A bottom-up approach to cell mechanics

The mechanical stability and integrity of biological cells is provided by the cytoskeleton, a semidilute meshwork of biopolymers. Recent research has underscored its role as a dynamic, multifunctional muscle, whose passive and active mechanical performance is highly heterogeneous in space and time and intimately linked to many biological functions, such that it may serve as a sensitive indicator for the health or developmental state of the cell. *In vitro* reconstitution of 'functional modules' of the cytoskeleton is now seen as a way of balancing the mutually conflicting demands for simplicity, which is required for systematic and quantitative studies, and for a sufficient degree of complexity that allows a faithful representation of biological functions. This bottom-up strategy, aimed at unravelling biological complexity from its physical basis, builds on the latest advances in technology, experimental design and theoretical modelling, which are reviewed in this progress report.

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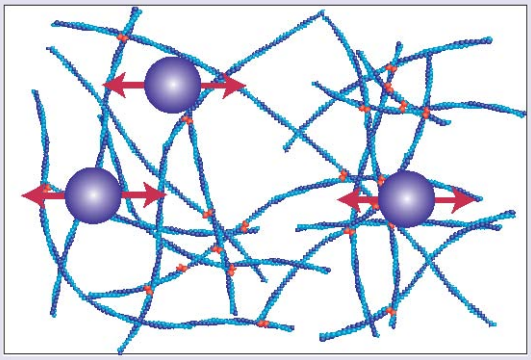
A crawling cell pushing its way through pores and obstacles, while constantly changing its shape and elasticity to adapt to a complex environment, is a fascinating demonstration of biological versatility. Underlying such remarkable active and passive mechanical performance is the cell's ability to control precisely and reorganize quickly the local structural and mechanical properties of its cytoskeleton — a protein fibre network spanning any eukaryotic cell and linked to the fluid lipid membrane that acts as its skin. It is composed mainly of three classes of protein fibres of very different stiffness: actin, microtubules and intermediate filaments, which are organized into higher-order assemblies by a myriad of 'helper' proteins known as crosslinkers, bundlers, capping and severing proteins, and so on, which bind to the scaffolding elements. The regulation of the on–off kinetics of these binding proteins, the polymerization–depolymerization dynamics of the polymers and the action of molecular motors is what turns the cytoskeleton from a merely static carcass into a multifunctional protean muscle.

Over the years, genetic, immuno-cytochemical and biochemical studies have identified many different players involved in the regulation of the cytoskeleton. Physical studies concentrating on the mechanical properties of cells or *in vitro* cytoskeletal model systems have been helpful in elucidating the complex synergies and redundancies of generic physical and specific biological mechanisms in establishing the overall mechanical performance of biological tissue. Unexpectedly, such studies have revealed a remarkable universality in the viscoelastic response functions of reconstituted networks and of whole cells of different types over a wide range of timescales, which is reminiscent of glassy materials[1–3]. At the same time, the overall cell stiffness has been demonstrated to be very sensitive to cytoskeletal dysfunction, a connection that lends itself to an extremely efficient and reliable automated detection (and may possibly also be the cause) of some diseases[4].

To gain a detailed understanding of how these highly universal and specific mechanical properties of cells originate from the molecular structure of the cytoskeleton, it is essential to probe cell mechanics on multiple scales. A growing arsenal of microrheological methods (Box 1), developed to resolve the local viscoelastic properties *in vitro* and *in vivo*, has helped to gather compelling evidence that the structure and elasticity of the cytoskeleton of living cells is generally much more localized and heterogeneous, and the relation between microstructure and overall cell mechanics is much less straightforward than simple idealized models pretend. Fascinating demonstrations of functionally relevant heterogeneities are provided by the formation of filopodia (finger-like extensions of the cytoplasm sent out by the cell in motion), in which

Box 1: Microrheology

In general, the mechanical properties of homogeneous materials are determined by applying stresses and measuring the resultant strains (or vice versa). In the regime of linear response, the ratios between stresses and strains — the viscoelastic moduli (or compliances) — are material properties that may show some frequency dependence. They may alternatively be determined from the spectrum of thermal fluctuations. Macroscopic rheological methods are widely used across many disciplines, but the softness of cytoskeletal networks, their limited availability, and the need to resolve the mechanical properties of their local heterogeneities have motivated the development of microrheological methods.



In the case of active microrheology, magnetic forces are applied and from the resulting displacements (in and out of phase) of magnetic particles in the system, the frequency-dependent suceptibilities are determined[60,61]. These may be transformed into 'local moduli' for a more direct comparison with the macroscopically measured moduli. Typically, forces applied are at the piconewton scale: only special setups allow the application of higher forces, up to a few nanonewtons[7].

Observing the induced motions of suspended non-magnetic beads yields the deformation field, and thus enables a more-detailed investigation of local heterogeneities and stress propagation[62]. This method (pictured), which is based on a combination of 'active' field-driven particles and passive tracer particles, was more recently developed into a purely passive technique, now widely known under the name of two-point microrheology[63]. The thermal motion of single particles is determined by either video microscopy or high-speed interferometric detection. Assuming a generalized Stokes–Einstein relation, the frequency-dependent moduli can be extracted[34,64]. Depending on the bead radius and detection limit, moduli from 10^{-3} to 10^{2} Pa are measurable.

When microrheological techniques were introduced, discrepancies between the measured network elasticity and macroscopically determined moduli were poorly understood. But it has since been shown that the local compliances depend on the size ratio of the colloidal particle and on some characteristic length scale of the network or of its constituents[39,65]. Paradoxically, a detailed theory of the microrheometry for such heterogeneous networks seems to require a complete understanding of the network mechanics that are to be studied by this method.

the local activation of different actin-binding proteins enables the dynamic generation of highly localized meso-structures[5,6] (Fig. 1). Yet, the sheer biological complexity of living cells makes microscopic approaches to cell mechanics a difficult task[2,7–9].

The isolation and study of purified subunits, which might be called 'functional modules', seem a promising way out of this dilemma[10]. Such an approach is not necessarily as unnatural as it may at first sound. In fact, a substantial specialization of the various constituents of the cytoskeleton seems to be instrumental for fulfilling all of its tasks. Whereas microtubules are mainly involved in transport and the process of cell division, actin and intermediate filaments are the main determinants of cell viscoelasticity. In cell motility, even the most complex orchestrated interplay of many different constituents of the actin cytoskeleton works independently of the nucleus and most organelles of cells — as dramatically demonstrated by chopped-off lamellipodia (sheet-like extensions of the cell) retaining their motility, and by bacteria and viruses exploiting parts of this cellular machinery for their own propulsion. Hence, functional modules of increasing complexity seem natural intermediates in a bottom-up reassembly of living matter. The physics of the simplest functional subunits, reconstituted *in vitro*, has over the last ten years attracted much interest from experimental and theoretical physicists. One of the current goals is, for instance, to achieve a physical understanding of well-defined composite networks confined by encapsulating lipid membranes. More-ambitious reconstructions

could include more energetic subunits, such as lamellipodia.

A related direction of research aims at what is often called *in silico* reconstitution of functional modules — the coarse-grained but faithful representation of parts of the cytoskeletal machinery on supercomputers. Among the favourite objects of study are filopodia[11] and the parasitic propulsion mechanisms of bacteria[12]. Such 'virtual labs' complement *in vitro* experiments in providing a unique testing ground for higher-level theoretical models aimed at identifying the generic features and mechanisms that physicists (and other reductionists) associate with 'understanding'.

In order of increasing complexity, and without the pretence of being comprehensive, we now summarize some of the major steps that have been taken in recent years to better understand and control the physics of functional modules of the cytoskeleton. After reviewing the current understanding of *in vitro* model systems, with an emphasis on the very recent literature, we focus on some emerging developments that might lead us closer to creating an 'assembly line' for biological cells and tissues.

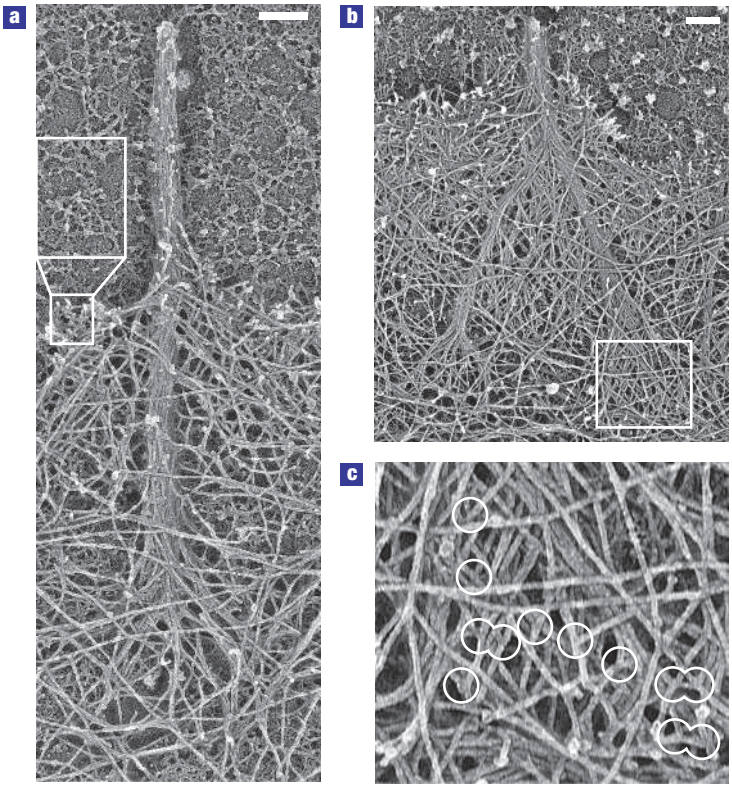


Figure 1 Biological complexity — the structural basis of cell motility. It is a major challenge to rebuild *in vitro* such complex heterogeneous and out-of-equilibrium structures as the filopodia, emerging at the leading edge of crawling melanoma cells, as shown in these electron microscopy images. **a**, A tight bundle of actin filaments, splaying apart at its root, becomes an integral part of the surrounding dendritic network of the lamellipodium. **b**, Fused filopodia, each having a splayed root. **c**, Enlargement of the boxed region in **b** at the root of the filopodium; branches at which filopodia originate are circled. Scale bars 0.2 μm. Reproduced from ref. 5 by copyright permission of The Rockefeller University Press.

ULTIMATE SIMPLICITY: PREVAILING THEORETICAL MODELS

In an effort to disentangle the complex reality, theorists like to distinguish entangled ('physical') polymer solutions, chemically crosslinked networks, and active gels. The last of these comprise biopolymer solutions and networks containing active, energy-consuming elements such as filaments undergoing treadmilling polymerization ('living polymers') or molecular motors. For each of these idealized systems, they have a minimal model that establishes a baseline against which to judge the much more complex reality. Although these minimal models are constructed in analogy with classical theories for flexible polymers, there are some subtle differences, which originate from the fact that most biopolymers resemble stiff rods rather than flexible coils. In contrast to flexible polymers, their local fluctuations and responses are highly anisotropic (Box 2), and it therefore becomes a difficult issue to decide to which deformation mode (longitudinal or transverse to the major axis) the surrounding network predominantly couples.

For entangled solutions, the minimal model follows the blob model for semidilute flexible polymer solutions[13]. The basic idea is that stiff polymers in semidilute solutions are confined to tube-like cages that suppress their thermal undulations of wavelengths longer than a length L_e, called the collision length or entanglement length. The confinement free-energy density f corresponding to this mutual entanglement is thus estimated as one unit of thermal energy, k_BT, per entanglement volume, or $f \sim k_BTca/L_e$, where c is the monomer concentration and a is the monomer (or backbone) diameter. The relation between L_e and c is established through the intuitive tube picture (Box 2), leading to $f \sim k_BTc^{7/5}$. Although additional assumptions are required[14,15] to relate f to mechanical properties such as the shear modulus of the solution, it is quite plausible and there is substantial experimental evidence that they are closely related[16,17].

In contrast, the minimal model for chemically crosslinked semiflexible polymer networks is constructed quite differently, in analogy with classical rubber elasticity[18]. In this approach, the origin of the mechanical response is assumed to lie within the affine stretching of single polymer strands of a certain length L_a. As a consequence of the much lower compliance of stiff polymers along their axis compared with the transverse direction, the chemically crosslinked networks are presumably much stiffer than entangled solutions, their elastic modulus being of the order of $k_BTca\ell^2/L_a^3$, where ℓ is the persistence length of the polymers (Box 2). All depends crucially on the choice of the scale L_a, of course, which separates the low single-polymer eigenmodes that follow the externally applied strain field affinely, from the stiff higher modes that remain essentially undisturbed. Rephrased in the language of solid-state physics, the problem is the choice of a proper (effective) unit cell. It is common practice to identify L_a with one of the entanglement length L_e, the mesh size ξ (Box 2) or an independent average crosslinker distance[19]. The inclusion of the various chemical on–off rates and compliances of common molecular crosslinkers into the description remains a major task for future modelling efforts.

All current theoretical modelling of the elasticity of both entangled and crosslinked networks is thus based on more or less plausible assumptions about how, and on which scale, the macroscopic strain field translates to the highly anisotropic individual

Box 2: The physics of stiff polymers

The minimal mathematical description of a stiff or semiflexible polymer is provided by the 'wormlike chain' (WLC) model. Compared with the common bead–spring models used to represent flexible polymers, it provides a more-faithful representation of a polymer in terms of an inextensible, continuous space curve, r_s, parameterized by arc length s. The equilibrium conformation is determined by the bending hamiltonian

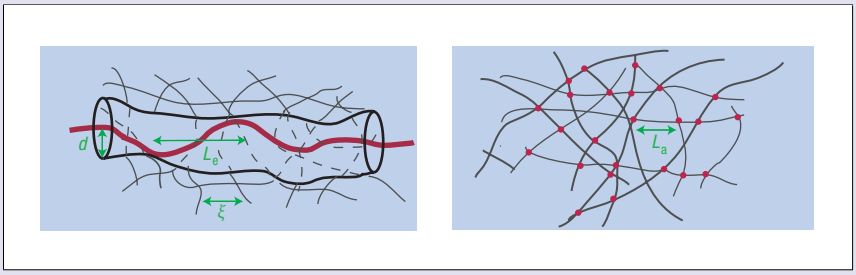
$$H = \frac{1}{2} k_B T \ell \int_0^L \mathrm{d}s (\partial^2 r_s / \partial s^2)^2$$

assigning an energy penalty of about $k_B T \ell / L$ to a curved conformation of contour length L (the term in parenthesis is simply the curvature of the contour). Here l is the persistence length, and $k_B T$ is the thermal energy.

For the relatively massive biopolymers, the product $k_B T \ell$ is often thought of as a constant material parameter characterizing the bending stiffness in the same manner as, for example, that for a chopstick. In the limit of a stiff polymer with $L \ll \ell$, the equilibrium conformation (for fixed $r^{\perp}_{s'}$) exhibits simple power-law correlations of the transverse fluctuations ($r^{\perp}_{s'}$) around the straight ground state,

$$\left\langle (r^{\perp}_{s'+s} - r^{\perp}_{s'})^2 \right\rangle \propto \frac{s^3}{\ell} \qquad (1)$$

which play an analogous role as the better-known self-similar correlations for flexible chains.



The longitudinal fluctuations of the contour are smaller than the transverse fluctuations by a factor s/ℓ. Accordingly, the response of stiff polymers to external forces is highly anisotropic too. The transverse spring constant is of the order of $k_B T \ell / L^3$, a result already known from slender rod mechanics; the longitudinal spring constant is $k_B T \ell^2 / L^4$. Because an inextensible rigid rod has vanishing longitudinal compliance, the longitudinal compliance of stiff polymers arises solely from their thermal undulations and is therefore of entropic origin — in contrast to their transverse compliance, which is purely mechanical.

Owing to the almost rod-like conformations, stiff solutions of polymer concentration n (often rephrased as $3/\xi^2 L$, which defines the mesh size ξ) have an extremely low overlap concentration $1/L^3$, corresponding to a polymer volume fraction $(a/L)^2$, where a denotes the backbone diameter. For actin, L/a is typically of the order of 10^3. According to Onsager's theory for the nematic transition, the thermodynamic properties should remain that of a dilute solution far beyond the overlap concentration up to polymer volume fractions of about a/L. As familiar from flexible polymers, entanglement effects modify this picture substantially as soon as the tube-like regions — that are, according to equation (1), required by the thermal undulations to fluctuate freely — start to overlap. The mutual steric hindrance of transverse undulations of wavelength longer than a certain length L_e, called the entanglement length, provides an effective backbone diameter $d \approx L_e^{3/2} / \ell^{1/2} \gg a$, called the tube diameter, which adjusts such that the volume fraction $nd^2 L = 3d^2/\xi^2$ of the tubes remains close to d/L_e.

In this so-called tube representation (pictured, left), the imaginary tubes are not able to fill the available space due to the steric constraints, but otherwise try to maximize their volume. It is believed that generic external deformations tend to make the overall tube arrangement less favourable, thus leading to a linear increase in confinement free-energy, which leads to the formula for the modulus quoted in the main text. The prefactor is sensitive to model assumptions concerning the coupling of the macroscopic strain field to the tubes[14–16].

For crosslinked stiff polymer networks, the prevailing picture is completely different[18] (pictured, right). One neglects the entanglement effects that govern the collective response of solutions, and assumes that the individual polymer strands are stretched affinely down to a so far unknown affinity scale L_a. The corresponding elastic modulus of a crosslinked network is then simply given by the longitudinal spring constant of the polymer strands of length L_a multiplied by a geometric factor L_a^2 and their number density. For very high frequencies of the applied deformation, the polymers will be tightly coupled to the surrounding solvent, so that L_a will drop below L_e, and the polymers will behave as if they were mutually independent on the relevant time scale[35–37].

elements. Theoretical attempts to get a firmer grip on this crucial question are so far limited to idealized random fibre networks[20,21]. Establishing a closer link between these artificial structures generated on the computer and the experimentally (or even biologically) relevant biopolymer networks is the subject of ongoing research, but the emerging bottom line seems to be that multiple elastic behaviours are possible within each model[20–23], and that competing explanations can account for the observed network response[3,24]. As relating model parameters to microstructure is still a challenging task in theoretical physics, it does limit the successful application of the models to biologically relevant questions — but it does not impair it. For example, extensions of the simple stiff-polymer rubber-elasticity model have been successful in rationalizing the nonlinear elastic and viscoelastic response of a broad selection of biopolymer gels and bundle networks[19,25,26].

Finally, active gels introduce a new level of complexity into the discussion. Not only do they inherit mechanical properties from both idealized equilibrium models (solutions and gels) and involve microstructure formation in an essential way, but also the permanent supply of mechanical energy keeps them far from equilibrium. To model active gels at a level that resolves single polymers, one has to decide how the active elements (representing the molecular motors or the polymerization–depolymerization kinetics) enter this game.

The most popular approaches[27,28] at the moment try to circumvent the substantial difficulties[29] by modelling the gels at a coarse-grained hydrodynamic level, which allows all of the intricate details to be subsumed into a set of phenomenological coefficients. This approach is routed in the hydrodynamics of flocking self-propelled particles[30], and it may help to classify the general scenarios of dynamic structure formation to be expected in such out-of-equilibrium systems. Its universal applicability relies on a strong coarse-graining, and it is not yet clear that cells exhibit a sufficiently strong scale separation to allow such a purely macroscopic hydrodynamic description. Even more than for the simple equilibrium models, it is necessary that the *a priori* comparatively complex models for non-equilibrium cellular dynamics are tested against *in vitro* or *in silico* reconstituted model systems to enable a reliable calibration of the model parameters.

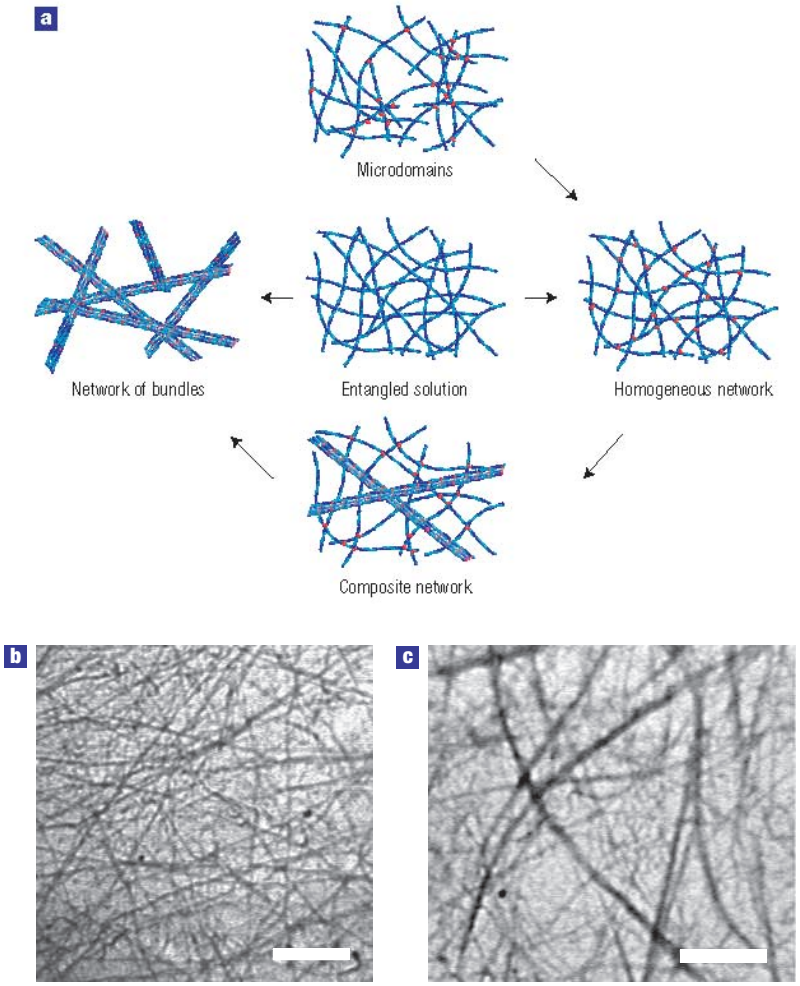


Figure 2 Physical simplicity — structural phase transitions. **a**, The addition of actin-binding proteins to polymerized actin solutions may induce various static and dynamic network heterogeneities. Depending on the specific type of crosslinker proteins (red), different microstructures are predicted and observed: microgels have been observed for actin networks crosslinked by α-actinin; bundles embedded in a continuous isotropic background network appear on addition of filamin; and depletion forces result in a direct transition from an isotropic solution into a purely bundled phase. It is possible that equilibrium phase diagrams could help to understand biological complexity. **b**,**c**, Electron micrographs of an entangled actin network (**b**) and a crosslinked composite network (**c**) in which bundles are embedded in an isotropic entangled background network. Scale bars 0.5 μm.

SIMPLICITY TO COMPLEXITY: *IN VITRO* MODEL SYSTEMS

In concert with theorists, many experimentalists have in recent years concentrated on the reconstitution of purely entangled actin solutions as close to equilibrium as possible. When physicists started to get interested in such *in vitro* actin solutions as an idealized paradigm for the more-complex biopolymer networks found inside living cells, the purification and maintenance of the purest actin solutions were already posing major difficulties[31].

Actin is a single-chain polypeptide (43 kilodaltons in molecular weight), which assembles into an asymmetric double-helical filament by a treadmilling-polymerization mechanism. The physics of actin polymer solutions and networks is determined by the long persistence length ℓ of about 16 μm, which is more than three orders of magnitude larger than the backbone diameter and comparable to, or even larger than, the typical contour length[32]. Yet, thermal undulations are not negligible but play a crucial role in endowing pure *in vitro* actin solutions with an appreciable rubber-like elastic response at volume fractions as low as 1%, which could neither be achieved with much stiffer nor with much more flexible molecules. This property is well explained by the 'tube' model for the confinement free-energy in entangled solutions, and it would be interesting to measure the osmotic compressibility of the solution as a function of concentration directly to establish

this connection more firmly. This would, moreover, serve the recently revived interest in the biological relevance of the longitudinal (osmotic compression) modes as opposed to the transverse (or shear) modes of the cytoplasm[33].

The frequency-dependence of the viscoelastic shear response of actin solutions, although of a quite common generic shape, has turned out to be quite difficult to understand. At the highest frequencies it is thought to originate from the longitudinal modes of effectively independent individual polymers[34–37], but the biologically more relevant crossover regime to the rubber plateau at around 0.5–2 Hz is still under investigation. The combination of complementary measurement techniques such as micro- and macrorheology (Box 1) and special-purpose designs[38] promise to provide more detailed insights into these puzzling questions.

A detailed comparison of micro- and macrorheological methods shows that classical one-point rheology is relatively insensitive to the polymer length, in contrast to the macroscopic strain field measured by the two-point techniques at intermediate frequencies. Yet, the results from both methods seem to converge at low frequencies with values obtained macroscopically[17,39]. This may imply that the different techniques measure the same plateau modulus at low frequencies, but couple differently to the polymers and to the surrounding solvent, and therefore probe different properties, at higher frequencies. This issue is complicated by the fact that polymers and solvent do not in general move coherently, so that beyond the much-debated question on which scale the network elasticity becomes homogeneous, it is also an open question on which scales and at which frequencies such peristaltic modes occur. Alternative explanations for the discrepancies in terms of a hypothetical depletion layer around the spheres are also discussed[14,40].

Experimentally, the next step of complication — adding chemical or biological crosslinks — is accomplished by addition of purified specific binding proteins. In general, this results in a complex (micro-) structure formation, with the occurrence of bundles or clusters, strongly depending on the molecular structure and concentration of the specific binding protein used (Fig. 2). Most experimental studies so far have concentrated on the effect of one single type of crosslinker at a time, but living cells use a large number of different binding proteins, which may interact and compete with each other. Despite attempts to quantify the concerted action of the actin-binding proteins fascin and α-actinin[41], or molecular crowding effects[42,43], we are admittedly still far from understanding the complicated structure formation observed in living cells (Fig. 1).

It is an ambitious — but unproven — physicist's conjecture that equilibrium phase diagrams that capture the generic physical mechanisms (such as spinodal decomposition, isotropic–nematic transitions and microphase separation) behind such structural rearrangements would help us to understand living structures and how they generate their local microstructures. Various structural phase transitions have been experimentally observed and schematically modelled[44–47], but detailed quantitative microscopic structural information is often elusive, owing to the difficulty or lack of adequate experimental techniques. Although it may sound rather straightforward, reliably extracting quantitative distributions of mesh sizes or bundle thicknesses with decent precision is a challenging and very laborious experimental task — especially in the most interesting, highly heterogeneous networks[48].

This is all the more frustrating, as the macroscopic and the local elastic properties are expected to depend strongly on the induced microstructures. Even if the network structure could be fully resolved, the mechanical properties of the individual constituents — such as the stiffness or internal friction of the bundles, or the compliance of individual crosslinkers — pose serious modelling challenges. For example, from classical elasticity theory, the bending stiffness of a tightly bound bundle increases with the fourth power of the bundle diameter, in sharp contrast to the quadratic increase expected for a loose assembly. With increasing thickness, thermal forces therefore become quickly irrelevant for a tight bundle, which suggests that, for example, the maximum force a filopodium can possibly withstand cannot exceed the critical Euler force for mechanical buckling of its stress fibres. A corresponding ideal behaviour of the bundle stiffness has been observed for actin filaments bundled by the protein scruin[11,49]. However, for stereocilia (the mechano-sensitive components of hair cells, such as those found in the ear), where mainly an isoform of plastin is bundling the filaments, only a quadratic relation of stiffness and diameter was observed — possibly suggesting some slip between the filaments[50].

Once the properties of the mesoscale constituents, such as the bundle thicknesses and concentrations are determined, modelling of the network of bundles can be based on the rubber elasticity model[18], at least if the crosslinks are tight and essentially permanent, as is the case for scruin[19,25]. Whether such approaches are also successful for networks crosslinked by different molecules, such as α-actinin or filamin, where an additional compliance is added to the network, remains to be seen.

Another way to approach more-realistic conditions is to consider geometric confinement effects: as most actin filaments inside cells have lengths of a few micrometres, and the cell height of a lamellipodium is of the order of 0.1 μm, geometrical confinement of the cytoskeletal networks by the plasma membrane will have severe effects on the mechanics and micro-structure formation in cytoskeletal networks. Thus the encapsulation of crosslinked actin networks into lipid vesicles[51] or other suitable cavities[52] is a next logical step in approaching a realistic biomimetic system.

TOWARDS ULTIMATE COMPLEXITY: ACTIVE GELS

Notwithstanding the difficulties discussed above, even-more-complex model systems are gradually

emerging, in which not only are more biologically relevant functionalities added to the systems but also non-equilibrium effects are deliberately introduced. From the point of view of classical polymer science, cytoskeletal networks including active elements such as the molecular motor Mysoin II, or 'living polymers' such as actin or microtubules, represent a very unusual class of polymeric systems with amazing transient and non-equilibrium properties[11,12,53–56]. Concerning their elastic properties, it has been shown that a motor-forced ballistic reptation is responsible for lowering the elastic moduli of such active gels[53]. So far, all experimental studies with such added activity are facing the major difficulty of obtaining highly purified motor preparations (with no rigor heads present) — clearly a case where faithful *in silico* models will be helpful.

The promise and success of the *in vitro* reconstitution strategy may be exemplified by the recent realization of propulsion driven purely by actin polymerization, observed in a minimal motility medium containing only an activated Arp2/3 complex for enhanced nucleation, actin depolymerizing factors (ADF, or cofilin), capping proteins (gelsolin), monomer-binding proteins (profilin), and adenosine triphosphate (ATP) as the energy source[57,58]. This opens the way for controlled quantitative study of such 'active modules'.

On the other hand, the current limitations and the comparatively primitive state of most *in vitro* and *in silico* systems is strikingly revealed by a closer look at the fascinating phenomenon of filopodia self-assembly from existing lamellipodia within living cells (Fig. 1). Cells clearly construct these network structures far from equilibrium: bundles are initiated by a filopodial tip complex; single actin filaments with individual lengths of a couple of micrometres are crosslinked by fascin, and actin bundles are formed by the convergence of filaments within a dendritic network with a strong local selection of actin-binding proteins[5]. Clearly, the impressive recent *in vitro* reconstitution of similar filopodia-like structures[59] is but the first step on the way to attaining full quantitative control over such startling complexity.

In summary, although the controlled design of biomechanical functions on a micro-assembly line may remain a distant goal for the foreseeable future, the concrete steps towards its realization in the lab and their theoretical modelling, though major tasks, are being addressed now. Tests *in vitro* and (increasingly) simulations will enable the quantitative study of functional modules with reduced and well-defined complexity. But our understanding of the physical principles underlying complex and delocalized biological functions will always rely on identifying the governing physical mechanisms, with the help of coarse-grained analytical theoretical models. The closer synthesis and convergence of the classical fields of theoretical and experimental physics, detailed numerical modelling, cell biology and biochemistry suggests itself as a natural condition for future progress.

doi:10.1038/nphys260

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Acknowledgements

We thank T. M. Svitkina for providing Fig. 1. We also thank Oliver Lieleg and Rainer Tharmann for providing figures and schematics. The work of ARB is supported by SFB413, also the support of the "Fonds der Chemischen Industrie" is gratefully acknowledged.
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Competing financial interests

The authors declare that they have no competing financial interests.