Harnessing biological motors to engineer systems for nanoscale transport and assembly

Living systems use biological nanomotors to build life's essential molecules—such as DNA and proteins—as well as to transport cargo inside cells with both spatial and temporal precision. Each motor is highly specialized and carries out a distinct function within the cell. Some have even evolved sophisticated mechanisms to ensure quality control during nanomanufacturing processes, whether to correct errors in biosynthesis or to detect and permit the repair of damaged transport highways. In general, these nanomotors consume chemical energy in order to undergo a series of shape changes that let them interact sequentially with other molecules. Here we review some of the many tasks that biomotors perform and analyse their underlying design principles from an engineering perspective. We also discuss experiments and strategies to integrate biomotors into synthetic environments for applications such as sensing, transport and assembly.

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By considering how the biological machinery of our cells carries out many different functions with a high level of specificity, we can identify a number of engineering principles that can be used to harness these sophisticated molecular machines for applications outside their usual environments. Here we focus on two broad classes of nanomotors that burn chemical energy to move along linear tracks: assembly nanomotors and transport nanomotors.

SEQUENTIAL ASSEMBLY AND POLYMERIZATION

The molecular machinery found in our cells is responsible for the sequential assembly of complex biopolymers from their component building blocks (monomers): polymerases make DNA and RNA from nucleic acids, and ribosomes construct proteins from amino acids. These assembly nanomotors operate in conjunction with a master DNA or RNA template that defines the order in which individual building blocks must be incorporated into a new biopolymer. In addition to recognizing and binding the correct substrates (from a pool of many different ones), the motors must also catalyse the chemical reaction that joins them into a growing polymer chain. Moreover, both types of motors have evolved highly sophisticated mechanisms so that they are able not only to discriminate the correct monomers from the wrong ones, but also to detect and repair mistakes as they occur[1].

Molecular assembly machines or nanomotors (Fig. 1a) must effectively discriminate between substrate monomers that are structurally very similar. Polymerases must be able to distinguish between different nucleosides, and ribosomes need to recognize particular transfer-RNAs (t-RNAs) that carry a specific amino acid. These well-engineered biological nanomotors achieve this by pairing complementary Watson–Crick base pairs and comparing the geometrical fit of the monomers to their respective polymeric templates. This molecular discrimination makes use of the differential binding strengths of correctly matched and mismatched substrates, which is determined by the complementarity of the base-pairing between them.

Figure 1b illustrates the assembly process used by the DNA polymerase nanomotor. A template of single-stranded DNA binds to the nanomotor with angstrom-level precision, forming an open complex. The open complex can 'sample' the free nucleosides available. Binding of the correct nucleoside induces a conformational change in the nanomotor which then allows the new nucleoside to be added to the growing DNA strand[1]. The tight-fitting complementarity of shapes between the polymerase binding site and the properly paired base pair guarantees a 'geometric selection' for the correct nucleotide[2]. A similar mechanism is seen in *Escherichia coli* RNA polymerase, where the binding of an incorrect monomer inhibits the conformational change in the motor from an 'open' (inactive) to a 'closed' (active) conformation[3].

Ribosome motors carry out tasks much more complex than polymerases. Instead of the four nucleotide building blocks used by polymerases to assemble DNA or RNA, ribosomes must recognize and selectively arrange 20 amino acids to synthesize a protein. This fact alone increases the chance of errors. Nevertheless, ribosomes obviously work (and do so along the same principles of geometric fit

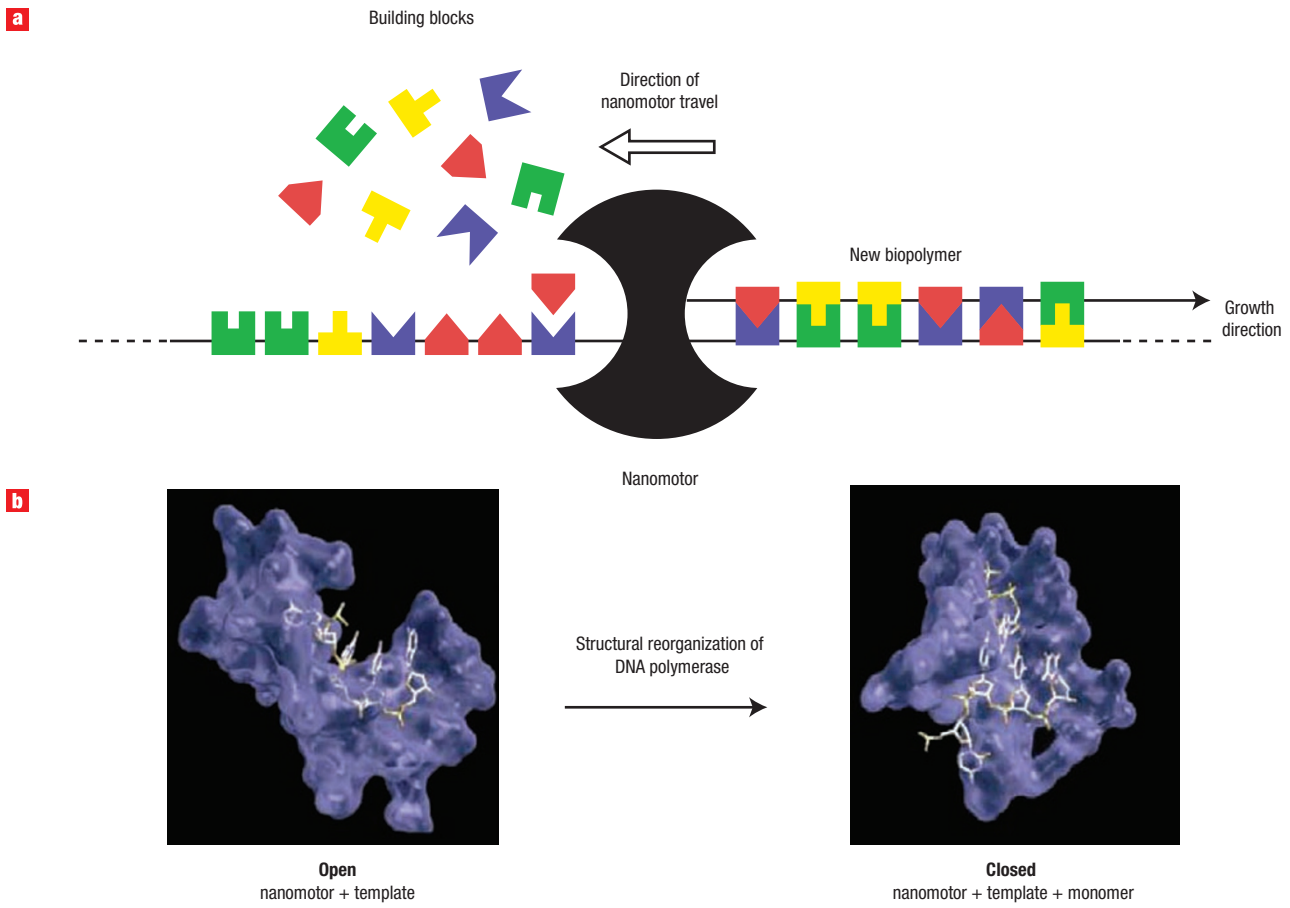

Figure 1 Molecular discrimination during sequential assembly. **a**, The polymerase nanomotor discriminates between four different building blocks as it assembles a DNA or RNA strand complementary to its template sequence. Molecular discrimination between substrate monomers that are structurally very similar is achieved by comparing the geometrical fit of the monomers to their respective polymeric templates. **b**, The T7 DNA polymerase motor undergoes an internal structural transition from an open state (when the active site samples different nucleotides) to a closed state (when the correct nucleotide is incorporated into the nascent DNA strand). Nucleotides are added to the nascent strand one at a time. This structural transition is the rate-limiting step in the replication cycle and is thought to be dependent on the mechanical tension in the template strand[2,9,107,116,121,127,128,131]. Figure adapted from ref. 127. Copyright (2001) PNAS.

and conformational change as do polymerases) and are able to build amino acid polymers that are subsequently folded into functional proteins. But ribosomal motors can be tricked, much more easily than DNA motors, into building the 'incorrect' sequences when supplied with synthetic amino acids that resemble real ones[4].

Engineering principle no. 1: Nanomotors used in the sequential assembly of biopolymers can discriminate efficiently between similar building blocks.

The structure of molecular machines can be visualized with angstrom-level resolution using X-ray crystallography, and the sequential assembly processes they drive can be probed in real time using single-molecule techniques[5–9]. By elucidating nanomotor kinetics under load, such nanoscale techniques provide detailed insights into the single-molecule dynamics of nanomotor-driven assembly processes. Techniques such as optical and magnetic tweezers, for example, have further elucidated the polymer properties of DNA[7,10–12] and the force-dependent kinetics of molecular motors[13–18]. Single-molecule fluorescence methods such as fluorescence energy transfer, in conjunction with such biomechanical tools, are illuminating the internal conformational dynamics of these nanomotors[19–21].

As the underlying design principles of assembly nanomotors are revealed, it will become increasingly possible to use these biomachines for *ex vivo* tasks. Sequencing and PCR are two such techniques that already harness polymerase nanomotors for the *ex vivo* replication of nucleic acids. The polymerase chain reaction, or PCR, is a landmark, Nobel prize-winning technique[22] invented in the 1980s that harnessed polymerase nanomotors to amplify a very small starting sample of DNA to billions of molecules. Likewise, there are many conceivable future applications that either use assembly nanomotors *ex vivo* or mimic some of their design principles. Efforts are already under way to control these nanomotors better and thus to improve such *ex vivo* sequential assembly processes for industrial use (see, for example, the websites www.cambrios.com; www.helicosbio.com; www.nanobiosym.com; www.pacificbiosciences.com).

In contrast, current *ex vivo* methods to synthesize block copolymers rely primarily on random collisions, resulting in a wide range of length distributions and much less control over the final sequence[23]. Sequential assembly without the use of nanomotors remains limited to the synthesis of comparatively short peptides, oligonucleotides and oligosaccharides[24–26]. Common synthesizers still lack both the precision of monomer selection and the inbuilt proofreading machinery for monomer repair that nanomotors have.

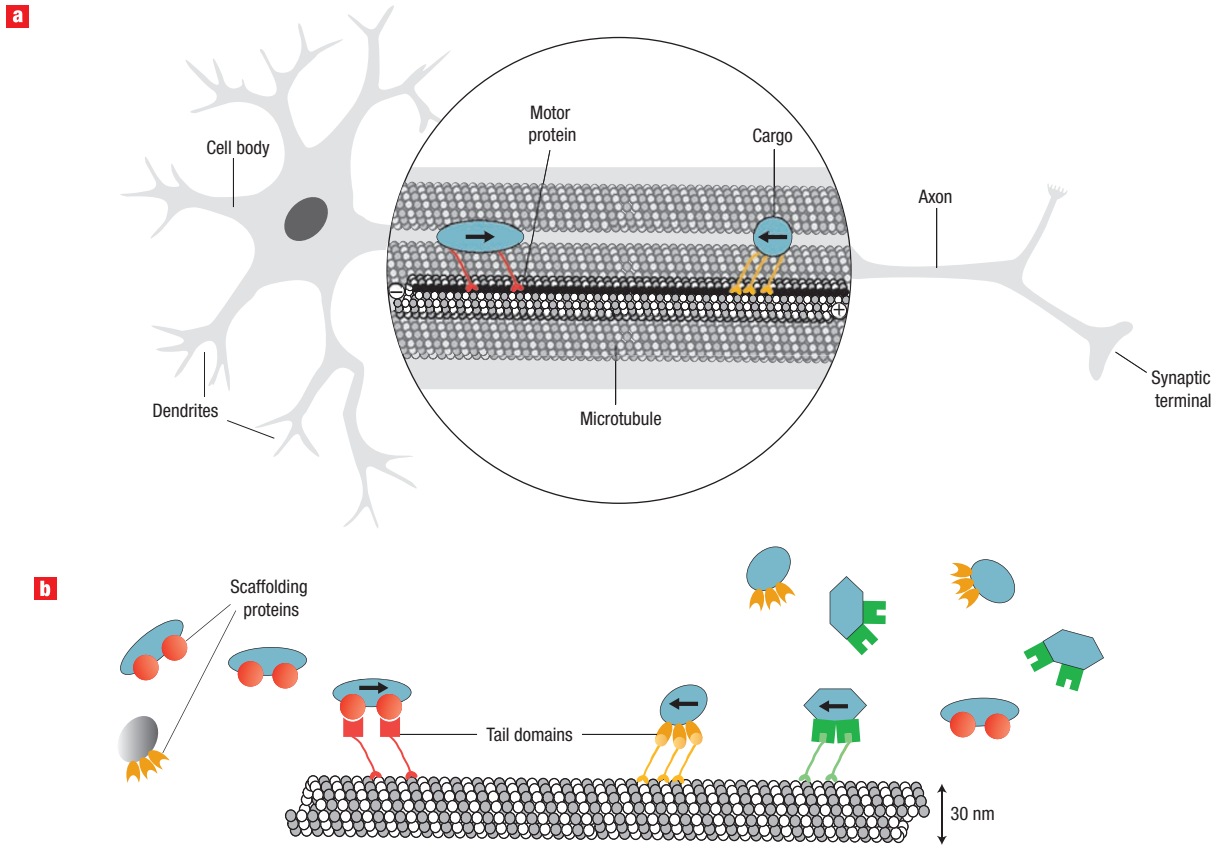

Figure 2 Motor-specific cargo transport in neurons. **a**, The axon of neurons consists of a bundle of highly aligned microtubules along which cargo is trafficked from the cell body to the synapse and vice versa. Most members of the large kinesin family (red) transport cargo towards the periphery, while other motors, including dyneins (yellow), transport cargo in the opposite direction. Motors preferentially move along a protofilament rather then side-stepping (one randomly selected protofilament is shown in dark grey). Protofilaments are assembled from the dimeric protein tubulin (white and grey spheres) which gives microtubules their structural polarity. The protofilaments then form the hollow microtubule rod. When encountering each other on the same protofilament, the much more tightly bound kinesin has the 'right of way', perhaps even forcing the dynein to step sidewise to a neighbouring protofilament[52–55]. **b**, Each member of a motor family selects its own cargo (blue shapes) through specific binding by scaffolding proteins (coloured symbols) or directly by the cargo's tail domains.

Building such copolymers with polymerase nanomotors *ex vivo* would yield much more homogeneous products of the correct sequence and precise length. Natural (for example, nanomotor-enabled) designs could inspire new technologies to synthesize custom biopolymers precisely from a given blueprint.

Ribosome motors have likewise been harnessed *ex vivo* to drive the assembly of new bio-inorganic heterostructures[27] and peptide nanowires[28,29] with gold-modified amino acids inserted into a polypeptide chain. These ribosomes are forced to use inorganically modified t-RNAs to sequentially assemble a hybrid protein containing gold nanoparticles wherever the amino acid cysteine was specified by the messenger RNA template. Such hybrid gold-containing proteins can then attach themselves selectively to materials used in electronics, such as gallium arsenide[28]. This application illustrates how biomotors could be harnessed to synthesize and assemble even non-biological constructs such as nanoelectronic components (see www.cambrios.com).

Assembly nanomotors achieve such high precision in sequential assembly by making use of three key features: (i) geometric shape-fitting selection of their building blocks (for example, nucleotides); (ii) motion along a polymeric template coupled to consumption of an energy source (for example, hydrolysis of ATP molecules); and (iii) intricate proofreading machinery to correct errors as they occur. Furthermore, nanomotor-driven assembly processes allow much more stable, precise and complex nanostructures to be engineered than can be achieved by thermally driven self-assembly techniques alone[30–32].

We should also ask whether some of these principles, which work so well at the nanoscale, could be realized at the micrometre-scale as well. Whitesides and co-workers, for example, have used simple molecular self-assembly strategies, driven by the interplay of hydrophobic and hydrophilic interactions, to assemble microfabricated objects at the mesoscale[33,34]. Perhaps the design principles used by nanomotors to improve precision and correct errors could also be harnessed to engineer future *ex vivo* systems at the nanoscale as well as on other length scales. Learning how to engineer systems that mimic the precision and control of nanomotor-driven assembly processes may ultimately lead to efficient fabrication of complex nanoscopic and mesoscopic structures.

CARGO TRANSPORT

Cells routinely use another set of nanomotors (that is, transport nanomotors) to recognize, sort, shuttle and deliver intracellular cargo along filamentous freeways to well-defined destinations, allowing molecules and organelles to become highly organized (see reviews[35–44]). This is essential for many life processes. Motor

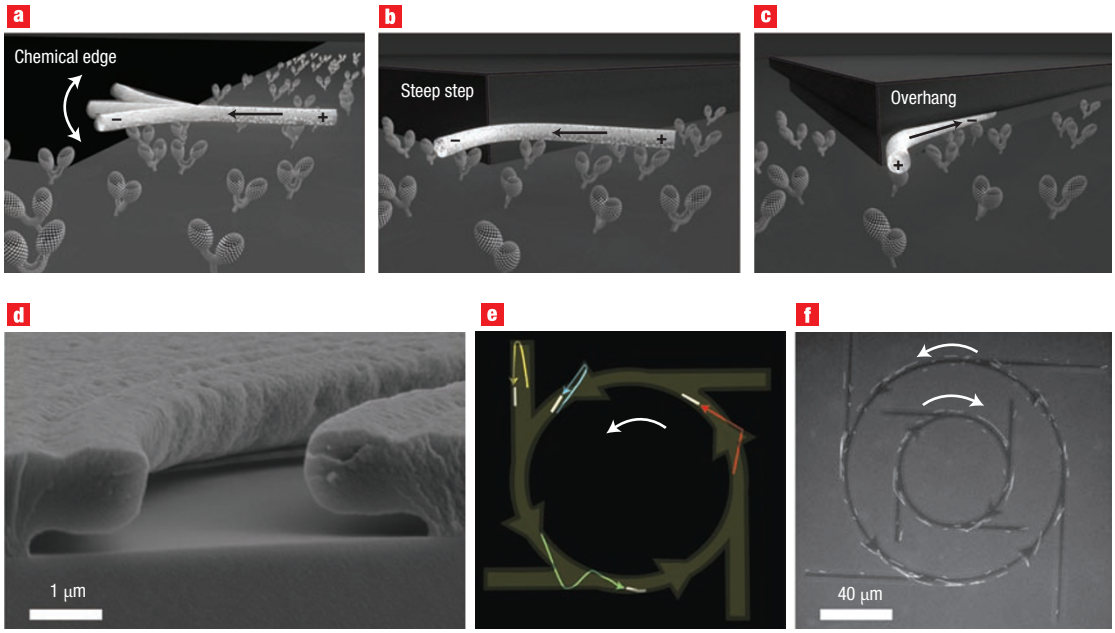

Figure 3 Track designs to guide nanomotor-driven filaments *ex vivo*. A variety of track designs have been used. **a**, A chemical edge (adhesive stripes coated with kinesin surrounded by non-adhesive areas). The filament crosses the chemical edge and ultimately falls off as it does not find kinesins on the non-adhesive areas[61]. **b**, Steep channel walls keep the microtubule on the desired path as they are forced to bend[61,65]. **c**, Overhanging walls have been shown to have the highest guidance efficiency[64]. **d**, Electron micrograph of a microfabricated open channel with overhanging walls[64]. **e**, Breaking the symmetry of micropatterns can promote directional sorting of filament movement[63,65,69,138]. The trajectories of four microtubules are shown: movement into reflector arms causes the tubule to turn around (yellow), an arrow-shaped direction rectifier allows those travelling in the desired direction to continue (red) and forces others to turn around (blue). At intersections tubules preferentially continue straight on (green). **f**, The complex microfabricated circuit analysed in **e** with open channels and overhanging walls demonstrating unidirectional movement of microtubules.

proteins transport cargo along cytoskeletal filaments to precise targets, concentrating molecules in desired locations. In intracellular transport, myosin motors are guided by actin filaments, whereas dynein and kinesin motors move along rod-like microtubules. Figure 2a illustrates how conventional kinesins transport molecular cargo along nerve axons towards the periphery, efficiently transporting material from the cell body to the synaptic region[45]. Dyneins, in contrast, move cargo in the opposite direction, so that there is active communication and recycling between both ends (see reviews[42,46]). In fact, the blockage of such bidirectional cargo transport along nerve axons can give rise to substantial neural disorders[47–50].

The long-range guidance of cargo is made possible by motors pulling their cargo along filamentous rods. Microtubules, for example, are polymerized from the dimeric tubulin into protofilaments that assemble into rigid rods around 30 nm in diameter[36]. These polymeric rods are inherently unstable: they polymerize at one end (plus) while depolymerizing from the other (minus) end, giving rise to a structural polarity. The biological advantage of using transient tracks is that they can be rapidly reconfigured on demand and in response to changing cellular needs or to various external stimuli. Highly efficient unidirectional cargo transport is realized in cells by bundling microtubules into transport highways where all microtubules are oriented in the same direction. Excessively tight bundling of microtubules, however, can greatly impair the efficiency of cargo transport, by blocking the access of motors and cargo to the microtubules in the bundle interior. Instead, microtubule-associated proteins are thought to act as repulsive polymer-brushes, thereby regulating the proximity and interactions between neighbouring microtubules[51].

Traffic control is an issue when using the filaments as tracks on which kinesin and dynein motors move in opposite directions. Although different cargoes can be selectively recognized by different members of the motor protein families and shuttled to different destinations, what happens if motors moving in opposite directions encounter each other on the same protofilament (Fig. 2b)? If two of these motors happen to run into each other, kinesin seems to have the 'right of way'. As kinesin binds the microtubule much more strongly, it is thought to force dynein to step sideways to a neighbouring protofilament[52]. Dynein shows greater lateral movement between protofilaments than kinesin[52–54] as there is a strong diffusional component to its steps[55]. When a microtubule becomes overcrowded with only kinesins, the runs of individual kinesin motors are minimally affected. But when a microtubule becomes overloaded with a mutant kinesin that is unable to step efficiently, the average speed of wild-type kinesin is reduced, whereas its processivity is hardly changed. This suggests that kinesin remains tightly bound to the microtubule when encountering an obstacle and waits until the obstacle unbinds and frees the binding site for kinesin's next step[56].

Engineering principle no. 2: Various track designs enable motors to pull their cargo along filamentous tracks, whereas others allow motors bound to micro- or nanofabricated tracks to propel the filaments which can then serve as carriers.

It is not a trivial task to engineer transport highways *ex vivo*, particularly in versatile geometries with intersections and complex shapes. Individual filaments typically allow only one-dimensional transport, as the motor-linked cargo drops off once the end of the filament is reached. Furthermore, conventional kinesin makes only a few hundred 8-nm-sized steps before dissociating from the microtubule[57,58], further limiting the use of such a system for *ex vivo* applications.

Instead of having the motors transport their cargo along filaments, motors have been immobilized on surfaces in an inverted geometry

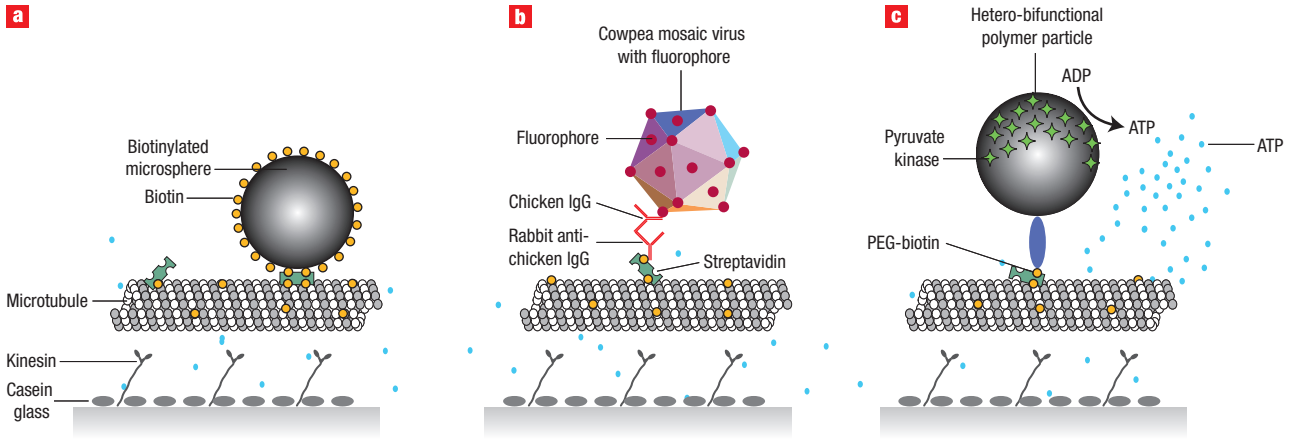

Figure 4 Selecting specific cargo by molecular recognition**.** A versatile toolbox exists by which synthetic and biological cargo can be coupled to microtubules. **a**, Biotinylated objects are coupled via avidin or streptavidin to biotinylated microtubules. **b**, Biological molecules, viruses[79,81] or cells can be coupled by antibody recognition. **c**, Backpacks of chemically or biologically active reagents can be shuttled around, including bioprobes[80] or tiny ATP factories[93] as shown here.

that enables the filaments to be collectively propelled forward[45]. The head domains of the kinesin and myosin motors can rotate and swivel with respect to their feet domains, which are typically bound in random orientations to the surface. These motor heads detect the structural anisotropy of the microtubules and coherently work together to propel a filament forward[59,60].

Various examples of such inverted designs for motor tracks have been engineered to guide filaments efficiently. Some of these are illustrated in Fig. 3. Inverted motility assays can be created, for example, by laying down tracks of motor proteins in microscopic stripes of chemical adhesive on an otherwise flat, protein-repellent surface, surrounded by non-adhesive surface areas. Such chemical patterns (Fig. 3a) have been explored to guide actin filaments or microtubules. The loss rate of guiding filaments increases exponentially with the angle at which they approach an adhesive/non-adhesive contact line[61]. The passage of the contact line by filaments at non-grazing angles, followed by their drop off, can be prevented by using much narrower lanes whose size is of the order of the diameter of the moving object. Such nanoscale kinesin tracks provide good guidance and have been fabricated by nanotemplating[62].

Alternatively, considerably improved guidance has been accomplished by topographic surface features (Fig. 3b). Microtubules hitting a wall are forced to bend along this obstacle and will continue to move along the wall[63–66]. The rigidity of the polymeric filaments used as shuttles thus greatly affects how tracks should be designed for optimal guidance. Whereas microtubules with a persistence length of a few millimetres can be effectively guided in channels a few micrometres wide as they are too stiff to turn around[61], the much more flexible actin filaments require channel widths in the submicrometre range[67,68]. Finally, the best long-distance guidance of microtubules has been obtained so far with overhanging walls[64,69](Fig. 3c). The concept of topographic guidance in fact works so well that swarms of kinesin-driven microtubules have been used as independently moving probes to image unknown surface topographies. After averaging all their trajectories in the focal plane for an extended time period, the image greyscale is determined by the probability of a surface pixel being visited by a microtubule in a given time frame[70].

But how can tracks be engineered to produce *uni*directional cargo transport? All the motor-propelled filaments must move in the same direction to achieve effective long-distance transport. When polar filaments land from solution onto a motor-covered surface, however, their orientations and initial directions of movement are often randomly distributed. Initially, various physical means, such as flow fields[71], have been introduced to promote their alignment. Strong flows eventually either force gliding microtubules to move along with the flow, or force microtubules, if either their plus or minus end is immobilized on a surface[72], to rotate around the anchoring point and along with the flow. The most universal way to control the local direction in which the filamentous shuttles are guided is to make use of asymmetric channel features. Figure 3d–f illustrates how filaments can be actively sorted according to their direction of motion by breaking the symmetry of the engineered tracks. This 'local directional sorting' has been demonstrated on surfaces patterned with open channel geometries, where asymmetric intersections are followed by dead-ended channels (that is, reflector arms), or where channels are broadened into arrow heads. Both of these topographical features not only selectively pass filaments moving in the desired direction, but can also force filaments moving in the opposite direction to turn around[65,69,73,74]. Once directional sorting has been accomplished, electric fields have been used to steer the movement of individual microtubules as they pass through engineered intersections[75,76].

In addition to using isolated nanomotors, hybrid biodevices and systems that harness self-propelling microbes could be used to drive transport processes along engineered tracks. Flagellated bacteria, for example, have been used to generate both translational and rotational motion of microscopic objects[77]. These bacteria can be attached head-on to solid surfaces, either via polystyrene beads or polydimethylsiloxane, thereby enabling the cell bodies to form a densely packed monolayer, while their flagella continue to rotate freely. In fact, a microrotary motor, fuelled by glucose and comprising a 20-μm-diameter silicon dioxide rotor, can be driven along a silicon track by the gliding bacterium *Mycoplasma*[78]. Depending on the specific application and the length scale on which transport needs to be achieved, integrating bacteria into such biohybrid devices (that work under physiological conditions) might ultimately prove more robust than relying solely on individual nanomotors.

CARGO SELECTION

To maintain intracellular contents in an inhomogeneous distribution far from equilibrium, the intracellular transport system must deliver molecular cargo and organelles on demand to precise destinations. This tight spatiotemporal control of molecular deliveries is critical for adequate cell function and survival. Molecular cargo or organelles

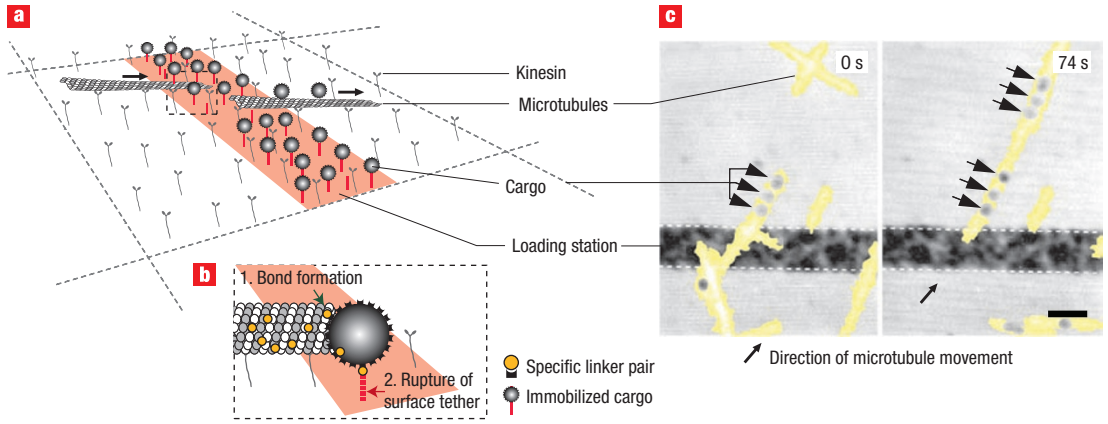

Figure 5 Cargo loading stations[93]. **a**, Stripes of immobilized cargo are fabricated by binding thiolated oligonucleotides to micropatterned lines of gold. Hybridization with complementary strands exposing antibodies at their terminal ends allows them to immobilize a versatile range of cargos that carry antibodies on their surfaces. **b**, The challenge is to tune the bond strength and valency to prevent thermal activation during cargo storage on the loading station. On collision with the shuttle (microtubule), the cargo must rapidly break off the bond it has formed with the station[88]. Fortunately, however, tensile mechanical force acting on a non-covalent bond shortens its lifetime. **c**, **d**, These concepts are used in the design of the loading stations shown here, where a microtubule moves through a stripe of immobilized gold cargo and picks up a few beads.

are typically barcoded so that they can be recognized by their specific motor protein (Fig. 4). Within cells, motors recognize cargo either from the cargo's tail domains directly, or via scaffolding proteins that link cargo to their tail domain[43].

Engineering principle no. 3: Engineered molecular recognition sites enable cargo to be selectively bonded to moving shuttles.

Although most cargo shuttled around by motors can be barcoded using the existing repertoire of biological scaffolding proteins, synthetic approaches are needed for all those *ex vivo* applications where the cargo has to be specifically linked to moving filaments. The loading and transport of biomedically relevant or engineered cargo has already been demonstrated (Fig. 4)[79–83]. Typical approaches are to tag the cargo with antibodies or to biotinylate microtubules and coat the cargo with avidin or streptavidin (Fig. 4) (for reviews, see refs 74,79), as done for polymeric and magnetic beads[84,85] (Fig. 4a), gold nanoparticles[86–88], DNA[87,89,90] and viruses[79,81] (Fig. 4b), and finally mobile bioprobes and sensors[80,81,91](Fig.4c). However, if too much cargo is loaded onto the moving filaments and access of the propelling motors is even partially blocked, the transport velocity can be significantly impaired[92]. Finally, the binding of cargo to a moving shuttle can be used to regulate its performance. In fact, microtubules have recently been furnished with a backpack that self-supplies the energy source ATP. Cargo particles bearing pyruvate kinase have been tethered to the microtubules to provide a local ATP source[93] (Fig. 4c). The coupling of multiple motors to cargo or other scaffold materials can affect the motor performance. If single-headed instead of double-headed kinesins are used, cooperative interactions between the monomeric motors attached to protein scaffolds increase hydrolysis activity and microtubule gliding velocity[59].

At the next level of complexity, successful cargo tagging, sorting and delivery will depend on the engineering of integrated networks of cargo loading, cargo transport and cargo delivery zones. Although the construction of integrated transport circuits is still in its infancy, microfabricated loading stations have been built[88] (Fig. 5). The challenge here is to immobilize cargo on loading stations such that it is not easily detached by thermal motion, yet to allow for rapid cargo transfer to passing microtubules. By properly tuning bond strength and multivalency, and most importantly by taking advantage of the fact that mechanical strain weakens bonds, cargo can be efficiently stored on micropatches and transferred after colliding with a microtubule[88]. Considerable fine-tuning of bond strength can be accomplished by using DNA oligomers hybridized such that the bonds are either broken by force all at once (a strong bond) or in sequence (a weak bond)[94].

As discussed above, filaments are most commonly used to shuttle molecular cargo in most emerging devices that harness linear motors for active transport. Alternatively, if the filamentous tracks could be engineered in versatile geometries, the motors themselves could be used to drag cargo coupled to the molecular recognition sites of their tail domains as in the native systems. We could thus make use of the full biological toolbox of already known or engineered scaffolding proteins that link specific motors to their respective cargoes[40,43]. So far, assemblies of microtubules organized into complex, three-dimensional patterns such as asters, vortices and networks of interconnected poles[95,96] have been successfully created in solution, and mesoscopic needles and rotating spools of microtubule bundles held together by non-covalent interactions have been engineered on surfaces[31]. All of these mesoscopic structures are uniquely related to active motor-driven motion and would not have formed purely by self-assembly without access to an energy source.

To increase the complexity of microtubule track networks, densely packed arrays of microtubules have been grown in confined spaces, consisting of open microfabricated channels with user-defined geometrical patterns[97]. The key to achieving directed transport, however, is for all microtubules within each bundle or array to be oriented in the same direction. This has been accomplished by making use of directed motility in combination with sequential assembly procedures (Fig. 6). First, microtubule seedlings have been oriented in open microfabricated and kinesin-coated channels that contain reflector arms. Once oriented by self-propelled motion, the seedlings were polymerized into mature microtubules that were confined to grow in the open channels until the channels were filled with dense networks of microtubules all oriented in the same direction[97]. Single kinesins take only a few hundred steps before they fall off, but the walking distance can be greatly increased if the cargo is pulled by more than one motor[98]. Such approaches to fabricating networks of microtubule bundles could be further expanded to engineer future devices that use either the full toolbox of native scaffolding proteins or new scaffolding proteins that target both biological and synthetic cargo.

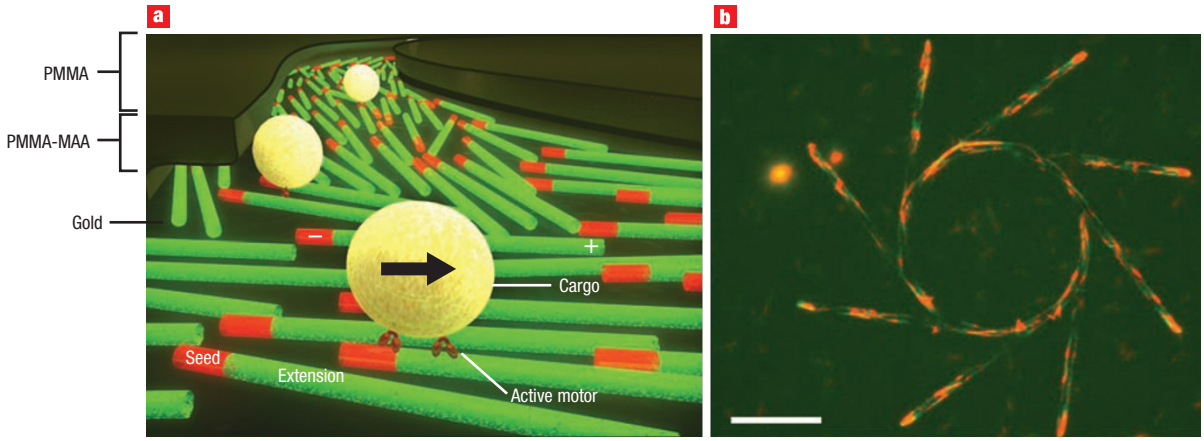

Figure 6 Filament tracks made from engineered bundles of microtubules[97]. Active transport is used to produce bundles of microtubules and confine them to user-defined geometries. **a**, Sequential assembly procedure: first, microtubule seedlings labelled in red are allowed to orient themselves in open kinesin-coated microfabricated channels that contained reflector arms. Second, and after mild fixation, the oriented seedlings are polymerized into mature microtubules through the addition of tubulin into the solution (labelled green) which preferentially binds to the plus-end (polymerizing end) of the microtubules. **b**, Fluorescence image of microtubules that have been grown in the confined space provided by the open channels until the channels were filled with dense networks of microtubules all oriented in the same direction[97]. Scale bar, 40 μm.

Nanoengineers would not be the first to harness biological motors to transport their cargo. Various pathogens are known to hijack microtubule or actin-based transport systems within host cells (reviewed in ref. 99). *Listeria monocytogenes*, for example, propels itself through the host cell cytoplasm by means of a fast-polymerizing actin filament tail[100]. Likewise, the vaccinia virus, a close relative of smallpox, uses actin polymerization to enhance its cell-to-cell spreading[101], and the alpha herpes virus hijacks kinesins to achieve long-distance transport along the microtubules of neuronal axons[102]. Signalling molecules and pathogens that cannot alter cell function and behaviour by simply passing the outer cell membrane can thus hijack the cytoskeletal highways to get transported from the cell periphery to the nucleus.

Engineering principle no. 4: By taking advantage of the existing cytoskeleton, tailored drugs and gene carriers can be actively transported to the cell nucleus.

Indeed, many viruses[37,103,104] as well as non-viral therapeutic gene carriers, such as polyethylenimine/DNA or other polymer-based gene transfer systems (that is, polyplexes)[105,106] take advantage of nanomotor-driven transport along microtubule filaments to accelerate their way through the cytoplasm towards the nucleus. Nanomotor-driven transport to the nucleus leads to a much more efficient nuclear localization than could ever be achieved by slow random diffusion through the viscous cytoplasm. Active gene carrier transport can lead to more efficient perinuclear accumulation within minutes[37,105,106]. In contrast, non-viral gene carriers that depend solely on random diffusion through the cytoplasm move much more slowly and thus have considerably reduced transfection efficiencies. Understanding how to 'hijack' molecular and cellular transport systems, instead of letting a molecule become a target for endosomal degradation[37,91], will ultimately allow the design of more efficient drug and gene carrier systems.

QUALITY CONTROL

Nanomanufacturing processes, much like macroscopic assembly lines, urgently need procedures that offer precise control over the quality of the product, including the ability to recognize and repair defects. Living systems use numerous quality control procedures to detect and repair defects occurring during the synthesis and assembly of biological nanostructures. As yet, this has not been possible in synthetic nanosystems. Many cellular mechanisms for damage surveillance and error correction rely on nanomotors. Such damage control can occur at two different levels as follows.

Engineering principle no. 5: Certain motor proteins recognize assembly mistakes and repair them at the molecular level.

DNA replication represents one of the most complex sequential assembly processes in a cell. Here the genetic information stored in the four-base code must be copied with ultra-high precision. Errors generated during replication can have disastrous biological consequences. Figure 7 illustrates the built-in mechanism used by the polymerase (DNAp) motor to repair mistakes made during the process of DNA replication[107]. When the DNAp motor misincorporates a base while replicating the template DNA strand, it slows down and switches gears from the polymerase to the exonuclease cycle. Once in exonuclease mode, it will excise the mismatched base pair and then rapidly switch back to the polymerase cycle to resume forward replication. Similar error correction mechanisms, known as 'kinetic proofreading', are conjectured to occur in RNA polymerases and ribosomal machineries[1,13,108–113].

Engineering principle no. 6: Integrated systems of motors and signalling molecules are needed to recognize and repair damage at the supramolecular level.

Nerve cells have evolved a highly regulated axonal transport system that contains an integrated damage surveillance system[114]. The traffic regulation of motors moving in opposite directions on a microtubule typically occurs in special 'turnaround' zones at the base and tip of an axon[43], but a zone for switching the organelle's direction can also be created when axonal transport is blocked at the site of nerve injury[46] (see Fig. 2). When irreparable, such blockages are often signatures of neurodegenerative diseases. For example, amyloid precursor protein[47] or tau[115] can give rise to the accumulation of protein aggregates that inhibit anterograde axonal transport, a mechanism potentially implicated in Alzheimer's disease.

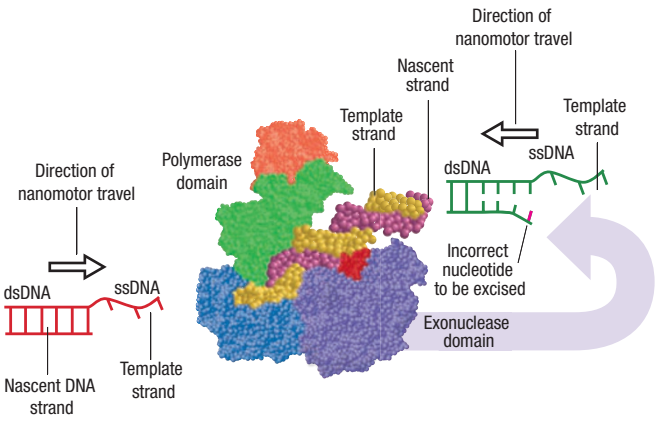

Figure 7 Quality control procedures for damage recognition and molecular repair. The DNA polymerase motor (DNAp) contains two active sites. It switches from polymerase (copying) to exonuclease (error correction) activity when it encounters a mismatched base. Mismatched bases are detected as they have weaker bonding interactions—the 'melting' temperature is lower—and this increases the chance of switching from the polymerase to the exonuclease active site[107]. In the exonuclease mode, the motor excises the incorrect base from the nascent DNA strand.

At present, there are no synthetic materials that can, in a self-regulated manner, recognize and repair defects at either the molecular or supramolecular level. Molecular recognition and repair is typically attributed to a tightly fitted stereochemical complementarity between binding partners. Nanoscale tools applied to the study of molecular recognition and repair are also elucidating the functional roles of the different structural conformations (and hence three-dimensional shapes) of the motors. For instance, the DNAp motor is in one particular conformation when it binds DNA in its copying (that is, polymerization) mode and in an entirely different conformation (that is, the exonuclease mode) when it binds DNA to proofread or excise a mistaken base from the replicated DNA strand[107]. In contrast, damage control at the supramolecular level (for example, during axonal transport) is achieved by the trafficking of signalling molecules. Deciphering the underlying engineering design principles of damage surveillance and error correction mechanisms in biological systems will inevitably allow better quality-control procedures to be integrated into nanoengineered systems.

EXTERNAL CONTROL

Engineering principle no. 7: As with macroscopic engines, external controls can regulate the performance of nanomotors on demand.

Learning how to control and manipulate the performance of nanomotors externally is another critical hurdle in harnessing nanomotors for *ex vivo* applications. By finding or engineering appropriate external knobs in the motor or its environment, its nanoscale movement can be tightly regulated, switched on and off, or otherwise manipulated on demand.

To achieve external control over the nanoscale movement of biological motors, it is important to identify the correct external parameters that can be used to control their dynamics. These external modulators of motor function ('handles') can be either naturally occurring or somehow artificially engineered into the motor to make it susceptible to a particular external control knob or regulator. Because the motion of nanomotors is typically driven by a series of conformational changes in the protein, mechanical load or strain on the motor molecule can also affect the dynamics of the motor. Nanomotors apply mechanical strain to their filaments or substrates as they go through various internal conformational changes. This mechanical strain is intimately related to their dynamics along the substrate and hence their functional performance. Certain interstate transition rates can depend, for example[107], on the amount of intramolecular strain in the motor protein. Applying a mechanical load to a motor perturbs key mechanical transitions in the motor's kinetic pathway, and can thereby affect rates of nucleotide binding, ATP hydrolysis and product release. Single-molecule techniques are beginning to elucidate how mechanical strain on a motor protein might be used to regulate its biological functions (for example, nanoscale assembly or transport)[13,55,107,116–120].

The single-molecule dynamics of the DNA polymerase (DNAp) motor, as it converts single-stranded (ss) DNA to double-stranded (ds) DNA, has been probed, for example, through the differential elasticity of ssDNA and dsDNA (see Fig. 8). The T7 DNA polymerase motor replicates DNA at rates of more than 100 bases per second and this rate steadily decreases with mechanical tension greater than about 5 pN on the DNA template[9]. The motor can work against a maximum of about 34 pN of template tension[9]. The replication rates for the Klenow and Sequenase DNA polymerases also decrease when the ssDNA template tension exceeds 4 pN, and completely ceases at tensions greater than 20 pN (ref. 121). Likewise, single-molecule techniques have allowed direct observation of the RNA polymerase (RNAp) motor moving one base at a time[122], and occasionally pausing and even backtracking[123]. Although RNAp motors are typically five- to tenfold slower than DNAp motors, the effects of DNA template tension on their dynamics are still being investigated[6]. Similarly, ribosome motors, which translate messenger RNA (mRNA) into amino acids at roughly 10 codons per second, have been found to generate about 26.5 ± 1 pN of force[124]. The underlying design principles by which these nanomotors operate are being further elucidated by theoretical models[107,116,125–128] that describe nanomachines at a level commensurate with single-molecule data. Furthermore, these molecular assembly machines can be actively directed, driven and controlled by environmental signals[107].

Consequently, an external load or force applied to the substrate or to the motor itself can be used to slow down a motor's action or stall its movement. The stalling forces of kinesin and dynein are 6 and 1 pN, respectively[58,129]. For example, the binding of two kinesin domains to a microtubule track creates an internal strain in the motor that prevents ATP from binding to the leading motor head. In this way, the two motor domains remain out-of-phase for many mechanochemical cycles and thereby provide an efficient, adaptable mechanism for achieving highly processive movement[130]. Beyond stalling the movement of motors by a mechanical load, other types of perturbations can also influence the dynamics of molecular motors, including the stretching of substrate molecules like DNA[13]. Although this external control over nanomotors has been demonstrated in a few different contexts *ex vivo*, a rich detailed mechanistic understanding of how such external control knobs can modulate the dynamics of the molecular motor is emerging from recent work on the DNA polymerase motor[9,107,116,121,127,128,131].

Remote-controlling the local ATP concentration by the photo-activated release of caged ATP can allow a nanomotor-driven transport system to be accelerated or stopped on demand[84]. External control knobs or regulators can also be engineered into the motors. For instance, point mutations can be introduced into the gene encoding the motor protein, such that it is engineered to respond to light, temperature, pH or other stimuli[43,85]. Engineering light-sensitive switches into nanomotors enables the rate of ATPase[43,132] to be regulated, thereby providing an alternate handle for tuning the motor's speed, even while the ATP concentration is kept constant and high. When additional ATP-consuming enzymes are present in solution, the rate of ATP depletion regulates the distance the shuttles move after being activated by a light pulse and before again coming to a halt[84].

Future applications could require that instead of all the shuttles being moved at the same time, only those in precisely defined locations

be activated, on demand. Some of the highly conserved residues within motors help to determine the motor's ATPase rate[43]. Introducing chemical switches near those locations might provide a handle for chemical manipulation of the motor's speed. In fact, this has already been realized for a rotary motor[132] as well as for a linear kinesin motor, where the insertion of a Ca^{2+}-dependent chemical switch makes the ATPase activity steeply dependent on Ca^{2+} concentrations[133]. In addition to caged ATP, caged peptides that block binding sites could be used to regulate the motility of such systems. Caged peptides derived from the kinesin C-terminus domain have already been used to achieve photo control of kinesin-microtubule motility[134]. Instead of modulating the rate of ATP hydrolysis, the access of microtubules to the motor's head domain can also be blocked in an environmentally controlled manner. In fact, temperature has already been shown to regulate the number of kinesins that are accessible while embedded in a surface-bound film of thermoresponsive polymers[135].

The nanomotor-driven assembly of DNA by the DNA polymerase motor provides an excellent example of how precision control over the nanomotor can be achieved by various external knobs in the motor's environment[107,116,127,128]. The DNAp motor moves along the DNA template by cycling through a given sequence of geometric shape changes. The sequence of shapes or internal states of the nanomachine can be denoted by nodes on a simple network[107,116,127,128]. As illustrated in Fig. 8, this approach elucidates how mechanical tension on a DNA molecule can precisely control (or 'tune') the nanoscale dynamics of the polymerase motor along the DNA track by coupling into key conformational changes of the motor[107].

Macroscopic knobs to precision-control the motor's movement along DNA tracks can be identified by probing how the motor's dynamics vary with each external control knob (varied one at a time). Efforts are currently under way to control even more precisely the movement of these nanomotors along DNA tracks by tightly controlling the parameters in the motor's environment (see www.nanobiosym.com). Concepts of fine-tuning and robustness could also be extended to describe the sensitivity of other nanomotors (modelled as simple biochemical networks) to various external control parameters[107]. Furthermore, such a network approach[107] provides experimentally testable predictions that could aid the design of future molecular-scale manufacturing methods that integrate nanomotor-driven assembly schemes. External control of these nanomotors will be critical in harnessing them for nanoscale manufacturing applications.

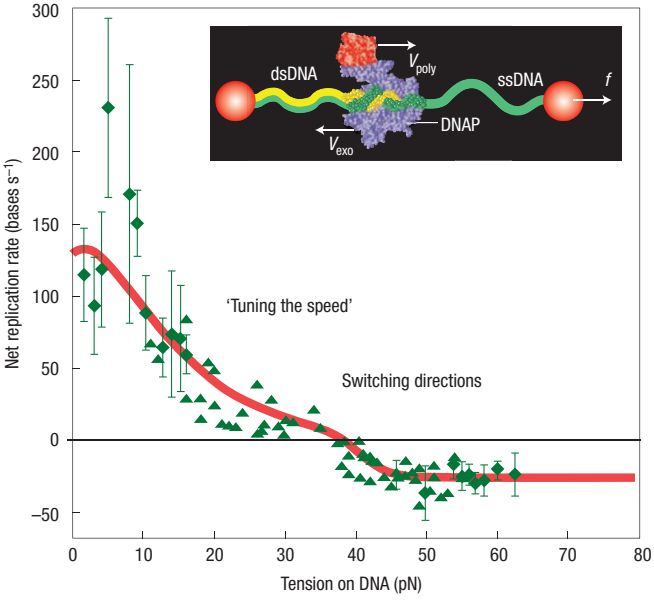

Figure 8 Precision control of nanomotors with external control 'knobs'. The net replication rate of a DNAp motor can be controlled by the mechanical tension on the DNA template strand. Single-molecule data for the motor's force-dependent velocity (two sets of data—diamonds and triangles—are shown, relating to constant force and constant extension measurements) can be described by a network model (red curve) as shown here. The change in net replication rate shows how external controls can change the dynamics of the nanomotor. This model illustrates how environmental control knobs can tune the dynamics of the nanomotor by altering the rate constants associated with its various internal transitions[106]. Tensions between 0 and 35 pN control the net replication rate, whereas tensions above 35 pN actually reverse the velocity of the nanomotor. Inset, experimental setup: a single DNA molecule is stretched between two plastic beads as the motor catalyses the conversion of single-stranded to double-stranded DNA. Figure adapted from ref. 106.

CONCLUDING REMARKS

We have reviewed several key engineering design principles that enable nanomotors moving along linear templates to perform a myriad of tasks. Equally complex biomimetic tasks have not yet been mastered *ex vivo*, either by harnessing biological motors or via synthetic analogues. Engineering insights into how such tasks are carried out by the biological nanosystems will inspire new technologies that harness nanomotor-driven processes to build new systems for nanoscale transport and assembly.

Sequential assembly and nanoscale transport, combined with features currently attributed only to biological materials, such as self-repair and healing, might one day become an integral part of future materials and bio-hybrid devices. In the near term, molecular biology techniques could be used to synthesize and assemble nanoelectronic components with more control (www.cambrios.com; see also ref. 29). Numerous proof-of-concept experiments using nanomotors integrated into synthetic microdevices have already been demonstrated (see reviews[74,136]). Among many others, these applications include stretching surface-bound molecules by moving microtubules[87,90]; probing the lifetime of a single receptor–ligand interaction via a cantilevered microtubule that acts as a piconewton force sensor[85]; topographic surface imaging by self-propelled probes[70]; and cargo pick-up from loading stations[88] as illustrated in Fig. 5.

Although much progress is being made in the synthesis of artificial motors (see review[137]), it has been difficult, in practice, to synthesize artificial motors that come even close in performance to their natural counterparts (see review[39]). Harnessing biological motors to perform nanoscale manufacturing tasks might thus be the best near-term strategy. Although many individual nanoparts can be easily manufactured, the high-throughput assembly of these nanocomponents into complex structures is still non-trivial. At present, no *ex vivo* technology exists that can actively guide such nanoscale assembly processes. Despite advances in deciphering the underlying engineering design principles of nanomotors, many hurdles still impede harnessing them for *ex vivo* transport and sequential assembly in nanosystems. Although the use of biological nanomotors puts intrinsic constraints on the conditions under which they can be assembled and used in biohybrid devices, many of their sophisticated tasks are still poorly mimicked by synthetic analogues. Understanding the details of how these little nanomachines convert chemical energy into controlled movements will nevertheless inspire new approaches to engineer synthetic counterparts that might some day be used under harsher conditions, operate at more extreme temperatures, or simply have longer shelf lives.

Certain stages of the materials production process might one day be replaced by nanomotor-driven sequential self-assembly, allowing much more control at the molecular level. Biological motors are already

being used to drive the efficient fabrication of complex nanoscopic and mesoscopic structures, such as nanowires[31] and supramolecular assemblies. Techniques for precision control of nanomotors that read DNA are also being used to engineer integrated systems for rapid DNA detection and analysis (www.nanobiosym.com). The specificity and control of assembly and transport shown by biological systems offers many opportunities to those interested in assembly of complex nanosystems. Most importantly, the intricate schemes of proofreading and damage repair—features that have not yet been realized in any manmade nanosystems—should provide inspiration for those interested in producing synthetic systems capable of similarly complex tasks.

doi:10.1038/nnano.2008.190

Published online: 27 July 2008.

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Acknowledgements

We thank Sheila Luna, Christian Brunner and Jennifer Wilson for the artwork, and all of our collaborators who contributed thoughts and experiments. At the same time, we apologize to all authors whose work we could not cite owing to space limitations.
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