The Cell: Strong, Soft Matter

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SUMMARY

In your body, cells are continuously squeezing themselves through holes of different shapes and sizes. For example, immune cells migrate through the polymer matrix of your tissues, hunting for intruders. This extracellular matrix is highly non-uniform, and cellular migration requires continuous shape adaptation. Similarly, cells change their shape dramatically during cell division and differentiation. Clearly, the cell needs to be highly deformable.

The same cell has a very stressful life mechanically. For example, the cell is under continuous risk of being blown up by osmotic pressure created by the large concentration of biomolecules inside. Furthermore, large forces are exerted on cells and their surrounding tissues, for example during embryonic development or in cartilage during the movement of joints. Therefore, apart from being highly deformable, a cell also needs to be very strong.

Typically, materials are either soft or strong. For example, a pudding is readily deformable but can easily be broken by hand, whereas the opposite holds true for a brick, which has a high rupture force, but still breaks at small deformations due to its high stiffness. In contrast to these synthetic materials, a cell combines strength with deformability. *Which material design principles allow the cell to meet these seemingly contradictory demands?* Answering this question will not only increase our understanding of biology, but can also inspire the design of new synthetic materials and possibly give insight into diseases where the mechanical properties of a cell are compromised.

The mechanics of a cell are controlled by the cytoskeleton, which consists of biopolymer filaments and accessory proteins like linkers and motors. We focus on actin, the most abundant cytoskelal subunit. Actin filaments form the cell cortex, which is the main cytoskeletal player in controlling cell shape and mechanics. In the cortex, actin filaments are transiently connected by linker proteins, with a bond lifetime of a few seconds. This design principle of transient linkers causes elastic rigidity on short-timescales, whilst allowing for viscoelastic flows on timescales longer than the crosslinker unbinding timescale.

The overarching goal of this thesis is to understand how the actin cortex allows cells to be soft yet strong. To be able to directly examine the mechanical properties of actin networks, we purify and reconstitute actin together with actin binding proteins. We combine experiments with theoretical modeling to unravel the governing principles which allow for both deformability and strength of actin networks.

In chapter 2 we investigate in detail the linker dynamics through a combination of theoretical modeling and several experimental techniques. We find that stress relaxation in an actin network occurs on a faster timescale than crosslinker redistribution. We rationalize this discrepancy, using a three-state model where crosslinkers are either bound to 0, 1 or 2 filaments. In this model, stress relaxation occurs as soon as a a doubly bound crosslinker unbinds one of two filament, whereas crosslinker redistribution requires unbinding from both filaments. Surprisingly, we find that the unbinding rate of a singly

bound crosslinker is more than an order of magnitude slower than when two filaments are attached. We attribute the increased unbinding rate of doubly bound crosslinkers to the stiff nature of biopolymers, which frustrates crosslinkers bound to two filaments. This chapter provides quantitative insight into the crosslinker dynamics in biopolymer networks.

In chapter 3 we examine how the linker dynamics affect the nonlinear mechanical properties of dynamically crosslinked actin networks. We find unexpectedly slow stress relaxation, similar to disordered systems close to the glass transition. We explain our experimental results using a theoretical model that accounts for the transient nature of the crosslinkers combined with the nonlinear force-extension behavior of the actin filaments. This chapter offers a microscopic mechanism for the universal glassy dynamics frequently observed in cellular mechanics.

In chapters 4 & 5 we propose a theoretical model that describes how fracturing occurs in transient networks such as the actin network. In chapter 4, we use stochastic modeling to investigate a collection of reversible bonds under load, and find a microscopic mechanism by which cracks emerge from the nonlinear local bond dynamics. We provide and numerically verify analytical equations for the dependence of the critical crack length on the bond kinetics and applied stress. In chapter 5, we extend the stochastic model to consider the effect of bond mobility after unbinding on the strength of a transient network. We find that bond mobility weakens transient networks. This is remarkable as bond mobility is common in biological transient networks such as the actin cortex, and in systems responsible for cellular adhesion. We speculate that cells trade fracture strength for the modularity and tight dynamic control offered by mobile linkers. These chapters reveal governing principles of the fracturing of transient networks such as the actin cortex.

In chapter 6 we study the effect of catch bonding on network strength. Catch bonds are proteins whose bond lifetime increases when moderate forces are applied via exposure of hidden binding sites. After full exposure at high force, catch bonds behave like normal (slip) bonds whose bond lifetime monotonically decreases with force. Using the stochastic model developed in chapter 4 & 5, we show that catch bonds provide vastly more long-lived networks than slip bonds. Surprisingly, we find that catch bond networks are stronger than slip bond networks even when the individual bonds are weaker. We verify our conclusion experimentally by combining single molecule and network experiments on actin crosslinked by either wild type α-actinin 4 (which forms catch bonds) or its slip bond counterpart, a mutant with a single point mutation referred to as K255E. We attribute the increased strength of catch bond networks to a self-assembly mechanism against force inhomogeneity. This chapter reveals the importance of catch bonds in preventing fracturing in highly deformable transient networks such as the actin cortex.

The work in this thesis gives novel insight into how cells manage to be highly deformable yet strong. The design principle of transient networks allows for substantial deformability over timescales longer than the typical crosslinker unbinding time. However, due to fluctuations in local linker density, transient networks are also prone to crack initiation and subsequent fracturing. Importantly, catch bond linkers self-assemble against force inhomogeneity, and thereby prevent crack initiation despite their faster unbinding

kinetics compared to slip bond linkers. These catch bond linkers therefore simultaneously increase both deformability and strength of transient networks. We have revealed this principle for actin networks, but expect the mechanism to occur for any transient network connected by catch bond linkers. Our finding of increased strength and deformability therefore suggests a novel evolutionary driving force behind catch bonds, and indeed we have found many biological examples of catch bonds connecting transient networks.

Strikingly, our work on the catch bond crosslinker and the constitutively active mutant is interesting from a biomedical perspective as well. The catch bond we considered, α-actinin 4, is the actin crosslinker in podocytes. These cells are under continuous pressure as they filter the blood in our kidneys. Whereas the catch bond crosslinker is the wild type version, the constitutively active mutant is known to cause the kidney failure disease focal segmental glomerulosclerosis 1 (FSGS1). Strikingly, FSGS1 cells expressing the constitutively active mutant linker are significantly more brittle than cells expressing the wild type catch bond linker. It will be interesting to study the importance of catch bonding in preventing failure of cells and tissues in the context of FSGS1 and other diseases.

Lastly, our work can provide inspiration for new design strategies of synthetic materials. Transient networks are not only common in biology, but are also often used to create synthetic viscoelastic materials such as colloidal gels and polyelectrolytes in food and cosmetics. These materials suffer from limited deformability due to crack initiation. For future work, it would be interesting to create synthetic analogues of catch bonds to create bio-inspired materials which are highly deformable yet durable under stress. Such materials would be ideal for applications in regenerative medicine.