Engineering with biomolecular motors

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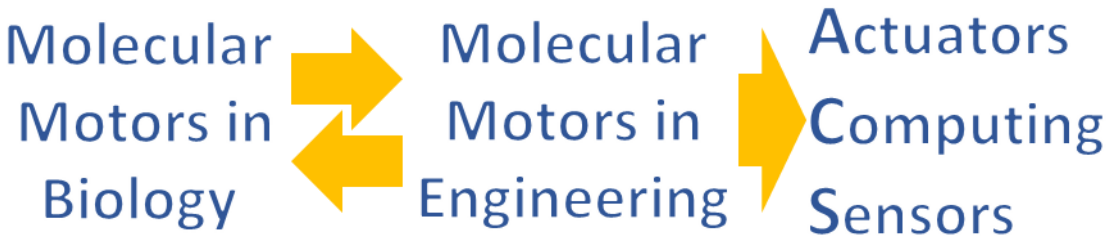
CONSPECTUS



Biomolecular motors, such as the motor protein kinesin, can be used as off-the-shelf components to power hybrid nanosystems. These hybrid systems combine elements from the biological and the synthetic toolbox of the nanoengineer and can be used to explore the applications and design principles of active nanosystems. The efforts to advance nanoscale engineering benefit greatly from biological and biophysical research into the operating principles of motor proteins and their biological roles. In return, the process of creating *in vitro* systems outside of the context of biology can lead to an improved understanding of the physical constraints creating the fitness landscape explored by evolution. However, our main focus is a holistic understanding of the engineering principles applying to systems integrating molecular motors in general.

To advance this goal, we and other researchers have designed biomolecular motor-powered nanodevices, which sense, compute and actuate. In addition to demonstrating that biological solutions can be mimicked *in vitro*, these devices often demonstrate new paradigms without parallels in current technology. Long-term trends in technology towards the deployment of ever smaller and more numerous motors and computers give us confidence that our work will become increasingly relevant.

Here, our discussion aims to step back and look at the big picture. From our perspective, energy efficiency is a key and underappreciated metric in the design of synthetic motors. Based on

an analogy to ecological principles, we submit that practical molecular motors have to have energy conversion efficiencies of more than 10% - a threshold only exceeded by motor proteins. We also believe that motor and system lifetime is a critical metric and an important topic of investigation. Related questions are if future molecular motors by necessity will resemble biomolecular motors in their softness and fragility and have to conform to the "universal performance characteristics of motors", linking the maximum force and the mass of any motor, identified by Marden and Allen. The utilization of molecular motors for computing devices emphasizes the interesting relationship between the conversion of energy, extraction of work and production of information. Our recent work touches upon these topics and discusses molecular clocks as well as a Landauer limit for robotics.

What is on the horizon? Just as photovoltaics took advantage of progress in semiconductor fabrication to become commercially viable over a century, one can envision that engineers working with biomolecular motors leverage progress in biotechnology and drug development to create the engines of the future. However, the future source of energy is going to be electricity rather than fossil or biological fuels, a fact that has to be accounted for in our future efforts.

In summary, we are convinced that past, ongoing and future efforts to engineer with biomolecular motors are providing exciting demonstrations and fundamental insights as well as opportunities to wander freely across the borders of engineering, biology and chemistry.

INTRODUCTION

A key feature of life is active movement. The discovery that molecular machines, specifically motor proteins, are responsible for the vast majority of these movements is a key advance in structural biology enabled by electron microscopy in the 1950's. The elucidation of the working mechanisms of these molecular machines is a major topic of biophysics since the 1990's. It led to a proliferation of single molecule techniques and coincided with the emergence of nanotechnology as a distinct field of research. Using motor proteins as off-the-shelf components to build nanodevices which integrate mechanical movement is a brilliant idea, which was conceived roughly simultaneously by several researchers around 1999. One of the authors had the good fortune to join a related project led by Viola Vogel and Jonathon Howard as a postdoctoral researcher in 2000, has been continuously working in this field since, and reviewed the technical progress in the field at regular intervals[1-7]. Here, we would like to highlight several insights and open questions which are at the forefront of our minds.

The discussion is organized as follows: First, biological examples of the use of molecular motors are briefly highlighted. These examples are followed by a discussion of the potential applications of molecular motors in technology, and the associated fundamental limits and requirements. Next is our perspective on the major roadblocks in the field, and the fundamental questions associated with these roadblocks. Finally, we will conclude with our perspective of the future in this field.

1. Molecular motors in biology

Biology is the proof-of-principle for nanotechnology[8] providing both, inspiration and a tool chest[9]. Biological systems rely on DNA and RNA to store and transmit information and use proteins to perform chemical and mechanical functions. Several protein families have motor functions,

meaning that their catalytic cycle is coupled to directed mechanical movement[10]. Rotary motors, such as F1-ATPase[11] and the bacterial flagellar motor[12], can be distinguished from linear motors, such as DNA polymerase[13], myosin[14] and kinesin (Figure 1a-c).

While viruses[15] and bacteria[16] have motors interacting with RNA and DNA, only eukaryotic cells have motors interacting with their cytoskeleton formed by actin filaments and microtubules[16]. Even unicellular eukaryotic organisms, such as yeast, utilize cytoskeletal motors from the dynein, kinesin and myosin families to assist in a wide range of processes, including cell division, cell motility and internal organization[17] (Figure 1d). However, the "killer applications" for linear motors emerged in animals, whose needs for large scale actuation and long range nervous signaling were met by the evolution of muscles and nerves. Myosin-II, the motor protein responsible for contraction in skeletal and cardiac muscle, is one of the most abundant proteins in the human body. Kinesin makes up about 0.3% of the protein mass in brain tissue (the tubulin to kinesin ratio in tissues is generally on the order of 100), which is about five-fold larger than in other tissues[18] and quite significant for a supporting actor. Consequently, native motor proteins and their associated cytoskeletal filaments can be extracted from muscle and brain tissue and are available from commercial sources. Exotic motor and cytoskeletal proteins with advantages for engineering applications can be found in specific organisms, such as thermophilic fungi[19], or genetically engineered[20] and expressed in bacterial or eukaryotic cells[21]. Due to the centrality of cardiovascular, musculoskeletal, cancer-related and neurodegenerative diseases to the modern practice of medicine, there is broad and sustained interest in the biomedical research community in these proteins and the associated mechanisms.

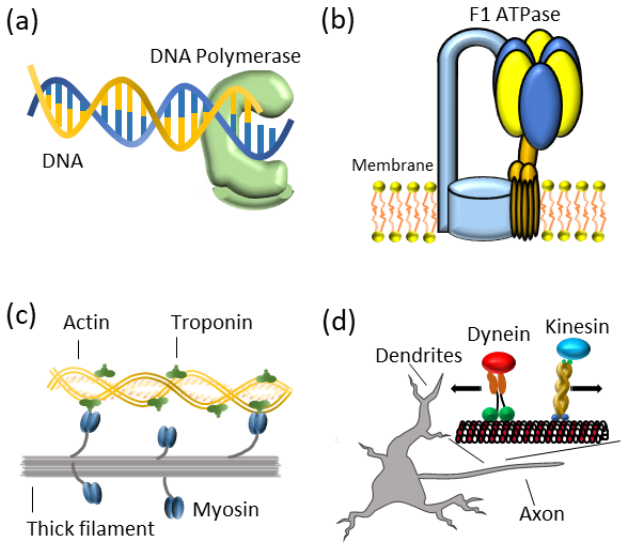


Figure 1: Illustrations of biomolecular motors. (a) DNA Polymerase synthesizing DNA. (b) F1 ATPase. (c) Myosin moving an actin filament. (d) Kinesin and Dynein "walking" on a microtubule in a neuron.

Given the evolutionary benefits of moving faster, over longer distances, with more force and less energy consumption, it is likely that e.g. F1-ATPase (responsible for ATP synthesis) and myosin-II (the motor in cardiac and skeletal muscle) are fully optimized as systems from the perspective of the species (which is subject to evolutionary pressures). However, the optimal system is typically achieved by balancing competing demands, and thus the properties of motor proteins may, but do not have to, represent an approximation of the fundamental limits for molecular motor performance in various metrics. The performance of these molecular machines can reach impressive heights: For example, F0F1-ATPase exhibits close to 100% efficiency[22] and the force-per-mass ratio of molecular motors is comparable to modern engines[23].

The trade-offs in the design of biological structures can be clarified if the design of synthetic structures from biological building blocks is attempted by multidisciplinary teams, an approach which gave rise to a branch in the field of synthetic biology[24]. Several authors described

putative engineering design principles for motors and associated systems[24-26], but additional work and verification is clearly needed.

Biology demonstrates the utility of biomolecular motors, but should they serve the engineer as inspiration for synthetic molecules or as building blocks of hybrid systems?

2. Biomolecular motors in technology

The initial demonstrations that motor proteins and their associated filaments can not only function in an *in vitro* environment[27] but can be induced to move along prescribed paths, carry cargo, and respond to external stimuli have generated sustained interest in the nanotechnology community[28-32]. The performance metrics of motor proteins exceed those of competing systems, in particular DNA walkers[33-35] and spiders[36,37] and synthetic molecular motors[38-41], by several orders of magnitude and thereby enable the construction of much more functional systems and devices. These motor protein-driven systems and devices can therefore be used to explore application concepts in the fields of sensing, actuation, and computing.

Sensors for the detection of molecules and microorganisms can be greatly improved by the integration of active nanoscale transport. These improvements can originate from accelerated sample collection[42-45], improved sample purification and identification[46,47], or energy savings and device simplifications (e.g. by removing the need for pumps and electric power)[48] (Figure 2a). Notably, active transport can overcome fundamental limitations of diffusive transport[1,44].

Actuators are, as described above, the key application of motor proteins in biology. Proof-of-principle demonstrations show that forces generated by motor proteins *in vitro* can move structures ranging from molecules[49-51] over nanoparticles[32,52] to microfabricated blocks[53] and facilitate

the formation and dissolution of bonds between these structures[54-57] (Figure 2b). The distinct scaling of motor-driven transport and force generation from thermally driven transport (diffusion) and thermal forces gives rise to new possibilities, such as the efficient assembly of large non-equilibrium structures[58]. As a result, "active self-assembly" can be considered to be conceptually different from "passive" or thermally driven self-assembly[59]. The generation of macroscopic forces, as achieved in muscles via the organization of up to 10^20 myosin motor proteins into hierarchically organized arrays, has proved to be a challenging goal. While hundreds of motors can collectively move a cytoskeletal filament, the directional alignment of a large number of cytoskeletal filaments is complicated[60], and therefore the largest structures moved *in vitro* are only tens of micrometers in size[61]. The solution, following the example of biology, may be to rely on self-organization mechanisms to dynamically assemble and maintain arrays of molecular motors. We have only seen the modest beginnings of this scientific journey[62].

Computing based on mechanical movement may seem to be a medieval concept embodied by the abacus or Zuse's Z3. However, if energy efficiency is the central goal rather than speed, there is no fundamental reason why electronic computing is superior to mechanical computing, where system states are encoded in positions and manipulated by the application of forces. Moreover, biological approaches to problem solving often involve the physical exploration of a solution space[63]. These points are beautifully illustrated by the work of an international collaboration of researchers, who implemented a computing device relying on the movement of cytoskeletal filaments propelled by surface-adhered motor proteins through a maze of channels encoding a mathematical problem[64] (Figure 2c). They used two types of junctions: junctions that will only allow the microtubule to continue straight and a junction where the microtubule can travel in one of two directions. Arranging these junctions in a specific array allowed the

microtubule to travel only in some possible routes, and thereby compute the solution for a combinatorial problem.

Of course, some systems combine aspects of sensing, actuation and computing. These include the melanophore-like optical device of Aoyama, Shimoike, and Hiratsuka[65], and the controllable swarm behavior of motor-propelled filaments studied by Kakugo et al.[66]. In addition, many studies have focused on overcoming specific technical challenges, on developing new technology elements, and on developing computational tools and scientific understanding. These important contributions are catalogued in recent reviews[67-69].

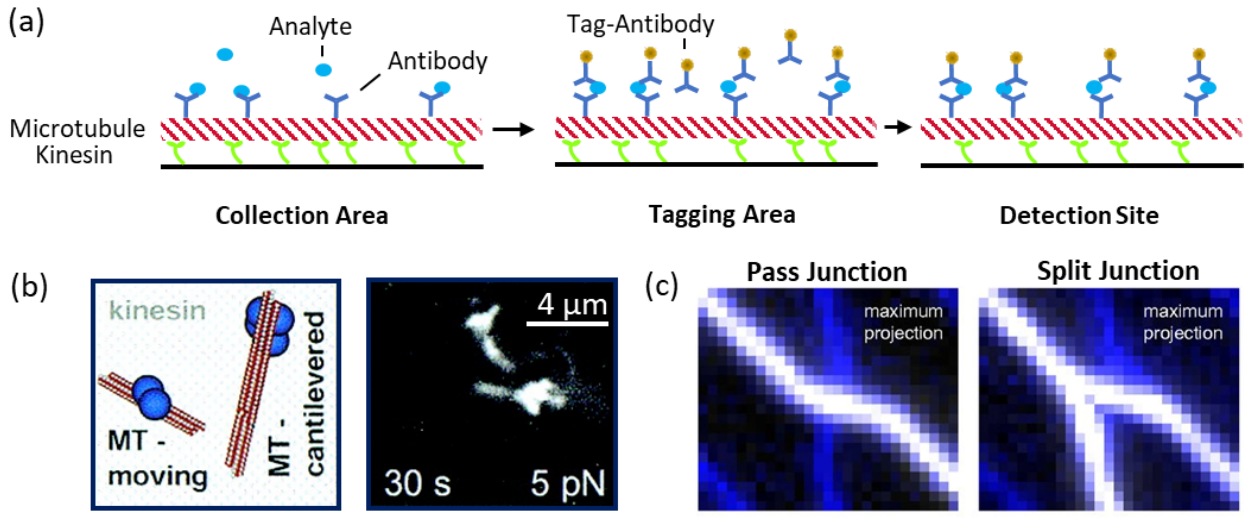


Figure 2: Biomolecular motor applications. (a) A schematic illustration of a sensor using active transport. Analytes are captured by specific antibodies attached to a microtubule. The microtubule is propelled by kinesin (bond to the surface) to a tagging area where tag-antibodies attach to the analytes. In the final step, at the detection area, the presence of the fluorescent tag is detected indicating the presence of the analyte.[43] (b) Kinesins can generate piconewton forces to stress a molecular bond and determine its rupture force in a microscopic forcemeter. Adapted with permission from Ref. 54. (c) Computations can be performed with microtubules and actin filaments propelled by surface-adhered motors as they repeatedly traverse X-junctions. Maximum projection image of microtubules passing through Left: a pass junction where the microtubules

will continue in the same direction. Right: a split junction where the microtubules can randomly travel left or right. Adapted with permission from Ref. 64..

3. Motivations, fundamental limitations, and open questions

A starting point for the discussion is the question: **Why engineer with biomolecular motors?** Two decades ago, it was simply astounding to be able to observe a molecule move, and even more astounding to be able to control its movement. We researchers and the public still marvel at this protean power, but the justification for continued research is the potential for biological insights (preferably of medical importance) and for advances in nano-engineering and materials science.

Biological insights arise often from the study of minimal systems of biological components which reconstitute biological processes, in a branch of synthetic biology[24]. At other times, the insights obtained by wrestling with hybrid systems, where biological components are used in a device context, also apply to fully biological systems. An example, is the idea that there is an optimal force to load an intermolecular bond, which maximizes the product of life time and force[26]. This insight can serve as guideline for the design hybrid and synthetic systems and also appears to apply to biological situations, such as the loading of actin-actin bonds in muscle or the kinesin-tubulin bond strained by a loaded kinesin. Interestingly, hybrid systems may provide insights into biological systems because the hybrid system is lacking certain features always present in the biological system. In a car, the importance of a radiator becomes only apparent when it is removed, and in a biological system the existence or importance of certain protective mechanisms may go unappreciated. Efforts to engineer hybrid systems may thus reveal the existence of critical subsystems and mechanisms. Finally, the refinement of the experimental techniques where biological components are closely interfaced with synthetic structures also benefits a variety of biophysical experiments[70].

Engineering advances are often academic at this stage, meaning that a new solution to an engineering problem is found which is interesting and inspiring but not practical in a commercial setting. A good illustration is the use of microtubules propelled by surface-adhered kinesin motors to map the topography[71,72] or even the local electromagnetic fields[73] of a surface in a Monte-Carlo fashion. This demonstrates an approach to obtain information about a surface which is completely orthogonal to scanning probe techniques. Academic engineering is worthwhile, but there is reason to believe that micro- and nanoscale motors will become commercially relevant since there is a historical trend towards simultaneously smaller and more abundant motors[67]. The applications of these ubiquitous smaller motors emerged in the context of other new technologies, and therefore surprisingly. For example, the smallest motors in widespread use are currently the motors controlling the read/write heads of hard drives, and these motors would not exist without the stunning advances in magnetic storage technology. Nevertheless, neither biomolecular nor synthetic molecular motors have found "killer application" in the technological domain. Further closing of the gap between biophysics and nanoengineering may help bring applications into focus.

Materials science is certainly searching for a material resembling muscle tissue, which can controllably adjust its linear dimensions and elasticity, is scalable, and consumes modest amounts of energy. Our work has so far more illustrated the challenges in re-creating force-producing structures from motor proteins and cytoskeletal filaments than demonstrated a path towards an "artificial muscle". However, by highlighting – for example – the importance of precise control over the arrangement of the molecular components for the efficient production of force[74], it holds some lessons for efforts to create contractile materials from synthetic molecular motors.

The discussion of the **fundamental limitations of biomolecular motors** – and molecular motors in general – in the pursuit of the applications mentioned above is now highly detailed with

respect to the attainable energy efficiency[75], but still rudimentary with respect to other important metrics, such as, maximum power and force per mass[6,67]. The ***theoretical limits to energy efficiency*** are, for example, discussed by Seifert[76]. In practical situations, biomolecular motors reach efficiencies in the conversion of chemical energy into mechanical work which are comparable to modern macroscale engines (40-60%). However, while losses increase dramatically for heat engines and electrical motors as they are scaled down, biological arrays of myosin motors (that is muscle) manage to retain their efficiency as they are scaled up. From our perspective, Figure 1 in Armstrong&Hess[67] communicates the key insight that – similar to biological organisms – the abundance of motors is roughly inversely proportional to their size. That means that small motors have to have energy conversion efficiencies comparable to those of large motors (~40%), or the small motors would consume a disproportionate and unaffordable share of the energy budget. Many of the current molecular motor concepts are intrinsically limited in their energy efficiency[77], which in our opinion makes them ineligible for widespread use. Just as an aside, molecular motors may be used as clocks rather than motors to deliver timing information rather than work[78]. Although the mechanical efficiency is zero, good use is made of the input energy to create information.

Marden and Allen identified ***universal limits with respect to the force generated per mass*** for linear and circular motors, but a mechanistic explanation of this observation is still missing[79] (Figure 3). Notably, current designs of synthetic molecular motors generate larger forces than expected for their mass, while estimates of the contractile forces generated by materials integrating synthetic molecular motors[80] conform closely to Marden and Allen's limits.

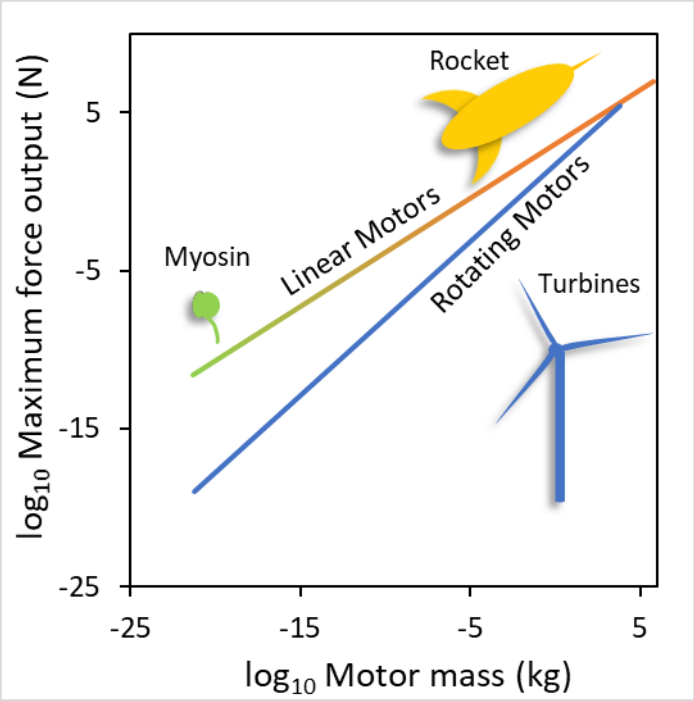


Figure 3: The relation between the maximum force (F) and the mass (m) of motors. For linear motors (full line) F is proportional to $m^{2/3}$ and for rotating motors (dashed line) F is proportional to m. The curves are plotted to fit the mean values calculated by Marden[23,79]. This relation applies from molecular motors to turbines and rocket engines.

Theoretical limits of motor lifetime, motor power, and controllability have, to our knowledge, not been identified. Initial studies of degradation processes in motor protein-driven *in vitro* systems have been conducted[81,82], but we do not yet understand why, for example, myosin motors are replaced in cardiac muscle every few days[83]. Removal of reactive oxygen species from the solution appears to extend the lifetime of motor proteins dramatically[84], but it seems logical to expect that the forces generated by a motor lead to failure with a non-zero probability in each catalytic cycle. Limits to power production at the macroscale are often arising from limitations to the dissipation of heat[85]. For molecular motors operating individually, heat dissipation is facilitated due to the increased surface-to-volume ratio, so limitations may rather arise from the challenge of delivering the input energy at a fast rate. Controllability, that is the ability to externally determine

and also modulate motor output, is critical for applications. Control of *in vitro* motor protein activity has been demonstrated by us using various mechanisms (e.g. by varying ATP concentration[32,86] or temperature[87]), but these approaches are often amazingly energy inefficient. Especially control via light, although easy to realize in a laboratory setting, is mismatched to the task, due to weak absorption, difficulty with molecular scale localization, photodamage, and - compared to the amount of work produced per cycle by motors - highly energetic photons. Even in biology, a significant fraction of the energy consumption of a muscle is devoted to the calcium-dependent activation mechanism[88]. Given that control involves manipulation of information, it is likely that fundamental thermodynamic limits, including Laundauer's principle of a minimum energy required for the erasure of a bit of information, not only exist but are actually relevant for molecular motors[89]. In principle, arrays of molecular motors are coupled dynamical systems which exchange information internally and are capable of exhibiting e.g. synchronization under suitable conditions. However, surface-adhered kinesins propelling a microtubule are found to not synchronize because the variability in the location of their surface attachment site relative to the microtubule axis creates large heterogeneity in their force production[74].

Fundamental limitations at the systems level may also be identified, e.g. for nanoscale robots powered by molecular motors. An example of such a limitation is the conjecture that doubling the rate of a chemical reaction by using a robot requires at least an amount of energy equal to kBTln2.[78] Dissipation due to frictional forces of course adds to this minimum. but even for a well-studied system such as muscle the magnitude of the energy loss to friction is controversial[90].

A key challenge is to dynamically adapt the force output of a system to the applied load. For example, when surface-adhered motor proteins propel a cytoskeletal filament, the viscous drag is on the order of femtoNewtons, while the combined action of the motors can produce many

picoNewtons[91]. The mismatch between load and capacity to produce force creates a very wasteful system, similar to using a truck to deliver a letter. We believe that systems in a dynamic equilibrium between assembly and disassembly hold great promise for the adaptation to external demands and the extension of system lifetime[62] (Figure 4).

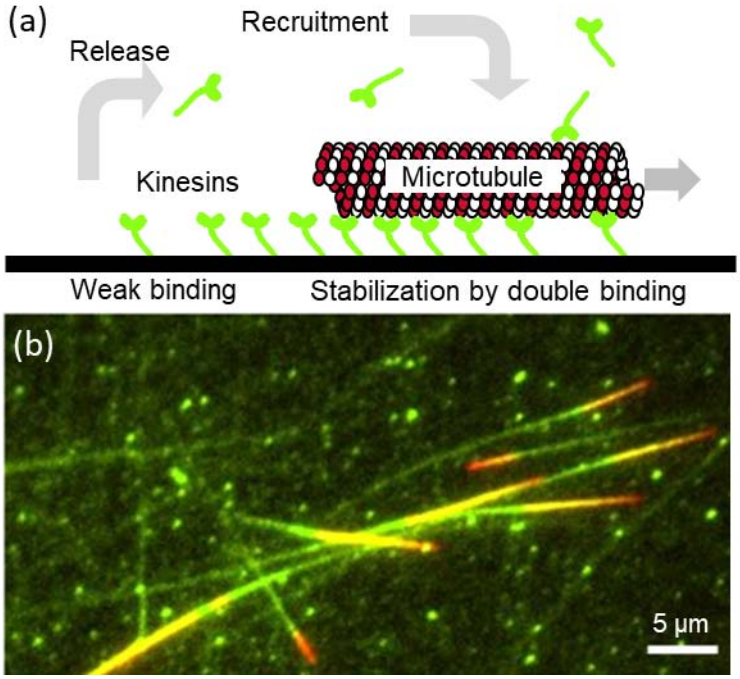


Figure 4: Dynamic force-producing systems. (a) An illustration of a microtubule assembling kinesins on a weakly-binding surface. (b) Fluorescence microscopy image of GFP-kinesin (green) and microtubules (red) showing the kinesin trails left by gliding microtubules. Adapted with permission from Ref. 62.

A central open question is if highly functional molecular motors have to resemble motor proteins not only in their strengths, such as efficiency and force output, but also in their shortcomings, such as softness and fragility. At the systems level, it is still unclear if the hierarchical organization of muscle has to be replicated, and if encapsulation of the force-producing unit in a membrane as described by Hagiya et al.[92] is required.

One could also argue that the extraction of motor proteins from their native environment in cells is an unnecessary complication, and that – as Hiratsuka et al. and Montemagno et al. demonstrated – force can be extracted from molecular motors acting within bacterial or mammalian cells[93,94]. However, the hyperbolic conclusion of this line of thinking is a return to the horse-drawn carriage. Engineers and materials scientists are used to man-made systems exceeding the capabilities of biological systems in key metrics, and it feels odd to settle for biological performance.

4. On the horizon

The largest structures assembled to date from microtubules and kinesin motors have a volume on the order of one milliliter and contain only up to 100 microgram of kinesin[95], but it is not unreasonable to consider the capability to mass-produce motor proteins. Although one can make the argument that the cost of an individual motor is negligible[96], the commercial price of one milligram of purified motor protein (myosin II or kinesin I) still exceeds a thousand dollars. Nevertheless, the continuing shift in drug development from small molecules to proteins provides the opportunity to advance the biotechnological production of motor proteins and their associated filaments. While it may seem implausible to piggyback on drug development, now ubiquitous solar cells have similarly benefitted from the technologies perfected for the production of high value microchips. Ultimately, we are not aware of an intrinsic reason why rather complex organic synthesis of molecular motors should be more scalable and cost-efficient than the production of motor proteins using biotechnology.

However, the ongoing and by now irreversible technological shift from fossil fuels to clean electric energy raises the question if molecular motors consuming chemical fuels are incompatible

with the future primary source of energy. Should we rather apply our creativity towards the development of miniaturized electric motors, or at least chemical motors based on the redox chemistry used in batteries? A split of the field into a biocompatible branch focused on the use of molecular motors for drug delivery and regenerative medicine applications and an industrial branch interfacing with the advancing solar economy and focused on motors driven by electrical energy is conceivable.

5. Conclusions

We are convinced that past, ongoing and future efforts to engineer with biomolecular motors are providing exciting demonstrations and fundamental insights as well as opportunities to wander freely across the borders of engineering, biology and chemistry. Technology is inexorably moving towards smaller and more numerous devices integrating active movement, and molecular motors of either biological or synthetic origin will find their breakthrough application

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