmann constant, and T is the absolute temperature.

FCS is a technique that measures diffusion coefficients by analyzing fluctuations in the fluorescence intensity of molecules. The fluctuations observed in FCS arise from the combined effect of both thermal and nonthermal forces. Using parameters obtained with FCS, specifically the effective volume length l and the correlation time representing the probabilistic equivalent of a particle passing through this volume τ_B , the average velocity u is expressed as

$$u = \frac{l}{\tau_B}. \quad (\text{VII} - 8)$$

From the macroscopic relationship between average particle velocity and force,

$$u = \mu f \quad (\text{VII} - 9)$$

where μ is the transfer coefficient, or mobility, indicating how easily particles move. From Eqs. (VII-8) and (VII-9), Eq. (VII-7) is rewritten as

$$f_A = \frac{l}{D\tau_B} \left(\frac{D}{\mu} - k_B T \right). \quad (\text{VII} - 10).$$

The second term on the right side of Eq. (VII-10) represents the thermal fluctuation component. By measuring the deviation of observed fluctuations from those predicted from thermal fluctuations, we can determine the nonthermal forces. Note that in the first term on the right side of Eq. (VII-10), for a spherical particle, μ is determined from the Stokes-Einstein relation, and the other parameters are obtained from FCS data. In other words, by analyzing the microscopic motion of particles subject to thermal and nonthermal forces, we quantitatively derive the macroscopic driving forces unique to biological systems. Unlike previous studies (VII.7,12,13,17–24), this approach does not require measuring passive responses of individual particles to externally applied mechanical stimuli.

VII-3.5 Analysis of diffusion heterogeneity

To further characterize the intracellular environment beyond the overall magnitude of active fluctuations, we analyzed the shape of individual FCS autocorrelation functions (ACFs) to assess the heterogeneity of diffusion dynamics. ACFs were categorized based on coefficient of determination R^2 to either a single-component diffusion model (Single), a two-component diffusion model (Double), or a third category (Other) included curves not well described by either model (Fig. VII-5a). We then examined how the distribution among these categories, as well as the characteristic diffusion times τ , derived from the fits, varied with cellular senescence and Myosin II inhibition.

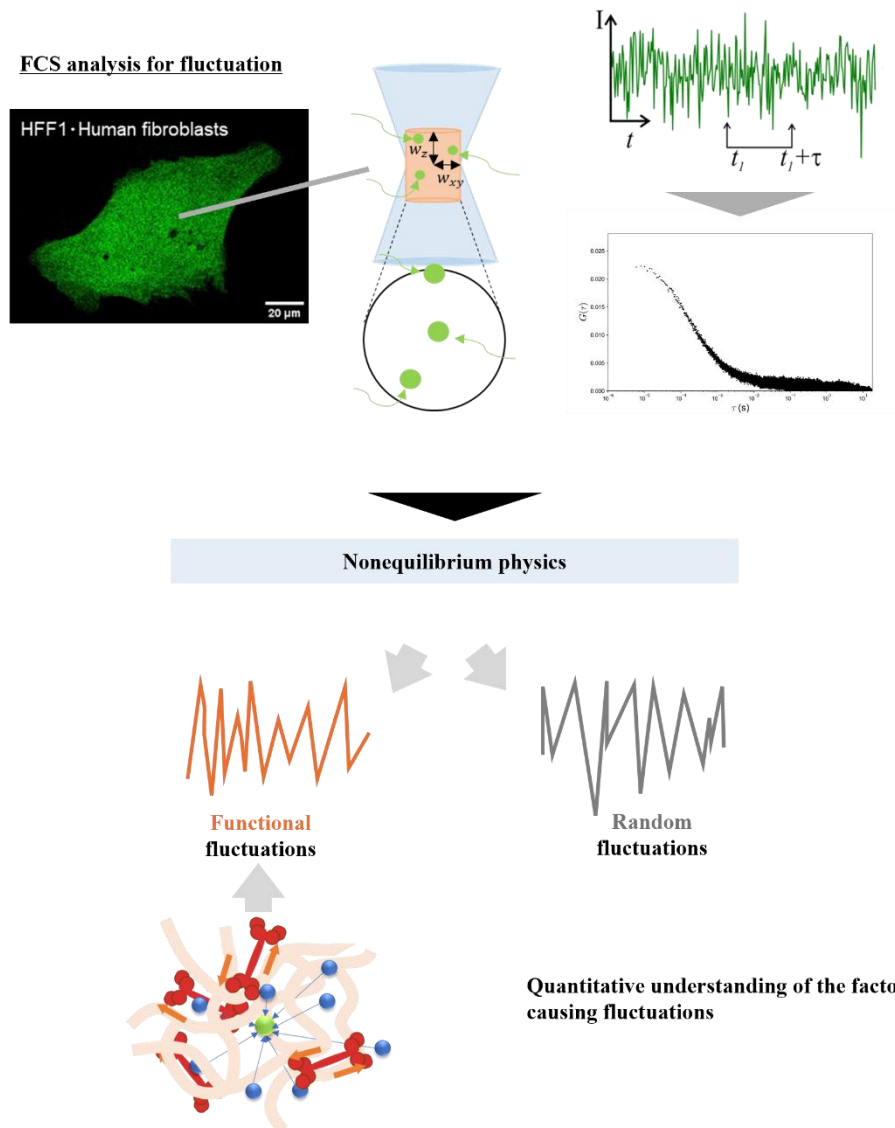


Fig. VII-1 Schematic overview of the theoretical framework and experimental approach. Diagram illustrating the nonequilibrium model used to quantify intracellular nonthermal forces acting on diffusing particles. Thermal fluctuations arise from Brownian motion, whereas nonthermal forces are generated by actomyosin activity. Fluorescence Correlation Spectroscopy (FCS) captures intensity fluctuations from fluorescent probes (mClover2 constructs) within a submicron observation volume, allowing extraction of diffusion coefficients and subsequent calculation of nonthermal active forces via violation of the Einstein relation.

VII-4. Results

VII-4.1 Modulation of myosin activity directly impacts cellular active fluctuations

To elucidate the sources of active fluctuations within the cytoplasm, we employed FCS and calculated the nonthermal active forces f_A derived from our nonequilibrium model (Eq. (VII-10)). In control cells, f_A driving active fluctuations was approximately 47 pN. It is known that individual molecular motors, such as actomyosin, typically generate forces on the order of 1-10 pN (VII.27–29). The diffusion of inert tracers like GFP within the cytoplasm is thought to be influenced by the combined effect of such forces, acting randomly and without a net direction from various active sources. Notably, the nonthermal forces in control conditions is roughly 5 to 10 times larger than the force generated by individual motors, suggesting that the fluctuations probed by FCS reflect collective or amplified effects arising from the active cellular machinery.

We investigated the specific role of myosin II, a key motor protein binding to actin filaments and undergoing an ATP-dependent power stroke (VII.30), by both inhibiting and increasing its activity. Treatment with blebbistatin, inhibiting myosin II, resulted in a statistically significant decrease in the calculated f_A compared to the control (Fig. VII-2a). Furthermore, treatment with calyculin A, a phosphatase inhibitor that promotes myosin II activity by increasing myosin light chain phosphorylation, also led to a significant reduction in f_A . This suggests that excessive contractility may lead to a frozen or overly rigid cytoskeletal state, thereby suppressing active fluctuations. Conversely, overexpression of myosin II led to a statistical increase in f_A . These results demonstrate that the level of myosin II activity directly and significantly determines the magnitude of the nonthermal force which controls active cytoplasmic fluctuations.

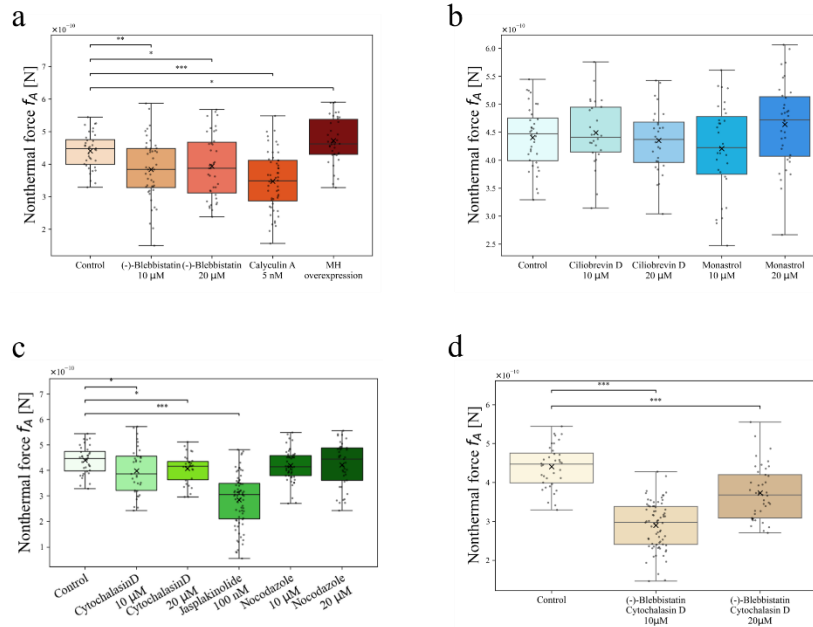


Fig. VII -2 Actomyosin contractility is the dominant origin of nonthermal intracellular forces. (a) Inhibition of myosin II with blebbistatin significantly reduces nonthermal active forces, while activation of myosin II increases them. (b) In contrast, inhibition of dynein (ciliobrevin D) or kinesin (monastrol) does not affect nonthermal forces. (c) Disruption of actin filaments with cytochalasin D decreases nonthermal forces, whereas microtubule depolymerization with nocodazole produces no effect. (d) Combined inhibition of myosin II and actin polymerization synergistically suppresses nonthermal forces. Together, these results demonstrate that the actomyosin network acts as the primary generator of intracellular nonthermal forces.

VII-4.2 Kinesin and dynein are not responsible for the fluctuations

To evaluate the contributions of the molecular motors, kinesin and dynein, to intracellular active fluctuations, we quantified f_A based on FCS and Eq. (VII-10). The cells were treated with specific inhibitors: ciliobrevin D, which inhibits dynein, and monastrol, which inhibits kinesin, at concentrations of 10 μ M and 20 μ M respectively (Fig. VII-2b). There was no statistically significant difference in f_A between the control group and any of the inhibitor-treated groups. This suggests that, unlike myosin, kinesin and dynein do not play a dominant

role in modulating the magnitude of active fluctuations in the cytoplasm under these conditions.

VII-4.3 Actin filaments, but not microtubules, regulates nonthermal forces

To investigate the role of cytoskeletal components in regulating intracellular active fluctuations, we examined the effects of actin and microtubule disruption on f_A (Fig. VII-2c). The cells were treated with cytochalasin D, an inhibitor of actin polymerization, and nocodazole, a reversible inhibitor of microtubule assembly. They target key cytoskeletal structures involved in cell shape maintenance, motility, intracellular transport, and mitosis. Treatment with cytochalasin D at both 10 μ M and 20 μ M led to a statistically significant reduction in f_A compared to the control group. This suggests that actin filaments play a crucial role in maintaining nonthermal forces generation in the cytoplasm. In contrast, cells treated with nocodazole did not exhibit significant changes in f_A , indicating that microtubule disruption alone does not strongly affect the magnitude of intracellular active fluctuations under these conditions. These results highlight the importance of actin-based structures in the regulation of nonthermal forces within the cytoplasm, consistent with the critical roles of actin in cell morphology and mechanical dynamics through fluctuations.

VII-4.4 Combined inhibition of myosin activity and actin polymerization

To further dissect the role of actomyosin dynamics in intracellular active fluctuations, we simultaneously treated cells with blebbistatin and cytochalasin D, which inhibit myosin II activity and actin polymerization, respectively (Fig. VII-2d). The magnitude of f_A was significantly reduced compared to control cells. Notably, the reduction in f_A observed in the co-treatment condition is greater than that seen with either blebbistatin or cytochalasin D treatment alone (Figs. VII-2a and b), suggesting a synergistic effect of inhibiting both actomyosin contractility and actin filament formation. These results indicate that both the structural integrity and contractile activity of the actin cytoskeleton contribute to the generation of intracellular nonthermal forces. Together, our findings highlight the central role of the actomyosin cytoskeleton in regulating active fluctuations within the cellular environment.

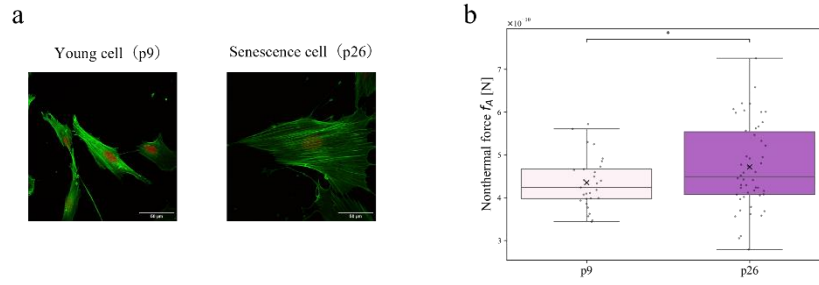


Fig. VII-3 Cellular senescence enhances nonthermal forces while simplifying diffusion dynamics. (a) Images for young (p9) and senescence cells (p26). They show different mechanical structure such as size and actin networks. (b) Senescent cells (p26) exhibit increased nonthermal active forces relative to young cells (p9).

VII-4.5 Cellular senescence leads to significantly increase active fluctuation

We compared f_A in young (p9) versus senescent (p26) cells. Senescent cells exhibited significantly higher f_A compared to young cells (Fig. VII-3). This result suggests that senescent cells possess stronger active fluctuations in the intracellular environment. Previous studies have reported an increased number of SFs in senescent cells, reflecting enhanced cytoskeletal organization and tension maintenance (VII.31). Our observation of elevated f_A is consistent with these findings, and implies that actomyosin activity is upregulated during senescence, thereby enabling the cells to sustain greater intracellular forces and accelerate active fluctuation.

VII-4.6 Smaller particles experience stronger fluctuations and respond more sensitively to actomyosin modulation

To investigate how the size of diffusing particles influences their interaction with intracellular nonthermal forces, we analyzed three structurally distinct fluorescent protein constructs, monomeric (1-mClover2), dimeric (2-mClover2), and trimeric (3-mClover2) forms, each representing progressively larger effective particle sizes (Fig. VII-4). Notably, the smallest probe, 1-mClover2, exhibited the highest levels of nonthermal forces under control, indicating

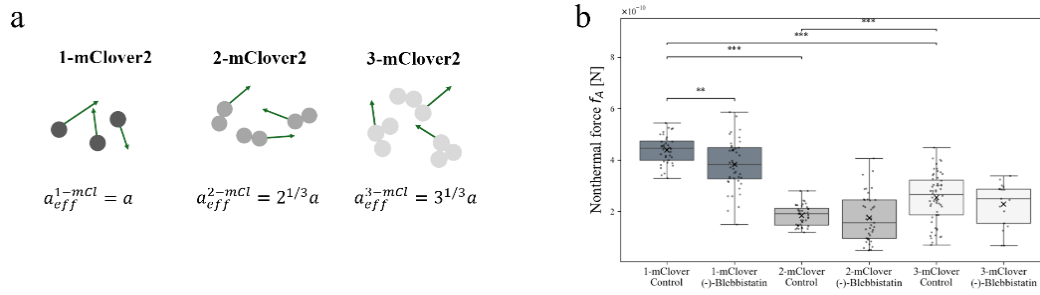


Fig. VII -4 Smaller particles experience stronger fluctuations and respond sensitively to actomyosin modulation. (a) Schematic diagram of fluorescent molecules and their effective diameters. (b) Nonthermal forces measured for monomeric (1-mClover2), dimeric (2-mClover2), and trimeric (3-mClover2) probes reveal a size dependence of intracellular fluctuations. 1-mClover2 shows the highest forces under control conditions, followed by 3- and 2-mClover2. Myosin II inhibition markedly decreases forces only for 1-mClover2, with little effect on larger constructs.

that smaller particles are subject to more pronounced active fluctuations within the cytoplasm. Interestingly, 3-mClover2 showed higher mean nonthermal forces and greater variability than 2-mClover2, despite its larger size. This apparent deviation from the expected monotonic trend likely reflects structural heterogeneity: the trimeric construct can adopt a range of conformations in the intracellular environment, thereby sampling distinct local mechanical landscapes.

Strikingly, upon inhibition of myosin II with blebbistatin, only 1-mClover2 displayed a statistically significant reduction in nonthermal forces, whereas the larger constructs showed minimal or no change. These results suggest that the mechanisms driving active fluctuations, and their sensitivity to cytoskeletal regulation, are particle-size dependent. In particular, smaller particles appear to be more directly influenced by dynamic actomyosin activity, possibly due to their ability to probe shorter length scales and faster timescales of force generation. Conversely, larger particles or extended molecular assemblies may require remodeling of broader cytoskeletal features, such as the actin meshwork, to experience comparable changes in fluctuation behavior. This size-dependent sensitivity highlights the multiscale nature of intracellular mechanics, where distinct physical regimes govern the coupling between nonthermal forces and diffusing entities.

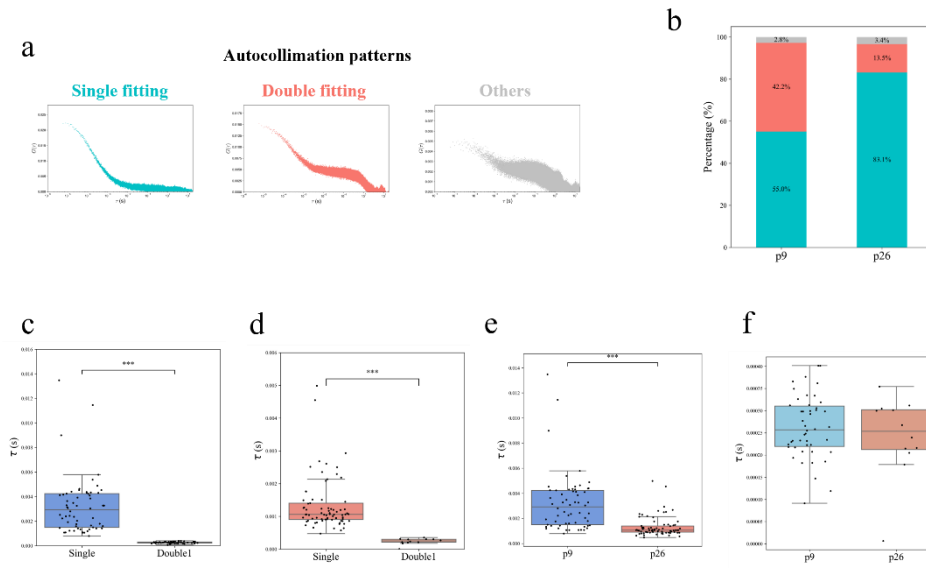


Fig. VII-5 Cellular senescence alters diffusion heterogeneity by shifting toward single-mode behavior. (a) Representative autocorrelation functions (ACFs) are classified into single-component, double-component, and other categories based on the coefficient of determination for each fitting model. (b) Percentage of fitting modes observed in young (p9) and senescent (p26) cells (c, d) Comparison of characteristic diffusion times between single and double fitting models shows significant differences, indicating that the multi-component mode reflects a distinct mechanical complexity within the diffusion process. (e) Characteristic diffusion times for the single-mode category in p9 and p26 cells⁴. (f) Characteristic diffusion times for the double-mode category in p9 and p26 cells⁵.

VII-4.7 Cellular senescence simplifies diffusion mode

Comparison of young (p9) and senescent (p26) cells revealed a marked divergence in their intracellular diffusion profiles (Fig. VII-5b). Senescent cells exhibited a significant increase in the proportion of ACFs best fitted by a single-component model, while the prevalence of double-component dynamics was concomitantly reduced. These findings indicate that senescence-associated changes promote a transition toward a more uniform cytoplasmic environment characterized by simplified fluctuation modes.

Further analysis focused on the diffusion timescales derived from these fits. The characteristic τ obtained from single fits were significantly different from those derived from double fits (Figs. VII-5c and VII-5d), suggesting the double mode reflects increased complexity affecting the diffusion process itself, not just superposition. The diffusion times associated with the second, slower component in double fits, showed greater variance compared to τ from others, possibly indicating contributions from multiple variable factors.

When comparing age groups, τ within the single category was found to be significantly smaller, implying faster diffusion in senescent cells compared to young cells, consistent with the reduced f_A observed in senescence (Fig. VII-5e). However, τ values associated with the double fits showed no significant difference between young and senescent cells, suggesting that while the probability of complex diffusion decreases with senescence, its characteristic timescale, when observed, is unchanged (Fig. VII-5f).

VII-4.8 Impact of myosin activities on diffusion modes

We next examined the effect of blebbistatin, which significantly impacted f_A , to further probe the factors influencing diffusion modes. Treatment with blebbistatin led to a significant increase in the proportions of both single and double fits, with a corresponding decrease in the proportion of others fits, compared to control cells (Fig. VII-6a). This suggests that myosin II inhibition distinctly influences the cellular conditions leading to both classified diffusion states, reducing the prevalence of poorly characterized fluctuations.

Analysis of diffusion times showed that, similar to young and senescent cells, τ values from single fits were significantly different from those associated with double fits in both control and blebbistatin treated conditions (Figs. VII-6b and c). blebbistatin treatment caused a significant increase in τ for ACFs classified in the single category, indicating that inhibition of myosin II slows the primary diffusion process (Fig. VII-6d). However, there was no significant difference in τ associated with the double fits between control and cells with blebbistatin (Fig. VII-6e). This suggests that while inhibition of myosin II alters the likelihood of observing both single and double diffusion states, it does not significantly alter the characteristic timescale of the double state when it occurs.

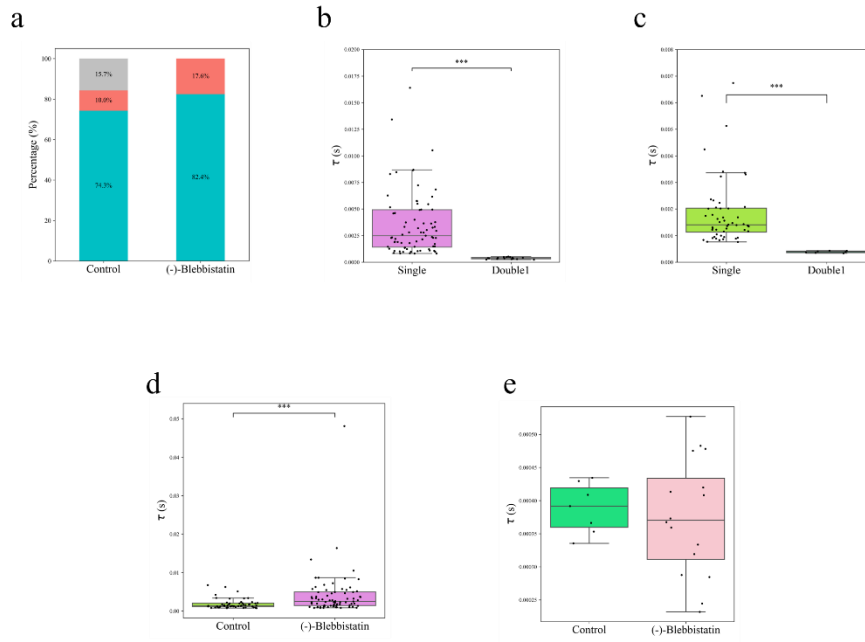


Fig. VII-6 Myosin II activity regulates the likelihood of distinct diffusion modes. (a) Proportions of fitting modes in control cells and cells treated with blebbistatin. (b,c) Characteristic diffusion times or single and double fitting models under control (b) and blebbistatin-treated (c) conditions. (d) Comparison of characteristic diffusion times for the single-mode category between control and blebbistatin-treated cells. (e) Comparison of characteristic diffusion times for the double-mode category between control and blebbistatin-treated cells.

VII-5. Discussion

In this study, we established a quantitative framework that integrates FCS with nonequilibrium physics to characterize the intracellular force landscape and its modulation by cellular state. By deriving nonthermal forces from the violation of the einstein relation, we successfully decoupled active cellular fluctuations from passive thermal noise without the need for external mechanical perturbations. Nonthermal forces generated by motor proteins such as actomyosin are essential for cellular adaptability and homeostasis (VII. 1,5,6,10,11,26). Our approach provides a statistical readout of the collective dynamics within the cytoplasm, offering an advantage over previous studies that required temporal tracking of individual particles under artificially applied mechanical stimuli (VII.7,12,13,15,17–19,33).

The primary finding of our work is that the actomyosin network serves as the dominant driver of active fluctuations in the cytoplasm. The calculated nonthermal force in control cells (~47 pN) significantly exceeds the force generated by individual actomyosin motors (1–10 pN), suggesting that FCS probes emergent collective dynamics. We propose that these fluctuations arise from stress propagation through a mechanically percolating actin network, where contractile events are amplified and transmitted across the cytoplasm. The synergistic reduction of f_A upon simultaneous inhibition of myosin activity and actin polymerization further underscores that both the contractile motor and the structural scaffold are indispensable for generating these functional fluctuations.

Our analysis of cellular senescence reveals a striking reconfiguration of this mechanical environment. Senescent cells (p26) exhibited significantly higher f_A compared to young cells (p9), a result consistent with enhanced stress fiber formation and elevated cytoskeletal tension (VII. 31). However, this increase in force magnitude was accompanied by a marked simplification of diffusion dynamics, evidenced by a shift toward single-component fluctuation modes. This transition toward a more uniform yet rigid cytoplasmic state may reflect a loss of regulatory flexibility, potentially explaining the reduced adaptive capacity characteristic of the senescent phenotype.

Furthermore, the discovery of size-dependent sensitivity to nonthermal forces highlights the multiscale organization of intracellular fluctuations. Smaller probes (1-mclover2) exhibit rapid and sensitive responses to subtle, myosin-driven changes, likely because their dimensions allow them to sample local, high-frequency dynamics within the actin meshwork. In contrast, larger molecular assemblies appear robust against background disturbances,

responding primarily to significant, global remodeling of the cytoskeletal architecture. These findings suggest a potential spatiotemporal hierarchy of fluctuations, where the sensitivity and response characteristics of intracellular components are tuned by their physical scale, distinguishing between fast local activity and stable mesoscale dynamics. This size-dependent sensitivity provides a mechanistic framework to interpret how diverse intracellular components experience and respond to mechanical noise.

In conclusion, our results position actomyosin-driven fluctuations as a central functional readout of the cellular physiological state. The transition from complex, heterogeneous fluctuations in young cells to simplified, high-magnitude forces in senescent cells provides a physical basis for understanding the senescence process. This nonequilibrium framework opens new avenues for exploring how cells regulate their internal physical environment and how this regulation fails in aging and disease.

VIII Conclusion

We have explored the fundamental mechanisms underlying cellular adaptability and tensional homeostasis, positing that these quintessential features of "livingness" are governed not only by biochemical signaling pathways but also by inevitable physical principles. Through a series of theoretical and experimental investigations spanning from molecular distributions to macroscopic cellular behaviors, we have demonstrated that the complex and often stochastic nature of cellular organization can be understood through the frameworks of statistical mechanics, thermodynamics, and nonequilibrium physics.

We first identified the theoretical requirements for maintaining tensional homeostasis, revealing that the probability distribution of actin filament lengths is physically constrained to maximize entropy while sustaining intracellular tension (Chapter II). Building on this, we elucidated how cells can transition between different homeostatic set-points. By modeling the force-dependent recruitment of α -SMA, we showed that the emergence of supermature focal adhesions and stress fibers is a bistable transition driven by mechanical parameters, allowing cells to adapt to varying substrate stiffness (Chapter III).

Furthermore, we addressed the dynamic aspects of cytoskeletal remodeling. Using percolation theory, we uncovered the physical origin of the asymmetric response between the formation and disintegration of stress fibers, demonstrating that the connectivity of the force-bearing network intrinsically dictates the speed of adaptation (Chapter IV). We also highlighted the functional role of structural disorder. Contrary to the intuitive view that randomness implies inefficiency, our analysis of sarcomere-like structures revealed that inherent structural variability increases entropy, thereby lowering the energetic barrier for remodeling and enhancing adaptive flexibility (Chapter V).

Extending this thermodynamic perspective to intracellular structure, we established that the stiffness-dependent reorganization of the cytoskeleton, from disordered meshes to aligned bundles, proceeds through a hierarchy of phase transitions. This finding suggests that cells utilize mechanical checkpoints governed by energy-entropy competition to coordinate intracellular order with environmental cues (Chapter VI). Finally, we linked these physical states to the physiological condition of the cell by quantifying nonthermal fluctuations. We demonstrated that actomyosin-driven active fluctuations are a hallmark of cellular vitality, and that cellular senescence is characterized by a "freezing" of these functional fluctuations, leading to a loss of regulatory flexibility (Chapter VII).

Based on these findings, we propose that a fundamental characteristic sustaining

"livingness" is the inherent "sloppiness" or imprecision arising from cellular fluctuations and noise. Artificial machines, engineered with high precision for specific goals, often exhibit fragility when facing inputs outside their design parameters. In contrast, while biological systems may exhibit lower precision in many contexts compared to machines, the degrees of freedom generated by this "surplus of sloppiness" provide the necessary buffer to maintain flexible adaptive functions in the face of unexpected environmental changes.

To quantitatively understand these phenomena beyond the scope of the current work, future inquiries using information thermodynamics may prove instrumental in elucidating the relationship between information flow arising from cellular fluctuations and adaptive functions. It is plausible to speculate that life utilizes the continuous flow of information inherent in noise fluctuations—not merely as interference to be suppressed like binary digital signals, but potentially as a vital energetic resource that fuels its functional resilience.

In conclusion, this work provides a unified physical framework for understanding how cells navigate the delicate balance between stability and plasticity. We argue that while biochemical signals provide the "possibility" for cellular responses, physical laws dictate their "inevitability." By viewing cellular phenomena through this lens of physical necessity, we not only deepen our understanding of basic cell biology but also offer new insights into the mechanical breakdowns associated with aging and disease, paving the way for future mechanobiological interventions.

References

- I .1. Chaudhuri O, Cooper-White J, Janmey PA, Mooney DJ, Shenoy VB. Effects of extracellular matrix viscoelasticity on cellular behaviour. *Nature* 2020 584:7822. 2020 Aug 26;584(7822):535–46.
- I .2. Rosen RS, Yarmush ML. Current Trends in Anti-Aging Strategies. *Annu Rev Biomed Eng.* 2023 Jun 8;25:363–85.
- I .3. Humphrey JD. Vascular Adaptation and Mechanical Homeostasis at Tissue, Cellular, and Sub-cellular Levels. *Cell Biochemistry and Biophysics* 2007 50:2. 2007 Oct 24;50(2):53–78.
- I .4. Prager-Khoutorsky M, Lichtenstein A, Krishnan R, Rajendran K, Mayo A, Kam Z, et al. Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. *Nat Cell Biol.* 2011 Dec;13(12):1457–65.
- I .5. Doss BL, Pan M, Gupta M, Greci G, Mège RM, Lim CT, et al. Cell response to substrate rigidity is regulated by active and passive cytoskeletal stress. *Proc Natl Acad Sci U S A.* 2020 Jun 9;117(23):12817–25.
- I .6. Wolfenson H, Bershadsky A, Henis YI, Geiger B. Actomyosin-generated tension controls the molecular kinetics of focal adhesions. *J Cell Sci.* 2011 May 1;124(9):1425–32.
- I .7. Vining KH, Marneth AE, Adu-Berchie K, Grolman JM, Tringides CM, Liu Y, et al. Mechanical checkpoint regulates monocyte differentiation in fibrotic niches. *Nature Materials* 2022 21:8. 2022 Jul 11;21(8):939–50.
- I .8. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nature Reviews Molecular Cell Biology* 2009 10:1. 2009 Jan;10(1):53–62.
- I .9. Humphrey JD, Schwartz MA. Vascular Mechanobiology: Homeostasis, Adaptation, and Disease. *Annu Rev Biomed Eng.* 2021;23(1):1–27.
- I .10. Fodale V, Pierobon M, Liotta L, Petricoin E. Mechanism of cell adaptation: When and how do cancer cells develop chemoresistance? *Cancer Journal.* 2011 Mar;17(2):89–95.
- I .11. Chantachotikul P, Liu S, Furukawa K, Deguchi S. AP2A1 is upregulated upon replicative senescence of human fibroblasts to strengthen focal adhesions via integrin β 1 translocation along stress fibers.

- I .12. Onck PR, Van der Giessen E. Micromechanobiology: A micromechanics approach to mechanobiology. *European Journal of Mechanics - A/Solids*. 2026 Mar 1;116:105893.
- I .13. Hohmann T, Dehghani F. The Cytoskeleton—A Complex Interacting Meshwork. *Cells* 2019, Vol 8, Page 362. 2019 Apr 18;8(4):362.
- I .14. Moujaber O, Stochaj U. The Cytoskeleton as Regulator of Cell Signaling Pathways. *Trends Biochem Sci*. 2020 Feb 1;45(2):96–107.
- I .15. Kasza KE, Zallen JA. Dynamics and regulation of contractile actin–myosin networks in morphogenesis. *Curr Opin Cell Biol*. 2011 Feb 1;23(1):30–8.
- I .16. Geeves MA, Fedorov R, Manstein DJ. Molecular mechanism of actomyosin-based motility. *Cellular and Molecular Life Sciences*. 2005 Jul 28;62(13):1462–77.
- I .17. Bernard Claude. An introduction to the study of experimental medicine. 2018;226.
- I .18. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, et al. Tensional homeostasis and the malignant phenotype. *Cancer Cell*. 2005 Sep 1;8(3):241–54.
- I .19. Stamenovic D, Smith ML, Zurich Michael E. Tensional homeostasis at different length scales *Soft Matter*. This journal is Cite this: *Soft Matter*. 2020;16:25.
- I .20. Brown RA, Prajapati R, Mcgrouter DA, Yannas I V, Eastwood AM. Tensional Homeostasis in Dermal Fibroblasts: Mechanical Responses to Mechanical Loading in Three-Dimensional Substrates. *J Cell Physiol* [Internet]. 1998
- I .21. Lazarides E, Burridge K. Alpha-actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. *Cell*. 1975;6(3):289–98.
- I .22. Meyer RK, Aebi U. Bundling of actin filaments by alpha-actinin depends on its molecular length. *Journal of Cell Biology*. 1990 Jun 1;110(6):2013–24.
- I .23. Gee E, Milkiewicz M, Haas TL. p38 MAPK activity is stimulated by vascular endothelial growth factor receptor 2 activation and is essential for shear stress-induced angiogenesis. *J Cell Physiol*. 2010 Jan 1;222(1):120–6.
- I .24. Dzeja PP, Terzic A. Phosphotransfer networks and cellular energetics. *Journal of Experimental Biology*. 2003 Jun 15;206(12):2039–47.
- I .25. Statistical Physics: Volume 5 - L D Landau, E.M. Lifshitz
- I .26. Cao J, Cusanovich DA, Ramani V, Aghamirzaie D, Pliner HA, Hill AJ, et al. Joint profiling of chromatin accessibility and gene expression in thousands of single cells. *Science* (1979). 2018 Sep 28;361(6409):1380–5.

- I .27. Goldstein RA, Luthey-Schulten ZA, Wolynes PG. Optimal protein-folding codes from spin-glass theory. *Proc Natl Acad Sci U S A*. 1992 Jun 1;89(11):4918–22.
- I .28. Bryngelson JD, Wolynes PG. Spin glasses and the statistical mechanics of protein folding. *Proc Natl Acad Sci U S A*. 1987 Nov 1;84(21):7524–8.
- I .29. Lynn CW, Cornblath EJ, Papadopoulos L, Bertolero MA, Bassett DS. Broken detailed balance and entropy production in the human brain. *Proc Natl Acad Sci U S A*.
- I .30. Lessey EC, Guilluy C, Burridge K. From Mechanical Force to RhoA Activation. *Biochemistry*. 2012 Sep 25;51(38):7420–32.
- I .31. Arnsdorf EJ, Tummala P, Kwon RY, Jacobs CR. Mechanically induced osteogenic differentiation – the role of RhoA, ROCKII and cytoskeletal dynamics. *J Cell Sci*. 2009 Feb 15;122(4):546–53.
- I .32. Kühn S, Geyer M. Formins as effector proteins of rho GTPases. *Small GTPases*. 2014;5(JUNE).
- I .33. Narumiya S, Tanji M, Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. *Cancer and Metastasis Reviews* 2009 28:1. 2009 Jan 22;28(1):65–76.
- I .34. Hotulainen P, Paunola E, Vartiainen MK, Lappalainen P. Actin-depolymerizing Factor and Cofilin-1 Play Overlapping Roles in Promoting Rapid F-Actin Depolymerization in Mammalian Nonmuscle Cells. 2004 Nov 17;16(2):649–64.
- I .35. Bamburg JR. Proteins of the ADF/cofilin family: Essential regulators of actin dynamics. *Annu Rev Cell Dev Biol*. 1999 Nov 1;15(Volume 15, 1999):185–230.
- I .36. Somlyo AP, Somlyo A V. Ca²⁺ Sensitivity of Smooth Muscle and Nonmuscle Myosin II: Modulated by G Proteins, Kinases, and Myosin Phosphatase. 2003 [cited 2026 Jan 11];83(4):1325–58.
- I .37. Burridge K, Wennerberg K. Rho and Rac Take Center Stage. *Cell*. 2004 Jan 23;116(2):167–79.
- I .38. Ridley AJ. Rho GTPases and cell migration. *J Cell Sci*. 2001 Aug 1;114(15):2713–22.
- II .1. Brown, R.A., Prajapati, R., McGrouther, D.A., Yannas, I.V., Eastwood, M. 1998. Tensional homeostasis in dermal fibroblasts: mechanical responses to mechanical loading in three-dimensional substrates. *J Cell Physiol* 175(3):323-332.

- II.2. Paszek, M.J., Zahir, N., Johnson, K.R., Lakins, J.N., Rozenberg, G.I., Gefen, A., Reinhart-King, C.A., Margulies, S.S., Dembo, M., Boettiger, D., Hammer, D.A., Weaver, V.M. 2005. Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8(3):241-254.
- II.3. Webster, K.D., Ng, W.P., Fletcher, D.A. 2014. Tensional homeostasis in single fibroblasts. *Biophys J* 107(1):146-155.
- II.4. Stamenović, D., Smith, M.L. 2020. Tensional homeostasis at different length scales. *Soft Matter*. 216(30):6946-6963.
- II.5. Mizutani, T., Haga, H., Kawabata, K. 2004. Cellular stiffness response to external deformation: tensional homeostasis in a single fibroblast. *Cell Motil Cytoskeleton* 59(4):242-248.
- II.6. Nagayama, K., Adachi, A., Matsumoto, T. 2012. Dynamic changes of traction force at focal adhesions during macroscopic cell stretching using an elastic micropillar substrate: tensional homeostasis of aortic smooth muscle cells. *J Biomech Sci Eng* 7(2):130-140.
- II.7. Kaunas, R., Usami, S., Chien, S. 2006. Regulation of stretch-induced JNK activation by stress fiber orientation. *Cell Signal*. 18(11):1924-1931.
- II.8. Chien, S. 2007. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. *Am J Physiol Heart Circ Physiol*. 292(3):H1209-1224.
- II.9. Humphrey, J.D. 2008. Vascular adaptation and mechanical homeostasis at tissue, cellular, and sub-cellular levels. *Cell Biochem Biophys*. 50(2):53-78.
- II.10. Hahn, C., Schwartz, M.A. 2009. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol*. 10(1):53-62.
- II.11. Kaunas, R., Deguchi, S. 2011. Multiple roles for myosin II in tensional homeostasis under mechanical loading. *Cell Mol Bioeng* 4 (2):182-191.
- II.12. Humphrey, J.D., Schwartz, M.A. 2021. Vascular mechanobiology: Homeostasis, adaptation, and disease. *Annu Rev Biomed Eng*.23:1-27.
- II.13. Pourati, J., Maniotis, A., Spiegel, D., Schaffer, J.L., Butler, J.P., Fredberg, J.J., Ingber, D.E., Stamenovic, D., Wang, N. 1998. Is cytoskeletal tension a major determinant of cell deformability in adherent endothelial cells? *Am J Physiol* 274(5):C1283-1289.
- II.14. Ingber, D.E. 2003. Tensegrity II. How structural networks influence cellular information processing networks. *J Cell Sci*. 116:1397-1408.
- II.15. Sato, K., Adachi, T., Matsuo, M., Tomita, Y. 2005. Quantitative evaluation of threshold fiber strain that induces reorganization of cytoskeletal actin fiber structure in osteoblastic cells. *J Biomech* 38(9):1895-1901.
- II.16. Deguchi, S., Ohashi, T., Sato, M. 2006. Tensile properties of single stress fibers

- isolated from cultured vascular smooth muscle cells. *J Biomech* 39:2603-2610.
- II.17. Svitkina, T.M., Verkhovsky, A.B., McQuade, K.M., Borisy, G.G. 1997. Analysis of the actin-myosin II system in fish epidermal keratocytes: mechanism of cell body translocation. *J Cell Biol* 139(2):397-415.
 - II.18. McGrath, J.L., Tardy, Y., Dewey Jr, C.F., Meister, J.J., Hartwig, J.H. 1998. Simultaneous measurements of actin filament turnover, filament fraction, and monomer diffusion in endothelial cells. *Biophys J* 75(4):2070-2078.
 - II.19. Deguchi, S., Matsui, T.S., Iio, K. 2011. The position and size of individual focal adhesions are determined by intracellular stress-dependent positive regulation. *Cytoskeleton* 68(11):639-651.
 - II.20. Mazloom-Farsibaf, H., Farzam, F., Fazel, M., Wester, M.J., Meddens, M.B.M., Lidke, K.A. 2021. Comparing lifeact and phalloidin for super-resolution imaging of actin in fixed cells. *PLoS One* 16(1):e0246138.
 - II.21. Huang, W., Matsui, T.S., Saito, T., Kuragano, M., Takahashi, M., Kawahara, T., Sato, M., Deguchi, S. 2021. Mechanosensitive myosin II but not cofilin primarily contributes to cyclic cell stretch-induced selective disassembly of actin stress fibers. *Am J Physiol Cell Physiol* 320:C1153-C1163.
 - II.22. Ishijima, A., Doi, T., Sakurada, K., Yanagida, T. 1991. Sub-piconewton force fluctuations of actomyosin in vitro. *Nature* 352:301-306.
 - II.23. Balaban, N.Q., Schwarz, U.S., Riveline, D., Goichberg, P., Tzur, G., Sabanay, I., Mahalu, D., Safran, S., Bershadsky, A., Addadi, L., Geiger, B. 2001. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat Cell Biol.* 3(5):466-72.
 - II.24. Tan, J.L., Tien, J., Pirone, D.M., Gray, D.S., Bhadriraju, K., Chen, C.S. 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc Natl Acad Sci USA* 100 (4):1484-1489.
 - II.25. van Hoorn, H., Harkes, R., Spiesz, E.M., Storm, C., van Noort, D., Ladoux, B., Schmidt, T. 2014. The nanoscale architecture of force-bearing focal adhesions. *Nano Lett* 14(8):4257-62.
 - II.26. Labeit, S., Ottenheijm, C.A.C., Granzier, H. 2011. Nebulin, a major player in muscle health and disease. *FASEB J* 25(3):822-829.
 - II.27. Oosawa, F., 1970. Size distribution of protein polymers. *J Theor Biol* 27(1):69-86.
 - II.28. Burlacu, S., Janmey, P.A., Borejdo, J. 1992. Distribution of actin filament lengths measured by fluorescence microscopy. *Am J Physiol Cell Physiol* 262(3):C569-C577.
 - II.29. Edelstein-Keshet, L., Ermentrout, G.B. 1998. Models for the length distributions of actin filaments: I. Simple polymerization and fragmentation. *Bull Math Biol.* 60(3):449-

475.

- II .30. Sept, D., Xu, J., Pollard, T.D., McCammon, J.A. 1999. Annealing accounts for the length of actin filaments formed by spontaneous polymerization. *Biophys J* 77(6): 2911-2919.
- II .31. Kawamura, M. Maruyama, K. 1970. Electron microscopic particle length of F-actin polymerized in vitro. *J Biochem* 67(3):437-457.
- II .32. Käs, J., Strey, H., Tang, J.X., Finger, D., Ezzell, R., Sackmann, E., Janmey, P.A. 1996. F-actin, a model polymer for semiflexible chains in dilute, semidilute, and liquid crystalline solutions. *Biophys J* 70(2):609-625.
- II .33. Ermentrout, G.B., Edelstein-Keshet, L. 1998. Models for the length distributions of actin filaments: II. Polymerization and fragmentation by gelsolin acting together. *Bull Math Biol* 60(3):477-503.
- II .34. Edelstein-Keshet, L., Ermentrout, G.B. 2001. A model for actin-filament length distribution in a lamellipod. *J Math Biol* 43(4):325-355.
- II .35. Biron, D., Moses, E. 2004. The effect of alpha-actinin on the length distribution of F-actin. *Biophys J* 86(5):3284-3290.
- II .36. Biron, D., Moses, E., Borukhov, I., Safran, S.A. 2006. Inter-filament attractions narrow the length distribution of actin filaments. *Europhys Lett* 73:464-470.
- II .37. Hu, J., Matzavinos, A., Othmer, H.G. 2007. A theoretical approach to actin filament dynamics. *J Stat Phys* 128:111-138.
- II .38. Edelstein-Keshet, L. 1998. A mathematical approach to cytoskeletal assembly. *Eur Biophys J* 27(5):521-531.
- II .39. Podolski, J.L., Steck, T.L. 1990. Length distribution of F-actin in *Dictyostelium discoideum*. *J Biol Chem* 265(3):1312-1318.
- II .40. Matsui, T.S., Kaunas, R., Kanzaki, M., Sato, M., Deguchi, S. 2011. Non-muscle myosin II induces disassembly of actin stress fibres independently of myosin light chain dephosphorylation. *Interface Focus* 1(5):754-766.
- II .41. Ehrlicher, A.J., Nakamura, F., Hartwig, J.H., Weitz, D.A., Stossel, T.P. 2011. Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature* 478(7368):260-263.
- II .42. Luo, T., Mohan, K., Iglesias, P.A., Robinson, D.N. 2013. Molecular mechanisms of cellular mechanosensing. *Nat Mat* 12:1064-1071.
- II .43. Liu, S., Matsui, T.S., Kang, N., Deguchi, S. 2022. Analysis of senescence-responsive stress fiber proteome reveals reorganization of stress fibers mediated by elongation factor eEF2 in HFF-1 cells. *Mol Biol Cell* 33(1):ar10.

- III.1. Balaban, N.Q., Schwarz, U.S., Riveline, D., Goichberg, P., Tzur, G., Sabanay, I., Mahalu, D., Safran, S., Bershadsky, A., Addadi, L., Geiger, B. 2001. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat. Cell Biol.* 3, 466–472.
- III.2. Biggs, M.J.P, Richards, R.G., McFarlane, S., Wilkinson, C.D.W., Oreffo, R.O.C., Dalby, M.J., 2008. Adhesion formation of primary human osteoblasts and the functional response of mesenchymal stem cells to 330nm deep microgrooves. *J. R. Soc. Interface.* 5, 1231–1242.
- III.3. Chien, S., 2007. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. *Am. J. Physiol. Heart Circ. Physiol.* 292, H1209–H1224.
- III.4. Deguchi, S., Ohashi, T., Sato, M., 2006. Tensile properties of single stress fibers isolated from cultured vascular smooth muscle cells. *J. Biomech.* 39, 2603–2610.
- III.5. Deguchi, S., Sato, M., 2009. Biomechanical properties of actin stress fibers of non-motile cells. *Biorheology.* 46, 93–105.
- III.6. Galbraith, C.G., Skalak, R., Chien, S., 1998. Shear stress induces spatial reorganization of the endothelial cell cytoskeleton. *Cell Motil. Cytoskeleton* 40, 317–330.
- III.7. Gardel, M.L., Schneider, I.C., Aratyn-Schaus, Y., Waterman, C.M., 2010. Mechanical integration of actin and adhesion dynamics in cell migration. *Annu. Rev. Cell Dev. Biol.* 26, 315–333.
- III.8. Geiger, B., Bershadsky, A., 2001. Assembly and mechanosensory function of focal contacts. *Curr. Opin. Cell Biol.* 13, 584–592.
- III.9. Goffin, J.M., Pittet, P., Csucs, G., Lussi, J.W., Meister, J.J., Hinz, B. 2006. Focal adhesion size controls tension-dependent recruitment of α -smooth muscle actin to stress fibers. *J Cell Biol.* 172, 259–268.
- III.10. Hahn, C., Schwartz, M.A., 2009. Mechanotransduction in vascular physiology and atherogenesis. *Nat. Rev. Mol. Cell Biol.* 10, 53–62.
- III.11. Hinz, B., Celetta, G., Tomasek, J.J., Gabbiani, G., Chaponnier, C., 2001. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. *Mol. Biol. Cell* 12, 2730-2741.
- III.12. Hinz, B., 2010. The myofibroblast: Paradigm for a mechanically active cell. *J. Biomech.* 43, 146–155.
- III.13. Huang, W., Matsui, T.S., Saito, T., Kuragano, M., Takahashi, M., Kawahara, T., Sato, M., Deguchi, S., 2021. Mechanosensitive myosin II but not cofilin primarily contributes to cyclic cell stretch-induced selective disassembly of actin stress fibers. *Am. J. Physiol. Cell Physiol.* 320, C1153–C1163.
- III.14. Humphrey, J.D., 2008. Vascular adaptation and mechanical homeostasis at tissue,

- cellular, and sub-cellular levels. *Cell Biochem. Biophys.* 50, 53–78.
- III.15. Kang, N., Matsui, T.S., Liu, S., Fujiwara, S., Deguchi, S., 2020. Comprehensive analysis on the whole Rho-GAP family reveals that ARHGAP4 suppresses EMT in epithelial cells under negative regulation by Septin9. *FASEB J.* 34, 8326–8340.
 - III.16. Kang, N., Matsui, T.S., Liu, S., Deguchi, S., 2021. ARHGAP4-SEPT2-SEPT9 complex enables both up- and down-modulation of integrin-mediated focal adhesions, cell migration, and invasion. *Mol. Biol. Cell* 32, ar28, 1–12.
 - III.17. Kaunas, R., Usami, S., Chien, S., 2006. Regulation of stretch-induced JNK activation by stress fiber orientation. *Cell. Signal.* 18, 1924–1931.
 - III.18. Kaunas, R., Deguchi, S., 2011. Multiple roles for myosin II in tensional homeostasis under mechanical loading. *Cell. Mol. Bioeng.* 4, 182–191.
 - III.19. Linder, S., Wiesner, C., Himmel, M., 2011. Degrading devices: Invadosomes in proteolytic cell invasion. *Annu. Rev. Cell Dev. Biol.* 27, 185–211.
 - III.20. Liu, B., Kilpatrick, J.I., Lukasz, B., Jarvis, S.P., McDonnell, F., Wallace, D.M., Clark, A.F., O'Brien, C.J., 2018. Increased substrate stiffness elicits a myofibroblastic phenotype in human lamina cribrosa cells. *Invest. Ophthalmol. Vis. Sci.* 59, 803–814.
 - III.21. Liu, H., Sun, Y., Simmons, C.A., 2013. Determination of local and global elastic moduli of valve interstitial cells cultured on soft substrates. *J. Biomech.* 46, 1967–1971.
 - III.22. Liu, S., Matsui, T.S., Kang, N., Deguchi, S., 2022. Analysis of senescence-responsive stress fiber proteome reveals reorganization of stress fibers mediated by elongation factor eEF2 in HFF-1 cells. *Mol. Biol. Cell* 33, ar10, 1–11.
 - III.23. Liu, Z., van Grunsven, L.A., Van Rossen, E., Schroyen, B., Timmermans, J.P., Geerts, A., Reynaert, H., 2010. Blebbistatin inhibits contraction and accelerates migration in mouse hepatic stellate cells. *Br. J. Pharmacol.* 159, 304–315.
 - III.24. Lu, L., Feng, Y., Hucker, W.J., Oswald, S.J., Longmore, G.D., Yin, F.C., 2008. Actin stress fiber pre-extension in human aortic endothelial cells. *Cell Motil. Cytoskel.* 65, 281–294.
 - III.25. Matsui, T.S., Deguchi, S., Sakamoto, N., Ohashi, T., Sato, M., 2009. A versatile micro-mechanical tester for actin stress fibers isolated from cells. *Biorheology* 46, 401–415.
 - III.26. Mitrossilis, D., Fouchard, J., Guiroy, A., Desprat, N., Rodriguez, N., Fabry, B., Asnacios, A., 2009. Single-cell response to stiffness exhibits muscle-like behavior. *Proc. Natl. Acad. Sci. U. S. A.* 106, 18243–18248.
 - III.27. Na, S., Meininger, G.A., Humphrey, J.D., 2007. Theoretical model for F-actin remodeling in vascular smooth muscle cells subjected to cyclic stretch. *J. Theor. Biol.* 246, 87–99.

- III.28. Saito, A.C., Matsui, T.S., Ohishi, T., Sato, M., Deguchi, S., 2014. Contact guidance of smooth muscle cells is associated with tension-mediated adhesion maturation. *Exp. Cell Res.* 327, 1–11.
- III.29. Sakamoto, Y., Buchanan, R.M., Sacks, M.S., 2016. On intrinsic stress fiber contractile forces in semilunar heart valve interstitial cells using a continuum mixture model. *J. Mech. Behav. Biomed. Mater* 54, 244–258.
- III.30. Stamenović, D., Smith, M.L., 2020. Tensional homeostasis at different length scales. *Soft Matter.* 16, 6946–6963.
- III.31. Stylianou, A., Gkretsi, V., Stylianopoulos, T., 2018. Transforming growth factor- β modulates pancreatic cancer associated fibroblasts cell shape, stiffness and invasion. *Biochim. Biophys. Acta Gen. Subj.* 1862, 1537–1546.
- III.32. Tan, J.L., Tien, J., Pirone, D.M., Gray, D.S., Bhadriraju, K., Chen, C.S., 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1484–1489.
- III.33. Trichet, L., Le Digabel, J., Hawkins, R.J., Vedula, S.R.K., Gupta, M., Ribault, C., Hersen, P., Voituriez, R., Ladoux, B., 2012. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6933–6938.
- III.34. van den Dries, K., Linder, S., Maridonneau-Parini, I., Poincloux, R., 2019. Probing the mechanical landscape - new insights into podosome architecture and mechanics. *J. Cell Sci.* 132, jcs236828.
- III.35. Wang, H.C., Lin, Y.L., Hsu, C.C., Chao, Y.J., Hou, Y.C., Chiu, T.J., Huang, P.H., Tang, M.J., Chen, L.T., Shan, Y.S., 2019. Pancreatic stellate cells activated by mutant KRAS-mediated PAI-1 upregulation foster pancreatic cancer progression via IL-8. *Theranostics.* 9, 7168–7183.
- III.36. Zaidel-Bar, R., Ballestrem, C., Kam, Z. and Geiger, B., 2003. Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. *J. Cell Sci.* 116, 4605–4613.
- IV.1. Alvarado J, Sheinman M, Sharma A, MacKintosh FC, Koenderink GH. Molecular motors robustly drive active gels to a critically connected state. *Nat Phys* 2013; 9:591-597.
- IV.2. Broadbent SR, Hammersley JM. Percolation processes: I. Crystals and mazes. *Mathematical proceedings of the Cambridge philosophical society.* Vol. 53. No. 3. Cambridge University Press, 1957.
- IV.3. Chen C, Krishnan R, Zhou E, Ramachandran A, Tambe D, Rajendran K, Adam RM,

- Deng L, Fredberg JJ. Fluidization and resolidification of the human bladder smooth muscle cell in response to transient stretch. *PLoS ONE* 2010; 5:e12035.
- IV.4. Chrzanowska-Wodnicka M, Burridge K. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J Cell Biol* 1996; 133:1403-1415.
 - IV.5. Deguchi S, Ohashi T, Sato M. Intracellular stress transmission through actin stress fiber network in adherent vascular cells. *Mol Cell Biomech* 2005; 2:205-216.
 - IV.6. Deguchi S, Matsui TS, Iio K. The position and size of individual focal adhesions are determined by intracellular stress-dependent positive regulation. *Cytoskeleton* 2011; 68:639-651.
 - IV.7. Forgacs G. On the possible role of cytoskeletal filamentous networks in intracellular signaling: an approach based on percolation. *J Cell Sci* 1995; 108: 2131-2143.
 - IV.8. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol* 2009; 10:53-62.
 - IV.9. Hirata H, Tatsumi H, Sokabe M. Dynamics of actin filaments during tension-dependent formation of actin bundles. *Biochim Biophys Acta* 2007; 1770:1115-1127.
 - IV.10. Hotulainen P, Lappalainen P. Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. *J Cell Biol* 2006; 173:383-394.
 - IV.11. Huang W, Matsui TS, Saito T, Kuragano M, Takahashi M, Kawahara T, Sato M, Deguchi S. Mechanosensitive myosin II but not cofilin primarily contributes to cyclic cell stretch-induced selective disassembly of actin stress fibers. *Am J Physiol Cell Physiol* 2021; 320:C1153-C1163.
 - IV.12. Kaunas R, Deguchi S. Multiple roles for myosin II in tensional homeostasis under mechanical loading. *Cell Mol Bioeng* 2011; 4:182-191.
 - IV.13. Kovács M, Thirumurugan K, Knight PJ, Sellers JR. Load-dependent mechanism of nonmuscle myosin 2. *Proc Natl Acad Sci U S A* 2007; 104:9994-9999.
 - IV.14. Labeit S, Ottenheijm CAC, Granzier H. Nebulin, a major player in muscle health and disease. *FASEB J* 2011; 25:822-829.
 - IV.15. Langley DP, Lagrange M, Nguyen ND, Bellet D. Percolation in networks of 1-dimensional objects: comparison between Monte Carlo simulations and experimental observations. *Nanoscale Horiz* 2018; 3:545-550.
 - IV.16. Lee CF, Haase C, Deguchi S, Kaunas R. Cyclic stretch-induced stress fiber dynamics - Dependence on strain rate, Rho-kinase and MLCK. *Biochem Biophys Res Commun* 2010; 401:344-349.
 - IV.17. Littlefield R, Almenar-Queralt A, Fowler VM. Actin dynamics at pointed ends regulates thin filament length in striated muscle. *Nat Cell Biol* 2001; 3:544-551.
 - IV.18. Liu S, Matsui TS, Kang N, Deguchi S. Analysis of senescence-responsive stress

- fiber proteome reveals reorganization of stress fibers mediated by elongation factor eEF2 in HFF-1 cells. *Mol Biol Cell* 2022; 33:ar10.
- IV.19. Macarelli V, Leventea E, Merkle FT. Regulation of the length of neuronal primary cilia and its potential effects on signalling. *Trends Cell Biol* 2023; 33:979-990.
 - IV.20. Matsui TS, Ito K, Kaunas R, Sato M, Deguchi S. Actin stress fibers are at a tipping point between conventional shortening and rapid disassembly at physiological levels of MgATP. *Biochem Biophys Res Commun* 2010; 395:301-306.
 - IV.21. Matsui TS, Kaunas R., Kanzaki, M., Sato, M., Deguchi, S. Non-muscle myosin II induces disassembly of actin stress fibres independently of myosin light chain dephosphorylation. *Interface Focus* 2011; 1:754-766.
 - IV.22. Matsui TS, Deguchi S. Spatially selective myosin regulatory light chain regulation is absent in dedifferentiated vascular smooth muscle cells but is partially induced by fibronectin and Klf4. *Am J Physiol Cell Physiol* 2019; 316:C509-C521.
 - IV.23. Miller CM, Korkmazhan E, Dunn AR. Extraction of accurate cytoskeletal actin velocity distributions from noisy measurements. *Nat Commun* 2022;13:4749.
 - IV.24. Ni Q, Wagh K, Pathni A, Ni H, Vashisht V, Upadhyaya A, Papoian GA. A tug of war between filament treadmilling and myosin induced contractility generates actin rings. *eLife* 2022; 11:e82658.
 - IV.25. Okamoto T, Matsui TS, Ohishi T, Deguchi S. Helical structure of actin stress fibers and its possible contribution to inducing their direction-selective disassembly upon cell shortening. *Biomech Model Mechanobiol* 2020; 19:543-555.
 - IV.26. Prager-Khoutorsky M, Lichtenstein, A, Krishnan R, Rajendran K, Mayo A, Kam Z, Geiger B, Bershadsky AD. Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. *Nat Cell Biol* 2011; 13:1457-1465.
 - IV.27. Pritchard RH, Huang YYS, Terentjev EM. Mechanics of biological networks: from the cell cytoskeleton to connective tissue. *Soft Matter* 2014; 10:1864-1884.
 - IV.28. Saito T, Huang W, Matsui TS, Kuragano M, Takahashi M, Deguchi S. What factors determine the number of nonmuscle myosin II in the sarcomeric unit of stress fibers? *Biomech Model Mechanobiol* 2021; 20:155-166.
 - IV.29. Saito T, Matsunaga D, Deguchi S. Analysis of chemomechanical behavior of stress fibers by continuum mechanics-based FRAP. *Biophys J* 2022; 121:2921-2930.
 - IV.30. Saito T, Matsunaga D, Deguchi S. Long-term molecular turnover of actin stress fibers revealed by advection-reaction analysis in fluorescence recovery after photobleaching. *PLoS ONE* 2022; 17:e0276909.
 - IV.31. Sato K, Adachi T, Shirai Y. Local disassembly of actin stress fibers induced by selected release of intracellular tension in osteoblastic cell. *J Biomech Sci Eng* 2006;

1:204-214.

- IV.32. Silveira PSP, Alencar AM, Majumdar A, Lemos M, Fredberg JJ, Suki B. Percolation in a network with long-range connections: Implications for cytoskeletal structure and function. *Phys A: Stat Mech Appl* 2009; 388:1521-1526.
- IV.33. Svitkina TM. Actin cell cortex: structure and molecular organization. *Trends Cell Biol* 2020; 30:556-565.
- IV.34. Trepac X, Deng L, An SS, Navajas D, Tschumperlin DJ, Gerthoffer WT, Butler JP, Fredberg J.J. Universal physical responses to stretch in the living cell. *Nature* 2007; 447:592-595.
- IV.35. Ueda Y, Matsunaga D, Deguchi S. A statistical mechanics model for determining the length distribution of actin filaments under cellular tensional homeostasis. *Sci Rep* 2022; 12:14466.
- IV.36. Ueda Y, Deguchi S. Emergence of multiple set-points of cellular homeostatic tension. *J Biomech* 2023; 151:111543.
- IV.37. Vignaud T, Copos C, Leterrier C, Toro-Nahuelpan M, Tseng Q, Mahamid J, Blanchoin L, Mogilner A, Théry M, Kurzawa L. Stress fibres are embedded in a contractile cortical network. *Nat Mater* 2021; 20:410-420.
- IV.38. Wang F, Kovács M, Hu A, Limouze J, Harvey EV, Sellers JR. Kinetic mechanism of non-muscle myosin IIB functional adaptations for tension generation and maintenance. *J Biol Chem* 2003; 278:27439-27448.
- IV.39. Wang S, Wolynes P.G. Tensegrity and motor-driven effective interactions in a model cytoskeleton. *J Chem Phys.* 2012; 136:145102.
- IV.40. Yamashiro S, Tanaka S, McMillen LM, Taniguchi D, Vavylonis D, Watanabe N. *Mol Biol Cell* 2018; 29:1917-2035.

- V.1. Zajac AL, Discher DE. Cell differentiation through tissue elasticity-coupled, myosin-driven remodeling. *Curr Opin Cell Biol.* 2008 Dec;20(6):609–15.
- V.2. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science.* 2005 Nov 18;310(5751):1139–43.
- V.3. Hove JR, Köster RW, Forouhar AS, Acevedo-Bolton G, Fraser SE, Gharib M. Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. *Nature.* 2003 Jan 9;421(6919):172–7.
- V.4. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nature Reviews Molecular Cell Biology* 2009 10:1. 2009 Jan;10(1):53–62.
- V.5. Henderson CA, Gomez CG, Novak SM, Mi-Mi L, Gregorio CC. Overview of the

Muscle Cytoskeleton. *Compr Physiol*. 2017 Jul 1;7(3):891–944.

- V.6. Purslow PP, Trotter JA. The morphology and mechanical properties endomysium in series-fibred muscles: variations with muscle length of. Vol. 15, *Journal of Muscle Research and Cell Motility*. 1994.
- V.7. Reconditi M, Brunello E, Fusi L, Linari M, Martinez MF, Lombardi V, et al. Sarcomere-length dependence of myosin filament structure in skeletal muscle fibres of the frog. *J Physiol*. 2014 Mar 1;592(5):1119–37.
- V.8. Ertbjerg P, Puolanne E. Muscle structure, sarcomere length and influences on meat quality: A review. *Meat Sci*. 2017 Oct 1;132:139–52.
- V.9. Gollapudi SK, Lin DC. Experimental determination of sarcomere force–length relationship in type-I human skeletal muscle fibers. *J Biomech*. 2009 Sep 18;42(13):2011–6.
- V.10. Herrera AM, McParland BE, Bienkowska A, Tait R, Paré PD, Seow CY. ‘Sarcomeres’ of smooth muscle: functional characteristics and ultrastructural evidence. *J Cell Sci*. 2005 Jun 1;118(11):2381–92.
- V.11. Peterson LJ, Rajfur Z, Maddox AS, Freel CD, Chen Y, Edlund M, et al. Simultaneous stretching and contraction of stress fibers in vivo. *Mol Biol Cell*. 2004 Jul 7;15(7):3497–508.
- V.12. Verkhovsky AB, Svitkina TM, Borisy GG. Polarity sorting of actin filaments in cytochalasin-treated fibroblasts. *J Cell Sci*. 1997 Aug 1;110(15):1693–704.
- V.13. Hotulainen P, Lappalainen P. Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. *Journal of Cell Biology*. 2006 May 8;173(3):383–94.
- V.14. Cramer LP, Siebert M, Mitchison TJ. Identification of Novel Graded Polarity Actin Filament Bundles in Locomoting Heart Fibroblasts: Implications for the Generation of Motile Force. *Journal of Cell Biology*. 1997 Mar 24;136(6):1287–305.
- V.15. Deguchi S, Sato M. Biomechanical properties of actin stress fibers of non-motile cells. Vol. 46, *Biorheology*. 2009. p. 93–105.
- V.16. Lazarides E, Burridge K. Alpha-actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. *Cell*. 1975;6(3):289–98.
- V.17. Coravos JS, Martin AC. Apical Sarcomere-like Actomyosin Contracts Nonmuscle *Drosophila* Epithelial Cells. *Dev Cell*. 2016 Nov 7;39(3):346–58.
- V.18. Russell RJ, Grubbs AY, Mangroo SP, Nakasone SE, Dickinson RB, Lele TP. Sarcomere length fluctuations and flow in capillary endothelial cells. *Cytoskeleton*. 2011 Mar 1;68(3):150–6.
- V.19. Goldspink G. Sarcomere length during post-natal growth of mammalian muscle

- fibres. *J Cell Sci.* 1968 Dec 1;3(4):539–48.
- V.20. Kolley F, Sidor C, Dehapiot B, Schnorrer F, Friedrich BM. Mechanisms of Sarcomere Assembly in Muscle Cells Inferred from Sequential Ordering of Myofibril Components. *PRX Life.* 2024 Jan 11;2(1):013002.
- V.21. Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. Vol. 10, *Nature Reviews Molecular Cell Biology.* 2009. p. 778–90.
- V.22. Conti MA, Adelstein RS. Erratum: Nonmuscle myosin II moves in new directions (*Journal of Cell Science* vol. 121 (11-18)). Vol. 121, *Journal of Cell Science.* 2008. p. 404.
- V.23. Schiffhauer ESS, Luo T, Mohan K, Srivastava V, Qian X, Griffis ERR, et al. Mechanoaccumulative Elements of the Mammalian Actin Cytoskeleton. *Current Biology.* 2016;26(11):1473–9.
- V.24. Greenberg MJ, Arpağ G, Tüzel E, Ostap EM. A Perspective on the Role of Myosins as Mechanosensors. Vol. 110, *Biophysical Journal.* Biophysical Society; 2016. p. 2568–76.
- V.25. Kolley F, Sidor C, Dehapiot B, Schnorrer F, Friedrich BM. Mechanisms of Sarcomere Assembly in Muscle Cells Inferred from Sequential Ordering of Myofibril Components. *PRX Life.* 2024 Jan 11;2(1).
- V.26. Friedrich BM, Fischer-Friedrich E, Gov NS, Safran SA. Sarcomeric pattern formation by actin cluster coalescence. *PLoS Comput Biol.* 2012;8(6).
- V.27. Katoh K, Kano Y, Masuda M, Onishi H, Fujiwara K. Isolation and contraction of the stress fiber. *Mol Biol Cell.* 1998 Oct 13;9(7):1919–38.
- V.28. Deguchi S, Ohashi T, Sato M. Tensile properties of single stress fibers isolated from cultured vascular smooth muscle cells. *J Biomech.* 2006 Jan 1;39(14):2603–10.
- V.29. Liu S, Matsui TS, Kang N, Deguchi S. Analysis of senescence-responsive stress fiber proteome reveals reorganization of stress fibers mediated by elongation factor eEF2 in HFF-1 cells. *Mol Biol Cell.* 2022 Jan 1;33(1).
- V.30. Crooks GE. Entropy production fluctuation theorem and the nonequilibrium work relation for free energy differences. 1999;
- V.31. Lovett DB, Shekhar N, Nickerson JA, Roux KJ, Lele TP. Modulation of nuclear shape by substrate rigidity. *Cell Mol Bioeng.* 2013 Jun 5;6(2):230–8.
- V.32. Newman J, oczka N, Schick KL. Dynamic light scattering measurements of the diffusion of probes in filamentous actin solutions. *Biopolymers.* 1989 Feb 1;28(2):655–66.
- V.33. Montague C, Rhee KW, Carlson FD. Measurement of the translational diffusion

- constant of G-actin by photon correlation spectroscopy. *J Muscle Res Cell Motil.* 1983 Feb;4(1):95–101.
- V.34. Kanzaki N, Uyeda TQP, Onuma K. Intermolecular interaction of actin revealed by a dynamic light scattering technique. *Journal of Physical Chemistry B.* 2006 Feb 16;110(6):2881–7.
- V.35. Kodama T, Woledge RC. Enthalpy changes for intermediate steps of the ATP hydrolysis catalyzed by myosin subfragment-1. *Journal of Biological Chemistry.* 1979;254(14):6382–6.
- V.36. Gajewski E, Steckler DK, Goldberg RN. Thermodynamics of the hydrolysis of adenosine 5'-triphosphate to adenosine 5'-diphosphate. *Journal of Biological Chemistry.* 1986;261(27):12733–7.
- V.37. Balnave CD, Davey DF, Allen DG. Distribution of sarcomere length and intracellular calcium in mouse skeletal muscle following stretch-induced injury. *Journal of Physiology.* 1997 Aug 1;502(3):649–59.
- V.38. Manabe Y, Ogino S, Ito M, Furuichi Y, Takagi M, Yamada M, et al. Evaluation of an in vitro muscle contraction model in mouse primary cultured myotubes. *Anal Biochem.* 2016 Mar 15;497:36–8.
- V.39. Burbaum L, Schneider J, Scholze S, Böttcher RT, Baumeister W, Schwille P, et al. Molecular-scale visualization of sarcomere contraction within native cardiomyocytes. *Nature Communications* 2021 12:1. 2021 Jul 2;12(1):1–12.
- V.40. Sanger JW, Sanger JM, Jockusch BM. Differences in the Stress Fibers Fibroblasts and Epithelial Cells between. Vol. 96, *THE JOURNAL OF CELL BIOLOGY.* 1983.
- V.41. Matsui TS, Kaunas R, Kanzaki M, Sato M, Deguchi S. Non-muscle myosin II induces disassembly of actin stress fibres independently of myosin light chain dephosphorylation. *Interface Focus.* 2011;1(5):754–66.
- V.42. Okamoto T, Matsui TS, Ohishi T, Deguchi S. Helical structure of actin stress fibers and its possible contribution to inducing their direction-selective disassembly upon cell shortening. *Biomech Model Mechanobiol.* 2020 Apr 1;19(2):543–55.
- V.43. Matsui TS, Deguchi S. Spatially selective myosin regulatory light chain regulation is absent in dedifferentiated vascular smooth muscle cells but is partially induced by fibronectin and Klf4. *Am J Physiol Cell Physiol.* 2019 Apr 1;316(4):C509–21.
- V.44. Costa KD, Hucker WJ, C-P Yin F. Buckling of Actin Stress Fibers: A New Wrinkle in the Cytoskeletal Tapestry. 2002;
- V.45. Katoh K, Kano Y, Masuda M, Onishi H, Fujiwara K. Isolation and contraction of the stress fiber. *Mol Biol Cell.* 1998 Oct 13;9(7):1919–38.
- V.46. Kentish JC, Ter Keurs HEDJ, Ricciardi L, Bucx JJ, Noble MI. Comparison between

the sarcomere length-force relations of intact and skinned trabeculae from rat right ventricle. Influence of calcium concentrations on these relations. *Circ Res*. 1986;58(6):755–68.

- V.47. Shannon CE, Weaver W. THE MATHEMATICAL THEORY OF COMMUNICATION. 1949;
- V.48. Loison O, Weitkunat M, Kaya-Çopur A, Nascimento Alves C, Matzat T, Spletter ML, et al. Polarization-resolved microscopy reveals a muscle myosin motor-independent mechanism of molecular actin ordering during sarcomere maturation. *PLoS Biol*. 2018 Apr 27;16(4):e2004718.
- V.49. Foley KS, Young PW. An analysis of splicing, actin-binding properties, heterodimerization and molecular interactions of the non-muscle α -actinins. *Biochemical Journal*. 2013 Jun 15;452(3):477–88.
- V.50. Meyer RK, Aebi U. Bundling of actin filaments by alpha-actinin depends on its molecular length. *Journal of Cell Biology*. 1990 Jun 1;110(6):2013–24.
- V.51. Wachsstock DH, Schwartz WH, Pollard TD. Affinity of alpha-actinin for actin determines the structure and mechanical properties of actin filament gels. *Biophys J*. 1993;65(1):205–14.
- V.52. Van Dijk J, Furch M, Lafont C, Manstein DJ, Chaussepied P. Functional characterization of the secondary actin binding site of myosin II. *Biochemistry*. 1999 Nov 16;38(46):15078–85.
- V.53. Maeda E, Kawamura R, Suzuki T, Matsumoto T. Rapid fabrication of tendon-like collagen gel via simultaneous fibre alignment and intermolecular cross-linking under mechanical loading. *Biomedical Materials*. 2022 Jun 7;17(4):045018.
- V.54. Heissler SM, Manstein DJ. Nonmuscle myosin-2: mix and match. *Cellular and Molecular Life Sciences* 2012 70:1. 2012 May 8;70(1):1–21.
- VI.1. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science*. 2005 Nov 18;310(5751):1139–43.
- VI.2. Friedrich BM, Buxboim A, Discher DE, Safran SA. Striated Acto-Myosin Fibers Can Reorganize and Register in Response to Elastic Interactions with the Matrix. *Biophys J*. 2011 Jun 8;100(11):2706–15.
- VI.3. Prager-Khoutorsky M, Lichtenstein A, Krishnan R, Rajendran K, Mayo A, Kam Z, et al. Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. *Nat Cell Biol*. 2011;13.

- VI.4. Gupta M, Sarangi BR, Deschamps J, Nematbakhsh Y, Callan-Jones A, Margadant F, et al. Adaptive rheology and ordering of cell cytoskeleton govern matrix rigidity sensing. *Nature Communications* 2015 6:1. 2015 Jun 25;6(1):1–9.
- VI.5. Doss BL, Pan M, Gupta M, Greci G, Mège RM, Lim CT, et al. Cell response to substrate rigidity is regulated by active and passive cytoskeletal stress. *Proc Natl Acad Sci U S A*. 2020 Jun 9;117(23):12817–25.
- VI.6. Greiner AM, Chen H, Spatz JP, Kemkemer R. Cyclic Tensile Strain Controls Cell Shape and Directs Actin Stress Fiber Formation and Focal Adhesion Alignment in Spreading Cells. *PLoS One*. 2013 Oct 28;8(10):e77328.
- VI.7. McGrath JL. Cell Spreading: The Power to Simplify. *Current Biology*. 2007 May 15;17(10):R357–8.
- VI.8. Ladoux B, Mège RM, Treppe X. Front–Rear Polarization by Mechanical Cues: From Single Cells to Tissues. *Trends Cell Biol*. 2016 Jun 1;26(6):420–33.
- VI.9. Thompson CJ. The statistical mechanics of phase transitions. *Contemp Phys*. 1978;19(3):203–24.
- VI.10. Shin Y, Brangwynne CP. Liquid phase condensation in cell physiology and disease. *Science* 2017 Sep 22;357(6357).
- VI.11. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, et al. Role of YAP/TAZ in mechanotransduction. *Nature* 2011 474:7350. 2011 Jun 8;474(7350):179–83.
- VI.12. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix Elasticity Directs Stem Cell Lineage Specification. *Cell*. 2006 Aug 25;126(4):677–89.
- VI.13. Valle-Orero J, Eckels EC, Stirnemann G, Popa I, Berkovich R, Fernandez JM. The elastic free energy of a tandem modular protein under force. *Biochem Biophys Res Commun*. 2015 May 1;460(2):434–8.
- VI.14. Frederick KK, Marlow MS, Valentine KG, Wand AJ. Conformational entropy in molecular recognition by proteins. *Nature* 2007 448:7151. 2007 Jul 19;448(7151):325–9.
- VI.15. Chen AL, Liang TL. Nonlinear dynamics and statistical physics of DNA You may also like Band structures of elastic waves in two-dimensional eight-fold solid-solid quasi-periodic phononic crystals. 2004;
- VI.16. Pande VS, Grosberg AY, Tanaka T. Statistical mechanics of simple models of protein folding and design. *Biophys J*. 1997 Dec 1;73(6):3192–210.
- VI.17. Ueda Y, Matsunaga D, Deguchi S. A statistical mechanics model for determining the length distribution of actin filaments under cellular tensional homeostasis. *Scientific Reports* 2022 12:1. 2022 Aug 24;12(1):1–10.

- VI.18. Nishizaka T, Seo R, Tadakuma H, Kinoshita K, Ishiwata S. Characterization of single actomyosin rigor bonds: Load dependence of lifetime and mechanical properties. *Biophys J*. 2000 Aug 1;79(2):962–74.
- VI.19. Akalp U, Schnatwinkel C, Stoykovich MP, Bryant SJ, Vernerey FJ. Structural Modeling of Mechanosensitivity in Non-Muscle Cells: Multiscale Approach to Understand Cell Sensing. *ACS Biomater Sci Eng*. 2017 Nov 13;3(11):2934–42.
- VI.20. Thomas W. For catch bonds, it all hinges on the interdomain region. *Journal of Cell Biology*. 2006 Sep 25;174(7):911–3.
- VI.21. Guo B, Guilford WH. Mechanics of actomyosin bonds in different nucleotide states are tuned to muscle contraction. *Proc Natl Acad Sci U S A*. 2006 Jun 27;103(26):9844–9.
- VI.22. Inoue Y, Adachi T. Role of the actin-myosin catch bond on actomyosin aggregate formation. *Cell Mol Bioeng*. 2013 Mar 27;6(1):3–12.
- VI.23. Vreven T, Hwang H, Pierce BG, Weng Z. Prediction of protein-protein binding free energies. *Protein Science*. 2012 Mar;21(3):396–404.
- VI.24. Huang W, Matsui TS, Saito T, Kuragano M, Takahashi M, Kawahara T, et al. Mechanosensitive myosin II but not cofilin primarily contributes to cyclic cell stretch-induced selective disassembly of actin stress fibers. *Am J Physiol Cell Physiol*. 2021 Jun 1;320(6):C1153–63.
- VI.25. Matsui TS, Deguchi S. Spatially selective myosin regulatory light chain regulation is absent in dedifferentiated vascular smooth muscle cells but is partially induced by fibronectin and Klf4. *Am J Physiol Cell Physiol*. 2019 Apr 1;316(4):C509–21.
- VI.26. Goffin JM, Pittet P, Csucs G, Lussi JW, Meister JJ, Hinz B. Focal adhesion size controls tension-dependent recruitment of α -smooth muscle actin to stress fibers. *Journal of Cell Biology*. 2006 Jan 16;172(2):259–68.
- VI.27. Ueda Y, Matsunaga D, Deguchi S. Asymmetric response emerges between creation and disintegration of force-bearing subcellular structures as revealed by percolation analysis. *Integrative Biology*. 2024 Jan 23;16.
- VI.28. Wolfenson H, Bershadsky A, Henis YI, Geiger B. Actomyosin-generated tension controls the molecular kinetics of focal adhesions. *J Cell Sci*. 2011 May 1;124(9):1425–32.
- VI.29. Ren XD, Kiosses WB, Schwartz MA. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO Journal*. 1999 Feb 1;18(3):578–85.
- VI.30. Cao X, Lin Y, Driscoll TP, Franco-Barraza J, Cukierman E, Mauck RL, et al. A Chemo-Mechanical Model for Extracellular Matrix and Nuclear Rigidity Regulated Size of Focal Adhesion Plaques. *Biophys J*. 2016 Feb 16;110(3):622a.

- VI.31. Trichet L, Le Digabel J, Hawkins RJ, Vedula RK, Gupta M, Ribault C, et al. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. *SCIENCES BIOPHYSICS AND COMPUTATIONAL BIOLOGY*. 2012;109(18).
- VI.32. Ibata N, Terentjev EM. Development of Nascent Focal Adhesions in Spreading Cells. *Biophys J*. 2020 Nov 17;119(10):2063–73.
- VI.33. Senju Y, Miyata H. The role of actomyosin contractility in the formation and dynamics of actin bundles during fibroblast spreading. *J Biochem*. 2009 Feb;145(2):137–50.
- VI.34. Prager-Khoutorsky M, Lichtenstein A, Krishnan R, Rajendran K, Mayo A, Kam Z, et al. Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. *Nat Cell Biol*. 2011 Dec;13(12):1457–65.
- VI.35. Friedrich BM, Safran SA. Nematic order by elastic interactions and cellular rigidity sensing. *Europhys Lett*. 2011 Feb 7;93(2):28007.
- VI.36. Garcia-Ojalvo J, Martinez Arias A. Towards a statistical mechanics of cell fate decisions. *Curr Opin Genet Dev*. 2012;22(6):619–626.
- VI.37. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nature Reviews Molecular Cell Biology* 2009 10:1. 2009 Jan;10(1):53–62.
- VI.38. Humphrey JD, Schwartz MA. Vascular Mechanobiology: Homeostasis, Adaptation, and Disease. *Annu Rev Biomed Eng*. 2021;23(1):1–27.
- VI.39. Chien S. Mechanotransduction and endothelial cell homeostasis: The wisdom of the cell. *Am J Physiol Heart Circ Physiol*. 2007 Mar;292(3):1209–24.
- VI.40. Kaunas R, Deguchi S. Multiple Roles for Myosin II in Tensional Homeostasis Under Mechanical Loading.
- VI.41. Deguchi S, Matsui TS, Iio K. The position and size of individual focal adhesions are determined by intracellular stress-dependent positive regulation. *Cytoskeleton*. 2011 Nov;68(11):639–51.
- VI.42. Deshpande VS, McMeeking RM, Evans AG. A bio-chemo-mechanical model for cell contractility. *Proc Natl Acad Sci U S A*. 2006 Sep 19;103(38):14015–20.
- VI.43. Olberding JE, Thouless MD, Arruda EM, Garikipati K. The Non-Equilibrium Thermodynamics and Kinetics of Focal Adhesion Dynamics. *PLoS One*. 2010 Aug 18;5(8):e12043.
- VI.44. Mammoto A, Ingber DE. Cytoskeletal control of growth and cell fate switching. *Curr Opin Cell Biol*. 2009 Dec 1;21(6):864–70.

- VI.45. Bun P, Liu J, Turlier H, Liu Z, Uriot K, Joanny JF, et al. Mechanical checkpoint for persistent cell polarization in adhesion-naïve fibroblasts. *Biophys J*. 2014 Jul 15;107(2):324–35.
- VI.46. Nicolas A, Geiger B, Safran SA. Cell mechanosensitivity controls the anisotropy of focal adhesions. *Proc Natl Acad Sci U S A*. 2004 Aug 24;101(34):12520–5.
- VI.47. Shemesh T, Geiger B, Bershadsky AD, Kozlov MM. Focal adhesions as mechanosensors: A physical mechanism. *Proceedings of the National Academy of Sciences*. 2005 Aug 30;102(35):12383–8.
- VI.48. Venkatachalapathy S, Sreekumar D, Ratna P, Shivashankar G V. Actomyosin contractility as a mechanical checkpoint for cell state transitions. *Scientific Reports* 2022 12:1. 2022 Sep 26;12(1):1–13.
- VI.49. Vining KH, Marneth AE, Adu-Berchie K, Grolman JM, Tringides CM, Liu Y, et al. Mechanical checkpoint regulates monocyte differentiation in fibrotic niches. *Nature Materials* 2022 21:8. 2022 Jul 11;21(8):939–50.
- VI.50. Aragona M, Panciera T, Manfrin A, Giullitti S, Michielin F, Elvassore N, et al. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell*. 2013 Aug 29;154(5):1047–59.
- VI.51. Doostmohammadi A, Ladoux B. Physics of liquid crystals in cell biology. *Trends Cell Biol*. 2022 Feb 1;32(2):140–50.
- VI.52. Liu S, Matsui TS, Kang N, Deguchi S. Analysis of senescence-responsive stress fiber proteome reveals reorganization of stress fibers mediated by elongation factor eEF2 in HFF-1 cells. *Mol Biol Cell*. 2022 Jan 1; 33(1):ar10.
- VI.53. Riback JA, Zhu L, Ferrolino MC, Tolbert M, Mitrea DM, Sanders DW, et al. Composition-dependent thermodynamics of intracellular phase separation. *Nature* 2020 581:7807. 2020 May 6;581(7807):209–14.
- VI.54. Vernerey FJ, Akalp U. Role of catch bonds in actomyosin mechanics and cell mechanosensitivity. *Phys Rev E*. 2016 Jul 11;94(1):012403.
- VI.55. Neupane K, Manuel AP, Woodside MT. Protein folding trajectories can be described quantitatively by one-dimensional diffusion over measured energy landscapes. *Nature Physics* 2016 12:7. 2016 Mar 7;12(7):700–3.
- VI.56. Nicolas A, Geiger B, Safran SA. Cell mechanosensitivity controls the anisotropy of focal adhesions. *Proc Natl Acad Sci U S A*. 2004 Aug 24;101(34):12520–5.
- VI.57. Shemesh T, Geiger B, Bershadsky AD, Kozlov MM. Focal adhesions as mechanosensors: A physical mechanism. *Proc Natl Acad Sci U S A*. 2005 Aug 30;102(35):12383–8.

- VI.58. Sartori P, Leibler S. Lessons from equilibrium statistical physics regarding the assembly of protein complexes. *Proc Natl Acad Sci U S A*. 2020 Jan 7;117(1):114–20.
- VII.1. Zajac AL, Discher DE. Cell differentiation through tissue elasticity-coupled, myosin-driven remodeling. *Curr Opin Cell Biol*. 2008 Dec;20(6):609–15.
- VII.2. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science*. 2005 Nov 18;310(5751):1139–43.
- VII.3. Hove JR, Köster RW, Forouhar AS, Acevedo-Bolton G, Fraser SE, Gharib M. Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. *Nature*. 2003 Jan 9;421(6919):172–7.
- VII.4. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nature Reviews Molecular Cell Biology* 2009 10:1. 2009 Jan;10(1):53–62.
- VII.5. Laevsky G, Knecht DA. Cross-linking of actin filaments by myosin II is a major contributor to cortical integrity and cell motility in restrictive environments. *J Cell Sci*. 2003 Sep 15;116(18):3761–70.
- VII.6. Kaunas R, Deguchi S. Multiple Roles for Myosin II in Tensional Homeostasis Under Mechanical Loading.
- VII.7. Umeda K, Nishizawa K, Nagao W, Inokuchi S, Sugino Y, Ebata H, et al. Activity-dependent glassy cell mechanics II: Nonthermal fluctuations under metabolic activity. *Biophys J*. 2023 Nov 21;122(22):4395–413.
- VII.8. Yusko EC, Asbury CL. Force is a signal that cells cannot ignore. *Mol Biol Cell* [Internet]. 2014 Nov 15 [cited 2024 May 27];25(23):3717–25.
- VII.9. Guolla L, Bertrand M, Haase K, Pelling AE. Force transduction and strain dynamics in actin stress fibres in response to nanonewton forces. *J Cell Sci*. 2012 Feb 1;125(3):603–13.
- VII.10. Yanagida T, Esaki S, Iwane AH, Inoue Y, Ishijima A, Kitamura K, et al. Single-motor mechanics and models of the myosin motor. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2000 Apr 29;355(1396):441–7.
- VII.11. Finer JT, Simmons RM, Spudis JA. Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* [Internet]. 1994 [cited 2024 May 27];368(6467):113–9.

- VII.12. Mizuno D, Tardin C, Schmidt CF, MacKintosh FC. Nonequilibrium mechanics of active cytoskeletal networks. *Science* (1979) [Internet]. 2007 Jan 19 [cited 2024 May 27];315(5810):370–3.
- VII.13. Nishizawa K, Honda N, Inokuchi S, Ebata H, Ariga T, Mizuno D. Measuring fluctuating dynamics of sparsely crosslinked actin gels with dual-feedback nonlinear microrheology. *Phys Rev E*. 2023;108:34601.
- VII.14. Kajita MK, Konishi Y, Hatakeyama TS. Active thermodynamic force driven mitochondrial alignment. *Phys Rev Res*. 2024;6.
- VII.15. Manrique PD, Huo FY, Oud S El, Johnson NF. Non-equilibrium physics of multi-species assembly: From inhibition of fibrils in biomolecular condensates to growth of online distrust. 2023 Dec 13.
- VII.16. Einstein A. Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. *Ann Phys*. 1905;322(8):549–60.
- VII.17. Mizuno D, Tardin C, Schmidt CF. Rapid local compression in active gels is caused by nonlinear network response. *Soft Matter* [Internet]. 2020 Oct 21 [cited 2024 May 27];16(40):9369–82.
- VII.18. Mizuno D, Head DA, Mackintosh FC, Schmidt CF. Active and Passive Microrheology in Equilibrium and Nonequilibrium Systems.
- VII.19. Nishizawa K, Bremerich M, Ayade H, Schmidt CF, Ariga T, Mizuno D. Feedback-tracking microrheology in living cells. *Sci Adv* [Internet]. 2017
- VII.20. Ahmed WW, Fodor É, Almonacid M, Bussonnier M, Verlhac MH, Gov N, et al. Active Mechanics Reveal Molecular-Scale Force Kinetics in Living Oocytes. *Biophys J*. 2018 Apr 10;114(7):1667–79.
- VII.21. Bertrand OJN, Fygenson DK, Saleh OA. Active, motor-driven mechanics in a DNA gel. *Proc Natl Acad Sci U S A* [Internet]. 2012 Oct 23 [cited 2024 May 27];109(43):17342–7.
- VII.22. Fodor, Guo M, Gov NS, Visco P, Weitz DA, Van Wijland F. Activity-driven fluctuations in living cells. *Europhys Lett* [Internet]. 2015 Jun 11 [cited 2024 May 27];110(4):48005.
- VII.23. Gallet F, Arcizet D, Bohec P, Richert A. Power spectrum of out-of-equilibrium forces in living cells: amplitude and frequency dependence.
- VII.24. Lau AWC, Hoffman BD, Davies A, Crocker JC, Lubensky TC. Microrheology, Stress Fluctuations, and Active Behavior of Living Cells. 2003;

- VII.25. Buenaventura A, Saito T, Kanao T, Matsunaga D, Matsui TS, Deguchi S. Intracellular Macromolecular Crowding within Individual Stress Fibers Analyzed by Fluorescence Correlation Spectroscopy. *Cell Mol Bioeng* [Internet]. 2024 Jun 7 [cited 2024 Jun 21];1–12.
- VII.26. Soares E Silva M, Depken M, Stuhmann B, Korsten M, MacKintosh FC, Koenderink GH. Active multistage coarsening of actin networks driven by myosin motors. *Proc Natl Acad Sci U S A* [Internet]. 2011 Jun 7 [cited 2024 May 18];108(23):9408–13.
- VII.27. Higuchi H, Muto E, Inoue Y, Yanagida T. Kinetics of force generation by single kinesin molecules activated by laser photolysis of caged ATP. *Proc Natl Acad Sci U S A* [Internet]. 1997 Apr 29 [cited 2024 Jun 21];94(9):4395–400.
- VII.28. Tyska MJ, Dupuis DE, Guilford WH, Patlak JB, Waller GS, Trybus KM, et al. Two heads of myosin are better than one for generating force and motion. *Proc Natl Acad Sci U S A* [Internet]. 1999 Apr 13 [cited 2024 Jun 21];96(8):4402–7.
- VII.29. Molloy JE, Burns JE, Kendrick-Jones B, Tregear RT, White DCS. Movement and force produced by a single myosin head. *Nature* 1995 378:6553 [Internet]. 1995 Nov 9 [cited 2024 Jun 21];378(6553):209–12.
- VII.30. Howard J. Mechanical signaling in networks of motor and cytoskeletal proteins. *Annu Rev Biophys*. 2009 Jun 9;38(1):217–34.
- VII.31. Chantachotikul P, Liu S, Furukawa K, Deguchi S. AP2A1 modulates cell states between senescence and rejuvenation. *Cell Signal*. 2025 Jan 21;111616.
- VII.32. Bacabac RG, Mizuno D, Schmidt CF, MacKintosh FC, Van Loon JJWA, Klein-Nulend J, et al. Round versus flat: Bone cell morphology, elasticity, and mechanosensing. *J Biomech*. 2008 Jan 1;41(7):1590–8.
- VII.33. Wang S, Shen T, Wolynes PG. The interplay of nonlinearity and architecture in equilibrium cytoskeletal mechanics. *Journal of Chemical Physics* [Internet]. 2011 Jan 7 [cited 2024 May 1];134(1).
- VII.34. Deguchi S, Ohashi T, Sato M. Intracellular stress transmission through actin stress fiber network in adherent vascular cells. Vol. 2, *MCB*. 2005.
- VII.35. Vignaud T, Copos C, Leterrier C, Toro-Nahuelpan M, Tseng Q, Mahamid J, et al. Stress fibres are embedded in a contractile cortical network. *Nat Mater*. 2021 Mar 1;20(3):410–20.
- VII.36. Svitkina TM. Actin Cell Cortex: Structure and Molecular Organization. Vol. 30, *Trends in Cell Biology*. Elsevier Ltd; 2020. p. 556–65.

- VII.37. Ueda Y, Matsunaga D, Deguchi S. Asymmetric response emerges between creation and disintegration of force-bearing subcellular structures as revealed by percolation analysis. *Integrative Biology*. 2024 Jan 23;16.
- VII.38. Stamenovic D, Smith ML, Zurich Michael E. Tensional homeostasis at different length scales *Soft Matter*. This journal is Cite this: *Soft Matter*. 2020;16:25.
- VII.39. Webster KD, Ng WP, Fletcher DA. Article Tensional Homeostasis in Single Fibroblasts. 2014 [cited 2024 May 27];
- VII.40. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, et al. Tensional homeostasis and the malignant phenotype. 2005;
- VII.41. Brown RA, Prajapati R, Mcgrouther DA, Yannas I V, Eastwood AM. Tensional Homeostasis in Dermal Fibroblasts: Mechanical Responses to Mechanical Loading in Three-Dimensional Substrates. *J Cell Physiol* [Internet]. 1998 [cited 2024 May 27];175:323–32.
- VII.42. Liu S, Matsui TS, Kang N, Deguchi S. Analysis of senescence-responsive stress fiber proteome reveals reorganization of stress fibers mediated by elongation factor eEF2 in HFF-1 cells. *Mol Biol Cell*. 2022 Jan 1;33(1).

Publication list

Peer-reviewed paper

- [1] **Yuika Ueda** and Shinji Deguchi. "Adaptive flexibility of cells through nonequilibrium entropy production." *BioSystems* (2025): 105594.
- [2] **Yuika Ueda**, Daiki Matsunaga, and Shinji Deguchi. "Asymmetric response emerges between creation and disintegration of force-bearing subcellular structures as revealed by percolation analysis." *Integrative Biology* 16, zya012, (2024).
- [3] **Yuika Ueda** and Shinji Deguchi. "Emergence of multiple set-points of cellular homeostatic tension." *Journal of Biomechanics* 151 (2023): 111543.
- [4] **Yuika Ueda**, Daiki Matsunaga, and Shinji Deguchi. "A statistical mechanics model for determining the length distribution of actin filaments under cellular tensional homeostasis." *Scientific Reports* 12 (2022): 14466.

Preprints

- [5] **Yuika Ueda** and Shinji Deguchi. "Intracellular order formation through stepwise phase transitions." arXiv preprint:2408.14242 (2024). (Under review process)
- [6] **Yuika Ueda** and Shinji Deguchi. "Nonthermal driving forces in cells revealed by nonequilibrium fluctuations." arXiv preprint:2408.06683 (2024).

Award

- [1] 16th JSPS Ikushi Prize, 2026
- [2] 2026 Biophysical Society Annual Meeting, Travel Award, 2025
- [3] 37th JSME Bioengineering conference, Poster award 2025
- [4] JSPS 16th HOPE Meeting with Nobel Laureates Fellow, 2025
- [5] 2024 JSME Women of future award, 2024
- [6] The 52nd Naito Conference, Poster Award for Excellence, 2024

Selected research grand and fellowship

- [1] JST ACT-X, "Life and Information"
- [2] The Great Britain SASAKAWA FOUNDATION
- [3] The University of Osaka, Global leader fellowship
- [4] JSPS Research Fellowship (DC1)
- [5] Nakatani Foundation for Advancement of Measuring Technologies in Biomedical Engineering, Fellowship
- [6] Japanese Society for Medical and Biological Engineering, Saito Fellowship
- [7] MEXT KAKENHI Grant-in-Aid for Transformative Research Area (A), Support for International Activities of Young Multimodal ECM Researchers
- [8] The ANRI Research Fellowship

Acknowledgement

I would like to express my sincere gratitude to everyone who inspired and supported me throughout my PhD study.

First, I would like to thank my supervisor, Professor Shinji Deguchi. His support since my undergraduate years has been exceptional and invaluable. He believed in my potential in science more than I did myself, which encouraged me to move forward with all my decisions. I hope to repay his kindness by pioneering good science. I also thank Associate Professor Daiki Matsunaga. Through our frank and valuable discussions, he always guided my research in a positive direction. I am also grateful to Assistant Professor Takumi Saito. He has been a role model I have looked up to since my undergraduate days.

Next, I would like to thank Professor Guillaume Charras at University College London. Accepting me as a visiting researcher, he showed me the excitement of biology and the inspiring reality of working at the forefront of science, which opened up my career. I am also grateful to Dr. Andreas Weber for his kind and detailed guidance on everything from experiments to presentations. I thank all members of the Charras Lab for their warm encouragement. My thanks also go to Dr. Reza Haqshenas for his generous support during my first research experience abroad and for helping me with my life in London.

I would like to thank my committee members, Professor Shigeo Wada and Professor Susumu Goto, for their insightful discussions and suggestions regarding my research.

I also thank all members of the Deguchi Lab. Our daily interactions were essential for this work. I would like to express my sincere gratitude to Dr. Pirawan Chantachotikul for kindly and thoroughly teaching me the basics of experiments, and for always encouraging me from the desk next to mine. I thank Intan Chairun Nisa for her contribution to the experiments in Chapter VI. I am also especially grateful to Ms. Junko Yamaguchi, not only for her administrative support but also for her kind and encouraging words.

I would like to express my gratitude to all the colleagues who have supported my career. I am particularly grateful to my colleagues from Mobi 1, K.R., S.S., F.Y., S.N., G.K., and T.M., and to S.K., who respected my decision to pursue a PhD and whose efforts around the world continue to inspire me. I also thank I.R. for encouraging me to make this decision. I extend my appreciation to N.Y. and K.K. for their great support, especially during my time in London. Finally, I would like to thank my family for their immense support.

This work was supported by JST ACT-X, The Great Britain Sasakawa Foundation, JSPS Research Fellowship (DC1), Nakatani Foundation, the Japanese Society for Medical Biological Engineering, and The ANRI Research Fellowship.