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Non-equilibrium kinetics and trajectory thermodynamics of synthetic molecular pumps†

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A major goal in the design of synthetic molecular machines is the creation of pumps that can use the input of energy to transport material from a reservoir at low chemical potential to a different reservoir at higher chemical potential, thereby forming and maintaining a chemical potential gradient. Such pumps are ubiquitous in biology. Some, including the Ca^{+2}-ATPase of the sarcoplasmic reticulum, and the (Na^+,K^+)-ATPase found in the membranes of almost all cells, use energy from ATP hydrolysis to accomplish this task. Others, such as bacteriorhodopsin, use energy from light. Here, we examine in the context of recent artificial molecular pumps, the kinetics and thermodynamics of both light or externally driven pumps on the one hand and pumps driven by chemical catalysis on the other. We show that even for formally similar mechanisms there is a tremendous difference in the design principles for these two classes of pumps, where the former can function as energy ratchets, and the latter must operate as information ratchets. This difference arises because, unlike optically or externally driven pumps, the transition constants for pumps in which the required energy is provided by catalysis of a chemical reaction obey the principle of microscopic reversibility. We use cycle kinetics developed by Terrell Hill in the analysis of energy driven pumping. This approach is based on the trajectory thermodynamics of Onsager and Machlup. The recent "stochastic thermodynamic" approach is shown to be fundamentally flawed and to lead to incorrect predictions regarding the behavior of molecular machines driven by catalysis of an exergonic chemical reaction.

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Introduction

Designing synthetic molecular systems for the transport of molecules is a major goal of synthetic chemistry, both for the practical benefits and for the tremendous insight that is and has been provided into the fundamental principles behind biological transport molecules. A giant step in this direction was taken by Jean-Marie Lehn[1,2] and his colleagues using supramolecular entities that can come together, undergo some transition such as transport across a membrane, and then dissociate. This mechanism is conceptually similar to biological ion transporters that can bind an ion from one side of a membrane and release it to the other side, thus allowing controlled transport of material from high to low electrochemical potential. Ion pumps such as the (Na^+K^+)-ATPase take this a step further and couple energy released by the hydrolysis of ATP to drive transport of ions from low to high electrochemical potential. Surprisingly, energy from an applied oscillating or fluctuating electric field can substitute for the energy from ATP hydrolysis to drive uphill transport.[3] This observation lead to the development of the theory of energy ratchets and stochastic pumping, originally known as electro-conformational coupling[4] in homage to Paul Boyer's conformational coupling mechanism for the FoF1-ATPsynthase.[5] Recent synthetic efforts towards imitating biochemical motors have focused on incorporating the ability to input energy and thereby cause transport from regions of low to high chemical potential thus storing energy.[6] In this paper we examine the kinetic and thermodynamic theory for these transport processes, with an eye toward delineating the difference between light[7,8] and external stimulus driven pumps[9] on the one hand, and pumps driven by energy released by catalysis of an exergonic chemical reaction – catalysis driven pumps – on the other hand.[10] A major result is the demonstration of a fundamental difference[11] between the design requirements for pumps depending on the energy source. This difference is based on the fact that mechanisms for catalysis driven pumps must obey the principle of microscopic reversibility.[12]

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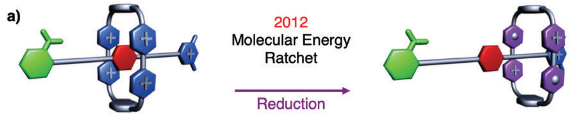
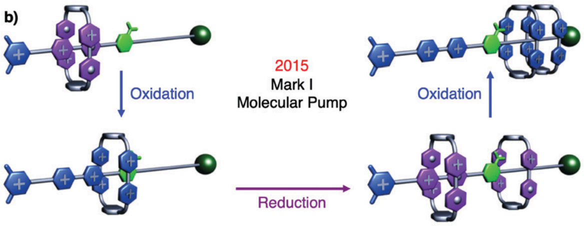
† This paper is dedicated to Jean-Marie Lehn on the occasion of his eightieth birthday.

Synthetic pumps

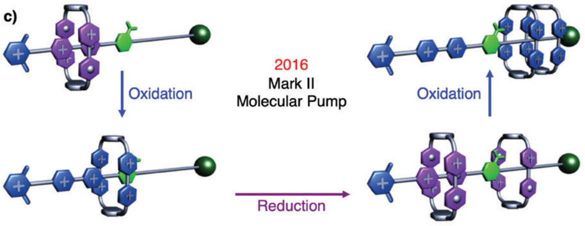
A first step toward the goal of creating synthetic pumps was the demonstration (Fig. 1a) of directional loading of a macrocyclic ring onto and off of a dumbbell (DB) shaped molecule in the assembly and disassembly of a pseudorotaxane.[13,14] The pseudorotaxane is a supramolecular entity, *i.e.*, a complex, and not a mechanically interlocked molecule since the moieties on the ends hinder but do not totally block dethreading of the ring. Under equilibrium conditions detailed balance requires that the rate of threading from the left must equal the rate of dissociating to the left, and that the rate of threading from the right must equal the rate of dissociation to the right. A corollary of this detailed balance is that the probability to thread from the left and dissociate to the right of the DB must be equal the probability to thread from the right and dissociate to the left of the DB. When the system is provided energy from light,[13] or by external modulation of the redox potential,[14] then directional threading was demonstrated – *i.e.*, detailed balance was broken (see Fig. 1a). The key design principle involved was having a ring with a switchable affinity for a binding site (red), along with a switchable barrier (blue/violet) on one side and a fixed barrier (green) on the other side. Directionality arises from the correlation between the affinity and the switchable barrier. If the barrier is small when the affinity is high and large when the affinity is low the ring will most likely thread over the switchable barrier and dissociate over the fixed barrier, and *vice versa*, as is the case in Fig. 1a. The affinity and barrier height modulation can be accomplished either with a fixed period, or randomly with Poisson distributed lifetimes,[15] allowing the overall process to be modelled as a single kinetic cycle.[16] There are countless variations on this theme, but the essential feature is that at least one barrier and one affinity must be modulated in order to cause directed transport. Schäfer *et al.*[17] synthesized a rotaxane based artificial molecular transporter. Another step toward realization of a synthetic pump was reported by Chen *et al.*[18] who incorporated a rotaxane into a membrane where a potassium (K^+) carrier was tethered to a mobile ring on the rotaxane. The thermally activated back and forth motion of the ring allowed passive transport of K^+ from high to low chemical potential, and finally, Cheng *et al.*[6,9] created an artificial pump that can use external energy to form (Fig. 1b) a highly non-equilibrium structure by pumping rings onto a collecting chain. Pezzato *et al.*[19] built on this idea in the design and synthesis of a duet pump (Fig. 1c and d) by which rings can be actively loaded from both sides, and Qiu *et al.*[20] created a dual pump (Fig. 1e) that actively pumps onto one side, and then off from the other side. Each of the individual elements – incorporation into a membrane, directional pumping, and energetically demanding transport against a free-energy gradient have been accomplished, but no single experimental system with all these elements has been reported yet. Now, let us look as a specific model for a DB molecule serving to transport rings across a membrane.



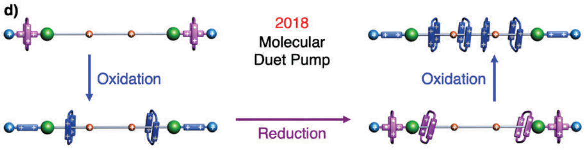
A redox-driven energy ratchet involving ON/OFF donor-acceptor recognition leading to the unidirectional movement of a ring with respect to a pseudo-dumbbell, fueled either by chemicals or by light.



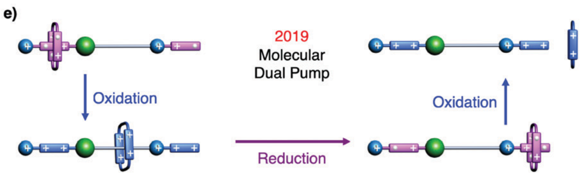
A redox-driven molecular pump (Mark I) whose pumping relies on radical-recognition, followed by Coulombic repulsion and thermal energy to produce a [2]- followed by a [3]rotaxane.



A redox-driven molecular pump (Mark II) in which the Coulombic repulsion following oxidation has been increased with reference to the Mark I version so that less thermal energy is required to produce a [2]- followed by a [3]rotaxane.



A molecular duet pump consisting of two individual Mark II molecular pumps capable under redox control of producing a [3]- followed by a [5]rotaxane.



A molecular dual pump consisting of two individual Mark II molecular pumps, linked in a head-to-tail fashion so as to realize the controlled capture and release of a ring through the formation of an intermediate [2]rotaxane

Fig. 1 Evolution of synthetic molecular pumps from 2012 to 2019.

Kinetics and thermodynamics of a simple transporter

Consider a DB-shaped molecule incorporated into a membrane such that one end of the DB is in contact with solution on one side of the membrane, and the other end of the DB is in contact with solution on the other side of the membrane. The solutions on the two sides of the membrane can be viewed as reservoirs in which the chemical potentials of a ring, inspired by cyclobis(paraquat-*p*-phenylene) (CBPQT, fondly known as blue box) are held at μ_{left} and μ_{right} (with concentrations [left] and [right], respectively) as shown in Fig. 2. We have drawn the

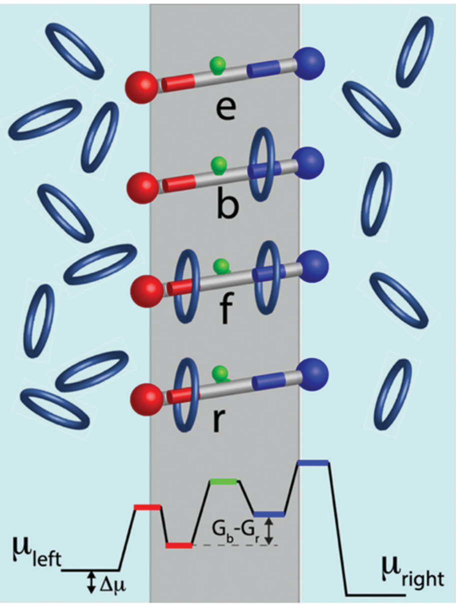


Fig. 2 Schematic illustration of a DB-shaped molecule spanning a membrane (gray region) with pseudo-stoppers at the liquid–membrane interface. The stopper in contact with the left-hand reservoir, as well as the recognition site is shown in red, and the stopper and recognition site near the right-hand reservoir is shown in royal blue. There is also a molecular "speed bump" shown in green that controls the transport between the two recognition sites. The DB provides a conduit by which rings (shown in dark blue – the rings were inspired by CBPQT blue-box) can move across the membrane by binding to a recognition site, undergoing transition over the speed-bump to the other recognition site, and then dissociating from the side opposite to that from which it first bound. The four possible states of the DB, indicating which recognition sites are occupied, are full (f), empty (e), blue (b), and red (r), and there are three in principle different pseudo-stoppers, green, red, and blue.

dumbbell molecule as asymmetric, with different stoppers and recognition sites on the two sides, shown in red and blue, with a speed bump, shown in green separating the two recognition sites.

Before we discuss externally driven pumping, let us consider the unpumped kinetics and thermodynamics of the molecular transport both at ($\mu_{\mathrm{left}} = \mu_{\mathrm{right}}$) and away from ($\mu_{\mathrm{left}} \neq \mu_{\mathrm{right}}$) equilibrium.

A kinetic diagram describing the transitions between the states of the DB/ring complexes is shown in Fig. 3. The ratios of the forward and backward rate constants for each transition can be given in terms of the state energies G_{b}, G_{r}, G_{f}, G_{e} and the chemical potentials of the rings μ_{left} and μ_{right} in the two reservoirs,

$$\frac{k_{\mathrm{bf}}}{k_{\mathrm{fb}}} = \underbrace{\mathrm{e}^{G_{\mathrm{b}}-G_{\mathrm{f}}+\mu_{\mathrm{left}}}}_{K_{\mathrm{bf}}\mathrm{e}^{\mu_{\mathrm{left}}}}; \quad \frac{k_{\mathrm{rf}}}{k_{\mathrm{fr}}} = \underbrace{\mathrm{e}^{G_{\mathrm{r}}-G_{\mathrm{f}}+\mu_{\mathrm{right}}}}_{K_{\mathrm{rf}}\mathrm{e}^{\mu_{\mathrm{right}}}}$$
$$\frac{k_{\mathrm{eb}}}{k_{\mathrm{be}}} = \underbrace{\mathrm{e}^{G_{\mathrm{e}}-G_{\mathrm{b}}+\mu_{\mathrm{right}}}}_{K_{\mathrm{eb}}\mathrm{e}^{\mu_{\mathrm{right}}}}; \quad \frac{k_{\mathrm{er}}}{k_{\mathrm{re}}} = \underbrace{\mathrm{e}^{G_{\mathrm{e}}-G_{\mathrm{r}}+\mu_{\mathrm{left}}}}_{K_{\mathrm{er}}\mathrm{e}^{\mu_{\mathrm{left}}}} \qquad (1)$$
$$\frac{k_{\mathrm{rb}}}{k_{\mathrm{br}}} = \underbrace{\mathrm{e}^{G_{\mathrm{r}}-G_{\mathrm{b}}}}_{K_{\mathrm{rb}}}$$

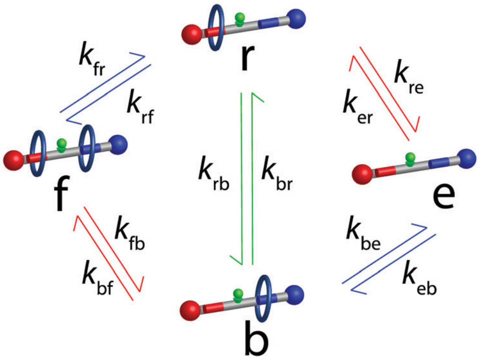


Fig. 3 Kinetic scheme describing possible transitions between states of the DB. When $\mu_{\mathrm{left}} = \mu_{\mathrm{right}}$ the net cycling rate for each cycle described by this kinetic scheme must, according to the second law of thermodynamics, be zero irrespective of the values of the state energies G_{b}, G_{r}, G_{f}, G_{e}. The colours of the arrows are chosen to indicate the barrier over which transition occurs.

where all energies and chemical potentials in this paper are given in units of the thermal energy $k_{\mathrm{B}}T$. The equilibrium constants $K_{ij} = \mathrm{e}^{G_i - G_j}$ obey the relation $K_{ij} = K_{ji}^{-1}$.

There are two cycles describing transport of a ring from left to right, $\mathcal{S}_1 \equiv \mathrm{e} \to \mathrm{r} \to \mathrm{b} \to \mathrm{e}$ and $\mathcal{S}_2 \equiv \mathrm{f} \to \mathrm{r} \to \mathrm{b} \to \mathrm{f}$, each with a microscopic reverse, $\mathcal{S}_1^\dagger$ and $\mathcal{S}_2^\dagger$, respectively, in which a ring is transported from right to left. The relative likelihood of clockwise *vs.* counter-clockwise cycles through the states depends only on the difference in chemical potentials in the two reservoirs $\mu_{\mathrm{left}} - \mu_{\mathrm{right}}$, *e.g.*

$$\frac{\pi_{\mathcal{S}_1}}{\pi_{\mathcal{S}_1^\dagger}} = \frac{\pi(\mathrm{e} \to \mathrm{r} \to \mathrm{b} \to \mathrm{e})}{\pi(\mathrm{e} \to \mathrm{b} \to \mathrm{r} \to \mathrm{e})} = \frac{k_{\mathrm{er}}k_{\mathrm{rb}}k_{\mathrm{be}}}{k_{\mathrm{eb}}k_{\mathrm{br}}k_{\mathrm{re}}} = \underbrace{K_{\mathrm{er}}K_{\mathrm{rb}}K_{\mathrm{be}}}_{1}\,\mathrm{e}^{\mu_{\mathrm{left}}-\mu_{\mathrm{right}}}$$
$$\frac{\pi_{\mathcal{S}_2}}{\pi_{\mathcal{S}_2^\dagger}} = \frac{\pi(\mathrm{f} \to \mathrm{r} \to \mathrm{b} \to \mathrm{f})}{\pi(\mathrm{f} \to \mathrm{b} \to \mathrm{r} \to \mathrm{f})} = \frac{k_{\mathrm{fr}}k_{\mathrm{rb}}k_{\mathrm{bf}}}{k_{\mathrm{fb}}k_{\mathrm{br}}k_{\mathrm{rf}}} = \underbrace{K_{\mathrm{fr}}K_{\mathrm{rb}}K_{\mathrm{bf}}}_{1}\,\mathrm{e}^{\mu_{\mathrm{left}}-\mu_{\mathrm{right}}} \qquad (2)$$

The identical kinetic scheme was analysed using "stochastic thermodynamics" in a recent *Nature Physics* perspective article.[21] The authors arrived at the very different, and transparently incorrect, conclusion in their box 1 – "A brief primer on local detailed balance and stochastic thermodynamics" – that if $\Delta G_{\mathrm{br}} \neq 0$ there is net cycling even when $\mu_{\mathrm{left}} = \mu_{\mathrm{right}}$, writing $\ln\left[\frac{\pi(\mathrm{e} \to \mathrm{r} \to \mathrm{b} \to \mathrm{e})}{\pi(\mathrm{e} \to \mathrm{b} \to \mathrm{r} \to \mathrm{e})}\right] = \mu_{\mathrm{left}} - \mu_{\mathrm{right}} + \Delta G_{\mathrm{br}}$. This equation is obviously contrary to the second law of thermodynamics. The correct theory for describing non-equilibrium steady-state behaviour of molecular systems is the trajectory thermodynamic formulation based on the work of Onsager and Machlup,[22,23] and cycle kinetics and thermodynamics as described by Terrell Hill in numerous articles and books.[24–26]

The fundamental equation for developing relationships based on cycle thermodynamics is that of microscopic reversibility[24,27]

$$\frac{\pi_{\mathcal{S}}}{\pi_{\mathcal{S}^\dagger}} = \mathrm{e}^{\mathcal{W}_{\mathcal{S}}} \qquad (3)$$

where $\mathcal{S}$ represents any single complete cycle starting and ending in the same state, $\mathcal{W}_{\mathcal{S}}$ is the net energy exchanged between the molecular machine and its environment in the cycle $\mathcal{S}$, and $\mathcal{S}^{\dagger}$ is the microscopic reverse of that cycle.

We calculate the ratio of left-right to right-left transport events as

$$\frac{\pi(\text{left} \rightarrow \text{right})}{\pi(\text{right} \rightarrow \text{left})} = \frac{\pi_{\mathcal{S}_1} + \pi_{\mathcal{S}_2}}{\pi_{\mathcal{S}_1^{\dagger}} + \pi_{\mathcal{S}_2^{\dagger}}} = \mathrm{e}^{\mu_{\text{left}} - \mu_{\text{right}}} \tag{4}$$

This simple result arises because the kinetic diagram describes only transport, with no energy input, and hence $\mathcal{W}_{\mathcal{S}_1} = \mathcal{W}_{\mathcal{S}_2} = \mu_{\text{left}} - \mu_{\text{right}}$, and $\frac{\pi_{\mathcal{S}_1}}{\pi_{\mathcal{S}_1^{\dagger}}} = \frac{\pi_{\mathcal{S}_2}}{\pi_{\mathcal{S}_2^{\dagger}}} = \mathrm{e}^{\mu_{\text{left}} - \mu_{\text{right}}}$. The average is more complicated in cases where not all ratios for forward and microscopic reverse trajectories are identical $\frac{\pi_{\mathcal{S}_i}}{\pi_{\mathcal{S}_i^{\dagger}}} \neq \frac{\pi_{\mathcal{S}_j}}{\pi_{\mathcal{S}_j^{\dagger}}}$ as when two processes (*e.g.*, transport and catalysis) are coupled. The transport stops at equilibrium, *i.e.*, when $\frac{[\text{left}]}{[\text{right}]} = \mathrm{e}^{\mu_{\text{left}} - \mu_{\text{right}}} = 1$, where [left] and [right] indicate concentrations of blue rings in the two reservoirs.

Turning a poor transporter into a good pump

The DB molecule shown in Fig. 2 is a very poor transporter, requiring multiple energetically uphill transitions over large barriers for a blue ring to make its way from the left (high chemical potential) to the right (low chemical potential) reservoir. These same characteristics of the energy landscape that make the molecule a poor transporter however offer the opportunity to design a very good pump to drive uphill transport of rings from the right to left reservoir by adding one ingredient, the ability to switch the DB molecule between two states with different kinetic and thermodynamic interactions with the blue ring.[7] This can be accomplished, *e.g.*, by changing the chemical composition of one of the reservoirs, or by coupling the transitions to catalysis of an exergonic reaction. The switching reaction is shown schematically in Fig. 4a where the low-affinity recognition site and large barrier (shown in blue) are switched to a high-affinity recognition site and smaller barrier (shown in violet). The energy profiles for the two states are shown in Fig. 4b. The predominant mechanism is highlighted in the context of the "kinetic cube" diagram[25] shown in Fig. 4c, where the bold lines indicate the main transport cycle

$$\cdots \rightleftharpoons \mathrm{e} \underset{\omega_{\mathrm{e}^*}}{\overset{\omega_{\mathrm{e}}}{\rightleftarrows}} \mathrm{e}^* \underset{k_{\mathrm{b}^*\mathrm{e}^*}}{\overset{k_{\mathrm{e}^*\mathrm{b}^*}}{\rightleftarrows}} \mathrm{b}^* \underset{\omega_{\mathrm{b}}}{\overset{\omega_{\mathrm{b}^*}}{\rightleftarrows}} \mathrm{b} \underset{k_{\mathrm{rb}}}{\overset{k_{\mathrm{br}}}{\rightleftarrows}} \mathrm{r} \underset{k_{\mathrm{er}}}{\overset{k_{\mathrm{re}}}{\rightleftarrows}} \mathrm{e} \rightleftarrows \cdots \tag{Scheme I}$$

The relative likelihood of transporting a blue ring from the left to right reservoir *vs.* from the right to left reservoir is given by the ratio

$$\frac{\pi(\text{left} \rightarrow \text{right})}{\pi(\text{right} \rightarrow \text{left})} = K_{\mathrm{er}} K_{\mathrm{rb}} K_{\mathrm{b}^*\mathrm{e}^*} \frac{\omega_{\mathrm{b}}\omega_{\mathrm{e}^*}}{\omega_{\mathrm{e}}\omega_{\mathrm{b}^*}} \tag{5}$$

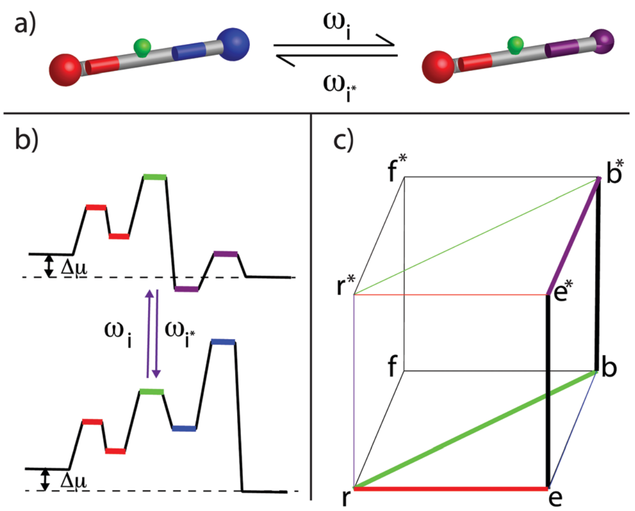


Fig. 4 Powering a synthetic pump. (a) Switching a dumbell between two states, illustrated as switching between blue pseudo-stopper and recognition site and violet pseudo-stopper and recognition site. For external driving the switching rates are independent of the state of the DB ring complex, but for catalysis-driven pumping the rates depend on whether the right-hand recognition site is or is not occupied. (b) Energy landscapes for transport of a blue ring along the two states of the DB rod. (c) Kinetic diagram for the transitions between the eight states of the DB-ring complex, where the energetically preferred cycle is shown in bold. Transitions between the upper and lower squares indicate switching transitions and the other transition are color coded according to the speed-bump over which the blue ring passes, with the switching transitions shown in black.

The most obvious and fundamental questions are what direction does the mechanism cycle, *i.e.*, is the ratio in eqn (5) greater than or less than one? Also, what thermodynamic and kinetic features determine that direction? In order to answer these question we focus on the ratios of the pumping transition constants $\frac{\omega_{\mathrm{b}}}{\omega_{\mathrm{b}^*}}$ and $\frac{\omega_{\mathrm{e}^*}}{\omega_{\mathrm{e}}}$, the key parameters in determining the directionality of the pump, for two cases, one where the pumping is accomplished by external modulation of the conditions and the other in which the transitions between the two energy surfaces arise as a result of catalysis.

Stochastic pumping by an energy ratchet[28,29]

For external modulation the switching rates can be chosen[15,27] to be $\omega_i = \omega_{i^*} = \gamma$ for all i. The mechanism of pumping is straightforward. Starting in the empty state e, the system is switched to state e* followed by exergonic binding of a ring to bring the system to state b*. The system is then switched to the original condition bringing the molecule to state b followed by exergonic transitions to r and then to e, thus completing a cycle in which a blue ring is transported from right to left. In this stochastic pumping the energy input occurs in the transition b* → b, and the subsequent exergonic transitions b → r → e can be very reasonably termed a power-stroke. From eqn (1) and (5) the ratio of the probability for left-right transport of a ring to the probability for right-left transport is

$$\frac{\pi(\text{left} \rightarrow \text{right})}{\pi(\text{right} \rightarrow \text{left})} = \mathrm{e}^{(G_{\mathrm{b}^*} - G_{\mathrm{b}})} \mathrm{e}^{(\mu_{\text{left}} - \mu_{\text{right}})} \tag{6}$$

The energy for uphill pumping comes from $G_{b^*} - G_b$ supplied when the molecule is switched from the violet to the blue state with a ring occupying the right-hand recognition site. Steady state is attained when [left]π(left → right) = [right]π(right → left), hence

$$\left.\frac{[\text{left}]}{[\text{right}]}\right|_{\text{ss}} = e^{(G_b - G_{b^*})} \tag{7}$$

The key design principle for stochastic pumping[29] by an energy ratchet is to create a molecule where both the energy of a state and the height of a barrier are modulated by changes in the chemical or thermodynamic environment of the bath.[3] If in the system shown in Fig. 4 only the barrier height changes then rings won't be recruited onto the recognition site under any environmental condition and there will be no pumping. If only the binding energy were to change, the rings would come onto and off of the DB from the same side, also accomplishing nothing. With modulation of both the barrier and binding energy as shown in Fig. 4b rings are recruited from the right (e* → b*) and dissociate to the left (b* → b → r → e), thereby pumping rings from right to left. A light driven pump works almost exactly like a stochastic pump but the transition constants are constrained by the Einstein relations for absorption and emission of light.[30]

At first glance it might seem that a similarly intuitive mechanism can be used to autonomously drive a pump by chemical catalysis, with the energy coming from a catalysed exergonic reaction $\text{S} \xrightleftharpoons{K_{\text{eq}}} \text{P}$. While it is indeed the case that a catalysed reaction can drive uphill transport, the principle by which it does so is entirely different than that for stochastic external or light driven pumping and is instead best described as an information ratchet.[31] A key point is that the directionality is governed by the relative specificities for S and P that allows gating and not by the free energies of the states, *e.g.* by $(G_b - G_{b^*})$.

Catalysis-driven pumping by an information ratchet

Let us take a closer look at how catalysis of the reaction $\text{S} \xrightleftharpoons{K_{\text{eq}}} \text{P}$ enters into the mechanism shown in Scheme I through the transitions $\text{e} \underset{\omega_{\text{e}^*}}{\overset{\omega_{\text{e}}}{\rightleftarrows}} \text{e}^*$ and $\text{b}^* \underset{\omega_{\text{b}}}{\overset{\omega_{\text{b}^*}}{\rightleftarrows}} \text{b}$. For a catalytic process these transitions ineluctably involve two possible paths, one for binding/release of substrate, S, and the other for binding/release of product, P, where the mechanism can be written as S + i ⇌ i* ⇌ i + P, where i is the "free", and i* the "bound" form of the molecular machine (MM).‡

$$\begin{aligned} \text{S} + \text{e} &\underset{k^{\text{S}}_{\text{e}^*\text{e}}}{\overset{[\text{S}]k^{\text{S}}_{\text{ee}^*}}{\rightleftharpoons}} \text{e}^* \underset{[\text{P}]k^{\text{P}}_{\text{ee}^*}}{\overset{k^{\text{P}}_{\text{e}^*\text{e}}}{\rightleftharpoons}} \text{e} + \text{P} \\ \text{S} + \text{b} &\underset{k^{\text{S}}_{\text{b}^*\text{b}}}{\overset{[\text{S}]k^{\text{S}}_{\text{bb}^*}}{\rightleftharpoons}} \text{b}^* \underset{[\text{P}]k^{\text{P}}_{\text{bb}^*}}{\overset{k^{\text{P}}_{\text{b}^*\text{b}}}{\rightleftharpoons}} \text{b} + \text{P} \end{aligned} \tag{Scheme II}$$

We can identify two independent parameters for each catalytic reaction. The thermodynamic affinities for S and P in states e and b are $K^{\text{S}}_{\text{ee}^*} = \frac{k^{\text{S}}_{\text{ee}^*}}{k^{\text{S}}_{\text{e}^*\text{e}}}$, $K^{\text{P}}_{\text{ee}^*} = \frac{k^{\text{P}}_{\text{ee}^*}}{k^{P}_{\text{e}^*\text{e}}}$, $K^{\text{S}}_{\text{bb}^*} = \frac{k^{\text{S}}_{\text{bb}^*}}{k^{\text{S}}_{\text{b}^*\text{b}}}$, $K^{\text{P}}_{\text{bb}^*} = \frac{k^{\text{P}}_{\text{bb}^*}}{k^{\text{P}}_{\text{b}^*\text{b}}}$. These affinities are measures of the tightness of binding and are constrained by microscopic reversibility such that $K^{\text{S}}_{\text{ee}^*}/K^{\text{P}}_{\text{ee}^*} = K^{\text{S}}_{\text{bb}^*}/K^{\text{P}}_{\text{bb}^*} = K_{\text{eq}}$. The kinetic specificities for S and P in states e* and b* are the ratios of the off rates, $s_{\text{e}^*} = k^{\text{S}}_{\text{e}^*\text{e}}/k^{\text{P}}_{\text{e}^*\text{e}}$ and $s_{\text{b}^*} = k^{\text{S}}_{\text{b}^*\text{b}}/k^{\text{P}}_{\text{b}^*\text{b}}$ and are measures of the relative rates of dissociation of S and P. The net rate constants are the sums of the rate constants for the two paths, $\omega_{\text{e}} = \left([\text{S}]k^{\text{S}}_{\text{ee}^*} + [\text{P}]k^{\text{P}}_{\text{ee}^*}\right)$, $\omega_{\text{e}^*} = \left(k^{\text{S}}_{\text{e}^*\text{e}} + k^{\text{P}}_{\text{e}^*\text{e}}\right)$, $\omega_{\text{b}} = \left([\text{S}]k^{\text{S}}_{\text{bb}^*} + [\text{P}]k^{\text{P}}_{\text{bb}^*}\right)$ and $\omega_{\text{b}^*} = \left(k^{\text{S}}_{\text{b}^*\text{b}} + k^{\text{P}}_{\text{b}^*\text{b}}\right)$, the ratios of which can be written

$$\begin{aligned} \frac{\omega_{\text{e}}}{\omega_{\text{e}^*}} &= [\text{S}]K^{\text{S}}_{\text{e}} \underbrace{\frac{\left[s_{\text{e}^*} + \text{e}^{-\Delta\mu}\right]}{\left[s_{\text{e}^*} + 1\right]}}_{\mathcal{A}_{\text{e}^*}} \\ \frac{\omega_{\text{b}}}{\omega_{\text{b}^*}} &= [\text{S}]K^{\text{S}}_{\text{b}} \underbrace{\frac{\left[s_{\text{b}^*} + \text{e}^{-\Delta\mu}\right]}{\left[s_{\text{b}^*} + 1\right]}}_{\mathcal{A}_{\text{b}^*}} \end{aligned} \tag{8}$$

where $\Delta\mu = \mu_{\text{S}} - \mu_{\text{P}}$ and $\mathcal{A}_{\text{e}^*}$ and $\mathcal{A}_{\text{b}^*}$ are kinetic asymmetry factors introduced by Astumian and Bier[32] that depend only on the ratio of "off" rates and on the difference in chemical potentials of substrate and product. These kinetic asymmetry factors do not depend on the free energies of the states e, e*, b, b* or on the difference in chemical potentials of the blue rings in the two reservoirs $(\mu_{\text{left}} - \mu_{\text{right}})$. Further, the ratios $\frac{\omega_{\text{e}}}{\omega_{\text{e}^*}}$ and $\frac{\omega_{\text{b}}}{\omega_{\text{b}^*}}$ cannot be expressed as the exponential of any thermodynamic quantity such as dissipation, entropy production, or "generalized thermodynamic force". Using eqn (8) the ratio in eqn (5) is seen to be

$$\frac{\pi(\text{left} \rightarrow \text{right})}{\pi(\text{right} \rightarrow \text{left})} = \mathcal{A}_{\text{b}^*}\mathcal{A}_{\text{e}^*}{}^{-1}\text{e}^{\left(\mu_{\text{left}} - \mu_{\text{right}}\right)} \tag{9}$$

since $K_{\text{er}}K_{\text{rb}}K^{\text{S}}_{\text{bb}^*}K_{\text{b}^*\text{e}^*}K^{\text{S}}_{\text{e}^*\text{e}} = 1$, and the non-equilibrium steady-state ratio is

$$\left.\frac{[\text{left}]}{[\text{right}]}\right|_{\text{ss}} = \mathcal{A}_{\text{b}^*}\mathcal{A}_{\text{e}^*}{}^{-1} \tag{10}$$

The states e* and b* can have different specificities (s_{e^*} and s_{b^*}). When $s_{\text{e}^*} = s_{\text{b}^*}$, there is no coupling between conversion of substrate to product and transport of the blue ring owing to the fact that in this case $\mathcal{A}_{\text{b}^*}\mathcal{A}_{\text{e}^*}{}^{-1} = 1$. The sole determinant of the direction of coupling induced by having $\mu_{\text{S}} > \mu_{\text{P}}$ is the ratio of the specificities $s_{\text{e}^*}/s_{\text{b}^*}$. If $s_{\text{e}^*}/s_{\text{b}^*} < 1$ then conversion of an S to P tends to pump a blue ring to the right and the steady state ratio is less than one, $\left.\frac{[\text{left}]}{[\text{right}]}\right|_{\text{ss}} < 1$. If $s_{\text{e}^*}/s_{\text{b}^*} > 1$ then conversion of an S to P tends to pump a blue ring to the left and the steady-state ratio is greater than one, $\left.\frac{[\text{left}]}{[\text{right}]}\right|_{\text{ss}} > 1$. The key principle in the design of a catalysis-driven pump is to use allosteric

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‡ Similar results hold for redox couples, where two separate reactions are written $\text{ox}_1 + \text{i} \overset{\rightarrow}{\rightleftharpoons} \text{i}^* + \text{red}_1$ and $\text{ox}_2 + \text{i} \overset{\leftarrow}{\rightleftharpoons} \text{i}^* + \text{red}_2$ and the over-arrows indicate the shift of the equilibrium based on the redox potentials of the two redox reactions $\text{ox}_1 \rightleftharpoons \text{red}_1$ and $\text{ox}_2 \rightleftharpoons \text{red}_2$. The net reaction can be written $\text{ox}_1 + \text{red}_2 + \text{i} \rightleftharpoons \text{i}^* \rightleftharpoons \text{i} + \text{red}_1 + \text{ox}_2$.

interactions to control the gating of the catalysed reaction such that half the reaction is fast in one state, *e.g.* "e", and the other half of the reaction is fast in a different state, *e.g.* "b", so that $\mathrm{S} + \mathrm{e} \overset{\text{fast}}{\rightleftharpoons} \mathrm{e}^* \overset{\text{slow}}{\rightleftharpoons} \mathrm{e} + \mathrm{P}$ and $\mathrm{S} + \mathrm{b} \overset{\text{slow}}{\rightleftharpoons} \mathrm{b}^* \overset{\text{fast}}{\rightleftharpoons} \mathrm{b} + \mathrm{P}$. The electrostatic repulsion that is so important in the design of externally driven pumps shown in Fig. 1 is not important at all for determining the effectiveness of pumping where the energy is supplied by catalysis of an exergonic reaction.

Power stroke or information ratchet?

The stochastic pumping mechanism works in a very intuitive way – energy is put into the system by an external change of the environment to switch the DB molecule and thus to drive the transition b* → b. Relaxation of the blue ring to the left is much faster than relaxation to the right so rings are pumped from right to left reservoirs. Then, the environment is externally switched back to the original condition to reset the system. Light driven processes work in a very similar way except that the switching is accomplished by absorption and emission of a photon and can proceed autonomously while the system is illuminated. The parts of the cycle can be conveniently broken up into energy input, power stroke, and reset, as shown in Scheme III.

$$\rightarrow \underbrace{\mathrm{e} \rightarrow \mathrm{e}^* \rightarrow \mathrm{b}^*}_{\text{reset}} \overbrace{\quad\rightarrow\quad}^{\text{energy input}} \underbrace{\mathrm{b} \rightarrow \mathrm{r} \rightarrow \mathrm{e}}_{\text{power stroke}} \rightarrow \qquad \text{(Scheme III)}$$

The overall process, an energy ratchet, is quite reasonably termed a power-stroke mechanism, and the individual components – energy input, power stroke, and reset – are all localized to specific transitions within the cycle, with the directionality governed solely by the sign of $(G_{\mathrm{b}^*} - G_{\mathrm{b}})$.

It would seem that a similar description could be given for pumping by chemical catalysis, with a localized energy input due to an exergonic chemical reaction depositing chemical energy in the system to promote the specific transition b* → b. This is not the case. As Hill pointed out,[23] free-energy transduction from a catalysed chemical reaction is a global property of the overall cycle. The asymmetry factors $\mathcal{A}_{\mathrm{b}^*}$ and $\mathcal{A}_{\mathrm{e}^*}$ involve the ratio of "off" rates for substrate and product, kinetic terms that do not depend on the free-energies of any of the states. While the most probable sequence of states and directionality when $\mathcal{A}_{\mathrm{b}^*}\mathcal{A}_{\mathrm{e}^*}{}^{-1} < 1$ is the same as that driven by stochastic pumping, describing the catalysis driven process as occurring by a power-stroke mechanism is problematic given that the directionality does not depend on the free-energies of the states, and is instead determined solely by the kinetic gating. Let us examine this dichotomy in the case of the simplest model for energy and information ratchets in which the energies of a single intermediate state surrounding barriers are pumped externally or by catalysis,[10] respectively.

Energy and information ratchets

Two distinct mechanisms for adaptation and transport have been discussed in the literature, energy ratchets and information ratchets.[31] Let us examine these two mechanisms in the context of a very simple single intermediate reaction.

Externally driven energy ratchet

In Fig. 5 two energy landscapes for a system are shown, one in violet and the other in royal blue. For external modulation or driving by light, the switching rate can be described by a single transition constant γ. In this case the two energy landscapes, together with the modulation, act as an energy ratchet. Starting in A on the violet landscape, the system almost certainly undergoes transition to I. When conditions are changed to favour a state of the molecule with the blue landscape, the external energy provided lifts the energy of state I to that of I*. In state I* the system undergoes spontaneous transition to B*. Returning the system to the original conditions with the violet landscape bringing the system to state B. Under the externally driven condition the ratio between the concentrations is

$$\frac{[\mathrm{B}]|_{\mathrm{ss}}}{[\mathrm{A}]|_{\mathrm{ss}}} = K_{\mathrm{AI}}K_{\mathrm{I^*B^*}} = \mathrm{e}^{G_{\mathrm{I}^*} - G_{\mathrm{I}}} \qquad (11)$$

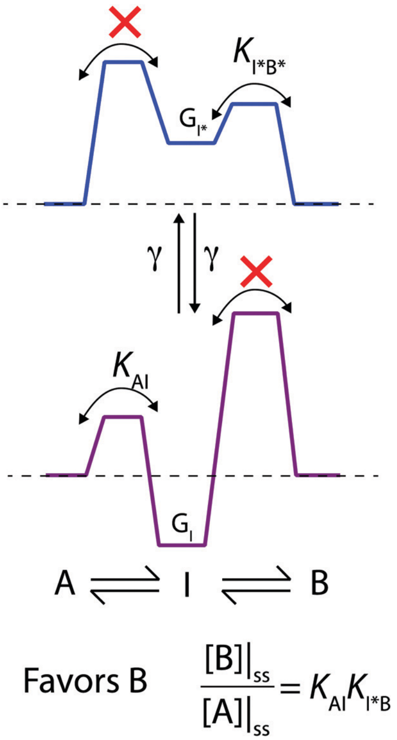


Fig. 5 Depiction of a generic single-intermediate energy ratchet scheme for driving formation of a non-equilibrium distribution between state A and B. The system undergoes the spontaneous transitions A → I on the violet landscape and I* → B* on the blue landscape. The response of the system to pumping is a non-monotonic function of frequency. If γ is too small, the system relaxes to equilibrium on each potential separately so the concentration ratio between B and A is one, and if γ is too large, the system experiences only the average of the two potentials where the stationary concentration ratio is also unity. In between these two extremes the pumping maintains a non-equilibrium steady-state ratio between the concentrations of A and B which is not unity and depends on $G_{\mathrm{I}^*} - G_{\mathrm{I}}$.

Can such a mechanism operate autonomously, using chemical free-energy released by catalysis of an exergonic chemical reaction?

Catalysis driven information ratchet

The answer is a partial yes – chemical fuel can indeed be harnessed to drive a system away from equilibrium, but the underlying principle by which it does so is very different than the energy ratchet with its "power stroke". The mechanism is known as an information ratchet.[31,33] Depending on the specificities of states I, and B for S and P the mechanism can favour either A or B. To see how this works let us first consider the case that state I is specific for substrate S (*i.e.*, that the rates for binding/dissociating P to I are very small, indicated by the red cross) and that state B is specific for P (*i.e.*, that the rates for binding/dissociating S to B are very small, indicated by the red cross). Starting in A the system will almost certainly undergo transition to state I which can bind S bringing the system to state I* from which transition to B* leads to dissociation of P and the system ending in state B. The overall process is

$$A \xrightarrow{k_{\mathrm{AI}}} I \xrightarrow{[\mathrm{S}]k^{\mathrm{S}}_{\mathrm{II^*}}} I^* \xrightarrow{k_{\mathrm{I^*B^*}}} B^* \xrightarrow{k^{\mathrm{P}}_{\mathrm{B^*B}}} B \qquad \text{(Scheme IV)}$$

$$\pi^{\mathrm{SP}}_{\mathrm{AB}} = [\mathrm{S}]k_{\mathrm{AI}}k^{\mathrm{S}}_{\mathrm{II^*}}k_{\mathrm{I^*B^*}}k^{\mathrm{P}}_{\mathrm{B^*B}}$$

The superscript on π indicates conversion of S to P, and the subscript indicates conversion of A to B. The microscopic reverse is

$$B \xrightarrow{[\mathrm{P}]k^{\mathrm{P}}_{\mathrm{BB^*}}} B^* \xrightarrow{k_{\mathrm{B^*I^*}}} I^* \xrightarrow{k^{\mathrm{S}}_{\mathrm{I^*I}}} I \xrightarrow{k_{\mathrm{IA}}} A \qquad \text{(Scheme V)}$$

$$\pi^{\mathrm{PS}}_{\mathrm{BA}} = [\mathrm{P}]k^{\mathrm{P}}_{\mathrm{BB^*}}k_{\mathrm{B^*I^*}}k^{\mathrm{S}}_{\mathrm{I^*I}}k_{\mathrm{IA}}$$

The ratio of the probabilities for these two cycles, and hence the ratio of steady-state concentrations, is

$$\frac{[\mathrm{B}]|_{\mathrm{ss}}}{[\mathrm{A}]|_{\mathrm{ss}}} = \frac{\pi^{\mathrm{SP}}_{\mathrm{AB}}}{\pi^{\mathrm{PS}}_{\mathrm{BA}}} = \mathrm{e}^{\mu_{\mathrm{S}}-\mu_{\mathrm{P}}} \qquad (12)$$

If the specificities are switched such that state B is specific for S and state I is specific for P (shown on the bottom right of Fig. 6) the sequence for converting A to B without using any "forbidden" transitions (*i.e.*, transitions with a red X) is

$$A \xrightarrow{k_{\mathrm{AI}}} I \xrightarrow{[\mathrm{P}]k^{\mathrm{P}}_{\mathrm{II^*}}} I^* \xrightarrow{k_{\mathrm{I^*B^*}}} B^* \xrightarrow{k^{\mathrm{S}}_{\mathrm{B^*B}}} B \qquad \text{(Scheme VI)}$$

$$\pi^{\mathrm{PS}}_{\mathrm{BA}} = [\mathrm{P}]k_{\mathrm{AI}}[\mathrm{P}]k^{\mathrm{P}}_{\mathrm{II^*}}k_{\mathrm{I^*B^*}}k^{\mathrm{S}}_{\mathrm{B^*B}}$$

and the microscopic reverse is

$$B \xrightarrow{[\mathrm{S}]k^{\mathrm{S}}_{\mathrm{BB^*}}} B^* \xrightarrow{k_{\mathrm{B^*I^*}}} I^* \xrightarrow{k^{\mathrm{P}}_{\mathrm{I^*I}}} I \xrightarrow{k_{\mathrm{IA}}} A \qquad \text{(Scheme VII)}$$

$$\pi^{\mathrm{SP}}_{\mathrm{BA}} = [\mathrm{S}]k^{\mathrm{S}}_{\mathrm{BB^*}}k_{\mathrm{B^*I^*}}k^{\mathrm{P}}_{\mathrm{I^*I}}k_{\mathrm{IA}}$$

with the ratio

$$\frac{[\mathrm{B}]|_{ss}}{[\mathrm{A}]|_{\mathrm{ss}}} = \frac{\pi^{\mathrm{PS}}_{\mathrm{AB}}}{\pi^{\mathrm{SP}}_{\mathrm{BA}}} = \mathrm{e}^{\mu_{\mathrm{P}}-\mu_{\mathrm{S}}} \qquad (13)$$

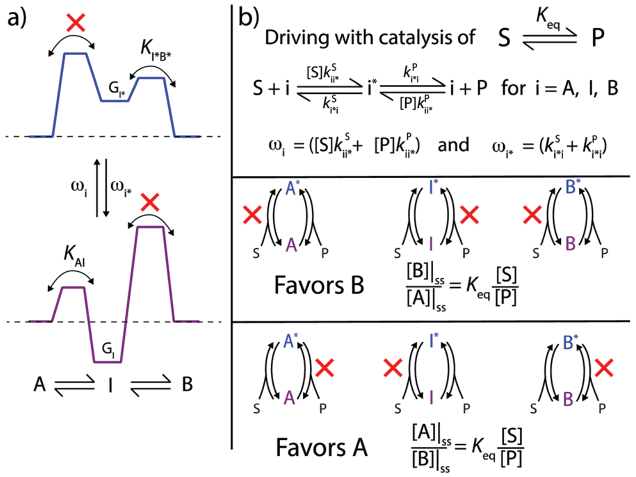


Fig. 6 (a) The identical landscapes as in Fig. 5, but now the switching rates ω_i and ω_{i^*} arise from catalytic process carried out by each state. (b) The adaptation due to the dis-equilibrium between S and P can be switched depending on the specificities of state I and B. If I is specific for S and B is specific for P the disequilibrium between S and P favours B, but if I is specific for P and B is specific for S the disequilibrium between S and P favours A.

By switching the specificities, the system goes from favouring B when $\mu_{\mathrm{S}} > \mu_{\mathrm{P}}$ to favouring A. Unlike the case of external pumping, $(G_{\mathrm{I^*}} - G_{\mathrm{I}})$ plays no role whatsoever in determining whether A or B is favoured. The expressions eqn (12) and (13) arise from taking "perfect specificity" limits to catalysis driven adaptation for the reaction A ⇌ I ⇌ B as shown in Fig. 6b, but this condition is easily relaxed to allow for a general expression $\frac{[\mathrm{B}]|_{\mathrm{ss}}}{[\mathrm{A}]|_{\mathrm{ss}}} = \frac{\pi^{\mathrm{PS}}_{\mathrm{AB}} + \pi^{\mathrm{SP}}_{\mathrm{AB}} + \pi^{\mathrm{SS}}_{\mathrm{AB}} + \pi^{\mathrm{PP}}_{\mathrm{AB}}}{\pi^{\mathrm{SP}}_{\mathrm{BA}} + \pi^{\mathrm{PS}}_{\mathrm{BA}} + \pi^{\mathrm{SS}}_{\mathrm{BA}} + \pi^{\mathrm{PP}}_{\mathrm{BA}}}$, which can be simplified using microscopic reversibility[27] eqn (3). It is very important to recognize that the work done in the process of transition from A to B is not a state function – the external work done can be $+\Delta\mu$, $-\Delta\mu$, or 0, and in calculating the overall probabilities to go from A to B *vs.* from B to A we must use an average weighted by the rate constants.

Stochastic thermodynamics is incorrect for catalytic processes

Stochastic thermodynamics is a recently emerging theory for describing stochastic systems.[21,34,35] A central assumption of this theory is a "local detailed balance" condition that the ratio of rates between any two states is given by the exponential of a generalized thermodynamic force or by an entropy production. For catalysis driven processes this condition is incorrect. As seen in eqn (9) the ratio of rates between a bound and free form of a catalyst is governed by the kinetic asymmetry[32,36] and not by a "generalized thermodynamic force". The errors in stochastic thermodynamics arise from a cavalier description of chemical catalysis, and from incorrectly treating as a state function the work done, energy dissipated, or entropy produced in undergoing transition between two states, which in general they are not.

According to the authors of ref. 21 a "central assumption of stochastic thermodynamics" leads to their eqn (1) for the ratio between forward and backward rates $\sigma(x,y) = \ln\left[\frac{r(x,y)}{r(y,x)}\right]$, where $r(x,y)$ is the "probability per unit time to jump from y to x", and $\sigma(x,y)$ is "the non-dimensional change in the entropy of the mediating reservoir". Irrespective of the details, this asserted relation makes a bold claim – that the ratio of rates depends only on the states y and x, a claim that is true at equilibrium but not generally true away from equilibrium. In the simplest model for catalysis, the Michaelis–Menten mechanism, at equilibrium the ratio for the probabilities of transition between x = "free" and y = "bound" forms is the same for binding/release of substrate and binding/release of product. Away from equilibrium, however, the ratio of net probabilities for the transition between "free" and "bound" forms is not a state function and depends strongly on the kinetic asymmetry of the system. It is important to note that any state that can bind substrate can also bind product, albeit with possibly very different rates. As discussed by Hill[25] it is not meaningful to assign separate chemical potentials to bound substrate and bound ligand. When either bind, they lose their individual identity. Any attempt to subdivide the states to eliminate the necessity of explicitly treating several distinct paths between bound and free states of an enzyme is futile.

Confusion arises from the fact that in the perfect specificity limit in Fig. 6 the correct general expression $\frac{\omega_{\mathrm{B}}\omega_{\mathrm{I}^*}}{\omega_{\mathrm{B}^*}\omega_{\mathrm{I}}} = \mathcal{A}_{\mathrm{I}}\mathcal{A}_{\mathrm{B}}^{-1}$ approaches[36] the "local detailed balance" forms, with $\frac{\omega_{\mathrm{B}}\omega_{\mathrm{I}^*}}{\omega_{\mathrm{B}^*}\omega_{\mathrm{I}}} \approx \mathrm{e}^{\Delta\mu}$, $\frac{\omega_{\mathrm{B}}\omega_{\mathrm{I}^*}}{\omega_{\mathrm{B}^*}\omega_{\mathrm{I}}} \approx 1$, or $\frac{\omega_{\mathrm{B}}\omega_{\mathrm{I}^*}}{\omega_{\mathrm{B}^*}\omega_{\mathrm{I}}} \approx \mathrm{e}^{-\Delta\mu}$, depending on the specificities. This limit is a good approximation for some biomolecular motors such as the FoF1 ATPase, a poor approximation for other motors such as kinesin, and fails miserably for a catalysis driven synthetic motor.[36]

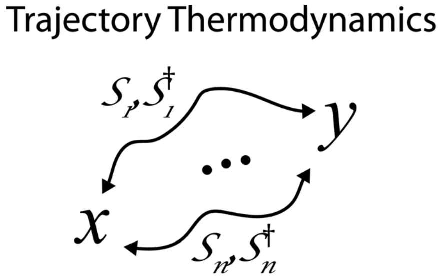
It is important to remember that kinetics – the relative heights of energy barriers – and not thermodynamics – the relative depths of energy wells – determines the direction of motion.[36,37] This essential role of kinetics, and the ineluctable importance of kinetic asymmetry,[32,36] is entirely absent in the formulation of stochastic thermodynamics.

Trajectory thermodynamics *vs.* stochastic thermodynamics

There are two competing theories for describing thermodynamics of molecular machines. The first is trajectory thermodynamics, a theoretical approach that has its origins in the work of Onsager and Machlup,[22,23] and subsequently of Terrell Hill.[24–26] Astumian has used trajectory thermodynamics as the basis of a general theory for molecular machines.[27] Trajectory thermodynamics focuses on the distinct trajectories between two states, say x and y (see Fig. 7), by which a transition from one state to another can occur. The theoretical underpinning of this theory is the principle of microscopic reversibility, eqn (3), and easily derived corollaries such as $\langle \mathcal{W}_{\mathcal{S}_i} \rangle_i \geq 0$ and $\langle \mathrm{e}^{\mathcal{W}_{\mathcal{S}_i}} \rangle_i = 1$, where the averages are over the trajectories $\mathcal{S}_i$.

The ratio of rates between x and y is

$$\frac{r(x,y)}{r(y,x)} = \frac{\sum_i \pi_{\mathcal{S}_i^\dagger}}{\sum_i \pi_{\mathcal{S}_i}} = \frac{\sum_i \pi_{\mathcal{S}_i}\mathrm{e}^{\mathcal{W}_{\mathcal{S}_i}}}{\sum_i \pi_{\mathcal{S}_i}} \tag{14}$$



$$\frac{\pi_{\mathcal{S}_i}}{\pi_{\mathcal{S}_i^\dagger}} = e^{\mathcal{W}_{\mathcal{S}_i}}$$

$$\frac{\pi_{\mathcal{S}_i}\mathcal{W}_{\mathcal{S}_i} + \pi_{\mathcal{S}_i^\dagger}(-\mathcal{W}_{\mathcal{S}_i})}{\pi_{\mathcal{S}_i} + \pi_{\mathcal{S}_i^\dagger}} = \frac{(1 - e^{\mathcal{W}_{\mathcal{S}_i}})}{(1 + e^{\mathcal{W}_{\mathcal{S}_i}})}\mathcal{W}_{\mathcal{S}_i} \geq 0$$

$$\frac{\pi_{\mathcal{S}_i}e^{-\mathcal{W}_{\mathcal{S}_i}} + \pi_{\mathcal{S}_i^\dagger}e^{\mathcal{W}_{\mathcal{S}_i}}}{\pi_{\mathcal{S}_i} + \pi_{\mathcal{S}_i^\dagger}} = \frac{\pi_{\mathcal{S}_i^\dagger} + \pi_{\mathcal{S}_i}}{\pi_{\mathcal{S}_i} + \pi_{\mathcal{S}_i^\dagger}} = 1$$

Fig. 7 Basic setup for discussion of the not necessarily equilibrium exchange between two molecular states, x and y by trajectory thermodynamics. There can be many (n) paths $\mathcal{S}_i$ from x to y. Each path from x to y has a microscopic reverse path from y to x. The ratios of the rates/probabilities for each forward and microscopic reverse trajectory, $\pi_{\mathcal{S}_i}$ and $\pi_{\mathcal{S}_i^\dagger}$ obey microscopic reversibility expressed mathematically as a Boltzmann-like relation involving the path dependent energy exchanged between the molecule and its environment, $\mathcal{W}_{\mathcal{S}_i}$, and where $\mathcal{W}_{\mathcal{S}_i^\dagger} = -\mathcal{W}_{\mathcal{S}_i}$. Two easily derived corollaries resulting from microscopic reversibility are also shown.

where the exchanged energy $\mathcal{W}_{\mathcal{S}_i}$ comprises a path independent term $\Delta G_{xy} = G_x - G_y$ common to all paths and a path dependent term $\mathcal{W}_{\mathcal{S}_i,\mathrm{pd}}$, with $\mathcal{W}_{\mathcal{S}_i} = \Delta G_{xy} + \mathcal{W}_{\mathcal{S}_i,\mathrm{pd}}$. Since the steady state condition is $[x]|_{\mathrm{ss}}r(x,y) - [y]|_{\mathrm{ss}}r(y,x) = 0$, we obtain the steady-state relation[38]

$$\frac{[y]|_{\mathrm{ss}}}{[x]|_{\mathrm{ss}}} = \mathrm{e}^{\Delta G_{xy}}\left\langle \mathrm{e}^{\mathcal{W}_{\mathcal{S}_i,\mathrm{pd}}} \right\rangle_i \tag{15}$$

where the factor $\left\langle \mathrm{e}^{\mathcal{W}_{\mathcal{S}_i,\mathrm{pd}}} \right\rangle_i$ describes how the concentrations adapt to non-equilibrium conditions.

Trajectory thermodynamics has been used to describe, among other phenomena, the effect of oscillating fields on membrane transport,[4,5] how external energy driven fluctuations can allow an enzyme to drive the reaction it catalyses away from equilibrium,[38] generically known as an energy ratchet, and how an enzyme can use energy to do mechanical, electrical, or osmotic work on the environment, generically known as an information ratchet.[31,39]

The theoretical grounding for trajectory thermodynamics is the Onsager–Machlup thermodynamic action theory.[22] A knee-jerk reaction of many physical scientists is that "Onsager's theory is only valid near equilibrium in the linear regime", and we all know that molecular machines and biological cells operate far from equilibrium. How can this near-equilibrium theory provide understanding of the far-from-equilibrium processes of molecular machines? If we read the Onsager–Machlup paper carefully we see that Onsager and Machlup provide guidance into what these terms actually mean, explaining that "The essential physical assumption about the irreversible processes is that they are linear; *i.e.*, that the fluxes depend linearly on the forces that cause them." This requirement is nothing other than that the dynamics be describable in terms of low Reynolds number motion,[40] *i.e.*, that the viscous forces acting on the individual molecules be much greater than the inertial forces, a condition that is very generally applicable to molecules in aqueous media. The overdamped nature of the dynamics leads to an "unreasonable effectiveness of equilibrium theory for describing non-equilibrium experiments".[41] The linearity that holds for single molecule dynamics has recently been shown to propagate to the level of phenomenological theories for macroscopic behaviour, being beautifully described as "the beneficent thickness of water".[42]

A recent theory known as stochastic thermodynamics[34,35] focusses on the states of the system and on net transitions between them, and not on individual trajectories. A key assumption in the formulation of stochastic thermodynamics is that a "local detailed balance" relation holds for net transition rates between any two states,[21] $\frac{r(x,y)}{r(y,x)} = \mathrm{e}^{\sigma(x,y)}$, where $\sigma(x,y)$ is the "entropy production". Away from equilibrium this assumption is simply wrong – the ratio of rates is not a state function. Seifert has saliently observed that a central idea behind stochastic thermodynamics is that entropy can be consistently assigned to a trajectory described in terms of a sequence of states of a system.[35] This assignment is precisely what cannot be done when catalysis is involved.

In many cases, especially for the description of externally driven mechanical motion, trajectory thermodynamics and stochastic thermodynamics make identical predictions. However, in some cases, such as in the description of catalysis driven processes, the two theories make very different predictions and in those cases the predictions of trajectory thermodynamics are shown to be correct. An explicit example is found in the recent molecular rotor of Wilson *et al.*[10] The rotor can be arranged such that stochastic thermodynamics predicts clockwise rotation while trajectory thermodynamics predicts counter-clockwise rotation. The rotor is shown to, in fact, rotate in the counter-clockwise direction confirming the prediction of trajectory thermodynamics and falsifying that of stochastic thermodynamics.[36]

Conclusions

The advent of the design and synthesis of artificial molecular machines, lauded by the award of the 2016 Nobel prize in chemistry,[43–45] has brought about a significant increase in our understanding of biomolecular machines.[46] This new perspective has been bolstered by strong theory based on the fundamental principle of microscopic reversibility, and by a re-appreciation[41,42] of the work of Lars Onsager and of Terrell Hill who developed what has come to be known as trajectory thermodynamics.

The theoretical pump described in this paper is a case in point. Even in equilibrium the DB molecular system shown in Fig. 2 is dynamic, with every possible transition occurring. Equilibrium is maintained because each transition is exactly as likely as the reverse of that transition – the binding of a ring to the red recognition site is as likely as dissociating a ring from the red site; transition of a ring from the blue recognition site to the red recognition site is as likely as transition of a ring from the red recognition site to the blue recognition site; *etc.* When the system is brought out of equilibrium by enforcing conditions such that $\mu_{\text{left}} \neq \mu_{\text{right}}$ the physical motion of the blue rings through the solution, or from one recognition site to another, does not change. Dissipation arises because of transport of a blue ring from the reservoir at high chemical potential to the reservoir at low chemical potential, not because of friction. We can go further by considering the effect of providing chemical energy with $\mu_{\text{S}} > \mu_{\text{P}}$. It is unreasonable to postulate that substrate, be it ATP, Fmoc-Cl, or some other molecule, violently kicks a blue ring onto or off of a recognition site,[47] or that the mechanism involves anything resembling a judo throw.[48] The non-equilibrium conditions instead change the relative likelihood of the many possible cycles. It is essential to understand that the constraints on the rate constants, and hence on the cycle probabilities, determine the principle by which the molecular machine functions. For external driving, or for driving by light, the mechanism is very simple – the system is "energized" followed by thermal relaxation (a power stroke) where the direction of relaxation is controlled by kinetic barriers. Such a simple picture is very easy to understand, but for catalysis driven pumping this explanation is wrong. The kinetic constants are constrained by microscopic reversibility.[12] The mechanism, while possibly incorporating a power stroke, is not a power stroke mechanism, and instead drives uphill transport by the selective opening and closing of gates[49,50] (switching selectivity) to shepherd the blue rings from one side to the other. These differences, based on the constraints appropriate for the type of driving used, are abundantly clear in the eqn (7) *vs.* (10) that describe the steady state condition for the concentrations of blue rings on the left and right side of the membrane for externally (or light) *vs.* catalysis driven pumps, respectively.

The error in Box 1 of the paper by Horowitz and Gingrich[21] results from a failure to incorporate microscopic reversibility into the kinetic description of the system. These authors introduce plausible ratios between rate constants consistent with "general local detailed balance", but which fail to reflect the fact that the two internal states ("r" and "b" in Fig. 3) have energies that are different than one another by an amount G_{br}. This mistake leads Horowitz and Gingrich to the conclusion, given as the last equation of their Box 1, that net cyclic flux occurs even when $\mu_{\text{left}} = \mu_{\text{right}}$, a flagrant violation of the second law of thermodynamics. This error is precisely the mistake against which Onsager[51] warned in 1932 in his analysis of a three-state cycle mechanism similar to that in ref. 21, and discussed in detail by Astumian, Mukherjee, and Warshel.[37]

Confronting molecular machines in terms of what they are – molecules in solution[42] rather than shrunken macroscopic devices comprising the cell as a machine[52] – will facilitate development of theoretical and computational descriptions of molecular machines[53] and will stimulate and inform the design of ever more complex devices operating away from thermodynamic equilibrium.[54,55] These advances may even provide insight into an answer for Jean-Marie Lehn's provocative question – ''How does simple matter become complex?''.[56]

Conflicts of interest

There are no conflicts to declare.

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