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Introduction: A material cell living in a material world
All living organisms are built up of basic units called cells. Although some organisms such as bacteria consist of only one cell, the more complex organisms consist of a large number of varied cells, differentiated to perform specific vital functions. Even if we single out biological functions of a mechanical nature, the main function of cells is highly diverse; certain cells provide structural support (e.g., epithelial cells), others generate forces (e.g., muscle cells) and yet others are highly mobile such as the white blood cells of the immune system that track down and eliminate pathogens.

The mechanical properties of animal and plant cells are largely due to their cytoskeleton, an assembly of networks of various biopolymers and associated regulatory proteins, as illustrated in Fig. 1.1. The cytoskeleton plays a central role in various biological functions, ranging from forming the machinery for intercellular transport of cargo to the spatial and temporal organization of the cell on a wide range of length-and timescales. Here we focus on its mechanical function: the fibrous networks of the cytoskeleton form a scaffold that protects the structural integrity of the cell by resisting both internal and external stresses. Frequently, however, the cell has to adapt its shape or move around, requiring the cytoskeleton to generate forces and to remodel its structure dynamically. Over billions of years of evolution, this has resulted in a finely tunable network structure, controlled by the varying expression of hundreds of different regulatory proteins.

When considering the structural and mechanical properties of the cellular cytoskeleton, it is extremely useful to take a physical perspective and view it as a material. We take this approach in this thesis and investigate the materials properties of various cytoskeletal networks as well as the extracellular matrix, a biopolymer network that forms a major component of the connective tissue surrounding cells. Biopolymer networks are highly disordered assemblies of a large number of components, and although the behavior of the individual constituents is understood in some cases, a comprehensive theoretical description of their collective behavior remains a formidable challenge.

What makes biopolymer networks particularly intriguing—apart from their biological relevance—is that they are fundamentally different from most other common materials. First of all, they are part of a living, active system. This is reflected, for example, through the action of motor proteins; fueled by chemical energy, they generate forces that drive the cytoskeleton away from thermal equilibrium. Despite some recent theoretical progress, only little is understood theoretically of such out-of-equilibrium behavior. In chapter 7 of this thesis we discuss the effects of force-generating molecular motors on the mechanics of biopolymer networks.

A second important aspect of biopolymer networks is that the relevant interaction energies that hold the material together and preserve its shape are small and often comparable in magnitude to the thermal energy in the system. As a result, thermal fluctuations play an important role and mechanical interactions and entropic
Figure 1.1 – Schematic of the eukaryotic cell highlighting the cytoskeleton, which contains three families of filaments, including filamentous actin (F-actin), intermediate filaments and microtubules along with various binding proteins for force generation, cross-linking and polymer growth regulation. The cytoskeleton largely controls the mechanical response and locomotion of living cells. Courtesy of Fred MacKintosh (Vrije Universiteit Amsterdam).
effects often compete, resulting in a rich and finely tunable mechanical and dynamical network behavior. In this respect, cells and their cytoskeletal networks are soft materials [18] that only weakly resist deformation. Nonetheless, they exhibit numerous unique properties that contrast them with other soft materials such as foams, granular matter, synthetic polymer gels and emulsions. One striking example that will appear throughout this thesis is their dramatic stiffening under deformation [19–28]; even under small relative deformations of 10-50 percent, their elastic stiffness can increase dramatically, in some cases reaching stiffnesses a thousand times larger than their stiffness at small deformations.

These and other aspects lead to unusual materials behavior, as borne out by numerous experimental studies on reconstituted biopolymer networks (see section 1.3). Such in vitro biological gels form simplified modules of the cytoskeleton and the extracellular matrix. The study of these in vitro systems is very important since they facilitate a systematic and quantitative characterization of cytoskeletal networks. Such studies provide insight into the basic physical principles that govern the mechanical behavior of these systems, which may help elucidate their biological function and may inspire the development of novel biomimetic materials.
1.1 Cytoskeletal biopolymers

The main structural components of the cytoskeleton are fibrous networks consisting of three families of filaments: microtubules, filamentous actin (F-actin) and intermediate filaments (Fig. 1.2). These families of filaments fulfill distinct biological functions and most regulatory proteins are specific to a particular type of filament. From a physical point of view, the main differences between the different families of filaments are their dimensions and mechanical response (Table 1.1). The bending rigidity $\kappa$ of these biopolymers is high compared to most synthetic polymers. Nonetheless, thermal fluctuations are capable of exciting weak, yet significant undulations in most biopolymers, such as F-actin and intermediate filaments. Comparing the bending rigidity $\kappa$ to the thermal energy scale $k_B T$ gives a lengthscale $\ell_p = \kappa / k_B T$, where $k_B$ is Boltzmann’s constant and $T$ is the temperature; this persistence length represents the distance measured along the filament over which the bending rigidity is sufficiently large to resist thermal fluctuations and keep the filament straight. More specifically, $\ell_p$ is the decay length of the angular correlations of a thermally fluctuating polymer. The persistence lengths of various biopolymers are listed in Table 1.1. An important aspect setting biopolymers apart from most synthetic polymers is that their persistence length is often comparable or larger than the relevant lengthscale on which the polymer is considered, such as its contour length or the cross-linking lengthscale of the network in which they are embedded. Such polymers are said to be semiflexible.

1.2 Semiflexible polymers

The minimal model for an inextensible semiflexible polymer is the worm-like chain (WLC) model \[29–31\]. In this model the filamentous protein structure is coarse-grained to a continuous space curve $r(s)$ along the arc length $s$. The WLC Hamiltonian that captures the bending energy of a polymer of length $L$ is given by

$$H_{WLC} = \frac{\kappa}{2} \int_0^L ds \left( \frac{\partial^2 r}{\partial s^2} \right)^2. \tag{1.1}$$

Thermal energy can excite Brownian undulations in a semiflexible polymer with a typical amplitude $\sim L/\ell_p^3 [31]$; this leads to a contraction of the polymer. A thermally contracted polymer can be extended by applying a tensile force, $f$, that stretches out these fluctuations. However, since such an extended state is entropically less favorable, the polymer will resist extensional deformations similar to a spring with an effective spring constant $\sim k_B T \ell_p^2 / L^4 [31]$. However, when the relative extension exceeds values $\sim L/\ell_p$, typically on the order of tens of percents, the system no longer responds as a simple Hookean spring. Instead the differential stiffness of
the polymer increases strongly with the applied forces as $f^{3/2}$ [19]. This entropic stiffening is the origin of the nonlinear stiffening response of cross-linked F-actin networks [19, 20], and in chapter 8 we provide evidence that intermediate filament networks cross-linked by divalent ions stiffen through a similar mechanism. Another interesting aspect of semiflexible polymers is their highly anisotropic response to a force. The ratio between the transverse and longitudinal spring constants is $\frac{L_{90}}{L_p} \ll 1$.

Although the single-filament properties are fairly well understood, the behavior of entangled solutions and cross-linked networks of semiflexible polymers still remains to pose a significant theoretical challenge. Biopolymers provide an excellent experimental model system to study the behavior of semiflexible polymers and their networks.

### 1.3 Reconstituted biopolymer networks

The use of reconstituted biological gels as a bottom-up approach to cell mechanics has developed rapidly since the 1980s and has proven very successful in unravelling the basic properties of the various components of the cytoskeleton and their interactions under well-controlled conditions [2–9, 36, 37]. One particular type of biopolymer that has received much attention in these studies is F-actin, which forms one of the major structural components of the cytoskeleton. Typically, reconstituted F-actin gels consist of less than $\sim 1$ volume percent of protein in an aqueous solution. When purified monomeric actin is polymerized in the presence of the cross-linking protein filamin it can form isotropic networks of individual actin filaments, mimicking the structure of the actin cortex (Fig. 1.1), as shown in Fig. 1.3. In contrast, at large filamin concentrations the system adopts a qualitatively different network structure composed of curved bundles of F-actin (Fig. 1.3b). The precise type of cross-linking protein and its concentration relative to actin can have a dramatic effect on the network architecture, and experiments have further shown that the cross-linking protein can strongly affect the dynamic mechanical response as well as the nonlinear elastic response [9, 19, 23, 38]. A large part of this thesis is dedicated to understanding how
1.3. RECONSTITUTED BIOPOLYMER NETWORKS

Figure 1.3 – (Color online) a) Electron micrograph of a fixed and rotary-shadowed filamin-F-actin network at an actin concentration 1 mg/ml, average filament length 15 µm, and a filamin:actin molar ratio of 0.005:1. b) Confocal microscopy image of a fluorescently labeled bundled filamin-F-actin network at high filamin concentrations. Courtesy of Karan Kasza (Harvard).

the microscopic properties of various types of cross-linkers control the mechanical behavior of actin and intermediate filament networks on the macroscopic scale.

Another important theme in this thesis concerns the mechanics of networks of fibers that are softer to bending than to stretching. Under shear, such networks exhibit highly nonuniform deformations. Both the linear mechanical response and the nonlinear elasticity at large shears of such systems remains poorly understood. Chapter 6 is dedicated to the elastic response of such non-affine stiff fiber networks. Examples of networks that exhibit such behavior may include bundled actin networks and collagen (one of the primary components of connective tissues). A microscopy image of a reconstituted network of collagen is shown in Fig. 1.4. These networks typically consist of thick fibers. One of the principle questions about such fiber networks is to what extent their behavior is governed by fiber stretching deformations. The entropic stretching stiffness of such fibers is likely to be very large. Consequently, the fibers may be much softer to bending deformations than stretching deformations.
1.4 Rheology: measuring the macroscopic dynamic mechanical response of biological gels

Biopolymer gels exhibit a mechanical response that in general depends on the time-scale on which the system is probed. Furthermore, these gels often exhibit a highly nonlinear elastic response under shear. A careful and quantitative characterization of both the linear and nonlinear viscoelastic response is experimentally challenging. Various rheological methods have been developed to characterize the mechanical behavior of reconstituted biopolymer networks including both micro- and macrorheological methods. Microrheological methods are based on tracking one or multiple beads embedded within the network. The response of the system can be inferred directly by driving the beads with external fields or indirectly by monitoring their thermal fluctuations, although the latter relies on the assumption of the network being in thermal equilibrium. However, most of the experiments presented in this thesis have been performed using a macrorheological approach. With such an approach we can directly probe the macroscopic mechanical response by establishing the relation between the shear stress $\sigma$ and the shear strain $\gamma$ in a sample (Fig. 1.5). If the sample has a purely elastic response, and if network deformations are sufficiently small such that the response of the system is linear,

$$\sigma = G\gamma,$$  \hspace{1cm} (1.2)
where $G$ is the shear modulus. However, biopolymer gels exhibit both an elastic and a viscous component, which depends on the timescale on which the system is probed. To quantify such a viscoelastic response, the material is probed at a frequency $\omega$, which allows us to determine the frequency dependent complex shear modulus $G(\omega) = G'(\omega) + iG''(\omega)$. The storage modulus $G'$ captures the in-phase elastic-like response and the loss modulus $G''$ captures the out-of-phase viscous-like response.

Already at moderate strains ($\gamma \sim 0.1 - 1$) most cross-linked biopolymer networks stiffen dramatically. Various rheological protocols have been developed to determine this nonlinear response \[19, 39–42\]. Although these methods are discussed in more detail in chapter 5, we will here briefly introduce the prestress method since it is widely used throughout this thesis. In this protocol, the applied stress is the control variable and a differential measurement is used to determine the materials' differential stiffness. A steady prestress, $\sigma_0$, is applied on top of which a small amplitude oscillatory stress, $\delta \sigma(t) = \delta \sigma e^{i\omega t}$ is superposed. For different frequencies $\omega$ one can determine the small oscillatory strain response, $\delta \gamma(t) = \delta \gamma e^{i\omega t}$. The complex differential or tangent viscoelastic modulus is defined as $K = \frac{\delta \sigma}{\delta \gamma}$.

1.5 Outline of this thesis

**Chapter 2: Cross-linked governed dynamics**

One essential feature setting biopolymer networks apart from rubber-like materials is the intrinsic dynamics of their cross-links. This has important implications for cells, which have constantly remodeling internal networks, reflecting in part the dynamic nature of their cross-links. Recent experiments on actin networks with transient linkers provide evidence of a complex viscoelastic behavior.

To describe these systems we develop a microscopic model for the long time network relaxation that is controlled by cross-link dynamics. This cross-link governed dynamics (CGD) model describes the structural relaxation that results from many independent unbinding and rebinding events. We derive a set of nonlinear stochastic differential equations describing the time evolution of the dynamics of the polymers in the network. Using a combination of Monte Carlo simulations and a mean field approximation, we show that this type of cross-link dynamics yields a novel power-law regime in the rheology. Our model is in excellent quantitative agreement with experiments.

**Chapters 3 and 4: Semiflexible polymer networks with flexible cross-links**

Recent experiments on F-actin with the physiological cross-linker filamin have demonstrated several striking features; while their linear modulus is significantly lower than for rigidly cross-linked actin systems, they can nonetheless withstand remark-
Figure 1.5 – (Color online) The viscoelastic response of reconstituted biological gels can be determined using macrorheological approaches. In such approaches, the resistance to a deformation is determined by probing the sample with an applied strain $\gamma$ or stress $\sigma$ to obtain the shear modulus $G = \sigma / \gamma$ (a). Most rheological experiments on biological gels use either a cone-plate (uniform strain) geometry (b) or a plate-plate geometry (non-uniform strain).
ably large stresses and can stiffen by a factor of 1000 with applied shear. We show quantitatively that this behavior can be accounted for by the highly flexible nature of the filamin cross-links. To describe these systems we develop a self-consistent mean field theory for the macroscopic nonlinear elasticity of these networks. The networks are modeled as a collection of randomly oriented rods connected by flexible linkers to a surrounding elastic continuum, which is required to self-consistently represent the behavior of the network. We have confirmed the main predictions of this model in close collaboration with the experimental group of D. Weitz.

Chapter 5: Nonlinear rheological methods for biopolymer gels
One of the hallmarks of biopolymer gels is their nonlinear viscoelastic response to stress, making the measurement of their mechanics very challenging. Using both strain ramp and differential prestress protocols, we investigate the nonlinear response of a variety of systems ranging from extracellular fibrin gels to intracellular F-actin solutions and F-actin cross-linked with permanent and physiological transient linkers. In particular, we designed a new protocol to investigate how both the linear and nonlinear mechanical response changes as the system creeps and deforms plastically under a large applied shear stress. In this protocol the differential response is determined under DC positive shear stresses of varying magnitude alternated with periods without load. The total strain and differential response are monitored continuously.

We find that the nonlinear response measured with the prestress protocol is remarkably insensitive to creep. This demonstrates that the nonlinear mechanical response of these biopolymer networks is robust, even when the network is flowing. To provide insight into these observations, we develop a simple, yet very general phenomenological model that includes the nonlinear elasticity of the network as well as network flow on long timescales.

Chapter 6: Criticality and isostaticity in fiber networks
The rigidity of elastic networks depends sensitively on their internal connectivity and the nature of the interactions between constituents. The isostatic point above which systems are rigid is captured by a classical argument introduced by Maxwell, which balances the degrees of freedom in the system against the number of constraints due to connectivity. Fibrous networks, such as those that form the cellular cytoskeleton or the extracellular matrix, exhibit rigidity at remarkably low connectivity, well below the Maxwell central force isostatic point. This rigidity is due to additional constraints provided by the fibers resistance to bending. However, the degree to which bending governs network mechanics remains a subject of considerable debate. We study disordered fibrous networks with variable coordination number, both above and below the central-force isostatic point. We find that this point controls a broad crossover from stretching- to bending-dominated elasticity. Strikingly, this crossover exhibits an
anomalous power-law dependence of the shear modulus on both stretching and bending rigidities. At the central-force isostatic point—well above the rigidity threshold—we find divergent strain fluctuations together with a divergent correlation length $\xi$, implying a breakdown of continuum elasticity in this simple mechanical system.

Chapter 7: Motor generated stiffening in fiber networks
Reconstituted active filamentous F-actin networks with motor proteins form a good model system for cellular mechanics. The motor proteins generate forces that drive the network far from equilibrium and strongly affect the network mechanics. In some cases, the macroscopic nonlinear response of a passive network to an external shear is due to a transition between soft bending modes to stiffer stretching modes. The question arises how stress generating molecular motors couple to such a network and how they affect the macroscopic elastic response.

To address these issues, we develop a lattice-based approach to design networks with a connectivity of 4 or less, mimicking the architecture of biopolymer networks with binary cross-links. We showed how heterogeneous internal stresses generated by motors can lead to stiffening in networks that are governed by filament bending modes. The motors are modeled as force dipoles that cause muscle-like contractions. These contractions "pull out" the floppy bending modes in the system. Through this mechanism, motor activity can strongly stiffen the networks’ mechanical response.

Chapter 8: Ionically cross-linked IF networks
Intermediate filament (IF) networks in the cytoplasm and nucleus are crucial for the mechanical integrity of metazoan cells. While filamentous actin and microtubules have been extensively studied, much less is known about IFs. In particular, the mechanism of cross-linking in these networks and the origins of their mechanical properties are not understood.

In close collaboration with the experimental group of D. Weitz, we have shown that divalent ions can mediate a cross-linking interaction between the negatively charged tail domains of intermediate filaments. We use an affine model for the nonlinear elastic response of these systems, which includes both the entropic stiffening and the enthalpic stretching of the individual filaments, as well as geometric affects that arise in networks under large shear deformations. This model allows us to extract microscopic parameters from the measured macroscopic rheological behavior, including the Young’s modulus and the persistence length of the filaments, and the cross-linking lengthscale of the network.
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