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Towards the application of cytoskeletal motor proteins in molecular detection and diagnostic devices

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Over the past ten years, great advancements have been made towards using biomolecular motors for nanotechnological applications. In particular, devices using cytoskeletal motor proteins for molecular transport are maturing. First efforts towards designing such devices used motor proteins attached to micro-structured substrates for the directed transport of microtubules and actin filaments. Soon thereafter, the specific capture, transport and detection of target analytes like viruses were demonstrated. Recently, spatial guiding of the gliding filaments was added to increase the sensitivity of detection and allow parallelization. Whereas molecular motor powered devices have not yet demonstrated performance beyond the level of existing detection techniques, the potential is great: Replacing microfluidics with transport powered by molecular motors allows integration of the energy source (ATP) into the assay solution. This opens up the opportunity to design highly integrated, miniaturized, autonomous detection devices. Such devices, in turn, may allow fast and cheap on-site diagnosis of diseases and detection of environmental pathogens and toxins.

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Introduction

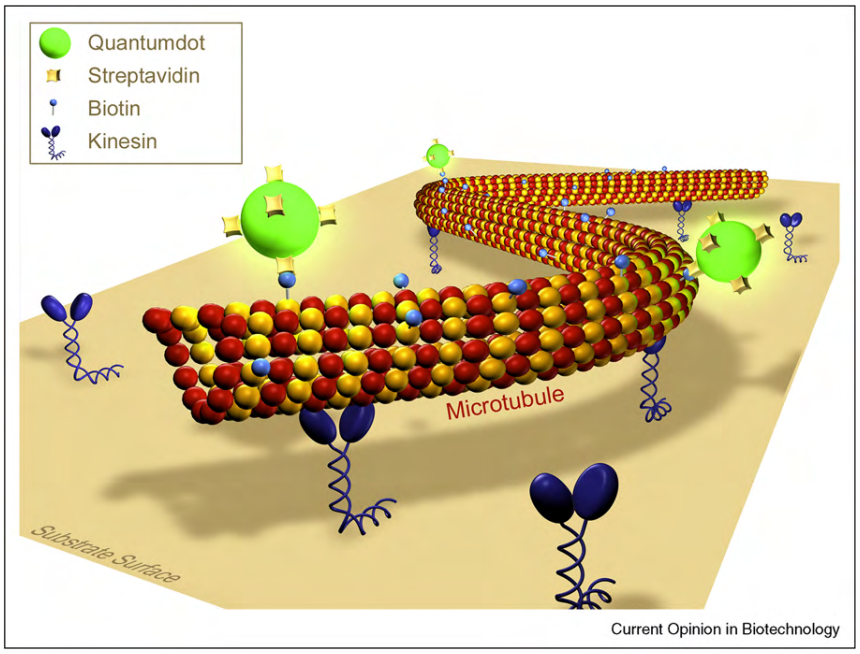
Technology has often made great advances by imitating biological systems. A prominent example of such an advance is the imitation of bird flight by airplanes. Moreover, imitating, or even using biological systems is especially promising for nanotechnology. Many proteins work as nanoscopic machines. They are usually only a few nanometres in size and have been optimized by evolution to fulfil their designated tasks with extremely high efficiency and specificity. With our growing understanding of the biophysics of many proteins, the wealth of these nano-machines can be tapped and used for nanotechnology. In particular, cytoskeletal motor proteins are highly promising for nanotechnological applications, because they perform mechanical work using the chemical energy of ATP-hydrolysis. Using biomolecular motors, proof-of-principle studies (elaborately reviewed in [1•]) have already successfully demonstrated transport [2•,3,4,5••,6–13], sorting [14,15], self-assembly [16,17] and detection [18•,19••,20,21••] of nano-sized cargo. In this review we will focus on the use of molecular motors for biosensing and diagnostics.

Motor proteins

In order to perform mechanical work, motor proteins act on filamentous structures within the cell. Thereby, they propel themselves and their cargo along the filament like a train moving along its track. In the case of cytoskeletal motors, such a track is provided by a cytoskeletal filament like an actin filament or a microtubule. So far, the most promising assay for using such a motor system in artificial nanoscale environments is the so-called 'gliding motility assay', a setup where cytoskeletal filaments are propelled by surface-attached motor proteins in a way reminiscent of crowd surfing (Figure 1) [22–25]. Probably the best studied and most well understood motor proteins – and therefore the most promising candidates for nanotechnological applications – are the microtubule-based kinesin-1 and the actin-based myosin-II motors.

Microtubules self-assemble, in the presence of GTP, from α-tubulin and β-tubulin heterodimers with a combined molecular weight of about 110 kDa. These dimers are arranged in protofilaments within the microtubule lattice (Figure 2a). Usually, thirteen of these protofilaments are associated laterally to form hollow tubes of 25 nm diameter. Because of this tubular structure, microtubules are quite stiff, having a persistence length on the order of millimetres [26]. While microtubules are highly dynamic *in vivo* [27], they can be stabilized for *in vitro* applications by growing them in the presence of the slowly hydrolysable GTP analogue guanosine 5′-[α,β-methylene] triphosphate (GMPCPP) or using the microtubule stabilizing drug taxol [28]. Owing to the asymmetric nature of the tubulin dimers, microtubules are polar with the fast-growing 'plus end' being terminated by β-tubulin and the slower-growing 'minus end' being terminated by α-tubulin. In addition to their structural functions in a cell, microtubules provide a functional scaffold for a whole class of microtubule-associated proteins. The best studied and most intriguing (especially with respect to nanotechnology) group of proteins that associates with

Figure 1



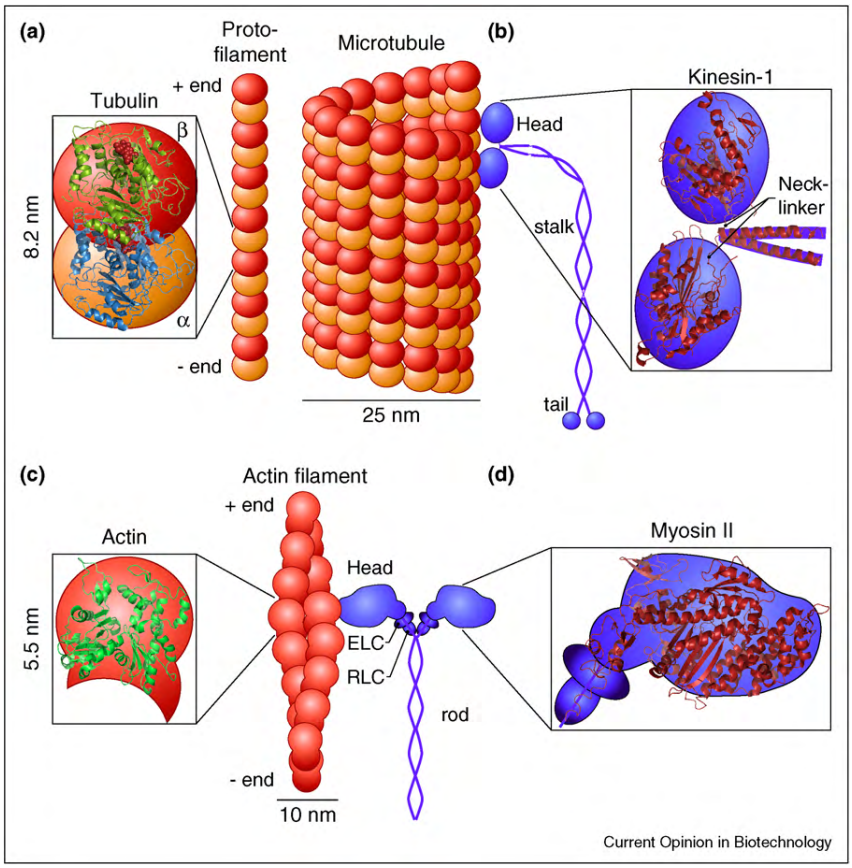
Gliding motility transport assay in an artificial environment. Microtubules transporting cargo (e.g. quantum dots) are propelled over the surface by immobilized kinesin-1 motors in the presence of ATP. Courtesy of Franziska Friedrich, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden. © Franziska Friedrich 2004.

microtubules are the motors of the dynein and kinesin families. These motor proteins move along the lattice of microtubules, transporting cargo (e.g. vesicles containing neurotransmitters) over large distances, positioning organelles, or helping to rearrange the microtubule network.

Kinesin-1 (Figure 2b; formerly called 'conventional kinesin') is the archetypal member of the kinesin family of motor proteins. It was first discovered in the giant axon of the squid, where it was shown to transport vesicles from the cell body to the axon terminal [29]. Kinesin-1 moves in a stepwise hand-over-hand mechanism comparable to our own walk [30–32]. Its motor domains hydrolyse ATP, moving the molecule in steps of 8.2 nm (the length of a tubulin dimer) towards the microtubule plus end. It has been shown that kinesin-1 can take approximately 100 steps before dissociating from its track, allowing single motors to transport cargo over long distances [33,34]. During its stepping cycle, a single kinesin-1 molecule can pull cargo against a force of 6 pN [35,36]. This force per 8.2 nm step corresponds to an energy production of 49×10^{-21} J (equivalent to 49 pN nm). At the same time, kinesin-1 hydrolyses one ATP molecule [37,38], which releases 100×10^{-21} J of energy at cellular concentrations of ATP, ADP, and P_i [39]. This means that this motor protein has an energy efficiency of almost 50%, which is better than the combustion engines currently used in cars (≈40% efficiency) [40,41]. These properties make kinesin-1 a highly promising molecular motor for novel nanotechnological applications.

Filamentous actin (F-actin) is composed of a large number of identical G-actin monomers each having a molecular weight of about 40 kDa (Figure 2c). Actin (for a review see [42]) is highly conserved among species, being similar to the α-actin from mammalian muscle recently employed for the development towards nanotechnological applications of actin and myosin II. The monomers in F-actin are arranged along two helical protofilaments that are wound around each other with a helical half-pitch of about 36 nm. The distance between the monomers along a protofilament is 5.5 nm and the diameter of the actin filament is ~10 nm. Owing to the small diameter and the geometrical arrangement of the actin monomers, the persistence length of actin filaments is about 10–20 μm [43,44], which is considerably lower than the persistence length of microtubules. However, the persistence length of actin is affected by the binding of various ligands. This includes the stabilizing peptide, phalloidin [44] that is used routinely to prevent actin depolymerization during *in vitro* studies. In the absence of other ligands, the binding of phalloidin increases the persistence length of actin filaments from about 10 μm to 15–20 μm. However, it was shown recently [45] that this effect was largely reversed for actin filaments propelled by heavy meromyosin motor fragments (HMM).

Figure 2



Structure and dimensions of cytoskeletal filaments and cytoskeletal motors. **(a)** Tubulin dimers (PDB ID: 1TUB) polymerize into protofilaments and microtubules. **(b)** Kinesin-1 (PDB ID: 3KIN) forms a homodimer. The motor 'heads' that interact with the microtubule are joined to the coiled-coil 'stalk' via a 'neck-linker'. **(c)** Actin monomers (PDB ID: 2ZWH) self-assemble to form a double helical filament. **(d)** Myosin II (PDB ID: 2JHR) heavy chains bind to actin filaments via the 'head' domains that are joined together by a coiled-coil 'rod'. The essential light chain (ELC) and the regulatory light chain (RLC) stabilize the α-helical neck region of the heavy chain and are of importance for motor function and regulation, respectively. Owing to asymmetries of the monomers, both actin filaments and microtubules have two distinct ends. The faster growing end is termed the '+ end' while the slower growing end is termed the '− end'.

Like microtubules, actin filaments are highly dynamic structures with periods of growth and shrinkage, processes that, in the cell, are carefully controlled by cell signaling and numerous actin binding proteins [46]. In further analogy to microtubules, hydrolysis of high-energy phosphates (in this case ATP) at the active sites of actin monomers, drives the addition of monomers during polymerization. Actin filaments, like microtubules are polar structures with addition of monomers predominantly at the plus end and loss of monomers during depolymerization, mainly at the minus end. Proteins that specifically bind to the plus end (e.g. Gelsolin and Cap Z) and contribute to the regulation of actin dynamics in the cell are of interest as linkers for cargo attachment [2•]. The reason is that the plus end corresponds to the trailing end of an actin filament propelled by myosin II and cargo attached to this end is less likely to hinder transport.

Myosin II is a molecular motor (Figure 2d; for a detailed review, see [47]) that may be obtained in large quantities from muscle. It is the myosin II motor that has been used most extensively in developments towards nanotechnological applications. In the myofilament lattice of muscle, the myosin II molecules form ordered arrays ('thick' myosin II filaments) allowing a team of motors to effectively propel actin filaments and develop macroscale force. Each myosin II molecule consists of a C-terminal, a ~160 nm long coiled-coil tail (rod) formed by α-helical parts of the two myosin II heavy chains being wrapped around each other. At their N-terminals, each heavy chain folds up into a globular motor domain with an actin binding site and an ATP binding catalytic site. Each motor domain is connected to the tail via an α-helical neck region that acts as a lever arm being stabilized by one essential and one regulatory light chain. Myosin II differs from kinesin-1 and several other myosin motors by

being non-processive. This means that a single myosin II motor domain detaches rapidly from an actin filament after performing its power-stroke, that is before its partner head binds to the next site along the filament. As a result, each myosin II molecule only takes one step at a time before detaching from actin. Therefore, in contrast to processive motors, many myosin II motors need to act in concert to ensure constant binding and continuous transportation of actin filaments. However, these motors use ATP as efficiently (maximum thermodynamic efficiency 50%; [48]) and produce a similar maximum force (6 pN per motor domain [49]) as kinesin-1. It is also of interest to note that a team of myosin II motors transports actin filaments at considerably higher maximal velocity (10 μm/s) than kinesin-1 and other processive motors.

Using gliding motility assays, great advancements have been made towards exploiting kinesin-1 and myosin II for nanotechnological applications. These advancements can be seen as a toolbox that future lab-on-a-chip devices may exploit. Particularly valuable are mechanisms for spatial guiding of gliding filaments using surface topography and chemical patterning of active motor proteins [50–54,55•,56–61]. Temporal control has been achieved using the slowly hydrolysable ATP-analogue adenosine 5′-[β,γ-imido] triphosphate (AMPPNP) to stop gliding filaments [62,63] or thermo-responsive polymers to release gliding filaments from the surface [64].

Molecular detection—immunoassays

Currently, biological samples are routinely analysed by immunoassays such as the enzyme-linked immunosorbent assay (ELISA). In these assays, antibodies are used for analyte capture and identification. The presence of the analyte is then reported by a signaling process, for example fluorescence or chemiluminescence. The sensitivity of immunoassays has been improved considerably by several methods: (i) combining the conventional ELISA assay with the sensitivity of a polymerase chain reaction (PCR) achieved femtomolar detection limits [65–67]. (ii) This assay was further improved both in terms of sensitivity and parallelization in the so-called bio-barcode assay that uses micro-beads coated with antibodies and DNA oligonucleotides in combination with quantitative PCR [68] or DNA microarrays [69] for attomolar detection limits. (iii) Silicon nanowires [70] or carbon nanotubes [71] coated with antibodies change their electrical properties upon antigen binding and have been reported to be able to detect single virus particles [72]. The most recent developments in diagnostics relying on the use of nanomaterials, are reviewed by Giljohann and Mirkin [73].

As with microprocessors, the miniaturization of immunoassays can greatly improve efficiency: Apart from making devices cheaper by reducing material consumption, small sample volumes help to considerably speed up processing times owing to shorter diffusion distances [74,75]. Taken together with the possibility of massive parallelization [76], this can facilitate increased throughput. Also, sensitivity can be greatly enhanced by decreasing the size of the detection area, because this avoids depletion of the analyte from solution [77] and reduces non-specific adsorption [70,78]. Great progress towards more miniaturized immunoassays has been made using microarrays where many different antibodies are attached to a surface in an array of spots [79–81]. However, as Sheehan and Whitman [77] pointed out, diffusion of the analyte to the detector area limits the number of molecules that reach the detector within a given time. Assuming desired detection times in the range of minutes and femtomolar sensitivities, this leads to a practical lower limit for the surface area of a detector of a few hundred μm^2. To a certain extent, this limitation can be overcome by actively transporting the analyte to the detector using microfluidics [76,82,83]. In the case of tissue samples, reducing the processing volume and avoiding dilution of the sample is even more important than increasing the sensitivity of the detection device. This is illustrated by the following considerations: A cell with a radius of 10 μm has a volume of ~4 pL. Just one molecule in such a small volume corresponds to a concentration of ~0.4 pM. Therefore, if dilution of the sample can be avoided, a detection limit in the femtomolar range is completely sufficient for single-cell or tissue protein analysis. This means that the improvements required for single-cell protein detection should focus on handling extremely small volumes rather than further lowering the detection limit. Nevertheless, in several other fields of diagnostics it can be of interest to achieve detection limits down to attomolar ranges [73].

Molecular motors for advanced detection devices

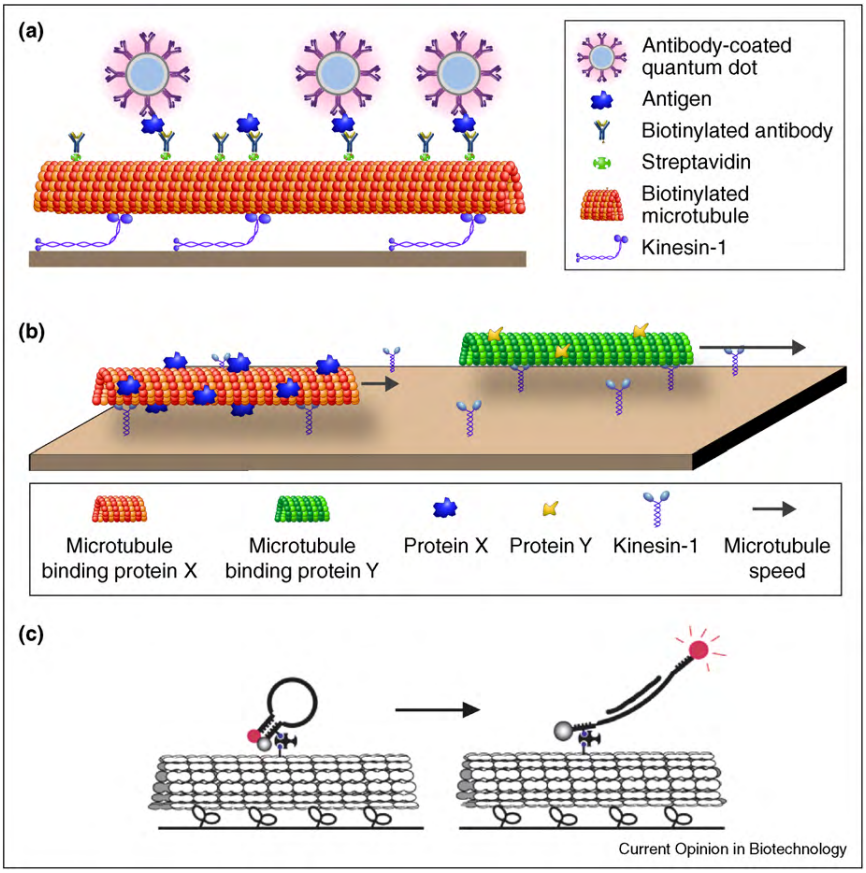
Microfluidics has greatly improved the efficiency of immunoassays by providing a means for active transport of the analyte through a small detection device. With decreasing dimensions of the detection devices, it becomes impractical to use microfluidics for active transport because of the need for external pumps and high pressure. For example, a 100 nm wide channel requires a pressure gradient of approximately 1 Pa/μm for a flow speed of 1 μm/s (see [84] equation 3). State of the art pumps able to achieve the required pressures on the order of kPa (for mm long channels) are themselves several cm in at least one dimension and require several volts to operate [85,86]. This is where motor proteins come in, having been optimized by evolution for just that purpose: transport of nanoscale cargoes in nanoscale environments. Used in detection devices, molecular motors could replace fluidic flow for analyte transport, thus facilitating further miniaturization of the detection area, resulting in a significant increase in efficiency and sensitivity [87•]. In addition to working on a nanometre scale, molecular

motors are powered by ATP [88]. Therefore, the energy supply of the detection device can be provided by adding ATP to the sample buffer, making the device completely independent of external power sources. This could potentially lead to lab-on-a-chip detection devices that are as easy to handle as a urine test strip or a home-use pregnancy test. Despite the ease-of-use, such devices could allow precise, quantitative detection of a multitude of analytes at the point-of-care.

Active transport of molecular targets has been demonstrated using gliding cytoskeletal filaments functionalized with biotin-streptavidin and/or antibodies [2•,3,7,89•, 90,91] (Figure 3a). Cytoskeletal filaments functionalized with antibodies can be used to specifically transport virtually any target. In addition to providing a means for active transport, the specific attachment of molecules to gliding filaments could serve to purify the target molecule if the molecule is transported from one compartment containing the analyte solution into another compartment. In principle, this concept [92] is similar to affinity purification, except that, the 'matrix' (i.e. the antibody carrying filaments) moves through the solution, not vice versa. Importantly, motor-based transportation can separate the location of analyte-binding from the detection area, eliminating any non-specific binding to the sensor area, and thus removing a key factor that may prevent very low detection limits.

Intriguingly, molecules attached to the filaments can influence the gliding speed [18•,93]. It has been shown that this effect depends on the density of molecules attached and can be used to detect the binding of macromolecules to gliding microtubules [18•] (Figure 3b). Because several, differently labeled populations of microtubules functionalized with different antigen binding

Figure 3



Analyte detection on gliding filaments. **(a)** Antibody sandwich assay: Microtubules functionalized with antibodies capture the analyte from a sample, and transport it along the kinesin-1-coated surface. The analyte can then be identified by the binding of antibody-coated quantum dots. Adapted from [89•] – Reproduced by permission of The Royal Society of Chemistry. **(b)** The speed of protein-coated microtubules gliding on a kinesin-1 surface is influenced by the density of the coating protein and can be used for differential detection. Adapted from [18•] – Reproduced by permission of The Royal Society of Chemistry. **(c)** Biotinylated molecular beacons are attached to biotinylated microtubules that are gliding on a kinesin-1-coated surface. The molecular beacons are in a quenched state until an unlabeled complementary DNA or RNA target hybridizes to their DNA loop, opening the beacons and enhancing their fluorescence. Adapted from [96•] – Reproduced by permission of John Wiley and Sons.

fragments could be used, this approach in principle allows the detection of multiple analytes from the same solution in parallel. Without the need for labeled analyte or secondary antibodies, this slow-down effect could be used in highly integrated devices. There, the presence of an analyte in a sample could be measured simply by letting the analyte-carrying microtubules run along a millimetre long, narrow channel and measuring the time until the different microtubule populations reach a detector. Nevertheless, detection can also be achieved by fluorescently labeling the analyte or by using a second, fluorescently labeled antibody. If the label is very bright, or if total internal reflection fluorescence microscopy is used, this method can be used to detect single analyte molecules [10,89•,94,95]. The fact that the analyte is transported along the surface by gliding filaments allows its distinction from molecules that are non-specifically adsorbed to the surface. This considerably increases the specificity and signal-to-noise ratio of the detection. Raab and Hancock [96•] successfully demonstrated the detection of ssDNA using microtubule-bound molecular beacons. These beacons consisted of small DNA oligonucleotides that have one end labeled with a fluorescent molecule and the other with a fluorescence quencher (Figure 3c). In the absence of the target ssDNA or RNA, the quencher and fluorophore are brought in close proximity owing to short complementary sequences at both ends of the beacon. Upon binding of a complementary strand of nucleic acid, the beacon unfolds, drastically increasing its fluorescence intensity. Another way to obtain high specificity is by labeling two different populations of actin filaments or microtubules, each with different fluorophores [97] and different recognition elements, for example monoclonal antibodies against two different epitopes on an analyte molecule (a virus, prostate specific antigen, etc.). If the analyte is present, the different filament populations would be cross-linked, causing them to be transported together by myosin II or kinesin-1 on a surface. This co-transport could be easily detected and would be evidence for the existence of analyte in the sample. A proof of principle for this approach is the observation of two biotinylated cytoskeletal filaments moving together for tens of micrometres after being cross-linked to each other via a streptavidin-coated quantum dot [3,16].

All assays discussed so far relied on random gliding of microtubules or actin filaments without spatial confinement or directionality. As reasoned by Katira and Hess [87•], the sensitivity and speed of detection can be increased by actively transporting cargo to a small detection area. This principle was implemented by Lin *et al.* [19••]: Using photolithography, they created petal shaped structures that allowed microtubule binding and gliding only in certain areas (Figure 4a). These areas were shaped such that gliding microtubules were guided towards a central detection area where they were trapped and concentrated. Using this device, Lin *et al.* detected fluorescently labeled streptavidin with a femtomolar detection limit. However, one caveat of this detection device is the need for fluorescently labeled analyte. This limitation was overcome by Fischer *et al.* [21••] in a device they termed a 'smart dust biosensor' (Figure 4b). Their device miniaturizes the well established principle of a double antibody sandwich assay: Analyte is captured by antibody-coated microtubules in the central area of the device (dark grey circle in Figure 4b). As soon as the device is exposed to light, ATP is released and the microtubules start moving randomly on the surface. When they accidentally move into the outer area (light grey circle) that contains fluorescently labeled second antibody, the labels are also picked up by the microtubules. Detection is then achieved in the outermost area (green circle) where the microtubules get stuck and thus are concentrated.

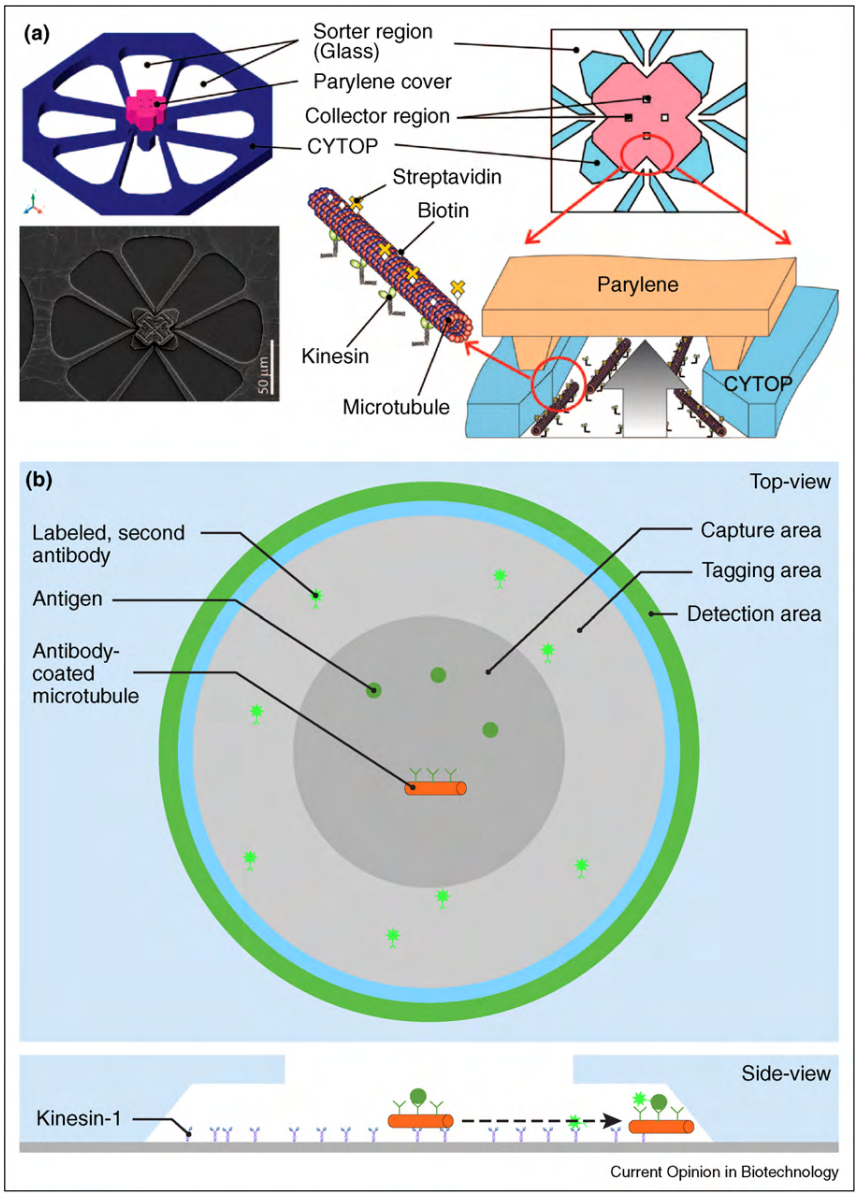
Actin-based detection has been demonstrated by measuring the gradual inhibition of myosin II by mercuric ions [98], or anti-myosin II antibodies [99]. However, these detection methods are very specific and cannot easily be generalized to the detection of arbitrary analytes.

Microtubule-based vs. actin-based transport in nanotechnological applications

In order to fully exploit existing cellular transport machineries, it is important to compare the pros and cons of the microtubule–kinesin-1 and the actin–myosin II systems for use in detection devices. Both systems have been explored extensively for this purpose but they represent quite distinct versions of cytoskeletal motor systems. Thus, whereas the microtubule–kinesin-1 system in the cell is specialized for long-distance cargo-transportation, the actin-myosin II system is specialized for production of high forces and velocities.

One difference between microtubules and actin that is very relevant for nanotechnological applications is *bending rigidity*: while microtubules are quite rigid, actin filaments are much more flexible. This is relevant especially for guiding and surface exploration. On an unstructured surface, microtubules usually glide on relatively straight paths over hundreds of μm. This has the advantage that they can be well-guided by chemical patterning of planar surfaces with kinesin-1 [50,53,100]. By contrast, actin requires relatively narrow tracks that combine surface structuring and chemical patterning for efficient guiding [60,101]. In return, actin can be transported in highly curved tracks (<300 nm radius of curvature [59,60]) allowing greater miniaturization when combined with nanostructured surfaces. Furthermore, if a small surface is to be efficiently explored by the gliding filaments, actin is more suitable [102], since the greater flexibility makes its paths on the surface more curved than the comparatively straight paths of microtubules. Other issues are: (i)

Figure 4



Molecular detection devices based on spatially confined filament motility. **(a)** 3D representation and SEM image of a sorting and concentrating structure. Bioconjugated microtubules land in the sorter regions and are transported by kinesin-1 towards the collector region. The total device area is 25,600 μm^2, and the central collector region measures 372 μm^2. A parylene layer on the top of the collector serves as cover to prevent microtubule loss from the collector region. Adapted from [19••] – reproduced by permission of American Chemical Society. **(b)** Schematics of a 'smart dust' detection device. Antibodies on microtubules capture antigens from solution. Kinesin-1 motors are activated, and collisions of antigen-loaded, gliding microtubules with fluorescent particles functionalized with a second antibody lead to pick-up and transfer of the fluorescent tags to the detection zone, indicating the presence of antigen. A basic device layout comprises a circular well created in photoresist on a coverslip. Analyte harvesting, tagging and detection are performed in different radial zones. Adapted from [21••] – reproduced by permission of Nature Publishing Group.

Motor processivity. The processivity of kinesin-1 enables effective transportation of microtubules at low motor density on the surface. This could be beneficial for detection by reducing non-specific interactions between motors and analyte molecules attached to the filaments. (ii) *Gliding speed*. As a requirement for processivity, kinesin-1 has to coordinate its two motor heads [103]. Therefore, microtubules propelled by kinesin-1 are considerably slower (velocity ~1 μm/s) than actin filaments propelled by the non-processive myosin II (velocity ~10 μm/s). (iii) *Filament structure*. Microtubules consist of ≈13 protofilaments. This offers the possibility to attach

cargo to the microtubule lattice in relatively high densities before transport fails [18•]. Moreover, the structure of a microtubule, namely the exact number of protofilaments, determines whether it rotates around the filament axis or not, and conditions where no rotation occurs can be found [104,105]. By contrast, the helical arrangement of myosin II binding sites along the actin filaments cause them to always rotate when propelled by myosin II on a surface [106,107]. It is obvious that the rotation can hamper transportation of nano-sized cargoes attached along actin filaments [3], and this difficulty is probably a major reason why only very few actin-based detection systems have been studied. However, there are methods [2,4,91] that may eliminate this problem. Further exploration and development of these approaches could allow extended use of actin and myosin II for detection, for example taking advantage of higher velocity and greater filament flexibility.

The selection of either microtubule-based or actin-based transport for different applications needs to be addressed further in experimental studies. Possibly, certain applications would benefit best from the use of kinesin-1 and microtubules whereas actin and myosin II would be advantageous for other applications. Finally, the possibility to combine both systems should be considered in order to exploit their respective advantages most effectively.

Conclusions and outlook

The efforts to functionally embed motor proteins into synthetic environments are beginning to bear fruits. Detection systems outlined in this review have demonstrated that molecular motor-based transport can replace microfluidics in highly integrated detection devices. The main advantages are: (i) Independence from external energy supplies, (ii) miniaturization beyond the limits of microfluidics and (iii) straightforward separation between the site of analyte-binding and the site of detection. These advantages offer the possibility of creating highly integrated, autonomously operating lab-on-a-chip devices that combine sample preparation and detection. Such devices could be as easy to operate as a urine test strip or a home-use pregnancy test, while offering highly in parallel detection of many blood markers at the same time. Because these devices could be used at the point-of-care, they would greatly speed up the time between diagnosis and treatment and enable advanced diagnoses even in remote areas without highly sophisticated laboratories. Also, miniaturization offers the possibility of ultra-sensitive detection devices that work highly parallel. Such devices may prove crucial in novel screening appliances. Nevertheless, a number of challenges still have to be met:

Shelf life

While it has been demonstrated that gliding motility assays can be stored in a frozen state over months [108] and in a refrigerated state up to two weeks [109], shelf life is still an issue that needs to be addressed. In particular, surface treatment has been shown to greatly influence the activity of motor proteins and affects different motor proteins differently [21••,57,64,110–115].

Specificity

Many cellular proteins naturally bind to cytoskeletal filaments. This could lead to an unwanted (i.e. independent of the antibodies used for detection) transport of such proteins or even loss of motor protein function. The latter possibility will require tests of actin-myosin II and microtubule–kinesin-1 motility under the conditions met in standard test situations. While internal controls can correct for effects related to unwanted transport, the signal-to-noise ratio may be severely affected if proteins that interact with cytoskeletal filaments are to be analysed. One solution would be use actin-myosin II when microtubule-binding proteins are to be analysed and vice versa.

Sensitivity

All of the molecular motor-based detection systems outlined in this review are still on a proof-of-principle level. Most of them have not yet demonstrated detection sensitivities that can compete with other state-of-the-art detection methods like the bio-barcode assay [68,69]. One inherent advantage of the bio-barcode assay is that the magnetic beads used for analyte capture can freely diffuse through the analyte solution and rapidly explore the whole sample volume. A rough estimate using equation 2 from [77] shows that at common bio-barcode assay conditions [69] approximately one sixth of all available target molecules are collected within the first second. In these assays the number of beads and thus the surface area available for analyte capture increases proportionally to the sample volume. This means that for large sample volumes, the bio-barcode assay collects analyte molecules significantly more efficient than any surface-based detection method. However, for small sample volumes, where the surface-to-volume-ratio is high, this advantage becomes less significant. After successful capture of the analyte, molecular motor-based devices can exploit the advantages of active transport on the nanoscale: Using cytoskeletal motor systems, captured analyte can be purified, concentrated and detected with increased sensitivity all in relatively short time owing to advanced miniaturization [87•].

The current development of highly sensitive devices for molecular detection still focuses on the optimization of microfluidic devices and microarrays. However, at the same time it is more than worthwhile to develop molecular motor-based techniques so that they can take over when the limits of microfluidics are reached. Initially this parallel development will be driven by autonomous lab-on-a-chip devices that do not need to compete with the sensitivity of cutting-edge devices but rather with other

point-of-care detection methods like test strips. Ultimately, molecular motor-based detection devices have the potential to be miniaturized down to the nanoscale and be optimized for protein detection from extremely small volumes like single cells or even subcellular compartments.

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Conflict of interest

Alf Månsson is a co-founder, co-owner and CEO of the start-up company ActoSense Biotech AB (Kalmar, Sweden) aiming to develop diagnostic devices based on the aggregation of cytoskeletal elements, particularly actin filaments, in solution. Moreover, Alf Månsson holds two Swedish patents in this field and application for one of these patents (about aggregation of actin filaments by analyte molecules) has also been filed in the US, Europe and India.

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- • of special interest
- •• of outstanding interest

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