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Review

Biomimetic actuators: where technology and cell biology merge

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Abstract. The structural and functional analysis of biological macromolecules has reached a level of resolution that allows mechanistic interpretations of molecular action, giving rise to the view of enzymes as molecular machines. This machine analogy is not merely metaphorical, as bio-analogous molecular machines actually are being used as motors in the fields of nanotechnology and robotics. As the borderline between molecular cell biology and technology blurs, developments in the engineering and material sciences become increasingly instructive sources of models and concepts for biologists. In this review, we provide a – necessarily selective – summary of recent progress in the usage of biological and biomimetic materials as actuators in artificial environments, focussing on motors built from DNA, classical cellular motor systems (tubulin/kinesin, actin/myosin), the rotary motor F_1F_O-ATPase and protein-based 'smart' materials.

Key words. Biomimetic actuators; biomimetic smart materials; contractility; cytoskeleton; molecular machines; motor proteins; protein-based polymers.

Introduction

Up to the end of the last century, the naive reductionistic notion of the cell as a 'bag full of chemicals' has slowly but steadily made way for a more realistic view which recognizes the crucial role of structure in the regulation of cellular activities [1–3]. Hardly any hypothesis in modern biology has been tested as rigorously as the chemiosmotic theory, resulting in the insight that the structural coherence of the cell and its organelles is an indispensable factor for the energetics of life as we know it [4, 5]. Similarly, the structural significance of the cytoskeleton, not only as the base of cell motility [2, 6, 7], mechanotransduction [8] and cell volume regulation [9, 10] but also as a spatial organizer of metabolic pathways [11] and signalling networks [12] has begun to emerge. The increased appreciation of the significance of structure is not restricted to the level of the single cell. Today, the structure of the supracellular environment appears more important than generations of cell biologists believed; many cells behave qualitatively different in two-dimensional (2D) as compared to 3D cultures [13–15], which may have dramatic consequences, e.g. for our interpretation of the behaviour of cultured cancer cells [16]. On the molecular level, structure as a determinant of possible functions was pushed into focus by two developments. First, immense progress has been made in molecular and structural biology, which facilitated the generation of 3D models of proteins and other macromolecules at resolutions which were beyond imagination a few decades ago.

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Second, devices such as the atomic force microscope (AFM), optical tweezers, and magnetic nanobeads have enabled direct measurement of forces exerted by single molecules; 'molecular mechanics' truely has made its way from the theorist's computer to the experimenter's bench [17–20]. It can be expected that soon sophisticated models of dynamic molecular structures [21–23] will be combined with empirical knowledge of the molecules' behaviour when exposed to defined forces, allowing detailed reconstructions of molecular events and interactions that take place in living cells.

One indicator of our deepened understanding of the mechanisms and mechanics of macromolecules is the increasing frequency of engineering terminology in the cell-biological literature [24]; the famous 'rotary engine' which produces ATP in mitochondria is a case in point (see [25] for the F_1F_O-ATPase described in explicit analogy to the Wankel-type combustion engine). Ever since Giovanni Borelli's seminal *De Motu Animalium* of 1680 [26], the interpretation of organismic structures as working machines has been a powerful tool in the analysis of organismic activities (it should be noted that equating organisms to machines is a different philosophical issue that needs not concern us here). However, as the muscles of humans and domestic animals actually were the motors of most machines at Borelli's time, the analogies he postulated were far from being mere metaphors. Their frequent use rather indicates a near perfect congruence of the concepts used in contemporary physics and physiology. Similarly, the currently fashionable interpretation of biological macromolecules as molecular machines (which is not at all restricted to classical motor proteins; see [27–35]) seems more than metaphorical, hinting at an amalgamation of concepts derived from engineering and molecular biological sciences. Hardly surprisingly, this development coincides with a surge of interest in the potential which biological materials might have in the emerging field of nanotechnology.

In this context, 'biomimetic' (literally: copying life) has become a must-word that ensures public interest and boosts grant proposals. While there certainly are good reasons to consider biological materials from an engineering viewpoint [36, 37], what biomimetic actually means beyond its role as a propitiative phrase needs to be carefully evaluated [38]. In our opinion, the simplistic view of biomimetics as the transfer to technical applications of insights gained from pure biological research fails to recognize an important aspect of the interplay of science and technology. Technical analogies are not rare in textbooks of cell biology, but they seldom, if ever, describe biomimetic technologies, that is, technologies developed in a purposeful attempt to mimic biology. The opposite is true. Consider, for example, membrane transport. We commonly describe membrane function using concepts (conductivity, insulation, capacitance and so on [39]) established previously in electrical engineering. A membrane patch in a patch-clamp experiment forms part of an elaborate electronic circuit. The electronic elements of this circuit, however, do not simply facilitate observation in the same sense as binoculars do for the field ornithologist. Rather, they also provide explanatory concepts for the interpretation of the membrane's behaviour by way of technical analogy. Clearly, biological explanation frequently followed the acquisition of the ability to construct and control technical devices that could – by hindsight – be interpreted as being bio-analogous. Viewed from this angle, the widespread reference to man-made rotary engines found in papers on the F_1F_O-ATPase seems to express the hope that clues regarding the function and regulation of this particular molecular machine might be hidden in our practical skills in building and operating analogous engines in the technical world. One may wonder whether there actually are cases of truly biomimetic advances in human technology. But beyond doubt, biology has benefitted from a technomimetic mode of explanation throughout its history.

Thus, biologists pursuing pure research into the functioning of cells and molecules will have much to learn from 'biomimetic' nanotechnology and material sciences, two inherently interdisciplinary areas which are currently progressing with breathtaking speed. In this review, we summarize recent developments in the field of biomimetic actuators, i.e. molecules and materials derived from living cells which may be employed technologically as sources of mechanic force and motion. Given the immense variety of approaches explored in this field, our choice of examples is necessarily incomplete and selective, and we apologize for any omission some reader may find regrettable.

DNA as a molecular actuator

In the cell, DNA is packaged into highly condensed conformations by binding to proteins that subject it to mechanical forces which bend, twist, pull and compress the double helix. These mechanical interactions need to be understood to gain a realistic picture of the functioning of DNA in vivo [19, 40]. Novel biophysical techniques were instrumental in determining intrinsic mechanical properties such as the forces that keep double-stranded DNA 'zipped' [41, 42]. The torsional stress-releasing activity of topoisomerases has also been observed directly [40, 43]. Moreover, we have come to realize that mechanical factors play regulatory roles in DNA replication [44], transcription [45] and DNA uptake into viral capsids [33], and that the enzymes involved are best envisaged as molecular motors.

Given the specific pairing ability of complementary stretches of DNA molecules, it is hardly surprising that

DNA attracted the attention of scientists interested in the production of nanostructured architectures by self-assembly [46]. Various kinds of 'clonable' 2D and 3D structures have been created which may serve as scaffolds for the alignment of functional macromolecules [46, 47]. Surface-attached DNA arrays have been employed as templates for conducting nanowires ('molecular lithography,' [48, 49]). Quite unexpectedly, at least when considering its genetic function, DNA has proven to be a promising material with which to construct nanomachines.

DNA nanoactuators have been built from DNA molecules which switch between two stable conformational states, depending on controllable parameters such as the ionic composition of the medium or the presence of complementary single-stranded molecules. The switching process is usually monitored by fluorescence resonance energy transfer (FRET) spectroscopy [50], using two different fluorescent dye molecules attached to the DNA at specific positions which have different distances between them in the two stable conformations. So far, three approaches have proved successful. The first one may be exemplified by the first DNA nanomachine reported [51]. Here, three DNA molecules were used to construct two linear DNA arrays stabilized mechanically by double crossover (DX) structures, and a connection between these two 'handles' by a conventional double-stranded portion. This linker consisted of the base-paired sequence $d(CG)_{10}$, which readily performs the conversion from the right-handed helical B-conformation to the left-handed Z-state at high ionic strength. As a result of this transformation, the two DX-stabilized handles rotate around their longitudinal axes, causing a change of twist of about 200° relative to each other.

The second approach exploits the ability of DNA to form complementary double strands, and the fact that double-stranded and single-stranded DNA differ greatly in flexural persistence length, a measure of bending stiffness [40]. The devices constructed consist of relatively stiff double-stranded portions, which alternate with more floppy single-stranded stretches. Addition of single-stranded 'fuel-DNA' (F) complementary to the single-stranded stretches of the nanomachine stiffens these stretches, thereby inducing alternative conformations. The original state is then reestablished by adding a single-stranded complement (F′) of the 'fuel'-sequence that competes with the actuator for binding F. Based on this principle, DNA tweezers [52] and an expansive nanoactuator [53] have been constructed. In the latter case, the nanomachine consisted of two rigid, double-stranded arms connected by a short flexible hinge on one side, and a long single-stranded linker on the other; this device had the shape of an irregular circle (fig. 1 A). Addition of the F-sequence induced a straightened linear conformation, which was reversed by adding F′. It should be noted that during each expansion-contraction cycle one molecule each of F and F′ are consumed, and that double-stranded F-F′ appears as a waste product (fig. 1 A). The forces generated by this actuator have been estimated to be well in the range of those exerted by biological motors such as RNA polymerase, or actin and tubulin polymerization [53].

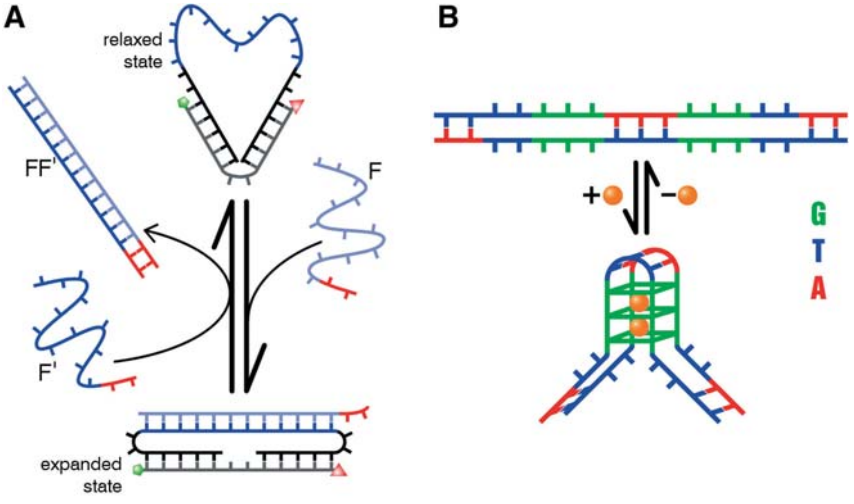

Figure 1. Molecular actuators built of DNA. (*A*) Expansive nanoactuator. In the relaxed state (top), the device consists of two rigid arms of double-stranded DNA (composed of three DNA molecules shown in black and grey) connected by a short single-stranded hinge and a long single-stranded linker (dark blue). Addition of fuel DNA (F; light blue) causes the circular molecule to expand, as the fuel and the linker form a rigid double strand (bottom). Complementary fuel (F′; dark blue) competes with the linker for F-binding and reestablishes the relaxed state of the circular molecule. Expansion of the molecule is monitored by FRET (chromophores shown as green pentagon and red triangle, respectively). Adapted from [53]. (*B*) DNA condensation by G-quartet formation. A stretch of double-stranded DNA with several base mismatches is shown on top (bases colour-coded as indicated on the right). Sr^{2+} (orange spheres) greatly stabilizes stacked G-quartets, and consequently leads to a more condensed conformation of the DNA (bottom). The process can be reversed by addition of a chelator which binds Sr^{2+}. Adapted from [55, 58]; see text for details.

An entirely different mechanism, which appears more economical as it does not necessarily rely on a supply of a macromolecular fuel, is employed in the third approach. Pairs of guanosin mismatches in double-stranded DNA can form stable guanine base quartets, in which four guanine moieties build a planar structure stabilized by eight hydrogen bonds [54]. The stability of stacked pairs of G-quartets is greatly enhanced by specific cations which fit into the cavity formed by the two quartets. This interaction is so specific that stacked quadruplexes can actually be used as ion-specific sensors [55]. The formation of intramolecular G-quadruplexes induces a compaction of the DNA molecule, as it establishes hairpin loops by clamping together more or less distant regions of the linear molecule (fig. 1 B). DNA contraction by reversible G-quadruplex formation has been demonstrated in experiments in which the reaction was controlled by the addition of complementary DNA sequences [56, 57] in a similar fashion as described above. However, controlling quadruplex formation by regulating the concentration of quadruplex-stabilizing cations is a more elegant method [55]. Fahlman et al. [58] have employed Sr^{2+}, levels of which are readily controlled using chelators, to reversibly induce DNA hairpin structures by the establishment of stacked G-quartets. This technique holds great promise, as the incorporation of quadruplex-forming sequences into DX-stabilized 3D-arrays of DNA is expected to result in smart materials that contract depending on ionic conditions [58].

Microtubule-dependent motor proteins

Microtubules are one of the three main classes of filaments of the eukaryotic cytoskeleton [6]. The building blocks of microtubules are dimers of α- and β-tubulin. The dimers attach to each other in a head-to-tail fashion to form protofilaments. In vivo, one microtubule consists of 13 protofilaments, but a variety of distinct conformations has been observed in vitro. Microtubules are hollow tubes of 25 nm diameter and a wall thickness of about 6 nm. They are polar, with the so-called plus-end at that side to which the β-subunits of the $\alpha\beta$-dimers point, and the minus-end on the opposite side. Due to the arrangement of the tubulin modules, the microtubule surface forms a slightly helical lattice of 8 nm periodicity. Microtubules are highly dynamic because of rapid polymerization or depolymerization of tubulin dimers; these processes usually occur at different rates on the two poles of the tube. In vivo, microtubule stability is regulated by several classes of microtubule-associated proteins (MAPs). In vitro, stabilization can be achieved by addition of chemicals such as taxol (for recent reviews on microtubule structure and dynamics, see [59–63]).

Microtubules play vital roles not only in cellular movements and cytokinesis, but also provide the tracks on which organelles, chromosomes, membrane vesicles and macromolecules are transported to their appropriate location within the cell [6]. Transport is mediated by motor proteins, which harness the chemical energy provided by ATP hydrolysis to move along the microtubule. The two major classes of microtubule-dependent motor proteins are distinguished by their polarity: kinesins (except for members of the ncd family) move towards the plus-end, while dyneins travel towards the minus-end of the microtubule [64]. Although dynein action has become better understood recently [65], the significance of this type of actuator for the development of biomimetic technologies has been limited so far [66], at least when compared to kinesin [36, 67, 68]. In the following, we therefore will concentrate on the latter.

The ever-growing family of kinesins includes a great number of structural and functional variants which are united by the common possession of highly conserved sequences in their motor domains [64, 69–71]. Conventional kinesin, the family's paradigmatic founding member, is probably the best-known microtubule-dependent motor protein to date. In the functional state, it is a homodimer. Each monomer consists of a motor domain or head which harbours one binding site each for ATP and the microtubule. The motor domain is connected to an α-helical neck by a highly flexible neck linker. On its other end, the linear neck connects to an even longer linear domain, the stalk, via a flexible hinge. The so-called tail on the far end of the stalk includes the cargo binding sites [72, 73]. Thus, the kinesin dimer has the shape of an elongate, very flexible clamp with attachment points for the track on which it travels and for the cargo it carries at its opposite ends. Kinesin motion proceeds in 8-nm steps by a processive hand-over-hand mechanism; the kinesin dimer virtually walks along the microtubule putting one motor domain before the other. In this process, the motor domains take turns in binding the microtubule, ensuring permanent contact of the motor with its track [73–75]. It is not well understood to date how the successive events of ATP binding, ATP hydrolysis and release of ADP/P_i at the ATP binding sites of the two motor domains are coordinated, so that conformational changes result which let the motors alternate in playing the role of the standing and the free leg [76, 77]. It seems clear, though, that the neck linker and the neck are crucial mechanical elements of the walking apparatus, and that their structure determines the directionality of kinesin movement [73–75, 78–81].

Under appropriate chemical conditions, kinesin moves along microtubules in vitro, which has provided a straightforward approach to analyzing kinesin activity in so-called motility assays. These assays exploit the tendency of microtubules and kinesin to adhere to certain surfaces (see [82] for a discussion of technical details). In principle, two types of motility assays can be distinguished (fig. 2 A). First, in the gliding geometry, micro-

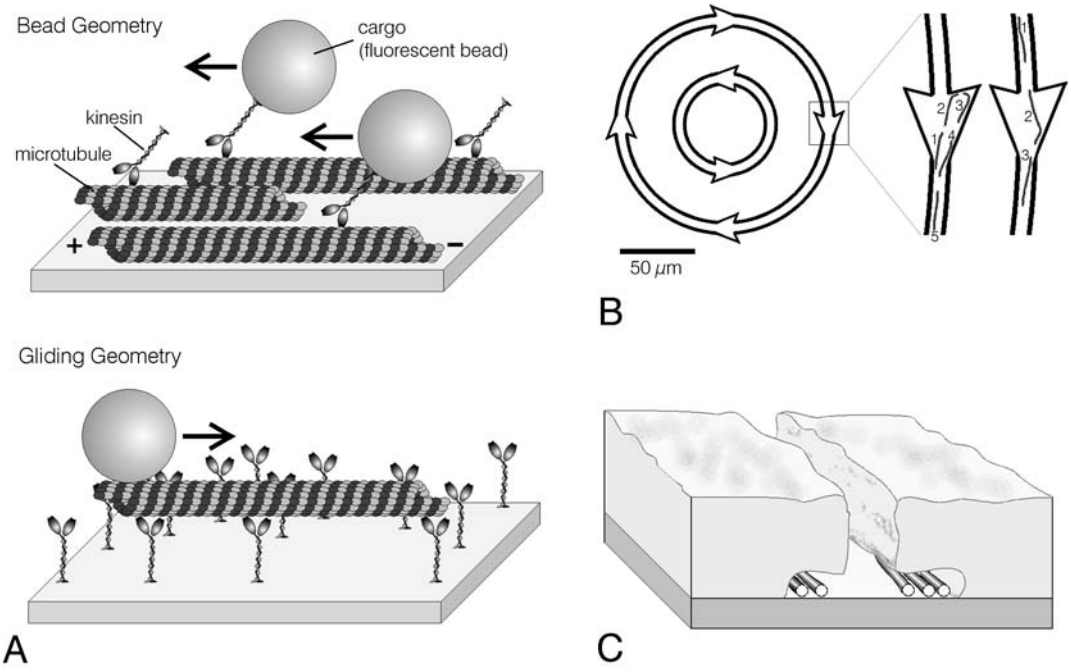

Figure 2. Microtubule-based motility assays. (*A*) The two possible geometries of motility assays; minus-ends of all microtubules shown point to the right. In the bead geometry (top), microtubules are adsorbed to an appropriate surface. The movement of kinesin motors attached to microscopically visible cargo can then be monitored. In the gliding geometry (bottom), the surface is covered by kinesin on which fluorescently labelled microtubules move. Cargo may be attached to these gliding microtubules. (*B*) In the gliding geometry, microtubules can be forced to move in a desired direction by structural features of the substrate. On the left, two circular microchannels are shown in which microtubules will move clockwise (outer circle) and counterclockwise (inner circle), respectively. Magnified sketches on the right show how the movement of microtubules is directed by the arrowhead structures of the channel side alls; the position of individual microtubules at consecutively numbered points in time is indicated. Adapted from [91]. (*C*) Topographical confinement of gliding microtubules to the bottom of a microchannel produced by microlithography. The undercuts at the bases of the side walls prevent microtubules (shown here as hollow tubes) from leaving the channel. Adapted from [96]; not to scale.

tubules glide along surfaces to which kinesin is bound. This process can be easily monitored if fluorescence-labelled microtubules are used. Second, in the so-called bead geometry, microtubules are bound to a surface and the movement of kinesin-coated microbeads on the microtubule tracks is monitored. The advantage of this approach is that relatively stable systems can be constructed, since kinesin readily moves along microtubules fixed with glutaraldehyde [83]. Moreover, observations and measurements on the level of individual motor protein molecules are possible in this setup (e.g. [84]).

Given the highly artificial setting of these experiments, it can hardly be surprising that efforts were made to develop motility assays into techniques to transport and deliver items at the micro and nano-scale [68]. Such 'nano-railway-systems' have to solve problems similar to real ones. The cargo must be loaded onto the vehicles at a given location, be transported to another specified location during a defined interval of time and be released from the vehicles in a regulated manner. With this concept in mind, biotin-streptavidin binding has been used to couple cargo to moving microtubules, and ultraviolet (UV)-mediated release of caged ATP has been tested as a means to control transport velocity [85]. However, the majority of recent studies focussed on the control of directionality in microtubule-kinesin transport systems.

In the bead geometry, a partial alignment of microtubules could be achieved on linearly patterned surfaces and under fluid flow fields [86]. Kinesin-covered silicon microchips were found to move along arrays of flow-aligned microtubules, but also to frequently rotate on the spot, indicating that the aligned microtubules differed in polarity (i.e. the plus-ends of different microtubules pointed to different directions [67]). The same group later demonstrated the generation of aligned and isopolar microtubule arrays by fluid flow, using antibodies specific for the microtubule minus-end by which freely floating microtubules were linked to the surface before the onset of flow [87]. Following an alternative approach, the group of E. Unger started with a gliding geometry setup to achieve a polarized array of microtubules for bead geometry experiments. They noticed that the leading minus-end of microtubules moving over a field of surface-bound kinesin motors frequently detached slightly from the surface before it made contact with new motor proteins. Strong fluid flow tended to drag the detached minus-ends in its direction, leading to a self-alignment of the moving microtubules with their plus-ends facing upstream [88].

In a second step, the ordered polar arrays were fixed with glutaraldehyde to stabilize the polar arrangement, and could then serve as tracks for kinesin movement [89]. Such a system can be used for kinesin-mediated transport over distances which greatly exceed the length of any individual microtubule.

The problem of directionality has also been addressed in gliding geometry architectures. Initially, poly-tetrafluoroethylene films onto which kinesin bound preferentially along linear scratches were tested [90], followed by polyurethane surfaces with micrometer-sized channels which acted as guard rails for travelling microtubules [85]. However, microtubules did not move unidirectionally, and frequently left the tracks they were supposed to follow. A solution to these problems was offered from the group of T. Uyeda, who let microtubules migrate in channel systems manufactured by microlithography [91]. Some of the photoresists used in this production procedure proved to bind functional kinesin very poorly. Fortunately, the walls but not the bottom of the microchannels consisted of this type of resist material, which tended to keep the microtubules inside the channels once they were in. Unidirectionality was achieved by special structures of the side walls, which redirected the movement of microtubules that bumped into them. Amusingly, arrowheads pointing into the desired direction proved particularly efficient (fig. 2B). This and alternative systems have been analyzed in depth in subsequent theoretical and empirical studies [92–95]. Most recently, the topography of the guiding channels has been modified by making undercuts at the bases of the channel walls (fig. 2C) [96]. Moving microtubules concentrate in these crevices to reach the high densities typical of axons, and show no tendency to leave the channel by climbing up the wall. However, it must be expected that the size of the cargo which can be transported is limited by the height of the undercuts (200 nm in [96]).

Practical applications of the microtubule/kinesin system are beginning to emerge. Moving microtubules were used to spread out individual DNA molecules to which they were connected via biotin/streptavidin [97]. This method may become important for the ‘molecular lithography’ mentioned above, which relies on accurate positioning of DNA strands. Fluorescent motile microtubules have been employed to create negative images of inaccessible regions of a microtopography [98], and a mixture of fixed and motile microtubules was used to quantify the molecular forces acting between microtubules and beads bound to them (pN forcemeter [99]). In the last two cases, it is not immediately obvious why microtubule-based systems should have advantages over existing technologies. However, the fact that biotechnologists have started to ‘play around’ with this system indicates that the exploration of the technological potential of microtubule/kinesin actuators has entered a new phase.

Actin-dependent motor proteins

The actomyosin system is ubiquitous in eukaryotes and provides the paradigm of organismic motility, the muscle [6]. Despite their biochemical, functional and regulatory distinctiveness, the actomyosin system resembles microtubules and their motor proteins in many ways. Actin filaments, also known as microfilaments, consist of two helically wound rows of actin monomers, and can show highly dynamic behaviour in vivo due to rapid polymerization and depolymerization (for recent reviews on actin dynamics see [100–102]). Microfilaments are polar, with a so-called barbed end and an opposite pointed end (sometimes referred to as plus- and minus-end, respectively). In cells, microfilaments may organize into bundles, stress fibres, or networks, and play important roles in cell motility, cytokinesis and cell attachment to substrates by focal adhesions [6]. Myosins are a highly diverse class of motor proteins that hydrolyse ATP to move along microfilaments [64, 103–105], mostly towards the barbed end (except for myosin VI; for recent reviews of myosin action, see [79, 81, 106–109]).

The technologically motivated development of classical motility assays into devices for controlled microscale transport has not been as rapid in actomyosin systems as in the case of microtubule/kinesin. This is due to inherent characteristics of actin and myosin. First, the vast majority of the studies published used the most readily available myosin, heavy meromyosin, a truncated version of myosin II from mammalian skeletal muscle. Myosin II is non-processive, that is, the motor detaches from the actin filament after every step [107, 110]. Thus, continuous transport is only possible if numerous myosin II molecules are linked to form a mechanically coherent structure (as in a muscle). This may be irrelevant in the gliding architecture, where filaments move on carpets of myosin, but it certainly complicates things in the bead architecture, where myosin-coated cargos move along actin tracks. Moreover, the actual step size of any non-processive motor protein molecule on a filamentous track obviously is undefined. Due to the helical surface structure of actin filaments, motors with step sizes that differ from the length of the actin helical repeat will tend to follow a helical path around the filament. In gliding architecture experiments, actin filaments rotate as they travel on myosin II layers [111]. Any cargo that might be attached to the moving filament will therefore sooner or later inhibit movement. These difficulties should be resolved if myosin II would be replaced by myosin V, a processive motor with a step size that matches the actin helical repeat [79, 108]. To our knowledge, there is only one report of myosin V being employed in a technologically motivated motility assay [112].

The second problem originates from the material properties of actin. The persistence length, a measure of bend-

ing stiffness, is several hundred times smaller in actin filaments than in microtubules [113]. For this reason it is much harder to guide actin filaments along microscale tracks, as their floppiness enables them to perform U-turns [92].

So far, some progress has been made in controlling the directionality of actin filament movement on myosin-coated surfaces. Filament motility has been studied on nanostructured surfaces [114–116], and the influence of material properties of the surface on myosin deposition has been scrutinized [116, 117]. Microscale patterns of surface hydrophobicity were shown to induce corresponding patterns of myosin density, resulting in a partial confinement of motile actin filaments to hydrophobic areas [118]. Noteworthily, actin filament alignment and movement could be directed by electric fields [112, 119].

F_1F_O-ATPase, a rotary motor

Several variants of the F_1F_O-ATPase are present in the membranes of mitochondria, chloroplasts and prokaryotic cells, where they convert transmembrane electrochemical proton gradients into ADP~P bonds and vice versa. To date, the F_1F_O-ATPase is probably the best-understood biological molecular motor. It consists of two major complexes, F_1 and F_O. F_O contains a 'roundabout' structure built of a variable number of c-subunits, which, together with the a-subunit, form a transmembrane proton channel (fig. 3A). Proton flux through this channel lets the roundabout rotate relative to the a-subunit. In the complete enzyme, the rotation is transmitted to the rod-shaped γ-subunit, which is rigidly linked to the c-subunit roundabout. The γ-subunit projects into the central cavity of the circularly arranged three $\alpha\beta$-dimers of the F_1 complex, which cannot follow the rotation as they are firmly connected to the a-subunit via the b-subunit (fig. 3A). Each $\alpha\beta$-dimer carries one catalytic site for ATP/ADP conversion; the cyclic conformational changes forced on the dimers by the eccentrically rotating γ-rod drive the synthesis of ATP. Thus, from an engineering viewpoint, F_1F_O-ATPases are rotary engines consisting of a rotor and a stator (not to be identified with F_1 and F_O! fig. 3A), which transform vectorial gradients of electrochemical potential across the membrane in which they are embedded into chemical energy via a mechanical intermediate (torque). Noteworthily, this engine works equally well in reverse mode, hydrolyzing ATP to generate a proton electrochemical gradient. For more detailed summaries, see [25, 120–126].

During the last decade, the F_1F_O-ATPase has become the cliché example of a molecular machine. It has been likened to structures as divergent as combustion engines [25] and rotaxane molecules [127], and there is no shortage of optimistic assessments of the enzyme's potential for biomimetic nanotechnology (e.g. [126]). However, amidst a wave of euphoric metaphors, one should realize what actually has been achieved so far. As with the motility assays discussed above, research into the technological potential of the F_1F_O-ATPase started from an experimental setup that had proved indispensable in the elucidation of the enzyme's mode of action. The fact that the stator and rotor portions of the F_1F_O-ATPase rotate with respect to each other was firmly established when the

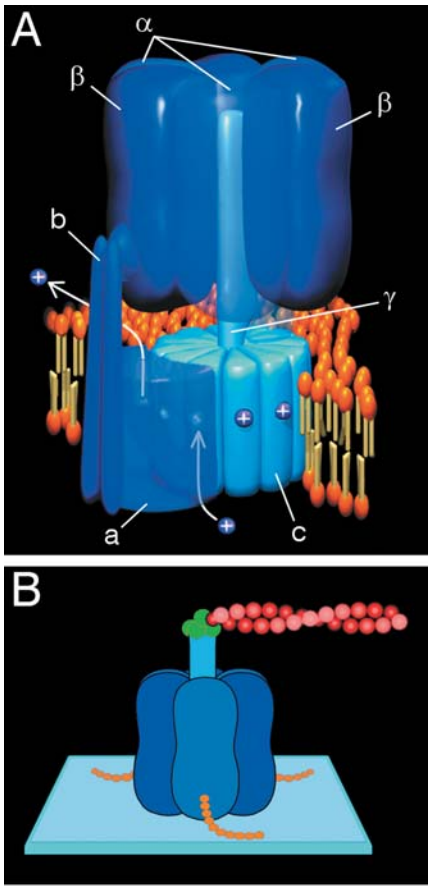

Figure 3. The F_1F_O-ATPase, a rotary engine. (*A*) Simplified model of the F_1F_O-ATPase from inner mitochondrial membranes. The F_O portion (subunit nomenclature is based on Arabic letters) is embedded in the membrane, while F_1 (subunits marked by Greek letters) protrudes into the mitochondrial matrix space. The elements shown in light blue can rotate continuously with respect to those shown in dark blue without damaging the structural coherence of the complex. In the ATP-synthesis mode, protons enter the enzyme from the intermembrane space and leave it into the matrix as indicated by arrows. This flux of protons along their electrochemical potential gradient in respiring mitochondria drives the rotation of the c-subunit 'roundabout' (anti-clockwise if viewed from top of this model). The γ-subunit stalk rotates eccentrically in the central cavity of the $(\alpha\beta)_3$-complex, forcing the conformational changes in the three ADP/ATP binding sites that drive ATP synthesis. (*B*) Cartoon of an isolated F_1 complex as used in nanotechnological studies. The complex is fixed headfirst on a surface by His tags (orange). The rotation of the γ-stalk after addition of ATP can be visualized, e.g. by a fluoroscently labelled actin filament (red) attached to it. Modified screenshots from an animation published in [163].

isolated F_1 complex was fixed headfirst on a glass surface by means of histidine tags attached to the $\alpha\beta$-subunits, and the ATP-driven rotation of the γ-subunit was made visible by attaching a fluorescent actin filament to it (fig. 3B; [128, 129]). C. Montemagno and his group have built on this approach [130] and combined inorganic and biological materials in a hybrid system to produce 'nanoventilators' [131, 132]. These devices consisted of F_1 complexes that rotated blades ($\sim 0.15 \times 1$ µm) made of silicon wafers (instead of actin as in the original experiments). To reduce undesirable interactions between the blade and the substrate on which the motor stood, the F_1 complexes were placed on Ni-posts (about 200 nm high) that had been created by electron-beam lithography. More recently, the same group introduced an artificial allosteric control element into their device, by which the F_1 complex could be switched on and off at will [133]. They took advantage of the fact that specific Zn^{2+} binding sites can be created in proteins by site-directed mutagenesis [134, 135], and inserted such a site at the interface between the α- and β-subunits where it interfered with the cyclic conformational changes occurring during ATP hydrolysis. The result was complete prevention of blade rotation in the presence of Zn^{2+}, which was reversed when Zn^{2+} was removed by a chelator [133]. This study marks a significant step, as it demonstrates a straightforward way to introduce a simple control mechanism of motor activity that is independent of the fuel supply (ATP, in this case).

Smart materials

In the fields of robotics and microscale technology, there is an increasing interest in actuators made of 'smart materials'. These materials contract and expand in response to chemical or physical stimuli such as changes of pH or the concentration of specific ions, irradiation, electric fields and others [136–141]. Smart behaviour has been studied in several classes of polymers, but polyelectrolyte gels, including polyacrylic acid and its derivatives, are particularly interesting in the present context. The molecules of these gels carry groups that may be ionized, which turns the gel into a Donnan phase with fixed charges that attract mobile ions of opposite charge. The resulting accumulation of ions causes osmotic swelling of the gel, the degree of which obviously depends on the degree of ionization of the polymer molecules. Any factor affecting the degree of ionization therefore controls gel swelling. When pH-mediated contraction of polyacrylic-acid derivatives was first described in the middle of the last century, the artificial contractile gel actually was presented as a possible model of muscle contractility [142–144; see also 145]. In contrast to the modern metaphor of smart materials being 'artificial muscles', this analogy originally meant to express a basic similarity of the molecular mechanisms of contraction, and defined a research strategy to elucidate the mechanism of muscular action. While it later turned out that the analogy did not hold, this episode provides another example of technomimetic reasoning in a supposedly biomimetic field.

Peptides have been used to produce smart actuators. Blocks of chemically cross-linked gels of actin and myosin were shown to slide past each other [146], but the most convincing cases of truly contractile peptide polymers with obvious technological potential are based on artificial peptide sequences. A large number of investigations into the mechanisms of energy transformations that can be performed using protein-based polymers have been published during the last decades (see [147–149], and references therein). These polymers are defined as repeating peptide sequences, where the repeating unit can be as few as two or as many as hundreds of amino acids, and in which the repeating unit may recur but a few times or as many as hundreds of times [148]. A natural prototype of such a peptide is found in the mammalian extracellular matrix component, elastin, the elastic properties of which are attributed to its repetitive stretches of the polypentapeptide $(\text{valine-proline-glycine-valine-glycine})_n$. In our present context, it is most important that such polymers were shown to exert mechanical forces by contraction in response to temperature shifts, pH changes, alterations of the ionic composition of the medium or changes of pressure [147]. D. Urry and colleagues have refined the theory of the energetics of these processes, which, as these authors suggest, may be of paramount importance for the interpretation of actuation by peptide polymers in general (see e. g. [148]), but which presently is hardly taken notice of by biologists. The theory is based on inverse temperature transition, a phenomenon frequently observed in protein-based polymers. Upon exceeding of a critical temperature (the transitional temperature, T_t), the molecular order of these polymers increases, as indicated by their crystallization from solution, fibril formation [147, 148] or the postulated development of intramolecular structures such as β-spirals [147] or β-sheets [150]. The apparent paradox of increased molecular order at raised temperature is resolved if the hydration water of the polymer is taken into account. In the vicinity of hydrophobic peptide residues, water forms highly ordered structures. Increased thermal energy destroys these 'nanoscopic icebergs', forcing the hydrophobic residues to reorganize into more densely packed clusters. Thus, the increase in molecular order of the polymer with increasing temperature is outweighed by the decrease in the order of the hydration shell, which is turned into bulk water. This process may result in reversible contractions of macroscopic polymer bodies [147–149, 151]. No-

tably, T_t of a given peptide polymer can be shifted significantly by alterations in physicochemical parameters such as pH, ionic strength and others, which is why peptide polymer contraction can be induced by these factors under isothermic conditions [147, 148].

Protein-based polymers have a number of advantages compared to other materials [148, 152]. However, the technological potential of polypeptides actually is hampered by what at first sight would seem a distinctive asset. The high diversity of monomers (20 biologically relevant amino acids) allows for practically unlimited variability of chemical structures, which is the reason for the wide variety in protein function observed in nature. On the one hand, this should enable the construction of peptide polymers with tailormade properties. On the other hand, because of the multitude of possible interactions between the building blocks of peptide chains, the properties of even the smallest artificial proteins are hardly predictable. Therefore, natural proteins are indispensable models for artificial contractile polypeptides with useful technological functionality.

In living organisms, motility is mostly based on mechanisms that rely on the hydrolysis of nucleoside triphosphates as a source of energy. While such systems have their merits in nanobiotechnology as discussed in previous sections, their dependence on a relatively strictly defined chemical environment can be a drawback [68]. However, the number of currently known biological polypeptides (other than elastin) that may serve as models for ATP-independent, 'smart' peptide polymer actuators is small. For example, nematode sperm cells crawl despite lacking a functional actin cytoskeleton, utilizing forces that result from the reversible, probably pH-regulated polymerization of the unique main sperm protein (MSP [153, 154]). Although the MSP filament network displays intriguing properties, it does not seem understood well enough to date to allow an evaluation of its potential value as a prototype of biomimetic actuators.

Two unusual biological contractile protein systems have been described which have the advantage that functional units of sizes that allow easy handling can be isolated directly from living cells. First, the spasmonemes of sessile ciliates are protein threads which enable these protozoans to rapidly reduce the effective length of their stalks, facilitating avoidance of adverse conditions or predators [155, 156]. Second, the so-called forisomes, protein bodies in the sieve tube system of leguminous plants, undergo contraction-expansion cycles (fig. 4), thus reversibly forming plugs in this long-distance transport network through which vascular plants accomplish the distribution of photo-assimilates throughout their body [157–159]. Contraction in spasmonemes as well as forisomes is driven by changes in the free concentration of calcium ions. Several facts point to a distinctiveness

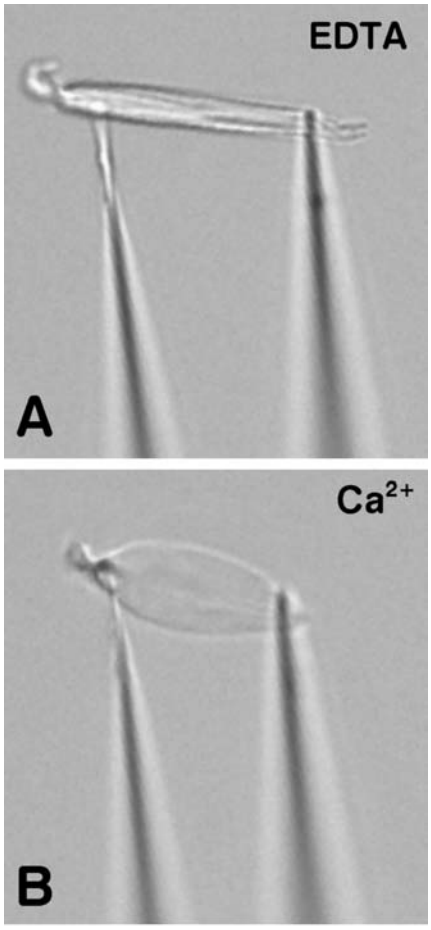

Figure 4. Micrographs of a contracting forisome as an example of a biological 'smart' material which transforms a change in the chemical potential of a particular ion species into mechanical work. (*A*) A forisome isolated from a sieve tube of a *Vicia faba* L. leaf is fixed between two micropipettes (protruding into the images from the bottom); in the presence of the chelator EDTA, the forisome shows its typical elongate shape with an aspect ratio (length/width) of about 10. (*B*) Addition of excess Ca^{2+} causes the forisome to contract anisotropically, changing its aspect ratio to about 3. Note that the contracting forisome bends the rigid glass pipettes; the distance between the tips of the pipettes is reduced from 13.3 µm in (*A*) to 10.2 µm in (*B*). This reaction can be completed in less than 0.1 s, and is reversible upon removel of Ca^{2+} by excess chelator. For experimental details, see [160].

of the two smart bioactuators: forisome contraction is anisotropic [160], whereas spasmonemes contract isotropically [161]. No pH-dependent contraction has been reported so far from spasmonemes, while unphysiologically high and low pH values cause forisomes to contract [160]. This feature allows forisome contraction to be electrically controlled by diffusional electrotitration [160], underlining the high technological potential of forisome proteins [162].

Concluding remarks

Our survey of the potential of biological macromolecules and polymers for use as actuators in technological contexts shows that current research does not generally follow the concept of biomimetic technology in the strict sense. Current projects range from modified motility assays in which biomolecules work in a fairly bioanalogous way, to the development of DNA-based smart materials that contract by ion-regulated G-quartet formation, a mechanism which – at least according to current knowledge – has no analogue in living cells. It is fascinating to see how not only experimental approaches but also theoretical concepts merge in this no-man's land between established disciplines. As biologists, we expect that our picture of the cell as a mechanically coherent structure will be strongly influenced by future developments in this truely interdisciplinary field.

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