Bio-inspired track-walking molecular motors (Perspective)[a)]

Zhisong Wang[b)]
Department of Physics, NUS Graduate School for Integrative Sciences and Engineering, and Center for Computational Science and Engineering, National University of Singapore, Singapore 117542, Singapore

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The emerging field of artificial track-walking molecular motors is reviewed. The author attempted to clarify the scientific and technological challenges that face the field. A comprehensive mechanistic diagram for molecular walkers was introduced, thereby the directions and possible routes for future development were suggested. © *2010 American Vacuum Society.*
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I. A BRIEF HISTORY

Heat engines and engine-propelled transport had played a crucial role in the great industrial revolution that has shaped the present machine-based human civilization. In parallel, molecular motors capable of sustained directional translocation or rotation will possibly play a strategic role in emerging nanotechnology at large. A clue to the molecular motor's technological potential is offered by a long and still growing list of Nature-made molecular motors (called motor proteins) that have been identified in living cells to carry out such diverse functions as intracellular transport,[1,2] muscle contraction,[3] synthesis of adenosine triphosphate (ATP) (energy currency of cells),[4] bacterial propulsion,[5] chromosome segregation,[6] etc. In fact, accumulating biological studies of the cellular motors over the past two decades served as a driving force for the recent thrust in development of artificial molecular motors. Prominent examples include molecular shuttles and rotors constructed from synthetic ring-shaped molecules,[7,8] and track-walking motors made of engineered DNA molecules[9–18] or synthetic molecules.[19] This short review will focus on track-walking motors. Readers interested in molecular shuttles and rotors can consult other excellent reviews.[7,8]

It is interesting to note that the possibility of making a molecular transporter was first suggested by Nobel-winning physicist Dr. Richard Feynman more than 50 years ago in a well-known lecture entitled "Plenty of room at the bottom."[20] In the lecture, Dr. Feynman argued that physical laws as he could see do not prevent the possibility of an infinitesimal machine like an automobile. A *de facto* proof for the feasibility of implementing self-propelled transporters at the molecular level came more than 20 years later, in the discovery[21] of motor proteins that autonomously and unidirectionally walk along the filamentous tracks in living cells by consuming chemical fuels, i.e., ATP. The first experimental breakthroughs[9–11] in artificial molecular walkers came as late as 2004.

Compared to localized motors like molecular shuttles and rotors, molecular walkers are capable of navigating a large distance in a self-propelling and self-directed way. Walker-based transport can be much more effective than random diffusion, especially when the cargo is big (e.g., ~micrometers across), the environment is crowded (e.g., the interior of a living cell), or the distance is long (over micrometers). Cytoskeleton-based molecular walkers in the form of bipeds are the primary means for moving large organelles over a long distance in living cells.[1,2] There are possibly many transport-based applications. Recent studies provided two notable examples, in which a motor-based nanoscale assembly line[17] and a motor-driven surface patterning[18] were respectively demonstrated. Besides, both studies went beyond a single linear track and accomplished motor operation on extended two-dimensional scaffolds made of DNA origami structures.

In principle, a molecular walker and derivatives may also find technological applications beyond transport. Multiple functionalities of molecular walkers are evidenced by a big family of biomotors called kinesin.[22] One member of this family, conventional kinesin,[3] is a bipedal walker capable of long-range intracellular transport against a load of ~7 pN.[23] Some other kinesin motors,[6] which are evolutionarily adapted by small modifications in molecular construction, can depolymerize their cytoskeletal tracks or manipulate their positions during cell division. Likewise, artificial molecular walkers and derivatives may be used to generate force, manipulate the track or control its chemistry, and convert energy.

II. SCIENTIFIC CHALLENGES OF MOLECULAR WALKERS

Molecular walkers presumably represent the highest level of mechanistic and functional integration at the current stage of nanomachine development. This is because a molecular walker must integrate a full set of working mechanisms for energy consumption, direction rectification, and coordination of multiple movable parts. If the walker operates autonomously like biomotors, all the mechanisms must be accommodated and regulated within a single molecular system. First, a molecular walker must be an artificial enzyme, be-

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[b)]Electronic mail: phywangz@nus.edu.sg

cause the energy supply, often in the form of decomposition of a fuel molecule, is consumed yet the walker remains chemically unchanged. Second, a structured track with a polarity is required to support a periodic array of binding sites for the walker's foot components. The walker-track binding must be asymmetric with respect to the track polarity, and the breaking of translational symmetry is a necessary condition for any directionality for the walker's movement. The walker-track binding asymmetry is local and static. Besides, the fuel consumption is stochastic in nature, but a walker's directional movement must be over a long range (many times longer than a stretch of the walker itself) and be dynamically sustained under the stochastic energy consumption. Besides, the walker must generate sufficient force against the viscous load of the transported cargo in an aqueous environment. Third, for the sake of consecutive walking steps for a long run, a walker must possess at least two foot components and coordinate their motions. A foot must play a double role—it either binds the walker to the track against fluctuation-induced dissociation or assumes a mobile phase to make up a walking step in response to energy injection. A minimum of two feet are required because the walker must remain a stable binding to the track by at least a foot when the other are in the mobile phase. More importantly, coordination between the individual feet is necessary to suppress the chance for them to be simultaneously in the mobile phase. Otherwise, the walker will entirely fall off the track to terminate walking. Due to stochastic nature of molecular processes (fuel consumption, foot dissociation and binding, etc.), the terminal falloff event is inevitable. The average number of consecutive steps (termed processivity in the biomotor research community) is a measure of the walker's ability for feet coordination.

It is tempting to regard molecular motors as a pure nanoengineering problem, but this oversimplified view misses the big, underlying science that remains elusive to date. At the heart of molecular walker science lie two emergence problems. (1) How does a dynamically sustained, long-range directional stepping emerge from local, static asymmetry at the walker-track binding interface under random driving in an isothermal environment? (2) How does a coordinated behavior emerge in a multicomponent molecular walker that is inherently prone to stochastic fluctuations? In order to create a working motor from scratch, the two emergence problems must be answered from physical principles and in a conceptually clear way. To seek theoretical and technical solutions to the emergence problems is a core scientific challenge in the field of molecular walkers.

The two emergence problems have far-reaching implications. It is not difficult to imagine that similar emergence problems will recur again and again in the future development of nanomachine systems that integrate ever more molecular components, working mechanisms, and functionalities. The field of molecular walkers may thus be regarded as a stepping stone toward the machine-based nanotechnology at large.

III. STATUS QUO OF RESEARCH ON ARTIFICIAL MOLECULAR WALKERS

On the experimental frontier, a handful of fabrication success[9–19] has been reported since 2004. All the demonstrated walkers to date are based on self-assembled DNA systems, except that a very recent study[19] demonstrated walker fabrication using full synthetic molecules. The walkers were assembled from multiple DNA strands of specially designed sequences, while the tracks were often relatively rigid double helices. The walkers contained footlike single strands that are sequentially complementary to single-stranded overhangs protruding out of the track. The foot strands spontaneously formed duplexes with the track overhangs by Watson–Crick base pairing to bind the walker to the track. Fuel strands were used to break the binding duplex to enable the walker's mobility, which was often in the form of intrachain diffusion. Covalent bonding formed between the walker and track in some cases, then enzymes and reactants were used to cleave the bonds. Most of the reported walkers may be regarded as bipeds because they contain two foot strands. Mostly, the feet of a walker were chemically different from each other. The walking gait was hand-over-hand[9,11,14–16,19] or inchworm.[12,13] In the former gait, the two feet alternately lead. In the latter, a foot invariably leads the other one.

The DNA walkers provide the first experimental proof of the feasibility of molecular walkers, and thereby ushered in the field of artificial track-walking molecular motors. The methods for design, fabrication, and fueling of the DNA walkers/tracks are truly masterful. Some of the methods were derived from previous research of DNA nanotechnology;[24] some were completely new addition to it (in fact, most of the walker fabricators were from the community of DNA nanotechnology). For example, the method of using fuel strands to break foot-track binding was borrowed from previous studies on switchlike DNA devices. The working principle is to use a longer duplex (i.e., one to be formed by the fuel strand and the foot strand) to replace a short one (i.e., the duplex previously formed between the foot strand and an overhang strand of the track). (Nice pictorial illustrations of the working principle can be found, e.g., in Refs. 14 and 15.) This duplex replacement is driven by the free-energy difference between the two duplexes and results in dissociation of the foot from the track. For a next round of binding with the track, the dissociated foot must be first set back to its single-strand state. For this purpose, a second fuel species is often needed to form an even longer duplex with the first fuel species so as to rid it of the foot. The two fuel-driven processes complete a chemical reaction between the two fuel species, which is catalyzed by the motor foot and produces a fuel-fuel duplex (waste).The free-energy difference between the fuel reactants and the final product is the amount of energy available to power the motor. For the sake of an autonomous operation, all of the fuel species must be present simultaneously. Reactions between them are to be prohibited before the motor track is introduced. This can be done by properly designing into each fuel sequence a unique "recog-

nition site" that is activated only in the presence of the motor. Fully autonomous operation was successfully demonstrated.[10,12,13,15,16,18]

Another notable technical development was achieved by Yin *et al.*,[15] in which a method (termed reaction graph abstraction) was introduced to encode into the nucleotide sequences not only the walker-track structures but also their self-assembly pathways. This method removes the need of often tedious, manually controlled stepwise assembly (and purification) of a walker-track system out of multiple strands, and allows instead a self-directed assembly of the whole system. Thus, this method may greatly reduce the difficulty of DNA walker fabrication.

As the first generation of molecular walkers made by man from scratch, the DNA walkers are no doubt a remarkable success. Yet equally clear is the big gap between them and the cellular counterparts in system sophistication, in performance, and in inner working mechanisms. Take kinesin for example, which is the smallest cellular walker found to date ($\sim$10 nm across). Kinesin[3] is an autonomous biped with two identical feet; its cytoskeletal track contains identical binding sites. Under normal physiological conditions, a single kinesin motor walks by an average speed of $\sim$1 μm/s and makes hundreds of consecutive steps (so covering a length of micrometers). The artificial walker-track systems mostly consist of chemically different feet in the walker and different binding sites in the track. The walkers can make only a few steps before falloff and run by a speed several magnitudes slower than that of kinesin.

More importantly, the direction rectification mechanisms for the reported artificial walkers are primitive from a physics perspective. A majority of the walkers[9–13,16–19] relied on the use of two different feet and multiple species of binding sites. Some walkers[9,11,19] attained a directionality by sequentially administering multiple fuel species in a proper order to dissociate feet in a position-selective way. This heterogeneity-based rectification is rather intuitional and resembles a worm using its distinct "head" and "tail" to select a direction along a branch. Other artificial walkers[10,12,13,16,18] relied on a "burn-the-bridge-behind" strategy to attain a directionality, namely, by chemically damaging each traversed binding site or by covering it with strongly bound fuels. In either case, the purpose is to block the backward binding site so as to bias a diffusing foot toward a forward binding site. This amounts to a ratchet effect that rectifies a forward motion of the walker as a whole. The ratchet effect came with a price—either the track was permanently damaged after each run of the motor or a reset operation was required to get rid of the many bound fuels. Besides, a high level of system heterogeneity adds to the difficulty of walker-track fabrication, especially for a long track.

A physically more sophisticated ratchet effect was developed by Green *et al.*[14] in a DNA walker-track system that demonstrated a position-selective consumption of fuel strands by chemically identical feet. Specifically, an overlap between adjacent binding sites was introduced to force a competition between the two feet, and thereby expose different parts of the feet depending on their positions. A fuel strand was designed to react more readily with the rearward than forward foot, leading to a bias favoring dissociation of the rearward foot. This leads to a second type of ratchet effect, and it again came with a price. The biased fuel binding was facilitated by a single-stranded DNA track, which must rotate freely to allow foot dissociation (namely, unwinding of the foot strand from a duplex which it formed with the track). However, a freely rotating single-stranded DNA track may pose a problem for practical applications. It is not clear how the second ratchet effect can be combined with the first one for a better motor.

Primitiveness of the direction rectification mechanisms of the present artificial walkers becomes clear in a comparison with the biomotor conventional kinesin.[3] More than a decade of single-molecule experiments on this archetypal bio-walker provided ample evidence for the existence of far superior mechanisms for direction rectification and intramolecular coordination, though full molecular details are still lacking. First, it is clear that both of the ratchet effects—one associated with a bias in foot dissociation and the other with a bias in diffusive foot binding—work combinatively in kinesin. This mechanistic combination may underlie kinesin's capability of walking a highly coordinated hand-over-hand gait[25] and making virtually zero backward steps under low load.[26,27] Second, kinesin is a homodimer with two chemically identical feet. The track has a single species of binding site, and kinesin does not modify the traversed sites either chemically or physically. These facts suggest that kinesin achieves the two ratchet effects neither by the intuitional heterogeneity-based rectification nor by the burn-the-bridge strategy. Third, kinesin's operation is fully autonomous once ATP is available in the buffer. The high level of system homogeneity and mechanistic integration and the capacity for autonomous operation and for maintaining chemical integrity of walker-track during fuel consumption may be regarded as four hallmarks of a good walker. Apparently, all of the reported artificial walkers fall far short of these standards of advancedness.

The theoretical problems concerning the emergence of direction rectification and feet coordination for such good walkers are nontrivial and a comprehensive understanding from physical principles is lacking. As a matter of fact, the field of molecular walkers is as acutely bottlenecked by limited techniques for experimental implementation as by an incomplete theoretical foundation.

There exists a large body of theoretical studies for direction rectification based on the concept of Brownian motors.[28–31] From bare physical principles, the Brownian motor theories reveal elegantly how sustained directional flow can emerge out of independent particles under random driving over a periodic potential field that possesses a minimum level of local asymmetry but lacks any long-range gradient. The Brownian motor theories were later extended from independent particles to multifeet walkers.[32–36] Such walker theories provided a line of physical understanding of directionality emergence, while the emergence of feet coordina-

tion appears more difficult to address within this conceptual framework. The walker theories[32–34,36] based on the Brownian motor concept often needed to introduce by hand a foot-foot coordination in order to reach the level of performance typical of kinesin.

There also exist walker theories following different lines of concepts. One example is a series of theoretical developments[37–40] for a rectification mechanism suitable for inchworm walkers. Another example is a group of theoretical studies[41–45] that explored a bio-inspired mechanism for directional rectification in homodimeric walkers.

The present status at the theory frontier is that only a few individual working mechanisms have been identified and clarified from physical principles sporadically on an *ad hoc* basis. It is still a long distance from a complete solution to the emergence problems for the aforementioned good walkers, which are desirable targets for future development of artificial walkers. As far as the reported experiments are concerned, they are largely decoupled from the existing theories, apparently because the latter provided insufficient mechanistic candidates for experimental implementation.

IV. A COMPREHENSIVE MECHANISTIC DIAGRAM FOR MOLECULAR MOTORS

What are the possible routes to break the bottleneck on walker development? An integrated mechanistic view of biomotors and artificial counterparts may provide some clues. In principle, different mechanisms for direction rectification may be mapped in a multidimensional landscape in terms of a finite number of properly chosen physical parameters for the motor-track systems. We may recall that the two parameters—temperature and pressure—are sufficient to represent working mechanisms for heat engines. What are the physical parameters suitable for a comprehensive representation of molecular motor mechanisms? This remains an open question to date. This fact certainly prevents a quantitative analysis of the molecular mechanisms. Nevertheless, a qualitative mechanistic analysis is possible and meaningful as we shall show below. As an example, we assume that a combination of two physical parameters can distinguish the rectification mechanisms for molecular walkers. Using the two parameters as x and y axes, a two-dimensional mechanistic diagram can be constructed (Fig. 1), in which each major rectification mechanism and its derivatives are represented as an area. Then, advancedness of the mechanisms may be added to the mechanistic diagram as the third dimension (z axis).

Advancedness of a mechanism is better measured by its intrinsic effectiveness for direction rectification than speed and other performances subject to external operational conditions (e.g., fuel concentrations). The rectification effectiveness can be generally quantified by a single quantity which we call directionality (D), namely, the ratio of the probability for the motor to make a forward step over the sum of probabilities for all possible outcomes (e.g., backward steps, futile step, etc.) per event of fuel consumption. The maximum directionality (D_{max}) achievable by a mechanism under the most ideal operation is largely determined by the essence of the mechanism independent of external operational conditions.[46]

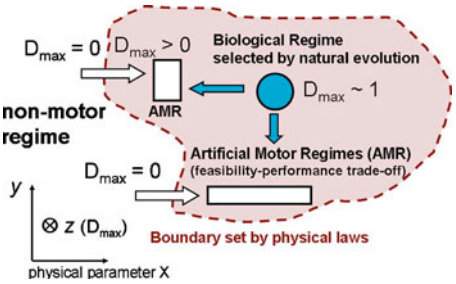

FIG. 1. (Color online) Mechanistic diagram of molecular walkers. The x and y axes are two physical parameters that supposedly characterize the mechanisms for directional rectification. A major mechanism and its derivatives are represented as an area in the x-y landscape. A third z axis is the maximum directionality (D_{max}) accessible to a rectification mechanism by the most ideal operation. D_{max} is a measure of the intrinsic rectification effectiveness of the mechanism. The shadowed area enclosed by the dashed line is the collection of the mechanisms capable of directional rectification ($D_{max} \neq 0$), while the area outside is the trivial nonmotor regime of $D_{max} = 0$, where net direction fails to occur despite the walker's energy consumption. The dashed line indicates the emergence of net directionality ($D_{max} = 0 \rightarrow D_{max} \neq 0$) and reflects the ultimate limits imposed by the second law of thermodynamics on molecular walkers. The black circle marks the rectification mechanisms selected by biological walkers. The empty and filled arrows indicate two basic strategies for identifying mechanisms suitable for implementation in artificial systems (see text for explanation). The two artificial motor regimes (empty rectangles) might derive from fundamentally different mechanisms or might be reduced versions of the same mechanism by different trade-offs between motor performance and technical feasibility.

The directionality remains zero ($D_{max}=0$) for the parameter values characterizing a physically trivial regime in which the walker's forward movement is canceled out by its backward movement, though the walker repeatedly consumes net energy inputs to enable dissociation and diffusive binding of individual feet. Any nonmotor molecular systems behave exactly this way amidst ubiquitous thermal fluctuations within the systems and in their environment, as dictated by the second law of thermodynamics. When the parameters are adjusted to certain values so that the perfect balance between the walker's forward and backward movement is broken, a rectification mechanism emerges. The maximum directionality then starts to attain a finite value ($D_{max}>0$). This defines the physically nontrivial regime in which directional rectification occurs and a molecular system is converted to a motor. The boundary between this rectification regime and the trivial regime (dashed line in Fig. 1) is determined by the ultimate limits of the second thermodynamic law on the directional rectification at molecular level. For the few known, specific mechanisms for directional rectification, the $D_{max} = 0 \rightarrow D_{max} \neq 0$ boundary can be well defined.[29,30,38,39,45] However, the formulation of the ultimate thermodynamic limits on molecular motors from a general physical perspective remains a difficult task despite some progress[47,48] in this direction.

Inside the rectification regime lies the subregime selected by natural evolution for cellular motors like kinesin. The biological regime, optimized by millions of years of natural

selection, presumably has a directionality close to unit, which was supported by experimental data[26,27] on kinesin.

Where lies the target regime for artificial motor development in the mechanistic diagram? In principle, it can be anywhere in the rectification regime, but the performance will be better away from the outer boundary. Desirably, it should be as close to the biological regime as possible, yet copying the biosystems can be technically formidable. A realistic choice is to target the artificial motor development to certain subregimes where performance may be less ideal but technical feasibility is optimized. A group of workable regimes selected through such a trade-off between technical feasibility and performance defines a roadmap for artificial motor development.

It is important to note that reproducing the superior performance of biomotors is not the only goal for artificial motors. From the perspective of technological applications, the ability to interface with other existing technological platforms can be as important. Besides, a motor's performance is measured by multiple aspects (e.g., autonomous operation, speed, load-bearing capacity, consecutive run length before the walker totally diffuses away from the track, etc.), and different applications may require a differing balance of performance. For example, a motor-based molecular assembly line[17] may prefer a manual operation to switch between multiple species of cargos but have a relatively low requirement for consecutive run length. Long-range drug delivery by molecular walkers may prefer a high consecutive run length and autonomous operation. Therefore, the function-performance balance is an important factor in the choice of the target mechanistic regimes for artificial motors.

V. TWO ROUTES TOWARD ADVANCED ARTIFICIAL WALKERS

From the mechanistic diagram, it becomes clear that there exist basically two routes to discover the workable mechanistic regimes for artificial motors. The first route is to seek the boundary where nonzero directionality emerges (i.e., $D_{\max}=0\rightarrow D_{\max}>0$) and identify some primitive rectification mechanisms that break the detailed balance of the walker-track system amidst stochastic thermal fluctuations. If the primitive mechanisms are optimizable, one might improve the performance up to the level of a workable regime for experimental development. This is an "uphill" strategy from $D=0$ to $D\sim 1$, by which one closes in on a specific target regime from a larger picture of general thermodynamic considerations.

All of the reported experiments[9–19] and a majority of existing theories[32,34–40,49,50] on molecular walkers appear to have followed this strategy. These experimental and theoretical developments suggest that it is possible to identify individual working regimes for artificial motors on an *ad hoc* basis by exploiting pure physical principles. Studies along this line also help to build up a complete knowledge of the $D_{\max}=0\rightarrow D_{\max}>0$ boundary.

Alternatively, one may identify an artificial motor regime by seeking to mimic the mechanistic phenomena of cellular motors. Single-molecule biophysical experiments on cellular motors have become increasingly successful in delivering a comprehensive phenomenological description, though the underlying molecular mechanisms are much more difficult to come by. Picking up specific phenomena or effects crucial to biomotors' rectification and exploring their physical origins from a general perspective can lead to rich and deep insights. A further generalization to engineered systems is often necessary to reveal possible mechanistic variations and assess technical feasibility. Because biomechanisms are often highly adaptable, the above strategy readily leads to a working framework for evaluating the performance-feasibility trade-off. The second strategy may be regarded as "downhill," because it reduces a biomechanism ($D_{\max}\sim 1$) to a target regime suitable for artificial motor development ($0<D_{\max}<1$).

An example of the biomimetic route is a series of theoretical developments[41–45] which we have sought following a molecular effect[3] identified in the biomotor kinesin (termed "necklinker zippering" in the kinesin community). These studies revealed physical mechanisms[41,45] by which a local conformational change may be amplified into a long-range bias for diffusive binding and by which a sustained directional movement in hand-over-hand gait may be further facilitated for a homodimeric walker. A generalization found a variation of the same mechanism in another biomotor myosin V,[44] and identified a reduced version that may be implemented in engineered systems.[45] Experimental development along this line has yet to come.

The two artificial motor regimes illustrated in Fig. 1 can be two reduced versions of the same biomechanism following two drastically different function-performance balances or are optimized versions of two fundamentally different, primitive rectification mechanisms. The downhill approach applies to the former case, and the uphill approach the latter.

VI. NANOSCIENCE OF ARTIFICIAL MOTORS AND THE BIOPHYSICS OF CELLULAR MOTORS AS A UNIFIED FRONTIER

The biomimetic strategy essentially calls for a unified physical study of artificial motors on one hand, and cellular motors on the other. This line of research will potentially lead to a new generation of artificial walkers that may deviate in molecular construction from the polypeptide systems of cellular motors, but essentially mimic the mechanistic phenomena of the biomotors. With fast advancement of the field of biomotor biophysics, the biomimetic strategy will become an increasingly viable route for a systematic development of advanced artificial walkers. The biomimetic strategy may combine with the first, physics-motivated strategy to form a joint effort for artificial walker development. Conversely, an artificial walker operating on a derivative of a biomechanism may serve as a simplified model system for the study of that biomechanism. So the physical sciences of artificial motors and the biological field of cellular motors may mutually benefit each other.

VII. FUTURE CHALLENGES AND OPPORTUNITIES

The mechanistic diagram not only helps put the previous walker research in perspective but also points to major future challenges and some viable strategies to address them. The first major challenge is to develop advanced walkers that rival biomotors in selected aspects (e.g., system homogeneity and mechanistic integration) and conveniently interface with other technological platforms for practical applications. The second challenge is to close in on ultimate solutions to the central scientific problems concerning the emergence of directional rectification and intramolecular coordination. A minimum, necessary solution to the emergence problems will quantitatively define the outer boundary of the motor regime, while sufficient and optimized solutions will help identify targets for advanced artificial walkers in the vicinity of the bio-walker regimes.

The two challenges can be met by several scientific and technological developments in the foreseeable future. First, it is necessary to identify more working mechanisms following either the biomimetic strategy or the physics-motivated strategy. The new mechanisms will add to the pool of mechanistic candidates for experimental implementation and will also fill the vast gaps in the mechanistic diagram (Fig. 1) that is merely a preliminary conceptual framework in its present form. Second, it is necessary to move beyond individual mechanisms to explore integration of multiple mechanisms in a walker-track system. Theoretical and experimental studies of mechanistic integration will be a key toward advanced walkers. Third, a push beyond DNA systems appears necessary to accommodate ever more advanced mechanisms and their integration, and also to reach applications on a wide range of technical platforms. The candidate systems include peptides and synthetic molecules. The recent success of von Delius *et al.*[19] in fabricating a bipedal walker with synthetic molecules is encouraging. Extension to more molecular systems will potentially widen the choice of technical means for walker driving. This will probably offer new opportunities to promote catalytic rates of the walker in its role of an artificial enzyme, and thereby help push walker velocity toward the level of biomotors.

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