Microstructure and mechanical behavior of cross-linked biopolymer networks

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Chapter 1

Introduction

A fundamental building block of biological life is the cell. Whether the organism is single-celled or multicellular, each individual cell performs various functions in accordance to the internal stimuli or interactions with its physical environment. Many of these stimuli and interactions involve mechanical forces, which cells need to recognize and resist. For this, cells require certain mechanical properties, e.g. stiffness.

Almost all eucharyotic living cells acquire essential mechanical properties from a scaffold of cellular filaments that constitute the cytoskeleton (Lodish et al., 2000; Mofrad and Kamm, 2006). The organization of cytoskeletal filaments —microtubules, intermediate filaments and actin filaments— is mediated by various "helper" proteins that nucleate, (de)polymerize, cross-link, branch, bundle, cap or severe the filaments in a cell (Chhabra and Higgs, 2007). Besides having a vital role in determining cell mechanical properties, the cytoskeleton is also involved in mechanosensing, transporting and signaling processes (Mofrad and Kamm, 2006).

Of special interest for the mechanical behavior of cells is the actin part of the cytoskeleton. Actin is mainly localized in the plasma region near the membrane, where actin filaments form a complex network-like structure, i.e. the cortex, that reinforces the otherwise highly flexible cell membrane, and is used by the cell to regulate its mechanical stability and drive shape change and movement (Fletcher and Mullins, 2010).

Actin is an abundant protein in all eucharyotic cells. Physiological actin concentrations range from $\sim$1 to $\sim$5 mg/mL. In its globular form, G-actin is a polypeptide chain $\sim$375 amino-acid residues long with a molecular mass of $\sim$42 kDa (Boal, 2002). G-actin monomers can polymerize into a helical slender filament, i.e. F-

actin, that has a diameter $t \approx 7\,\text{nm}$ and contour length l_0 in the range between $\sim$1 μm (*in vivo*) and $\sim$21 μm (*in vitro*) (Liu and Pollack, 2002; Gittes et al., 1993). F-actin is nearly inextensible, and despite its slenderness quite resistant to bending when interacting with its thermal environment. Because of this, it is commonly regarded as a semi-flexible chain (MacKintosh et al., 1995) with persistence length $\sim 17\,\mu$m (Ott et al., 1993), that is of the same order of magnitude as its contour length l_0. The persistence length of a molecular chain, defined as $l_\text{p} = \kappa/k_\text{B}T$, is a measure of its bending stiffness κ relative to the thermal energy $k_\text{B}T$, with k_B being the Boltzmann constant and T the temperature (Doi and Edwards, 2007). Unlike flexible chains commonly associated with synthetic polymer materials, a semi-flexible chain is less susceptible to the thermal bending and thus, adopts relatively extended conformations. The large persistence length and axial stiffness of semi-flexible biopolymers like F-actin are one of the key prerequisites for the remarkable mechanical behavior of biopolymer networks that will be studied here.

1.1 Bioploymer networks *in vitro*

In order to circumvent the full cytoskeleton complexity, most investigations of mechanical properties of actin cytoskeleton, and biopolymer networks in general, are carried out on *in vitro* reconstituted networks. Reconstituted networks are relatively simple hydro-gels that are self-assembled *in vitro* by entangling, branching and/or cross-linking biopolymer filaments. *In vitro* F-actin networks exhibit a rich mechanical behavior (Bausch and Kroy, 2006; Lieleg et al., 2010) that is comparable to the elasticity of the cells (Gardel et al., 2006).

Generally, the mechanical behavior of *in vitro* biopolymer networks reflects the properties of the constituents, i.e. biopolymer filaments (Shin et al., 2004; Lin et al., 2010b; Vader et al., 2009) and cross-linking macro-molecules (Gardel et al., 2006; Wagner et al., 2006), but is also highly dependent on the microstructure (Shin et al., 2004; Lin et al., 2010b; Vader et al., 2009; Gardel et al., 2004a; Lieleg et al., 2007; Kasza et al., 2010). In the case of "stiff" cross-linking proteins, such as scruin (Gardel et al., 2004a) or heavy meromyosin (Tharmann et al., 2007), the mechanical behavior of the actin network originates mainly from the F-actins themselves and their spatial organization.

Cross-linked actin networks are representative of the polymorphic nature of biopolymer networks (Lieleg et al., 2010). Various high resolution imaging and rheological techniques have been used to obtain an insight into the properties of the microstructure of *in vivo* (Svitkina et al., 2003) as well as *in vitro* actin networks (Shin et al., 2004; Tharmann et al., 2007; Schmoller et al., 2009). De-

pending on both type and concentration of the cross-linking molecule, different morphologies exist. Commonly, the cross-linking molecules at their low to moderate concentrations reorganize weak and entangled F-actin solutions into a more stiffer isotropically cross-linked networks of individual filaments. For sufficiently high cross-link concentrations however, the majority of cross-linking molecules act to bundle F-actins into a composite networks that comprise individual and/or bundled filaments (Lieleg et al., 2010). In addition, the interaction between F-actins themselves can induce an isotropic-to-nematic transition (Furukawa et al., 1993), thereby leading to a more parallel alignment of filaments in the network. Also, due to the transient nature of some of the cross-links and their finite binding lifetimes, the network microstructure could be a subject of constant remodeling (Xu et al., 2000).

Each of the above mentioned network microstructures has intrinsic geometrical length scales associated to it, e.g. mean filament (or bundle) contour length and diameter, mean cross-linking distance and/or network mesh size, which can be used to rationalize the mechanical behavior (Tharmann et al., 2007; Shin et al., 2004; Lieleg et al., 2007). For example, mesh size is a measure of the average distance between the filaments in the network; it depends on the filament concentration, i.e. mesh size decreases as the concentration of the filaments increases (Schmidt et al., 1989), and it has been shown that networks generally become more stiffer for decreasing mesh size (MacKintosh et al., 1995; Gardel et al., 2004a; Tharmann et al., 2007; Schmoller et al., 2009). The mean distance between cross-links is another important network parameter that is expected to affect the network stiffness, i.e. as the mean distance between cross-links decreases the network stiffness should increase (MacKintosh et al., 1995; Gardel et al., 2004a,b; Tharmann et al., 2007). However, unlike the mesh size, it is difficult to experimentally measure the mean cross-linking distance. Therefore, it has been postulated that mean cross-linking distance in addition to being dependent on the concentration of cross-linking molecules, also depends on the entanglement length (MacKintosh et al., 1995; Tharmann et al., 2007), i.e. a measure commonly used in polymer physics to define the length scale of the steric (excluded-volume) interactions between chains entangled in a network (Doi and Edwards, 2007).

In addition, numerical simulations of 3D networks hinted that the network topological properties, e.g. the connectivity, whether is simply considered as a static property of the microstructure (Huisman et al., 2007) or a dynamic strain-driven remodeling factor (Astrom et al., 2008), play also an important role in the network mechanical behavior. Although it is somewhat intuitive to expect that "more" or "better" connected network should be stiffer, a quantitative understanding of how connectivity can be characterized and how it is related to the other parameters of

the network microstructure is poorly understood.

As mentioned above, the complex network microstructure and its geometrical and topological properties are very important for the mechanical behavior of biopolymer networks. Despite much research in the recent past, the mechanical properties of biopolymer networks still hold many mysteries. The conjecture in this thesis is that they are mostly hidden in the network architecture or microstructure.

1.2 Biopolymer networks strain stiffen

Rheological experiments on single or bundled F-actin networks at time scales smaller than the binding lifetime of the cross-linking proteins, have shown linear elastic network behavior at small strains followed by highly nonlinear strain stiffening. The degree to which biopolymer networks can strain-stiffen is remarkable: at large strains the shear modulus can increase by two to three orders of magnitude (Gardel et al., 2004a, 2006; Yao et al., 2010). While bundled F-actin networks have a much larger initial elastic modulus than networks of single F-actins, the strain stiffening can be similar and follows a seemingly universal trend characterized by a power-law dependence of the elastic shear modulus on macroscopic stress with exponent 3/2 (Gardel et al., 2004a,b). More recently, the same power-law dependence has been observed in ionically cross-linked networks of intermediate cellular filaments (Yao et al., 2010; Lin et al., 2010b).

The network model, proposed by MacKintosh et al. (1995) and Storm et al. (2005) and based on the physics relevant for a free standing single filament, predicts that the strain stiffening characterized by the power-law exponent 3/2 is entropic in origin. The network in this model is considered as a collection of randomly oriented filaments, represented as thermal semi-flexible polymers, that are stretching affinely with the overall deformation. The response of the whole network then, is associated to the "mean" filament response and is obtained by averaging the response of an individual filament over all possible orientations. In this way, the network response inherits the entropic nature of the semi-flexible polymer behavior. In particular, interaction of the semi-flexible filament with the thermal environment introduces fluctuating undulations that continuously perturb the filament shape. Such fluctuating filament explores a set of conformations (shapes) which can be characterized by the mean distance between filament ends, e.g. r. A force required to change the filament end-to-end distance then, e.g. in the case of filament stretching, will be opposed by the change in entropy associated to the filament conformational space. In the limit of nearly full filament extension when the end-to-end distance approaches the contour length, $l_0 - r \to 0$, the applied force at

filament ends, e.g. f_r, is found to diverge following a power law $f_r \propto (l_0 - r)^{-2}$, and thereby leading to a power-law dependence of the network elastic shear modulus on macroscopic stress with exponent $3/2$[1]. Hence, it has been argued that strain-stiffening exponent $3/2$ observed in experiments during large network deformation originates from straightening of the thermal filament undulations.

However, the assumption that the network microscopic deformation field is affine, is a rather controversial issue, mainly due to numerical studies that have clearly shown strong non-affine trends (Onck et al., 2005; Hatami-Marbini and Picu, 2008; Huisman et al., 2008). In addition, once the filament is cross-linked and becomes part of the network, its thermally fluctuating nature is expected to be suppressed due to constraints imposed by cross-links (Ghosh et al., 2007). In another words, multiple cross-linking partitions the filaments into segments whose length for *in vitro* networks can be at least an order of magnitude below the filament persistence length (Tharmann et al., 2007; Yao et al., 2010). Thus, it is to be expected that the segments of the filament are more straight then the whole filament itself.

Based on 2D network simulations, Onck et al. (2005) proposed an alternative mechanism for strain stiffening, where the network microstructure plays a key role in mediating the transition from a regime dominated by the filament bending stiffness at smaller strains to a large-strain regime dominated by the filament axial stiffness. From the 2D network models, these authors concluded that filament undulations do not change the network stiffening but only postpone it, thereby suggesting that the presence of thermal filament undulations should not be essential for understanding the strain-stiffening network behavior. This point of view was further substantiated by the theoretical study by van Dillen et al. (2008).

If the network strain-stiffening is independent of the nature of the filament behavior (i.e. thermally undulated vs. athermal filaments), then there must be something else, more fundamental about the microstructure, that causes nonlinear behavior in biopolymer networks. The work presented in this thesis is set to investigate, by means of computer simulations, the fundamental relationships between properties of the network microstructure and the associated mechanical behavior of biopolymer-like networks.

1.3 In this thesis

Because single filaments like F-actin are too thin to be experimentally observed within the network, many details of the network microstructure cannot be directly

[1]The details of this derivation are addressed in Chapter 5 and Chapter 6

evaluated experimentally. However, in most general sense, the microstructural characteristics of cross-linked networks of individual F-actins follow from the assumptions: (i) that the filaments are finite in length and (ii) that the cross-linking proteins commonly have only two actin binding sites, i.e. a single cross-linking macromolecule is binary and can cross-link only two filaments.

Starting from these two simple assumptions, this thesis is dedicated to the numerical study of the microstructure and the response of a discrete three-dimensional isotropic network of cross-linked and unbundled filaments. It is considered the most simple case of a network where the filaments are athermal and the cross-links are static (no network remodeling). By neglecting filament fluctuations, the applicability of the model used here becomes limited to networks for which mean cross-linking distance is much smaller that the filament persistence length, i.e. a filament segment in between two cross-links is straight and mechanically equivalent to a beam.

After briefly reviewing the used finite element network model in Chapter 2, in Chapter 3 the geometrical and topological properties of the randomly generated network microstructures are studied. It is shown that the key length scale of an isotropically cross-linked network, i.e. the mean distance between the cross-links l_c depends only on the macroscopic concentrations of the network constituents and the mean filament length l_0.

The expression for the mean distance between cross-links l_c, obtained in Chapter 3, together with the scaling relation for the small strain network response in the limit of rigid cross-links, derived in Chapter 4, allows a direct comparison for the initial network shear modulus between the networks generated here and experimentally studied F-actin networks cross-linked by heavy-meromyosin (HMM) (Tharmann et al., 2007).

By studying the large strain response of networks with rigid cross-links in Chapter 5, the fundamental strain stiffening mechanism leading to the power-law dependence with exponent 3/2 between the network shear modulus and macroscopic stress is uncovered. It is observed that large network deformation leads to the formation of a percolating path composed of highly axially stressed filament segments, i.e. a so–called stress path, that dominates the response. The concept of a stress path, is not only key for understanding the strain-stiffening of rigidly cross-linked networks, but also for the networks with compliant cross-links which are studied in Chapter 6.

By means of a parametric study of the dependence of the nonlinear network behavior on mechanical properties of network constituents, e.g. filaments and cross-links, and network microstructure, it is shown in Chapter 6 that strain-stiffening generally originates from two fundamental mechanisms. The first mechanism is a

bending-dominated, it originates from the pulling-out of stress path undulations, and it can be characterised by a power-law dependence with exponent 3/2 of the shear modulus $\tilde{G}$ on macroscopic stress $\tilde{\mathrm{T}}$. The second mechanism is a finite strain effect induced by reorientation of the stress path, it is a stretching-dominated, and it gives rise to a power-law relation between $\tilde{G}$ and $\tilde{\mathrm{T}}$ with exponent 1/2.

It is proposed in Chapter 6 that the nonlinear strain-stiffening behavior of a cross-linked network can be quantified by a single parameter, i.e. characteristic ratio $\bar{l}_\mathrm{b}/l_\mathrm{c}$, that relates material properties (bending and axial stiffnesses) of the network constituents to the key length scale of the microstructure. As such, the characteristic ratio $\bar{l}_\mathrm{b}/l_\mathrm{c}$ reflects the relative importance between the two stiffening mechanisms, and depending on the mechanical behavior of the filamentous constituent, the cross-link and the network microstructure, a variety of possible stiffening behaviors can be obtained. The results obtained in Chapter 6 suggest a novel and unified way in interpreting the experimentally observed nonlinear elasticity of different biopolymer networks, comprising different types of biopolymer filaments, cross-links and of different microstructure.