1. Active mechanics of the cytoskeleton

Cells can resist but also actively exert mechanical forces. This ability allows them to perform many essential tasks. Some animal cells can crawl across surfaces and through small pores, pulling themselves forward while pushing against their environment. Some cells such as sperm cells swim by beating long appendages which push the surrounding fluid. Most cells proliferate by dividing into two daughter cells, requiring drastic changes in cell shape. Many cells maintain their internal components organized by a combination of internal pushing and pulling forces. Combinations of cell growth, divisions, and shape changes allow embryos to develop into organisms with a well-defined anatomy. Given the importance of mechanical forces in life, understanding the mechanisms that determine how cells exert and withstand forces is crucial.

In order to accomplish mechanical tasks, cells rely on *polymers*. The kind of polymer used depends on cell type. Many plant, yeast, and bacterial cells maintain relatively constant, rod-like shapes. These cells possess an outer cell wall composed of rigid polymers, which provide robust mechanical stability. In contrast, animal cells are usually soft and deformable. This allows them to move and change shape. Rather than possessing a static, rigid cell wall, animal cells rely on the cytoskeleton to provide resistance to external forces. However, at the same time the cytoskeleton is dynamic and adaptable and actively generates forces. Understanding the physical properties of biological polymers like cytoskeletal filaments is thus crucial in order to resolve the role of forces in cell mechanics.

One popular approach to study how biological polymers regulate cell shape and mechanics is to reconstitute purified biological polymers in a simplified, cell-free environment (Bausch and Kroy 2006; Fletcher and Geissler 2009). The advantage of such biomimetic systems is that their molecular and structural complexity can be precisely controlled. The reduced complexity

compared to living cells makes it easier to perform quantitative experiments that can be linked to quantitative physical theories that predict the macroscopic physical properties in terms of the molecular properties and interactions of the components.

In this chapter, we will provide a background on theory used in describing the mechanical properties of cytoskeletal polymers and summarize results of experiments with a focus on biomimetic approaches. First we introduce some examples of cytoskeletal structures found in cells. We then discuss the mechanical properties of single polymers, networks of polymers, and crosslinked networks. Finally, we will discuss how molecular motors allow these networks to actively generate force, which results in fascinating, self-deforming materials known as active gels (Joanny and Prost 2009).

1.1 The cytoskeleton

The *cytoskeleton* is a network of biological polymers which provides cells with mechanical strength and the ability to generate active forces. Cytoskeletal polymers associate with a variety of accessory proteins to form different supramolecular structures that are tailored for distinct tasks. Despite the large number of possible cytoskeletal structures, the cytoskeleton primarily comprises only three types of polymers. In this section, we will introduce these cytoskeletal polymers and highlight some of the structures they form. In later sections, we will investigate the properties of these polymers and some of their accessory proteins in more detail.

Actin filaments are somewhat flexible polymers that can form either fine random meshworks, branched networks, or stiff bundles. In many cases, actin filaments form contractile networks or bundles, in cooperation with myosin motor proteins. The most well-known example of such a contractile array is found in muscle, where actin and myosin form extremely well-organized arrays known as sarcomeres (Rayment et al. 1993). Non-muscle cells also possess contractile

actin-myosin structures, but these tend to be less well-ordered. Right underneath the membrane, actin and myosin form a thin *cortex* composed of a random meshwork of crosslinked actin filaments. Myosin motors actively generate contractile stress within this cortex, which can change cell shape (Salbreux, Charras, and Paluch 2012). These shape changes can take place at the individual cell level as well as on the tissue scale. During cytokinesis in yeast and animal cells, cortical actin filaments and molecular motors transiently organize into a *contractile ring*, which constricts to pinch off the mother cell into two daughter cells (Guertin, Trautmann, and McCollum 2002; West-Foyle and Robinson 2012; McMichael and Bednarek 2013). The actin cortex also assists yeast and animal cells during *endocytosis*, the process whereby cells internalize foreign objects or fluids (Engqvist-Goldstein and Drubin 2003). One example is *phagocytosis*, where immune cells engulf and destroy invasive pathogens like bacteria (May and Machesky 2001). During *embryogenesis*, epithelial cell monolayers collectively generate forces, maintaining tissue integrity (Cavey and Lecuit 2009), homeostasis (Guillot and Lecuit 2013), and shape (Rauzi and Lenne 2011). Apart from a thin cortex, some large cells such as oocytes additionally have a three-dimensional, cytoplasmic network of actin filaments (Field and Lénárt 2011) which can be used for transporting chromosomes (Lénárt et al. 2005). Crawling cells like fish keratocytes, amoebas, and metastatic cancer cells can move across surfaces using a combination of different actin-based structures (Abercrombie 1980; Rafelski and Theriot 2004; Ananthakrishnan and Ehrlicher 2007). At the front of crawling cells, a thin, two-dimensional branched array of actin filaments called the *lamellipodium* pushes the cell membrane forward. Membrane protrusions reinforced by tightly bundled actin filaments called *filopodia* often extend from the lamellipodium, which are thought to sense environmental cues and guide the direction

of cell motion (Davenport et al. 1993; Mattila and Lappalainen 2008). Similar actin-filled membrane protrusions are also found in specialized cell types, such as inner hair cells in the inner ear, which project *stereocilia* that participate in the transduction of sound waves to neuronal impulses (Manor and Kachar 2008). At the back of crawling cells, a network of actin filaments and molecular motors exerts retraction forces, which allows cell detachment from the substrate and forward motion of the cell body. In strongly adherent cells, actin and myosin form contractile *stress fibers* that span the cell and connect to focal adhesions(Naumanen, Lappalainen, and Hotulainen 2008). There is mounting evidence that cells can switch between different migration mechanisms. In dense tissues, some cells use mechanisms based on myosin-induced membrane *blebbing* for migration instead of polymerization-based motility(Paluch and Raz 2013). Interestingly, the actin cytoskeleton in plant cells has a completely different organization and function than in animal cells. The most prominent actin structure is provided by cytoplasmic actin cables composed of actin filaments bundled by accessory proteins. These *actin cables* usually radiate from the nucleus towards the cell membrane (Hussey, Ketelaar, and Deeks 2013) and assist in properly positioning the nucleus (Starr and Han 2003). Moreover, actin cables are used as tracks for transport by molecular motors, for instance for pollen tube growth (Kroeger and Geitmann 2012). Intriguingly, these roles are reminiscent of positioning and transport roles of microtubules in animal cells (see below).

Microtubules are much stiffer polymers than actin filaments. In animal cells, they play a crucial role in organizing the cell interior. Microtubules act as tracks for kinesin and dynein motors, which move along microtubules to transport intracellular cargo. In interphase cells, microtubules usually emanate from a *microtubule-organizing center* positioned near the nucleus, and grow radially outward toward the cell membrane, enabling long-range transport through the crowded

cytoplasm (Vale 2003; Barlan, Rossow, and Gelfand 2013). A striking example is provided by cells called *melanophores*, which allow many amphibians and fish to change color (Tuma and Gelfand 1999). This is accomplished by motor-driven transport of vesicles containing the pigment melanin across microtubules. When cells divide, the microtubules reorganize to form the *mitotic spindle*, a specialized assembly of microtubules, molecular motors, and many other accessory proteins that reliably separates chromosomes to the two daughter cells (Walczak and Heald 2008). Similarly to animal cells, fission yeast cells also use microtubules and molecular motors to separate chromosomes and transport cargo (Hagan 1998). However, unlike in animal cells, interphase microtubules are organized into a small set of bundles that extend to the two poles of the rod-shaped yeast cell. This allows molecular motors to deliver growth factors specifically to these two ends, maintaining yeast cells' rod-like shape (F. Chang and Martin 2009). Some cells, such as algae and certain bacteria, swim by beating *flagella* or *cilia*. These are long appendages which comprise an ordered arrangement of microtubules that slide past one another, causing the entire appendage to lash back and forth (Brokaw 1994; Kantsler et al. 2013). In plant cells, microtubules are again very differently organized than in animal cells. They usually form an ordered *cortex* underneath the cell membrane, that helps guide the ordered production of the cell wall, which is essential in maintaining plant cells' elongated shape (Gutierrez et al. 2009; Bringmann et al. 2012). Intriguingly, the cortical localization of microtubules mirrors the cortical localization of actin filaments in animal cells. When plant cells divide, the cortical microtubule array transforms into a *preprophase band* with the nucleus anchored at the center, thus determining the division plane (Van Damme 2009).

Intermediate filaments are a third set of cytoskeletal filaments that are present in animal cells,

but not in yeast or plant cells. They are so-named because their diameter (~10 nm) is intermediate between the diameters of actin filaments (~6 nm) and microtubules (~25 nm). Surprisingly, they are ten-fold more flexible than actin filaments (see below), indicating that their internal structure affects their mechanical properties. Intermediate filaments are encoded by 70 genes in the human genome, which are often categorized into six sequence homology classes (class I-VI), or, alternatively, into three assembly groups (I, II, III) that can co-exist as three separate IF systems within the same cell (Szeverenyi et al. 2008). One type of intermediate filaments, the *lamins*, forms a basket that provides the cell nucleus with mechanical strength, and also regulates nuclear events such as chromosome replication and cell death (Gruenbaum et al. 2000). The other intermediate filaments are found in the cytoplasm, and are expressed in a tissue-dependent and developmentally regulated manner (Helfand, Chang, and Goldman 2003; Herrmann et al. 2007). For example, epithelial cells such as skin cells resist deformation by a network of *keratin* filaments (Omary et al. 2009), neuronal axons are reinforced with *neurofilaments* (Lepinoux-Chambaud and Eyer 2013), and astrocytes have a cytoplasmic network of *glial fibrillary acidic protein* (Middeldorp and Hol 2011). Fiber cells of the vertebrate eye lens contain *beaded filaments*, which not only provide the lens with mechanical strength, but also maintain its transparency (Song et al. 2009). Intermediate filaments are generally thought to provide mechanical protection against large deformations (Fudge et al. 2009), and also to serve as a platform for signal transduction.

Septin filaments have only recently begun to gain recognition as a fourth component of the cytoskeleton (Mostowy and Cossart 2012). Septins were originally discovered in budding yeast, where they form rings at the bud neck that form a diffusion barrier between the membranes of the mother and daughter cell (Hartwell 1971; Byers 1976). Septins were later found to be

conserved across the animal kingdom. All eukaryotes have multiple septins, ranging in number from 2 in nematodes to 13 in humans. Functional studies of cells involving septin deletion or mutation suggest that septin assemblies play three key roles, which are likely interrelated: they maintain cortical integrity, act as diffusion barriers for membrane proteins, and serve as scaffolds for cytoplasmic proteins. During cytokinesis, septins are core components of the contractile ring, where they may act both as a diffusion barrier and as a scaffold (Glotzer 2005). In motile cells such as T-cells, septins form cortical arrays which contribute to cell rigidity and to regulation of cell motility (Gilden and Krummel 2010). However, given the complex composition of the cortex much of the evidence that septins fulfills these functions is indirect. It is for instance unknown whether septin exerts its functions independent of or in concert with actin. Moreover, there is a complete lack of quantitative evidence of the biophysical properties of septins.

The amino acid sequences of actin and tubulin proteins are surprisingly well conserved across many eukaryotic species (Sheterline and Sparrow 1994; Mitchison 1995). Intermediate filaments and septin filaments also maintain a large degree of evolutionary conservation, although species-specific variation is greater than with actin and tubulin. Throughout this chapter, we will focus on these eukaryotic cytoskeletal filaments. However, we note that various actin, tubulin, and intermediate-filament homologues have now been identified in prokaryotes (Shih and Rothfield 2006), whose structural and mechanical roles are starting to become more clear.

1.2 Single cytoskeletal filaments

Cells employ biological polymers that are built up from different building blocks, including sugars, nucleic acids, and amino acids. The cell wall of plant cells is made up of *cellulose fibers*, built from linked glucose chains (Somerville 2006). Bacterial colonies secrete extracellular polysaccharide chains, which maintain cohesion and contribute to the formation of *biofilms* such

as dental plaque (Costerton, Stewart, and Greenberg 1999). Cells store their genetic information in the form of *deoxyribonucleic acid* (DNA), which are chains built from four different types of interchangeable nucleic acids (Alberts 2008). Cells express DNA to produce *proteins*, which are macromolecules composed of one or more polypeptide chains, which themselves are long chains of up to twenty interchangeable amino acids (Alberts 2008). Due to the covalent bonds that link subunits together, macromolecular polymers typically form stable, long-lasting structures which maintain their structure and shape. In the case of cell walls or biofilms, these sturdy polymers allow cells or colonies to resist mechanical deformation.

Cytoskeletal polymers are supramolecular polymers built up from many individual protein subunits. These subunits are typically linked via weak interactions such as electrostatic interactions, hydrophobic interactions, and hydrogen-bonding. The specificity of these interactions results in highly ordered structures, while their non-covalent nature allows cytoskeletal protein polymers to assemble and disassemble dynamically in response to biochemical or mechanical signals. This adaptability is crucial for enabling cells to form dynamic and adaptable structures for cell movement and shape change. This dynamic character contrasts with the covalent bonds that usually link the subunits of biological polymers such as cellulose. Another interesting consequence of the dynamic nature of cytoskeletal filaments is that it allows them to actively exert forces (in conjunction with the consumption of chemical energy).

Cytoskeletal polymer structure. Actin filaments comprise globular actin protein monomers, which themselves comprise two domains separated by a cleft that binds a divalent cation together with either adenosine triphosphate (ATP) or adenosine diphosphate (ADP) (Carlier et al. 1994). Monomers assemble head-to-tail to form linear filaments. The ligand-binding cleft is directed toward the so-called “minus end” or “pointed end” of the filament. The opposite side is directed

toward the “plus end” or “barbed end”. Apart from assembling head-to-tail, actin monomers also associate via side-by-side contacts, forming a double-stranded helical structure with a 37-nm pitch (Selby and Bear 1956; Galkin, Orlova, and Egelman 2012a). Actin monomers incorporated in filaments exhibit multiple conformational states (Galkin, Orlova, Schröder, et al. 2010), potentially allowing them to act as tension sensors (Galkin, Orlova, and Egelman 2012a). Microtubules comprise α- and β-tubulin proteins, which form stable heterodimers. β-tubulin proteins bind guanine triphosphate (GTP) or guanine diphosphate (GDP) (Gardner, Zanic, and Howard 2013). Although α-tubulin proteins bind guanine triphosphate (GTP), this binding site is buried at the dimer interface. Dimers of α-β-tubulin assemble head-to-tail to form linear protofilaments, with α-tubulin at the “plus end” and β-tubulin at the “minus end” (Nogales, Wolf, and Downing 1998). Typically thirteen protofilaments associate side-by-side to form a hollow, cylindrical microtubule. This stable, tubular structure makes microtubules stiffer than actin filaments by a factor of approximately 300 (Gittes et al. 1993).

Intermediate filaments have a rather different structure from actin filaments and microtubules, because their subunits are fibrous rather than globular. IF proteins are rod-shaped double-stranded parallel dimers with a length of 40-50 nm and a diameter of 2 nm. The dimers possess a central, largely α-helical rod domain consisting of four coiled-coil segments, flanked by non-α-helical N- and C-terminal end domains. Cytoplasmic IF proteins assemble into filaments via a multi-step pathway, where dimers first associate to form anti-parallel, approximately half-staggered tetramers, which laterally aggregate into unit-length filaments, which in turn

longitudinally anneal to form filaments (Herrmann et al. 2007). The fibrils subsequently mature by a compaction process. Nuclear lamins follow a different assembly pathway, involving head-to-tail polymerization.

(FIGURE 1)

The notion of head-to-tail assembly indicates a special property of actin filaments and microtubules. *Structural polarity* refers to the fact that the two ends of the filament can be distinguished from each other. This is in strong contrast to intermediate filaments and septin filaments, which exhibit *structural symmetry* (Herrmann et al. 2007): both ends of the filament are identical and indistinguishable. The structural polarity of actin and microtubules has two important functional consequences: they can polymerize in a directional manner, and they are recognized by molecular motors that take advantage of their polarity to move in a processive manner. Given the structural symmetry of IF filaments and septins, it is believed that there are no motors interacting with them.

Polymerization and enzymatic activity. The process by which monomer subunits join a polymer is called *polymerization*. For supramolecular polymers such as actin, this process is characterized by the rate of monomer addition, k_{on}, as well as the rate of monomer dissociation, k_{off}. In equilibrium, these rates are identical. For septins and intermediate filaments, this equilibrium description is sufficient. Actin and microtubules, however, are non-equilibrium polymers because their assembly is coupled with enzymatic activity. Shortly after ATP-bound actin or GTP-bound tubulin joins a growing actin filament or microtubule, hydrolysis of the bound nucleotide occurs. This hydrolysis provides chemical energy that maintains different on/off rates at the two filament ends. The end with the higher on-rate is conventionally called the "plus end", while "minus end" refers to the end with the slower on-rate.

Continuous addition of fresh ATP- or GTP-bound monomers results in a so-called *ATP cap* or *GTP cap* at the plus end, while the rest of the filament contains ADP- or GDP-bound monomers. The presence of such a cap allows for interesting non-equilibrium processes such as actin *treadmilling* (Pollard and Borisy 2003) and microtubule *dynamic instability* (Gardner et al. 2011). These dynamic processes allow cytoskeletal filaments to exert polymerization forces, which we will discuss later in this chapter. With the help of specialized tip-binding molecules, actin filaments and microtubules can also generate depolymerization forces. Microtubule plus ends are targeted by so-called end-binding (or tip-tracking) proteins (Maurer et al. 2012; Bowne-Anderson et al. 2013; Seetapun et al. 2012), while actin filament plus ends are targeted by formins (Jegou, Carlier, and Romet-Lemonne 2013).

Although intermediate filaments lack structural polarity and enzymatic activity, evidence suggests that intermediate filaments exhibit fast polymerization and depolymerization kinetics, which is regulated by (de)phosphorylation (Helfand, Chang, and Goldman 2003). Septin filaments also lack structural polarity but do exhibit enzymatic activity (Weirich, Erzberger, and Barral 2008). Septin subunits bind and slowly hydrolyze GTP, and septin subunits can form filaments via the GTP-binding domain (or also at an interface containing N- and C-termini). Fluorescence recovery after photobleaching experiments in cells have revealed that septins are rather dynamic. This may be related to their enzymatic activity, but post-translational modifications may also play a role.

Worm-like chain model. So far we have seen how molecular structure can determine many of the special properties of cytoskeletal filaments. Yet physical models of cytoskeletal mechanics often ignore the fine structural details of cytoskeletal filaments. The worm-like chain model is the most common coarse-grained model used to describe the mechanical properties of

cytoskeletal polymers. This model was originally developed by Kratky and Porod (Kratky and Porod 1949). It approximates polymers by a smooth linear contour that resists bending with a quantity κ called the *bending modulus*. High values of κ indicate a stiff polymer that resists deformation. Polymers with low κ deform more easily and appear soft. In the absence of thermal fluctuations, linear polymers would assume a straight shape. At finite temperatures, however, random forces from thermal fluctuations will cause cytoskeletal polymers to bend. These thermal bending undulations have been observed experimentally for actin filaments, microtubules, and intermediate filaments by fluorescence microscopy (Ott et al. 1993; Isambert et al. 1995; Gittes et al. 1993; Noding and Köster 2012), and have been used to measure κ. This was achieved by measuring a length scale l_p called the *persistence length*, which is defined as the decay length of angular correlations along the polymer contour. Roughly speaking, the persistence length is the distance over which the polymer contour appears approximately straight. The persistence length is related to the bending modulus by the relation $\kappa = kT\, l_p$, where k is Boltzmann's constant and T is temperature.

Based on the persistence length l_p relative to the contour length L, we can distinguish between three classes of polymers. If the polymer backbone offers little resistance to bending ($l_p \ll L$), thermal fluctuations dominate, bending the polymer so strongly that it crumples to a highly bent conformation well described by a fractal contour (de Gennes 1979). Such polymers are called *flexible polymers*, and are suitable for describing many synthetic macromolecular polymers. In the opposite scenario ($l_p \gg L$), *stiff polymers* strongly resist thermal fluctuations and can be

modeled as rigid rods (Landau et al. 1986). A third, and intermediate, regime occurs when $l_p \sim L$. In this regime, thermal fluctuations cannot be neglected, though the polymer retains a well-defined, mostly straight shape with long, wavelike undulations. Polymers in this intermediate regime are called *semiflexible polymers*. Cytoskeletal filaments fall in this intermediate regime, having contour lengths of several μm, and relatively large persistence lengths that range from 0.5-1 μm for intermediate filaments, to 8-15 μm for actin filaments and several mm for microtubules (Kasza et al. 2007). The small persistence length of intermediate filaments is surprising, given that their diameter is intermediate between those of actin filaments and microtubules. This suggests that the bundle-like architecture of intermediate filaments plays a key role in their mechanics. Sliding between subunits may for instance act to lower the bending rigidity.

(FIGURE 2)

Response to pulling forces. So far we have seen that the mechanical properties of semiflexible filaments can be characterized by the persistence length. This quantity describes how filaments respond to thermal forces. But how do semiflexible polymers respond to external pulling forces? Given an infinitely strong force, we should expect the polymer to assume a straight shape: such a taut filament would not bend due to thermal forces. Theoretical models have accounted for the reduction of thermally-induced bends due to external pulling forces (MacKintosh, Käs, and Janmey 1995). The amplitude of thermally induced bends in the polymer depends on wavelength, typically quantified through the wave vector $q = n \pi / L$, where $n = 1, 2, 3\ldots$ If the polymer experiences tension due to an external pulling force f, the amplitude u_q of bending mode

q is given by

$$\left\langle |u_q|^2 \right\rangle = \frac{2kT}{L(\kappa q^4 + f q^2)}$$

Long-wavelength bends (lower q) have the largest bending amplitudes, while short-wavelength bends (higher q) decay quickly, as q^{-4}. This formula also shows that applying a pulling force f to the polymer reduces the transverse bending amplitudes u_q. This reduction in thermal modes results in an effective restoring force:

$$f \sim \frac{l_p \kappa}{L^4} x$$

where x denotes the displacement of the end-to-end-distance vector of the polymer contour from its equilibrium length. The effective spring constant of a semiflexible polymer is thus $l_p \kappa L^{-4}$.

Because semiflexible polymers bend in response to thermal forces, their response to a pulling force is entropic in origin. This effect is often called the *entropic spring*. The force-extension relation was experimentally verified with optical tweezers for DNA (Bustamante et al. 1994) as well as for actin and microtubules (van Mameren et al. 2009).

The above equations are only valid under the assumption of small forces and *linear responses*. For strong pulling forces, once the thermal undulations are pulled out, this assumption breaks down. In this case, we must account for stretch deformations of the polymer backbone itself. This has been accomplished by introducing in the worm-like chain model an enthalpic quantity μ called the *stretch modulus*.(Odijk 1995; Storm et al. 2005). The result is the emergence of two different force-response regimes with distinct spring constants. For small deformations, the effective spring constant is dominated by the bending modulus κ according to the entropic spring. For large deformations, the effective spring constant is dominated by the stretching

modulus μ, corresponding to the enthalpic stretch of the polymer contour. Experimental evidence suggests that actin filaments and microtubules break before they are substantially extended, whereas intermediate filaments are highly stretchable (Kreplak and Fudge 2007; Y.-C. Lin et al. 2010).

Response to pushing forces. We have investigated how individual semiflexible polymers respond to pulling forces. But how do they respond to pushing forces? Given small forces and linear responses, the stretch modulus μ determines the compressive deformation of elastic rods.

Long, semiflexible rods can readily undergo a buckling instability when pushing forces exceed the critical *Euler force* f_c (Landau et al. 1986):

$$f_c \sim \kappa L^{-2}$$

This force sets the maximal protrusive force that a rod can exert. Since microtubules are rather stiff at the length scale of the cell, they can withstand high compressive forces (Dogterom and Yurke 1997). Actin filaments buckle at force levels that are 300-fold lower, owing to their smaller bending stiffness (Gittes et al. 1993; Footer et al. 2007). This buckling response results in an asymmetric mechanical response: single actin filaments can withstand and propagate pulling forces but not pushing forces. However, bundling can overcome this limitation, and actin bundles can exert substantial pushing forces (see §1.5). In cells, coupling to the surrounding cytoskeleton can further reinforce filaments against compressive loads (Brangwynne et al. 2006; Das and MacKintosh 2010).

(FIGURE 3)

1.3 Filament networks

In cells, cytoskeletal polymers are generally present at high density where filaments overlap and entangle. In this section, we will describe the collective properties of materials composed of many filaments. We will begin by summarizing theoretical models for solutions of rigid rods, a limit that has been theoretically well characterized.

Rigid rod solutions. Consider a rigid rod of length L and thickness a diffusing freely in solution. As it translates and rotates, it sweeps out a volume $\sim L^3$. Thus, for a suspension of rods with concentration (number density) $c \ll L^{-3}$, neighboring rods are spaced far enough apart that they do not significantly interfere with each other's motions. In this *dilute regime*, the rotational diffusion constant $D_{\circlearrowleft}$ scales with rod length (with a prefactor that depends on temperature and viscosity) (Riseman and Kirkwood 1950):

$$D_{\circlearrowleft,dilute} \sim L^{-3} \ln(L/a)$$

If the rod concentration c increases beyond $\sim L^{-3}$, rods start to interact via *steric repulsion* (excluded volume): two rods cannot overlap in space, and therefore repel each other upon contact. In this *entangled regime*, the diffusion of a rod is constrained by its neighbors. Early theory by Doi and Edwards modeled the effect of entanglements for concentrated suspensions of rods by proposing the *tube model* (Doi and Edwards 1978). In this model, a rod of interest cannot diffuse freely in a volume $\sim L^3$, but is rather confined to an elongated virtual tube formed by the presence of neighboring rods. This results in a drastically reduced rotational diffusion constant in the entangled concentration regime:

$$D_{\circlearrowleft,\text{entangled}} \sim L^{-6} D_{\circlearrowleft,\text{dilute}}$$

In the dilute and entangled concentration regimes, the rod orientations are isotropically distributed to maximize rotational entropy. If the rod concentration c increases further towards $c^* \sim a^{-1}L^{-2}$ (where a L^2 is the volume of a single rod), however, packing effects cause spontaneous rod alignment. Although this orientational alignment decreases the rotational entropy, this loss is compensated by an increase in translational entropy due to a decrease in the mutual excluded volume. This entropically driven isotropic to nematic phase transition was first predicted by Onsager (Onsager 1945). In the limit of infinitely long rods, the critical concentration c^* depends only on the inverse of the rod aspect ratio a / L. The orientational anisotropy of rods in the nematic phase results in an anisotropy in optical properties, giving the appearance of a crystal. Hence, materials in the nematic phase form one of many possible *liquid-crystalline regimes*.

Polymer networks. The above equations were derived assuming rods that are perfectly rigid and of uniform diameter and length. These assumptions have been experimentally validated for monodisperse rod-shaped viruses, specifically tobacco-mosaic virus (Graf and Löwen 1999) and bacteriophage fd (Dogic and Fraden 2006). However, cytoskeletal polymers are neither rigid nor uniform in length. Semiflexibility and length polydispersity cause quantitative changes to the phase behavior and dynamics, which can be accounted for theoretically (Khokhlov and Semenov 1982; Odijk and Lekkerkerker 1985; Odijk 1986; Glaser et al. 2010). However, qualitatively the above description for rigid rods is consistent with the dynamics and phase behavior observed for purified actin filaments and microtubules. For entangled actin filament networks with actin

concentrations between 0.1 to 2 mg mL^{-1}, the tube model was confirmed experimentally (Käs, Strey, and Sackmann 1994): labeled filaments were observed to fluctuate within a virtual, confining tube formed by the unlabeled surrounding filaments. Filaments slide back and forth along the tube in a snake-like motion called *reptation*. The width of the virtual tube decreases with increased actin concentration (Käs et al. 1996). Above concentrations of ~2 mg mL^{-1}, networks of actin filaments can form nematic phases (Suzuki, Maeda, and Ito 1991; Käs et al. 1996). Shortening actin filaments by adding the capping protein gelsolin increases c^*, consistent with Onsager's theory (Suzuki, Maeda, and Ito 1991).

(FIGURE 4)

Mechanical properties. Entangled polymer networks are *viscoelastic materials*, which exhibit behavior characteristic of both fluids (viscous) and solids (elastic). The viscoelasticity of soft materials such as polymer networks can be conveniently measured by *rheology* experiments. In these experiments, networks are allowed to form between two large, flat surfaces, which are moved relative to one another to apply a shear stress. The response of a viscoelastic material to a shear stress is given by the complex *shear modulus*, $G = G' + i\,G''$ (Meyers and Chawla 2009). The complex modulus has two components: the *storage modulus* G', which measures elastic, or solid-like behavior; and the *loss modulus* G'', which measures viscous, or fluid-like behavior. The shear moduli are usually determined by applying a small oscillatory shear stress of controlled frequency, and measuring the resultant oscillatory shear strain response. The magnitude of the shear modulus follows from the stress/strain ratio, while the extent of viscous dissipation is

reflected in a phase shift between the stress and strain signals.

(FIGURE 5)

For entangled actin networks, the primary determinant of the shear modulus is the filament density. The storage modulus was measured to scale with concentration according to $G' \sim \varphi^{7/5}$, where φ is the polymer volume fraction (Hinner et al. 1998; Gisler and Weitz 1999). Theoretical scaling arguments, which consider two length scales, can account for this experimental result (MacKintosh 2011). The first length scale is the *mesh size* ξ, which is defined as the typical spacing between filaments. For random networks of rigid filaments, the mesh size scales as $\xi \sim \varphi^{-1/2}\, a$, where a is the thickness of a single filament (Schmidt et al. 1989). The second relevant length scale is the *entanglement length* l_e, which describes the typical length over which filament entanglements restrict thermal fluctuations. It scales as $l_e \sim (a^4\, l_p)^{1/5}\, \varphi^{-2/5}$ (Isambert and Maggs 1996; Hinner et al. 1998). Together, these two length scales determine the storage modulus according to $G' \sim kT / (\xi^2\, l_e)$. Substituting this expression with the expressions for ξ and l_e yields the experimentally validated scaling relation $G' \sim \varphi^{7/5}$. Interestingly, the two length scales, and thus the storage modulus G', do not depend strongly on the bending stiffness of the filaments, given by l_p. At small frequencies, below a time scale set by the reptation time of the filaments, entangled solutions of actin filaments behave as viscous liquids. This time scale is on the order of minutes to hours, depending on the filament length.

Physical forces. In cells, the organization of actin filaments is not only affected by mutual

repulsion, but also by steric interactions with other cytoplasmic components and with the cell membrane. Soluble cytoplasmic components such as globular proteins can in principle act to introduce an effective *depletion attraction* between filaments. From a modeling point of view, globular proteins can be thought of as diffusing, impenetrable spheres that exclude some volume. Above a critical protein concentration c^*, filaments spontaneously bundle in order to maximize the free volume available to the globular polymers, thereby maximizing translational entropy (Lekkerkerker and Tuinier 2011). Experiments have shown that actin filament networks indeed become bundled when sufficient amounts of inert polyethylene glycol (PEG) polymers are added (Hosek and Tang 2004). Depletion forces can effectively crosslink actin filament networks and increase the shear modulus (Tharmann, Claessens, and Bausch 2006).

External boundaries such as the plasma membrane can affect filament organization by *spatial confinement*. Models of rigid rods predict that rods in an isotropic suspension in contact with an impenetrable planar surface will align along the surface, forming a so-called orientational wetting layer (van Roij, Dijkstra, and Evans 2000). This entropic effect only occurs for rods close to the surface, with an effective layer depth on the order of one rod length. However, walls give also give rise to a depletion layer, again due to entropic (volume exclusion) effects. For actin filaments, such a depletion zone with reduced actin density was experimentally shown (Fisher and Kuo 2009). Yet, when filaments are confined in three dimensions to emulsion droplets or liposomes, they form a cortex-like layer when droplets are smaller than the persistence length of actin filaments (Limozin, Bärmann, and Sackmann 2003; Claessens et al. 2006). Similarly, microtubules grown in confining microchambers were found to coil and wrap around the chamber edges (Cosentino Lagomarsino et al. 2007). This effect can be explained by enthalpic effects: cortical localization minimizes the energy penalty associated with filament

bending. Microrheology measurements showed that confinement also affects the mechanical properties of entangled actin solutions, inducing stiffening (Claessens et al. 2006). It is still poorly understood how entropic and enthalpic effects together determine the organization of entangled (and liquid crystalline) solutions of cytoskeletal filaments in confinement.

Note that the depletion attraction and confinement effects induce only effective interactions between filaments, mediated by the maximization of entropy and/or the minimization of bending enthalpy. These indirect, physical forces likely contribute to the organization of cytoskeletal structures inside cells. The environment inside most cells is crowded with soluble proteins which comprise 20–30% of the cytoplasmic volume (Ellis 2001). For this reason, the depletion interaction (often referred to as *crowding*) has been suggested to contribute to actin filament bundling, amyloid fibril formation, and DNA looping (Marenduzzo, Finan, and Cook 2006). Similarly, confinement effects ~~may~~ play a significant role in cytoskeletal organization (Chen 1997). But the physical mechanisms underlying confinement effects remain poorly understood. Even simplified experiments of dense reconstituted actin filament networks remain challenging to predict theoretically (Soares e Silva 2011). Cytoskeletal filaments have contours and persistence lengths that are often comparable to cellular dimensions, especially in thin compartments such as lamellipodia and filopodia. Understanding how these length scales interact should lead to a physical framework which quantifies confinement effects in cells.

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1.4 Crosslinks

The extent to which physical forces determine the organization and mechanics of cytoskeletal networks remains poorly understood. One reason for this is that cytoskeletal organization is regulated by a myriad of *accessory proteins*, including cytoskeletal crosslinks, cytoskeleton-membrane linkers, and nucleating and length-controlling factors. By tightly regulating the density and activity of these accessory proteins, cells can create different cytoskeletal structures without significantly affecting the physical properties of the filaments themselves. In this section, we will focus on actin-binding proteins that bind to actin filaments and connect them into crosslinked networks or bundles. We will describe the molecular properties of these *crosslinks* and the consequences of these molecular properties for the organization and mechanical properties at the network level.

Crosslink proteins create connections between actin filaments by binding to two separate filaments with two different actin-binding domains. Different types of actin-binding domains have been identified. The most common type is the *calponin-homology domain*, which is found across a broad class of crosslink proteins, including *spectrin*, *filamin*, *fimbrin*, and *α-actinin* (Korenbaum 2002). These crosslinks are all homodimeric. *Fascin* proteins are unusual: they are monomers with two actin-binding domains, and bind actin filaments through β-trefoil domains (Jansen et al. 2011). Fascin as well as fimbrin are compact, globular proteins that prefer to bind to tightly apposed filaments under a small angle. As a result, they generate tight, unipolar

bundles. In contrast, larger crosslink proteins such as α-actinin and filamin can bind actin filaments over a wide range of angles, forming isotropic networks at low crosslink density and mixed network/bundle phases at high crosslink density (Courson and Rock 2010; Stossel et al. 2001).

Crosslink binding. Except for the acrosomal protein *scruin* (Gardel et al. 2004), actin crosslinks bind transiently, with typical dissociation constants of 0.1–3 μM (Skau et al. 2011; Yamakita et al. 1996; Ono et al. 1997; Chen et al. 1999; Goldmann and Isenberg 1993; Meyer and Aebi 1990; Wachsstock, Schwarz, and Pollard 1993). This corresponds to binding free energies of 32–42 kJ mol^{-1}, or 13–17 kT at room temperature. (For comparison, the energy released by hydrolyzing ATP to ADP is approximately 50 kJ mol^{-1}, or 20 kT. One kT roughly corresponds to the average energy from thermal agitation.) In equilibrium, crosslinks unbind with typical timescales of several seconds (Courson and Rock 2010). Stresses acting on crosslinks usually accelerate crosslink unbinding (E. Evans and Ritchie 1997). Such crosslinks are known as *slip bonds*. However, α-actinin 4 exhibits different stable conformations (Galkin, Orlova, Salmazo, et al. 2010), which can expose cryptic actin binding domains (Volkmer Ward et al. 2008) when subject to stress (Yao et al. 2011). Remarkably, these crosslinks therefore bind more tightly under tension, which is known as *catch bond* behavior (Thomas, Vogel, and Sokurenko 2008).

Crosslinked meshworks and bundles. Adding crosslinks to entangled actin networks can result in a variety of structures, including fine crosslinked meshworks, pure bundle networks, bundle cluster networks, and composite meshwork-bundle networks (Lieleg, Claessens, and Bausch 2010). However, predicting network structure given the crosslink molecular structure and its

binding kinetics remains elusive. This challenge is further compounded by the observation that slow kinetics can give rise to nonequilibrium network structures (Lieleg et al. 2011; Falzone et al. 2012).

Mechanical properties. Introducing crosslinks in an actin filament network introduces a new length scale that determines the network mechanics, called the *crosslink distance* l_c. In particular, l_c determines whether a macroscopically imposed network deformation locally results in filament stretching or filament bending. When a macroscopic shear strain results predominantly in filament stretching, the network experiences *affine deformations* (or *uniform deformations*). In this case, provided that thermal fluctuations are appreciable, the storage modulus of an isotropic, randomly crosslinked network depends on the concentration (molar ratio of crosslinks to actin monomers) c_x of crosslinks according to (Gardel et al. 2004):

$$G'_{\text{affine}} \sim c_x \kappa l_p l_c^{-3}$$

When the filaments are very stiff (for instance when the network consists of bundled actin (Lieleg et al. 2007)), or when the network connectivity is low, the filaments (or bundles) can locally bend when a macroscopic shear stress is applied, resulting in *nonaffine deformations*. In this case, the storage modulus of the network is insensitive to the concentration of crosslinks and instead depends strongly on the concentration of actin filaments c (Kroy and Frey 1996):

$$G'_{\text{nonaffine}} \sim \kappa \xi^4 \sim c^2$$

(FIGURE 6)

Nonlinear response. A unique property of actin networks as well as other protein polymer networks such as extracellular collagen is their strongly nonlinear response to an applied stress. This nonlinearity is evident when pulling on human skin (for instance, the earlobe). For small

deformations, skin appears soft and deforms easily. Yet after pulling past a certain amount, skin resists deformation and therefore appears stiffer. Crosslinked actin networks also exhibit this *stiffening* effect. Past a critical stress σ_{crit}, the network becomes stiffer as the stress increases.

The strain-stiffening response of actin networks contrasts with the rather linear response of conventional synthetic polymers, which are flexible and act as linear springs under both compression and tension (Storm et al. 2005). However, recent work demonstrated that supramolecular synthetic polymers can be designed to mimic the remarkable nonlinear rheology of cytoskeletal networks (Kouwer et al. 2013).

The highly nonlinear viscoelastic response of actin networks poses technical challenges for measuring quantitative rheological properties. When a sinusoidal stress is applied, the oscillatory strain response tends to deviate strongly from a sinusoidal shape and the shear modulus extracted from the stress-strain ratio represents only the first harmonic component of the response. Most studies on actin networks therefore report instead the *differential storage modulus*, $K' = [\delta\sigma / \delta\gamma] |\sigma_0$, which is the local tangent of the stress-strain curve. K'-values can be directly compared with theoretical predictions based on semiflexible polymer models (Gardel et al. 2004). K' is usually obtained by a differential prestress protocol, where small amplitude oscillations are superimposed on a steady-state shear flow. An alternative protocol that can be more suitable for materials exhibiting creep is a strain rate ramp protocol, where K' is extracted by differentiating the stress/strain curve measured at different strain rates (Semmrich et al. 2007; Broedersz et al. 2010). For crosslinked actin networks, differential moduli obtained from strain-ramp and prestress protocols have been shown to agree, whereas for entangled solutions of actin

filaments deviations have been reported.

The origin of the strong nonlinearity of protein polymer networks is the large thermal persistence length of the polymers. Strain-stiffening can result from the entropic force-extension behavior of the filaments, which are easily buckled but strongly resist stretching (MacKintosh, Käs, and Janmey 1995; Storm et al. 2005). Alternatively, strain-stiffening can emerge as a consequence of a shear-induced transition from bending- to stretching-dominated elasticity (Onck et al. 2005; Broedersz et al. 2011). For actin networks, the critical stress where stiffening first occurs, and the stress-dependence of the modulus in the nonlinear regime depend on the type of crosslink. With the permanent and rigid crosslink scruin, the elastic modulus increases with stress as according to $K' \sim \sigma^{3/2}$ as a direct consequence of entropic elasticity (Gardel et al. 2004). In this case, networks likely fail due to the rupture of actin filaments. The larger and more flexible crosslink protein filamin A causes stiffening with a markedly different stress dependence ($K' \sim \sigma^{1}$) (Gardel et al. 2006), which has been ascribed to the entropic compliance of this crosslink molecule (Kasza et al. 2009). Bundle networks obtained by polymerizing actin filaments in the presence of fascin strain-stiffen by a different mechanism based on non-affinity (Lieleg et al. 2007). (FIGURE 7)

1.5 Force Generation

Passive physical forces already lead to a rich mechanical response of cytoskeletal polymer networks. Networks of actin filaments and microtubules gain additional complexity because these polymers are inherently out of equilibrium due to the continuous consumption of chemical energy in the form of the nucleotides ATP and GTP. Directional (de)polymerization allows actin

filaments and microtubules to exert forces as they grow or shrink. Furthermore, certain motor proteins can slide filaments past one another, leading to generation of pushing and pulling forces. In this section, we review the mechanisms whereby cytoskeletal polymers can actively exert forces.

Polymerization and depolymerization forces. Actin filaments and microtubules polymerize asymmetrically due to differences in the free energy of monomer binding at the plus-end and the minus-end. This free energy difference can be harnessed as filaments grow against a barrier to exert *pushing forces* (Hill 1981; Theriot 2000). Single microtubules are stiff and can exert forces of up to 3–4 pN as they polymerize (Dogterom and Yurke 1997), though theoretical arguments suggest that forces of up to ~50 pN should be possible (Dogterom et al. 2005). These forces are essential for maintaining the internal organization of the cell (Laan et al. 2012; Tolić-Nørrelykke 2008), including the proper positioning of the kinetochore and chromosomes in the mitotic spindle in animal cells (Inoué and Salmon 1995). Actin, too, exerts pushing forces. Despite the fact that single actin filaments alone are more flexible than microtubules and buckle readily under compressive loads, actin filaments can exert polymerization forces of up to 1 pN provided that they are sufficiently short (Footer et al. 2007). The branched architecture of actin networks in the *lamellipodium* of migrating cells such as crawling fish keratocytes indeed ensures the presence of a dense array of short actin filaments right underneath the leading edge (Mogilner and Oster 1996; Mogilner and Oster 2003b). Crawling cells can thus exert forces of about 100 pN (Roure et al. 2005). A similar polymerization-based propulsion mechanism is used by the bacterium Listeria monocytogenes (Tilney and Portnoy 1989; Cameron et al. 2001). This pathogen uses the actin machinery of infected cells to propel itself with forces of 10–100 pN

(Wiesner 2003; McGrath et al. 2003). Bundling of actin by crosslink proteins allows for even larger pushing forces. *Filopodia* in the growth cones of migrating neurons, which contain fascin-mediated bundles that consist only of about 10 actin filaments can exert pushing forces of up to 3 pN (Cojoc et al. 2007). These forces may enable filopodia to sense mechanical cues and guide preferential extension of neuronal growth cones along soft substrates (Betz et al. 2011). More dramatically, during the *acrosomal process* of the horseshoe crab *Limulus polyphemus*, a stiff bundle of 15–79 actin filaments crosslinked by the protein scruin extends from sperm cells to break open the egg cell wall with a force of 2 nN (Tilney 1975; Shin et al. 2003; Shin et al. 2007). The actin homologue *ParM* in *Escherichia coli* bacteria can similarly polymerize and generate forces to push the chromosomes apart to the cell poles before division (Bork, Sander, and Valencia 1992; Garner et al. 2007; Wickstead and Gull 2011; Galkin, Orlova, and Egelman 2012b).

Surprisingly, there are also mechanisms by which structurally symmetric cytoskeletal polymers can exert pushing forces. Sperm cells of the nematode *Ascaris suum* and also sperm cells of *C. elegans* migrate using *major sperm protein* (MSP) polymers, which elongate and pack in a similar fashion as actin filaments in the lamellipodium (Roberts and Stewart 2000; Miao et al. 2008; Batchelder et al. 2011). A "push-pull" model has been proposed that relies on a pH gradient that regulates gelation and solation of MSP filaments, but the molecular details remain poorly understood (Miao et al. 2003; Mogilner and Oster 2003a).

Apart from exerting pushing forces during polymerization, actin filaments and microtubules can also exert *pulling forces* during depolymerization. In the case of microtubules, these forces are transmitted through *tip-tracking proteins*, which selectively bind the plus end of microtubules

(Schuyler and Pellman 2001). As microtubules shrink, tip-tracking proteins can remain bound to the retreating plus end (Lombillo, Stewart, and McIntosh 1995). The resulting pulling forces are believed to contribute to proper positioning of chromosomes during cell division (Hill 1981; Dickinson, Caro, and Purich 2004; Joglekar, Bloom, and Salmon 2010; McIntosh et al. 2010). In the case of actin filaments, depolymerization was shown to be essential for *actomyosin ring constriction* in dividing budding yeast *Saccharomyces cerevisiae* (Mendes Pinto et al. 2012). Recent experimental evidence suggests that formins anchored at actin plus ends allow for the generation of pulling forces (Jegou, Carlier, and Romet-Lemonne 2013).

Molecular motors. In addition to the intrinsic ability of actin filaments and microtubules to exert forces via (de)polymerization, cells also possess specialized proteins called molecular motors. These proteins can exert forces by again coupling the free energy of ATP hydrolysis to mechanical work. This mechanical work can be harnessed for a wide variety of tasks, including DNA replication and expression, protein translocation, cell migration, chromosome separation, and cytokinesis (Bustamante et al. 2004).

Here we focus on the cytoskeletal motor proteins, which can exert forces while moving along cytoskeletal filaments. There are three classes of cytoskeletal motor proteins (Joe Howard 1997). *Myosin* motors bind actin filaments and most of the ca. 20 different types of myosins move towards the plus-end (Korn and Hammer 1988). *Kinesin* and *dynein* motors bind microtubules and move towards the plus- and minus-end, respectively. Although there can be considerable variation among molecular motor types (Goodson, Kang, and Endow 1994; Thompson and Langford 2002), cytoskeletal motor proteins share a few common design principles (Joe Howard 1997; Schliwa and Woehlke 2003). They possess one or two head domains which bind filaments as well as ATP or ADP. Upon ATP hydrolysis, motor proteins undergo conformational changes,

manifested in a *power stroke* that results in step-wise motion of the motor along the filament. Step sizes typically vary between 8 and 30 nm, generating forces of up to ~10 pN (Finer, Simmons, and Spudich 1994; Ishijima et al. 1998; Mehta et al. 1999; La Cruz et al. 1999; Visscher, Schnitzer, and Block 2000; Burgess et al. 2003). Motor proteins also possess tail domains, which can bind to the tail domains of other motors to form oligomeric motor complexes (Bresnick 1999), or to the cell cortex (Dujardin and Vallee 2002), or to intracellular cargo (Hirokawa 1998).

Many cells and organisms rely on molecular motors to exert forces that are stronger than by polymerization or depolymerization alone. Unicellular organisms such as the alga *Chlamydomonas reinhardtii* beat two long *flagella* composed of microtubules and dynein and kinesin motors (Bernstein and Rosenbaum 1994), allowing the cell to propel itself with a force of 30 pN (McCord, Yukich, and Bernd 2005). Fish keratocytes glide on surfaces powered by myosin contraction, exerting traction forces of 45 nN (Harris, Wild, and Stopak 1980; Oliver, Jacobson, and Dembo 1995). Similar traction forces between kidney epithelial cells maintain tissue integrity and reach 100 nN (Maruthamuthu et al. 2011). Even higher forces can be achieved by muscle cells, which organize actin filaments and myosin motors in a *sarcomeric structure* dedicated to integrating the power strokes of many myosin motors (A. F. Huxley and Niedergerke 1954; H. Huxley and Hanson 1954; Gautel 2011). Individual cardiac muscle cells have been measured to exert forces of 10 μN (Tarr, Trank, and Goertz 1983; G. Lin, Pister, and Roos 2000; Yin et al. 2005).

1.6 Active gels

The ability of molecular motors to exert forces on cytoskeletal polymers allows for the existence

of a fascinating class of materials called *active gels*. These gels can deform themselves by coupling internal enzymatic activity to mechanical work. Such internal driving allows cells to move and change shape without relying on external forces. In this section, we will explore some of the properties of active gels.

Motor activity and spatial organization. Apart from exerting forces on their surroundings, cells use molecular motors to organize transient internal structures such as the mitotic spindle (Tolić-Nørrelykke 2008; Dumont and Mitchison 2009). Understanding how forces produced by single motors translate into cell-scale forces and cell-scale spatial organization remains an enormous challenge. Forces re-organize the cytoskeleton, but the spatial organization of the cytoskeleton in turn influences force generation. Addressing this feedback in living cells is hindered by their inherent complexity. Recent experiments with reconstituted cytoskeletal networks driven by molecular motors have started to address the feedback between spatial organization and force generation.

Microtubules driven by kinesin or dynein motors exhibit fascinating structural patterns in solution, including vortices and asters (Nédélec et al. 1997) and active liquid crystals (Sanchez et al. 2012). Similar asters have also been reported in the case of actin bundles driven by myosin motors (Backouche et al. 2006). In confined geometries, microtubule asters can be reliably centered by a delicate combination of pushing forces from microtubule polymerization and pulling forces from dynein motors (Holy:1997uq; Laan et al. 2012). In all these cases, self-organization arises from a feedback between force generation and the motion of stiff filaments. Compared to microtubules, single actin filaments are relatively flexible and readily buckle under compressive forces. This property is likely the reason why actin filament meshworks driven by

myosin motor complexes have not been reported to exhibit the same pattern formation as microtubules (Soares e Silva et al. 2011). Buckling of actin filaments under compressive loads leads to an asymmetry in the response of actin networks to local internal forces generated by motors (Lenz et al. 2012; Murrell and Gardel 2012; S. K. Vogel et al. 2013). Such an asymmetry biases motor forces in favor of pulling rather than extensile forces, thus causing network contraction (Liverpool et al. 2009). Other effects, such as re-arrangements of myosin motors within the actin network, may also contribute to bias activity towards contraction (Dasanayake, Michalski, and Carlsson 2011).

Material properties of active gels. Apart from exerting forces and affecting spatial organization, molecular motors can also strongly affect the material properties of the polymer systems with which they interact. Rheology experiments have shown that the stresses induced by myosin motors stiffen crosslinked actin networks by a factor of 100 or more (Mizuno et al. 2007). In the case of actin networks crosslinked by filamin, it was shown that internally generated stresses by myosin motors stiffen the network in a similar way as an externally applied stress from a rheometer. In both cases, the stiffness increased with stress according to the scaling relationship $K' \sim \sigma^1$ (Koenderink et al. 2009). Remarkably, the same scaling relationship was also observed in whole fibroblasts which were stretched axially (Fernández, Pullarkat, and Ott 2006).

(FIGURE 8)

Myosin activity furthermore causes enhanced fluctuations in crosslinked actin networks that violate the fluctuation-dissipation theorem (Mizuno et al. 2007) and cause strong non-Gaussian displacements of embedded probe particles (Stuhrmann et al. 2012). These enhanced fluctuations

are typically observable at frequencies below ca. 10 Hz, which reflects the typical on-time of the transiently binding motors (MacKintosh and Levine 2008). Microrheology experiments on whole cells have revealed very similar violations of the fluctuation-dissipation theorem (Lau et al. 2003; Balland, Richert, and Gallet 2004; Wilhelm 2008). In suspensions of clusters of actin bundles, myosin motors can maintain dynamic steady states where clusters continuously grow and shrink (Köhler, Schaller, and Bausch 2011a) and actin bundles move superdiffusively (Köhler, Schaller, and Bausch 2011b). Similar superdiffusive behavior was recently observed in active microtubule-kinesin solutions (Sanchez et al. 2012).

Active gel theories. The ability of force-generating elements to bring active gels out of equilibrium has led to a lot of recent theoretical effort in predicting the phase behavior of actively driven matter using generalized statistical-mechanical frameworks (Joanny and Prost 2009). These frameworks derive from general principles (force balance, conserved quantities, and constitutive relations) to describe behavior over long length- and time-scales. This feature often carries a drawback in that microscopic details such as filament semiflexibility are neglected, complicating a direct comparison with experiments on in-vitro model systems as well as living cells. Also, these models generally assume small, linear perturbations from equilibrium. Whether and when this assumption holds is unclear. Nevertheless, these models have been successfully applied to several different biological contexts. For instance, a two-dimensional model of lamellipodia has predicted gel thicknesses, flow profiles, and cell velocity consistent with experiment (Kruse et al. 2006), and models of active fluids have been applied to explain the origin of cortical actomyosin flows that establish polarity in *C. elegans* zygotes (Mayer et al. 2010). A similar combination of active-gel models and experiment has also revealed that

actomyosin contraction and ensuing membrane blebbing at the cell poles stabilize cleavage furrow positioning during cytokinesis (Sedzinski et al. 2011).

1.7 Towards a mechanical understanding of cellular forces

Cytoskeletal polymers allow cells to actively move, change shape, and exert forces. In this chapter, we have primarily focused on recent insights into the origin of the passive and active mechanical properties of cells gained from in-vitro model systems. The advantage of such systems is that they allow one to directly, and quantitatively, compare experimental findings to theoretical models. Increasingly, biophysicists turn to experiments on living cells and model organisms, and develop coarse-grained theoretical models. In future years there will be an important challenge to bridge from an understanding of these simplified systems to an understanding of cells in their full complexity. One interesting route to create such a bridge is to study systems of intermediate complexity, such as cell extracts (Pinot et al. 2012). Another interesting and important avenue is to reconstitute composite cytoskeletal networks to the effect of the highly disparate bending rigidities of the three cytoskeletal filament systems on the passive and active mechanics of the cytoskeleton (Y.-C. Lin et al. 2011). ∑∑

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Figure Captions

Figure 1. Three kinds of cytoskeletal polymers. Actin monomers (white circles) assemble to form actin filaments with a double-stranded helical structure. The α-β tubulin dimers (joined white circles) form long protofilaments, which assemble laterally into a hollow tube. Intermediate filaments comprise elongated tetramer subunits (I-shaped structures), which associate laterally and stagger to form fibrils. Note that actin filaments and microtubules are structurally polar, and therefore have two different ends, designated + and –. Intermediate filaments are structurally symmetric and their ends are thus indistinguishable.

Figure 2. The worm-like chain. **a**: Polymers can be modeled as linear contours in space (bold black line). They possess contour length L and persistence length l_p. **b**: The relationship between L and l_p determines whether the polymer is classified as flexible, semiflexible, or rigid.

Figure 3. Response of semiflexible polymers to pushing and pulling. **a**: Schematic of a polymer bound at one end and deformed at the other end, undergoing buckling, linear response (entropic spring), and contour stretching. **b**: Force-extension curves for the three types of response. Polymers cannot exert compressive forces higher than the critical Euler buckling force f_c.

Figure 4. Entangled rods and polymers. **a**: Schematic of an entangled rod solution. The black rod cannot rotate freely because it cannot penetrate the surrounding gray bars. Rather, it moves in a virtual "tube" (black dotted line), whose width decreases with increasing rod density. The rod cannot rotate freely, but can still diffuse back and forth (bold arrows). **b**: Time-lapse image of a fluorescently labeled actin filament entangled by neighboring unlabeled filaments. The filament is confined to a virtual tube (thin white lines) and slides back and forth inside this tube in a snake-like motion called reptation (note the location of the filament ends with respect to the tube).

Figure 5. Shearing an entangled network. **Left**: the undeformed network. **Right**: applying a shear stress σ results in a shear strain γ.

Figure 6. Shearing a network can result in two kinds of deformations. When a network deforms affinely, filaments stretch and compress in such a way that the strain field at every scale matches the macroscopically applied strain (top right). Filament bending or rotations result in deviations from affinity (bottom right).

Figure 7. The type of crosslink determines the nonlinear response of entangled actin filament networks. **Left**: networks crosslinked by the small, rigid crosslink scruin result in power-law stiffening as $K' \sim \sigma_0^{3/2}$. **Right**: networks crosslinked by the large, flexible crosslink filamin stiffen with a different power-law according to $K' \sim \sigma_0$.

Figure 8. The nonlinear response of reconstituted active acto-myosin gels and living fibroblasts exhibit comparable scaling relations. Left: actin networks crosslinked by filamin and actively stressed by muscle myosin II motors stiffen with stress according to $K' \sim \sigma_0$. **Right**: A similar stiffening behavior was observed for fibroblasts that were individually stretched between two glass plates. A linear relationship between the stretching modulus Θ and the applied stress σ_0 was found.