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# Re-engineering of protein motors to understand mechanisms biasing random motion and generating collective dynamics

Ken'ya Furuta and Akane Furuta

A considerable amount of insight into the mechanisms of protein-based biomolecular motors has been accumulated over decades of research. However, our knowledge about the design principles of these motors is still limited. Even less is known about the design of multi-motor systems that perform various functions within the cell. Here we focus on constructive (or synthetic) approaches to biomolecular motors that could make a breakthrough in our understanding. Recent achievements include studies at different hierarchical levels of complexity: re-engineering of individual motors, construction of multi-motor systems, and generation of large-scale complex behaviour. We then propose a strategy where the collective behaviour can be repeatedly tested upon modifying individual motors, which may provide important clues about how biomolecular motors and their systems are designed.

**Address**
Advanced ICT Research Institute, National Institute of Information and Communications Technology, Kobe, Hyogo 651-2492, Japan

Corresponding author: Furuta, Ken'ya (furutak@nict.go.jp)

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## Introduction

Since the Industrial Revolution, a variety of machines based on top-down control systems have been made and prevailed in the world. However, these traditional machines, including engines, robots, computers and their networks are showing weaknesses such as difficulty adapting to dramatic changes in the environment. As alternative strategies, increasing attention has been focused on the bottom-up strategies that life adopts.

## Autonomous molecular motors that life uses

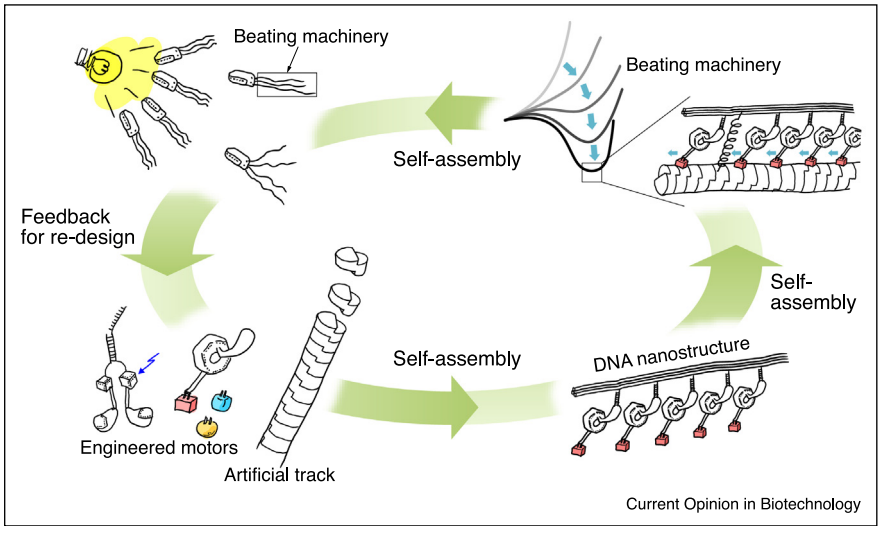
In living organisms, systems at all levels naturally perform incredibly advanced distributed processing, from the level of ant societies, to individuals, tissues, and down to the cellular level. At the cellular level, the motile machineries such as muscle, mitotic spindle, and cilia/flagella use nanometre-scale molecular machines made of proteins, called biological molecular motors (biomolecular motors), which move along cytoskeletal tracks. These motors capture ATP molecules dissolved in solution as energy sources, and produce cooperative phenomena such as contraction, elongation and oscillatory motion, on a scale of a centimetre to a few metres, six to eight orders of magnitude larger than the motors themselves. Such collective behaviour appears to be programmed into the properties of the individual motors and tracks. Biomolecular motors and cytoskeleton together therefore offers an ideal system for understanding the non-obvious relationship between the local interactions and collective behaviour frequently found in biology (Figure 1).

Biomolecular motors comprise two different types: linear and rotary molecular motors. Linear molecular motors include cytoskeletal motors, such as myosin, kinesin, and dynein that drive directional movement along cytoskeletal filaments [1], DNA and RNA polymerases [2], chaperones that unfold polypeptides [3], ribosomes [4], and tubulin/actin that hydrolyzes GTP/ATP to produce cellular movement [5]. Rotary molecular motors, including $F_1F_O$ ATP synthase, V-ATPase [6], and bacterial flagellar motors [7], are usually immobilized within a membrane and driven by the flow of ions across the membrane.

## Key questions in the biomolecular motor field

Remarkably, these tiny machines directly convert chemical energy into directional movement, which makes these motors distinct from man-made macroscopic machines and potentially useful as nanometre-sized actuators [8,9••]. However, the essential mechanisms that enable such a difficult task is currently unknown. The problem is that biomolecular motors are working in a stochastic environment where the energy of thermal fluctuations is comparable to the energy that can be obtained from ATP hydrolysis or ion translocation across a membrane. This leads to the idea that operating principles of nanoscale machines in thermal fluctuations are completely different from those of man-made macroscopic machines [10] that regard thermal fluctuations as 'noise', and suppress them by devoting much larger energy. This raises a question about what factors are critical for a nanoscale motor to simply move or rotate unidirectionally in a storm of thermal fluctuations.

**Figure 1**




An illustrated example of the hierarchical levels observed in biomolecular motor studies. We propose that an experimental system is needed that enables repetitive experimental cycles with quick feedback, in which the behaviour of the entire system can be tested upon modifying individual motors.

To address these key questions, many studies on biomolecular motors have been carried out where the motors were divided into pieces and analysed in detail. Such analytical approaches are powerful tools to identify important sites in molecules, and have provided many key insights. Yet, presently, we are still unable to design a new biomolecular motor from scratch. This might be partly because the analytical approaches alone would not provide practical information about how one should design and assemble a new molecule that works as a simple motor. For example, even if we identify many mutations at different specific sites in a motor that cause serious malfunctions and hence appear important for its functioning, it would not provide us with sufficient information to design a simple motile machine.

## Constructive (synthetic) approaches to biomolecular motors

One effective way to overcome this problem is to take a constructive (or synthetic) approach [11]. The basic idea of the approach is to design (or re-design) and construct new biological components and systems in order to understand life processes. In the case of biomolecular motors, one strategy would be to construct a molecular machine that captures the essence of biomolecular motors. By constructing diverse motile machines that correspond to a single function of directional motility and comparing them with each other, one could abstract common architecture in an inductive manner. This could include geometric arrangements of protein building blocks and time scale of track binding/unbinding function. Nevertheless, we could not draw a clear dividing line between analytical and constructive (synthetic) approaches. Biologists have developed diverse protein engineering methods including mutagenesis of specific amino-acid residues, deletion/insertion/replacement of domains, intra and intermolecular disulfide crosslinking, and generation of chimeric constructs. In this review, we subjectively classify recent studies according to the methodology, the extent of engineering, and the aim of the study, focusing mainly on protein-based motors. We cover studies on the design of individual biomolecular motors and studies aimed at generating collective dynamics across multiple layers of scales using molecular motors.

Unlike typical analytical studies, some studies sought to reveal the mechanisms of biomolecular motors by reconstruction of the whole motor [12] or its part [13], treating the functional subdomains as building blocks. DNA duplexes were also used as building blocks to construct individual dimeric motors [14]. Other studies aimed to improve the motility or reverse the directionality of biomolecular motors. For example, processivity, that is, the ability to undergo multiple steps before dissociating from the track, was improved by inserting charged amino acid residues in the neck region of kinesin [15]. Other examples are related to the directionality determinants of kinesin and myosin motors. The directionality determinants are central to our understanding of biomolecular motors, whose main function is to generate an asymmetry of motion. The reversal of directionality of kinesin was achieved by making chimeric constructs containing two naturally occurring opposite polarity motors, kinesin-1 and kinesin-14 [16–19]. These studies have revealed that the neck residues near the conserved motor core are