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Atomic-Scale Insights into Physical Mechanisms Driving Enzymes' "Working Cycles"

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Consumption and utilization of available free energy for the purpose of homeostasis is one of the fundamental properties of all living organisms, and it is carried on by a vast number of different biological molecular machines that form the basis of life. During recent years, it has become increasingly clear that the performance and function of such machines are intricately linked to mechanical movements altering their conformations. Although for a long time it has been known that directional conformational changes compose the main part of the working cycles of so-called motor proteins, it only recently has been shown in a study by Slochower and Gilson (1) that very similar molecular mechanisms drive the functioning of an even wider class of nonmotor enzymes whose main working cycles can be nicely described by the two-state model originally proposed for motor proteins (2). Using examples of three different nonmotor enzymes—adenosine kinase, protein kinase A, and HIV-1 (human immunodeficiency virus type 1) protease—the authors have demonstrated that by switching between the apo- and substrate-bound states, these molecules can utilize slight differences in the energy landscapes corresponding to these two states, sliding in the direction toward smaller free-energy point (Fig. 1). As a result, this leads to the appearance of directional motion in enzymes that is tightly coupled to their main working cycles driven by the energy coming from the difference in chemical potentials of the substrate and product complexes.

Even more surprisingly, it has been revealed by the authors that the working sequences of the studied proteins actually involve considerable directional cyclic movement of their smaller parts, including individual amino acid residues, which are coordinated by some yet-unknown mechanisms whose concerted action underlies the enzymes' function. Thus, it seems likely that there exist higher-order variables describing the collective motion of various enzyme parts, which are coupled to their progression along the reaction path, thus warranting future studies of general molecular mechanisms responsible for the emergence and kinetics of such multidimensional variables.

Although the above questions may provide important insights into the working mechanisms of various enzyme types, the study reported in (1) may be interesting for a broad range of research for a slightly different reason. Recent years have seen extensive development of nanotechnology and single-molecule fields, resulting in the emergence of a vast variety of artificial nanodevices that have very different functions (3). However, despite a wide range of fabricated constructs, there exists one feature that unites them all: to perform their functions, these nanoscale devices have to make discrete transitions between a number of well-defined molecular states that form the working sequence of such constructs. Whereas the simplest nanoscale devices are one-time runners that go through the working sequence only once and eventually stop in the last state of the sequence, more advanced mechanisms that have been synthesized so far can go through many repetitive working cycles while being driven by an external force or via utilizing available free energy (4). In the latter case, the energy input required for device operation may be provided through degradation of various chemical compounds or modification of complex biomolecules, thus reminding macroscopic engines that use chemical fuel to power their work.

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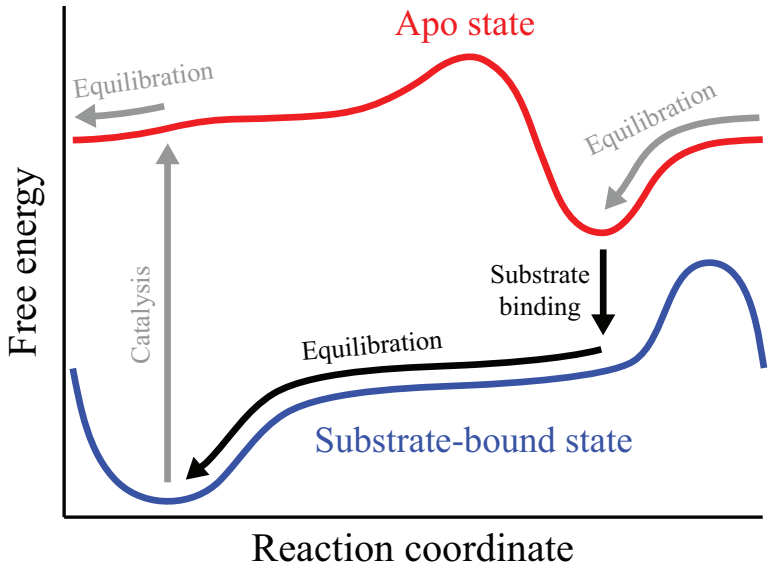

FIGURE 1 Enzyme working cycle from a physical point of view. By switching between the apo- and substrate-bound states, enzymes utilize slight differences between the energy landscapes of these two states to produce directional conformational changes that are tightly coupled to the main working cycle/reaction pathway responsible for the conversion of substrate molecules into the respective products. To see this figure in color, go online.

In light of such strong similarity between microscopic devices and macroscopic engines, many scientists have started to ask themselves a question: are there universal laws that limit the performance of nanoscale constructs in a way that might be similar to the well-known Carnot formula for heat engines? Besides providing better insight into the potential role of thermodynamic laws in governing the dynamics of microscale systems, finding the answer to this question may also lead to the development of a guideline for improving the performance of existing nanodevices. Although various approaches have been previously proposed to solve this question (4), it seems that all of the studies agree on one point: the performance of micro- and nanoscale systems is subject to limitations imposed by the first law and, especially, the second law of thermodynamics. The latter states that for a system to undergo any type of directional motion, including through a sequence of chemical states, it has to dissipate a finite amount of free energy into heat (5). In fact, based on chemical kinetics theory, it has even been shown that there exists a universal formula that tells exactly how much energy should be wasted into heat to produce directional motion for any nanoscale device at the desired level of irreversibility, which is closely related to device capability to maintain its working cycle amid ubiquitous stochastic thermal fluctuations that constantly try to derail the microscopic construct from its working path (5).

Interestingly, the generality of the discovered physical boundaries for nanoscale devices grants applicability of the above findings not only to man-made constructs but also to all naturally evolved protein complexes, many of which (such as motor proteins) have been found to operate very closely to the thermodynamic limits predicted by the above studies. This provides an exciting opportunity to obtain insight into molecular mechanisms that allow such protein complexes to operate so closely to physically possible limits; this knowledge may be then used in the future to optimize man-made nanoconstructs.

The only problem so far has been that all of the theoretical studies aimed at understanding the high efficiency of natural motor proteins have been dealing mainly with rather abstract entities like chemical states and fluxes, providing very limited connection to the processes that take place at the atomic scale. For this reason, whereas the general limitations put on microscale systems have been quite well characterized, the underlying mechanisms, which help protein complexes to approach such limits, have been rather poorly understood.

This is where the study done by Slochower and Gilson (1) can make a substantial contribution; in their work, the authors have proposed a new numeric method that allows one to bridge chemical kinetics theory to atomic-scale simulations via the following two-step algorithm: 1) applying molecular dynamics simulations to compute energy landscapes of the studied protein in different configurations (i.e., apo- or substrate-bound states) and 2) binning the obtained energy landscapes into discrete protein states to solve the standard system of kinetic master equations and find chemical fluxes between the states, thus gaining insight into the working cycle of the studied protein.

Although by using such a method the authors mainly focused their attention on studying the emergence of directional motion in individual amino acid residues coupled to the enzyme working sequence, the developed method has strong potential to help investigate molecular mechanisms underlying high thermodynamic efficiency of known motor proteins, both at the atomic scale and in terms of chemical fluxes. In the future, this type of research may help to better explain how the concerted collective motion of various amino acid residues taking place during the motor protein working cycle leads to generation of highly directional chemical fluxes responsible for strong performance of such biological complexes, providing an important set of guidelines for the

development of a new generation of artificial nanodevices.

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