time to useful timescales. By manipulating the ratio of these modified monomers, Rant and co-workers created nanopores that contained only a single nitrilotriacetic acid group within the sensing zone of the nanopore, which could be used to detect the reversible binding of single proteins.

In another part of the study, Rant and colleagues functionalized a nanopore with three nitrilotriacetic acid groups and showed that this nanopore could tightly capture Protein A, which is a clinically relevant pathogenic molecule that evades the immune system by binding to different antibodies with different affinities. The nanopores that contained Protein A were used as an assay to discriminate between different types of rodent antibodies — each type of antibody that entered the nanopore would bind to Protein A with a different affinity, resulting in a characteristically different electrical fingerprint. This forms a useful assay for studying protein–protein interactions.

A major limitation of this technique is that most naturally occurring proteins do not have polyhistidine tags. Although the mechanics and biophysics of key proteins can still be investigated once the protein is given such a tag, this method in its current form cannot be used as a biosensor to detect clinical targets directly. Despite this limitation, direct observation of single proteins has great potential for many applications, including ultrasensitive detection and enzyme kinetic measurements. Furthermore, the assay can be used to quantify the strength of molecular interactions, and could offer improvements over traditional immunoblotting methods.

This is the first time that reversible detection of single proteins has been demonstrated using a synthetic nanopore, and this technique helps solidify synthetic nanopores as an important research tool. ❐

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MOLECULAR MOTORS

Myosin shifts into reverse gear

A motor protein can be made to walk in either direction along a filamentous track by adjusting the concentration of calcium ions in the surrounding solution.

Wilhelm J. Walter and Stefan Diez

Cytoskeletal motor proteins moving along filamentous tracks perform a variety of functions within cells in a highly energy-efficient manner, and can be easily modified by the tools of molecular biology. This makes them promising candidates for use as force-generating elements in nanotechnology. However, controlling them in an artificial environment is a significant challenge. For example, a molecular motor usually moves in just one direction, and it would be useful to be able to make it move along its track in both directions. Writing in *Nature Nanotechnology*, Zev Bryant and colleagues[1] at Stanford University now report a controllable, bidirectional motor protein based on myosin VI.

Myosin VI is a motor protein that plays a role in intracellular processes such as endocytosis, secretion and anchoring of subcellular compartments[2]. The motor converts chemical energy obtained from ATP hydrolysis into mechanical force to move along actin filaments, which are long twisted polymers of the protein actin. Most myosins walk towards the plus (barbed) end of the filament. Myosin VI, however, walks towards the minus (pointed) end.

The general mechanisms of force generation in myosin motors are well understood. During the ATP-hydrolysis cycle small conformational changes in the filament-bound motor 'head' drive the rotation of a 'converter' region[3]. This rotation is amplified by a rigid coiled-coil, termed 'lever arm', into a large directed motion, the 'power stroke' (Fig. 1a). Notably, for all myosins, independent of their walking direction, the sense of the rotation is identical. In myosin VI, a small 'unique insert' generates a 180° kink at the base of the lever arm. As a result, the lever arm crosses the centre of rotation and on ATP hydrolysis performs a power stroke directed towards the filament minus end[4] (Fig. 1b). The importance of this geometrical arrangement for motor directionality has been demonstrated in a study where the unique insert was mimicked by incorporating a small four-helix bundle into the converter domain of myosin V, thus making it move towards the minus end of the filament rather than towards the plus end[5].

Based on their previous studies showing that the length of both the lever arm[6] and unique insert[7] can influence the directionality of myosin VI, Bryant and colleagues now followed a two-step strategy to engineer a controllable, bidirectional motor. First, they shortened the unique insert of myosin VI from seventeen amino acids to seven. This increased the tilting angle between the lever arm and the actin filament. Consequently, the centre of the lever arm came closer to the rotation axis of the converter domain. Second, they replaced the lever arm by two consecutive IQ motifs — binding domains for the protein calmodulin — from myosin V. These IQ motifs form a collapsible element whose length can be controlled by Ca^{2+}-dependent binding and unbinding of calmodulin[8]. At low Ca^{2+} concentrations the full-length lever arm is stabilized by calmodulin, whereas at high Ca^{2+} concentrations calmodulin unbinds and the lever arm collapses (Fig. 1c).

The directionality of the engineered myosin was determined using filament gliding assays, where fluorescent actin filaments with specifically labelled plus

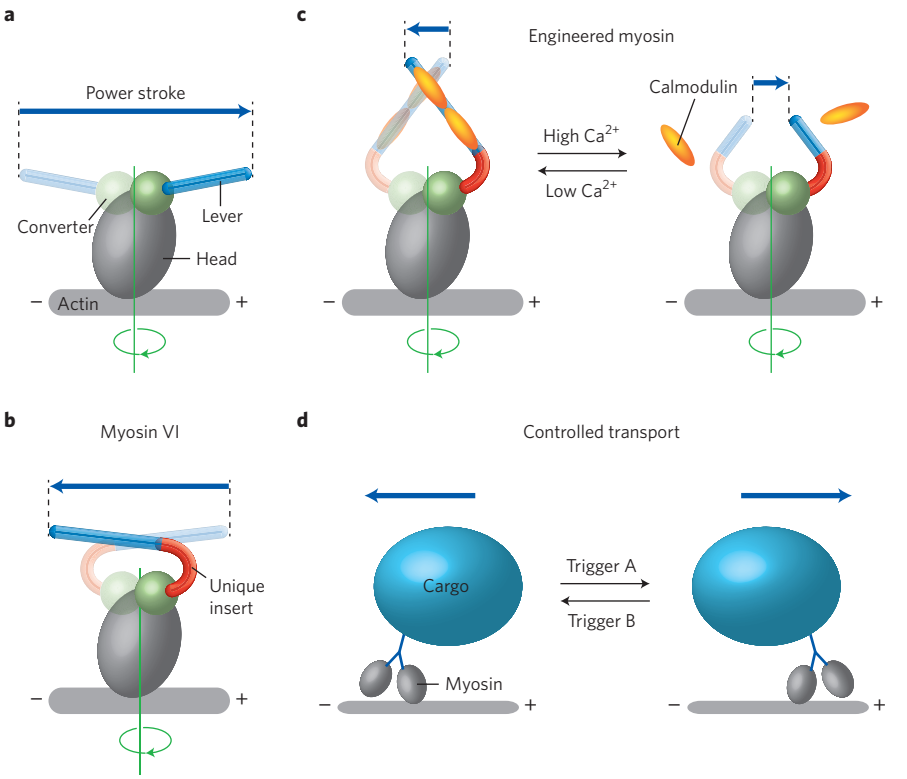

Figure 1 | Controlling the direction of myosin motors. **a**, Myosin motors that are involved in intracellular cargo transport walk along actin filaments by alternating steps of their two head domains. Each step requires the hydrolysis of one ATP molecule in the actin-bound head, leading to a rotation of the converter region. For plus-end directed myosins, such as myosin V, rotation of the converter region (around the green axis) is amplified by the lever arm to generate a large power-stroke towards the plus end of the polarized actin filament. (The translucent elements show the state of the motor before the power stroke.) **b**, For myosin VI, the unique insert reverses the direction of the power stroke by a 180° turn close to the converter. **c**, For the bidirectional motor, the unique insert was modified to generate a larger angle between the lever arm and filament. Binding of calmodulin to the IQ motifs in the lever arm depends on the concentration of Ca^{2+} in the surrounding solution. Long lever arms with stabilized IQ motifs generate small minus-end directed power strokes, whereas short lever arms with collapsed IQ motifs generate small plus-end directed power strokes. **d**, Potential application of engineered motors for the switchable, bidirectional transport of nanoscale cargo.

ends were combined with monomeric motor molecules attached to a surface. Motors with stabilized lever arms stepped towards the filament minus ends, whereas motors with collapsed lever arms stepped towards the plus ends. As intended, the gliding direction could be dynamically switched by changing the amount of free Ca^{2+} between nanomolar and micromolar concentrations. The Stanford team also obtained similar results for the stepping of artificially dimerized motors in single-molecule assays.

Bryant and colleagues have elegantly shown how a strictly controllable bidirectional motor can be created. Although the underlying mechanisms have been individually described before, the work provides an impressive demonstration of hypothesis-driven protein engineering. Nevertheless, a number of mechanistic details remain unclear. The gliding velocities of the engineered motors were significantly smaller (~3 nm s^{-1}) than of myosin VI motors with native converter regions (~50 nm s^{-1})[9]. Such low velocities may present a severe limit in the applications of the motor in nanoscale devices. These low velocities are likely to be due in part to the increased tilting angle between the lever arm and actin filament, which leads to a comparatively small step size. However, Bryant and colleagues also speculate about further factors. In particular, they highlight the fact that a comparable reduction in velocity was previously observed for similar constructs as a result of a prolonged actin-bound state in the ATP-hydrolysis cycle[6]. One of the major future challenges for the field will be to identify the structural determinants that lead to disturbed motor kinetics.

Despite the low velocity of the engineered motors, the underlying mechanical concepts are general and can serve as a basis for the future design of bidirectional transport systems to be employed in nanotechnology (Fig. 1d). Moreover, the concept of a lever arm with switchable length should be adaptable in multiple ways, and depending on the desired application, could be used to make various other motor properties controllable. For example, using lever-arm lengths different from those employed by Bryant and colleagues, one could switch motors between a fast mode (based on large power-strokes) with a given force and a slow mode (based on small power-strokes) with a potentially higher force, just like switching between two different gears in a car's gearbox.

Furthermore, it would be intriguing to extend the range of signals used for motor control. Although Ca^{2+} is a physiological regulator of myosin function, it has distinct limitations in terms of switching speed and localization. Optical signals[10] are predestined to provide the best possible spatial and temporal resolution. With such signals, the highly controlled and differential switching of motor-driven transport processes could then become feasible in artificial *in vitro* systems, and, due to the non-invasive nature of light, within living cells. A plethora of methods to manipulate and study intracellular mechanosystems may then become available. ❐

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