**Insights from an information thermodynamics analysis of a synthetic molecular motor**

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Information is physical, a realization that has transformed the physics of measurement and communication. However, the flow between information, energy and mechanics in chemical systems remains largely unexplored. Here we analyse a minimalist autonomous chemically driven molecular motor in terms of information thermodynamics, a framework that quantitatively relates information to other thermodynamic parameters. The treatment reveals how directional motion is generated by free energy transfer from chemical to mechanical (conformational and/or co-conformational) processes by ‘energy flow’ and ‘information flow’. It provides a thermodynamic level of understanding of molecular motors that is general, complements previous analyses based on kinetics and has practical implications for machine design. In line with kinetic analysis, we find that power strokes do not affect the directionality of chemically driven machines. However, we find that power strokes can modulate motor velocity, the efficiency of free energy transfer and the number of fuel molecules consumed per cycle. This may help explain the role of such (co-)conformational changes in biomachines and illustrates the interplay between energy and information in chemical systems.

Understanding how and why a machine works in the way it does is crucial for optimizing designs and inventing new ones. For macroscopic machines, such an understanding can be deduced from engineering principles and Newtonian physics. In contrast, at the molecular level there is no simple explanation for why individual components of biomachines move in a particular way. Is the movement of a specific amino acid in adenosine triphosphate synthase a key requisite for the mechanism, or does it occur incidentally as part of an evolutionary pathway that was overall successful? Biomolecular machines operate autonomously, apparently through Brownian ratchet mechanisms. They use energy and information to rectify the directionality of random thermal movements of their components so that work can be performed. These types of machines include biological pumps and motors, in which the energy is generally provided in the form of a chemical potential gradient. Chemists are learning how to design synthetic analogues of such machines, with examples including a minimalist autonomous chemically fuelled molecular rotary motor (Fig. 1).

Kinetic models have proved useful in describing the behaviour of molecular machines, but such analysis is inherently unable to give an account of free energy transfer from the fuel to the machine. The consideration of thermodynamics is essential to understand free energy transduction, how this drives directional motion and generates the capacity to perform work and, hence, how to optimize the motor’s design. Up to now, attempts in designing molecular motors have been led by chemical intuition, with little opportunity to reliably judge the effectiveness of a machine’s design or performance unless and until it has been realized experimentally.

Recently, stochastic thermodynamics has emerged as a method for studying systems that operate at energies of the order of thermal fluctuations. The theory is valid even when systems are driven far from equilibrium and has been used to study nonequilibrium nanoscale systems. A major achievement in this field was the connection made between stochastic thermodynamics and information theory, which gave birth to ‘information thermodynamics’. Information thermodynamics relates information to other thermodynamic quantities, such as free energy and entropy, and has proved particularly successful in resolving apparent thermodynamic paradoxes, such as Maxwell’s demon.

In this Article we develop a quantitative understanding of the processes that drive an autonomous chemically fuelled molecular motor using an analysis that incorporates concepts from information thermodynamics within the framework of nonequilibrium thermodynamics of open chemical reaction networks. Contrary to common models in stochastic thermodynamics, our framework neglects fluctuations and describes macroscopic ensembles of chemical species characterized by experimentally measurable concentrations. The approach is consistent with kinetic models, but goes further by introducing a quantitative thermodynamic understanding of how autonomous molecular motors work. Two features—information flow and energy flow—contribute to the transfer of free energy from the fuel to the machine that is the origin of current (that is, the net rate of displacement of the macrocycle directionally along the track; Box 1) in the motor. The effect of changing chemical gating, power strokes (a viscoelastic, free energy-releasing, large-amplitude conformational change) and overall rates on current and efficiency (Box 1) are examined through simulations, revealing design principles for molecular motors. Particular insight is gained in terms of the role of power strokes in tuning a motor’s performance while remaining consistent with core aspects of kinetic models, informing the current debate concerning the role of power strokes in biomolecular machines.

As a result, the mechanism of operation of the rotary motor can be understood in several different ways: through chemical design, reaction kinetics, molecular dynamics and, now, nonequilibrium information thermodynamics. Accordingly, this minimalist molecular motor can act as a Rosetta Stone for relating these disparate frameworks, aiding the translation...
of concepts and relationships among energy, information, kinetics and molecular structure.

Results and discussion
A bipartite chemical reaction network for the minimalist rotary motor. The rotary motor in Fig. 1 comprises a cyclic track with two degenerate binding sites for a macrocycle[16]. One site is deuterium labelled to distinguish it from the other by 1H NMR spectroscopy, although deuterium does not influence the chemical properties. Fluorenylmethoxycarbonyl (Fmoc) groups, which sterically prevent passage of the macrocycle, can be attached to hydroxy residues on the track (Fig. 2). When only one barrier is in place, macrocycle shuttling enables exchange between two co-conformers (co-conformers are structures that differ in the relative positions of the components[35]) that have the macrocycle either proximal (p) or distal (d) to the free hydroxy group. The fuelling reaction kinetically discriminates between the two co-conformers, favouring the reaction of the distal co-conformer over the proximal co-conformer. Under basic conditions, the waste-forming reaction removes barriers without any chemical gating. The chemical gating afforded by the biased fuelling reaction, and the free energy supplied by the fuel-to-waste conversion, result in directional movement of the macrocycle around the track. The rotary motor can be represented by a chemomechanical network of reactions (Fig. 2) in which mechanical and chemical transitions are coupled, as in common models for biological molecular motors[44] and minimal Brownian motors with external dichotomous noise[45]. Because no transitions enable simultaneous change in the mechanical and chemical states, this network is said to be bipartite[46].

Information thermodynamic analysis. As detailed in Supplementary Section I, the rotary motor is modelled as the isothermal open bipartite chemical reaction network[46,47] shown in Fig. 2. The concentrations of the six motor species, 2H, 2D, 1H_D, 1D_D, 1H_H and 1D_H (see Fig. 2 caption) evolve according to the rate constants of each reaction following mass-action kinetics. The system is open because the concentrations of fuel (Fmoc-Cl) and waste (HCl) are kept constant through addition from, or removal to, an external source. An important quantity for our analysis is the chemical potential difference between the fuel and waste species, \( \mu_0 - \mu_W \), which we denote as the free energy generated during the waste-forming reaction. For any thermodynamically consistent set of parameters (Methods), the system will evolve towards a stationary state in which the concentrations of all the motor species are constant in time, as are the thermodynamic properties of the system. The entropy production rate (the entropy changes in the system and in the reservoirs per unit time), denoted \( \Sigma \), measures how far from equilibrium the system operates and has to be non-negative by virtue of the second law of thermodynamics[47]. When multiplied by temperature (T), this corresponds to the amount of free energy that is instantaneously dissipated as part of the fuelling reaction and subsequently neutralized by KHCO₃, which is present to produce KCl, CO₂ and H₂O—and dibenzofulvene and CO₂. The fuelling reaction is catalysed by a pyridine-based nucleophilic catalyst (for example, 4-dimethylaminopyridine or the bulky catalyst shown in Fig. 4a). Note that both reactions are considered reversible, even when the backward reactions (that is, regeneration of fuel via barrier removal and waste products reacting to give the barrier) are extremely rare events[66].
dissipated by the motor \((TΣ)\). In a stationary state (the only type of state considered in this Article), all the free energy from the conversion of the fuel to waste is dissipated by the motor: \(TΣ = I_F(μ_F - μ_W) \geq 0\), where \(I_F\) is the rate at which the fuel is consumed by the motor (we neglect the fuel-to-waste background reaction)\(^{26}\). This indicates that a non-null chemical potential gradient between fuel and waste species (we consider the case where \(μ_F \geq μ_W\)) is necessary to drive the system out of equilibrium and produce directed motion. However, this expression gives no information as to how free energy is consumed nor the amount of dissipation specifically devoted to sustain directional motion of the components.

To obtain a more in-depth understanding, we use information thermodynamics to split the free energy dissipation rate into two separately non-negative contributions (Fig. 1b), one due to the chemical transitions \((TΣ^{\text{chem}})\) and the other due to the mechanical transitions \((TΣ^{\text{mech}})\). This is possible because the Fmoc-motor chemical reaction network is bipartite\(^{40}\):

\[
TΣ = I_F(μ_F - μ_W) - \dot{E} - RT\dot{I} + \dot{E} + RT\dot{I} \geq 0. \tag{1}
\]

This dissection of terms in equation (1) underlines that the free energy supplied by the fuel is only partially dissipated via the chemical processes as \(TΣ^{\text{chem}}\), while the remaining part is transferred to the mechanical processes and dissipated as \(TΣ^{\text{mech}}\). This transfer of free energy is composed of a standard free energy part, denoted ‘energy flow’:

\[
\dot{E} = J(μ_{H^1}^0 - μ_{H^1}^0 + μ_{H^1}^0 - μ_{H^1}^0), \tag{2}
\]

and a mutual information part\(^{38}\), denoted ‘information flow’:

\[
RTI = JRT\log \left( \frac{[H^1][H^1]}{[H^1][H^1]} \right) \tag{3}
\]

where \(R\) is the gas constant and \(J\) is the stationary clockwise (as viewed in Fig. 2) current at which the motor operates (Box 1). This current can be expressed as

\[
J = k_{+Δ}^D[1H^1] - k_{-Δ}^D[1H^1] = k_{+Δ}^D[1H^1] - k_{-Δ}^D[1H^1] = \Gamma(K_r - 1) \quad (4)
\]

where both \(\Gamma\) and \(K_r\) are positive quantities (for the derivation of equation (4) and the definition of \(\Gamma\), see Supplementary Section V-B4 and Supplementary equation (69)), the latter denoting the ratcheting constant (recently applied in the context of dissipative self-assembly\(^{17,18}\), which quantifies the kinetic asymmetry of the motor\(^{14,16,56}\) (Box 1).

Under the experimental conditions in which the motor was originally operated\(^{14}\), the rotary motor is driven purely by information flow as the macrocycle binds with equal affinity to fumaramide stations adjacent to a hydroxy group and an Fmoc group (within the detection limits of 1H NMR measurements), so there is no energy flow. However, when the standard chemical potentials of the distal and proximal co-conformers differ, energy flow arises according to equation (2). An example of this could arise if there was, say, a stabilizing interaction between the macrocycle and the Fmoc group, which would lead to an increase in energy of the macrocycle upon removal of the Fmoc-barrier close to it. As a consequence, the standard chemical potential would decrease, leading to the release of heat, each time net mechanical displacement occurs in the forward direction. This fits the definition of a ‘power stroke’\(^{45-47}\) (Box 1). Therefore, according to equation (2), the energy flow accounts for the part of the fuelling free energy that contributes to destabilizing the macrocycle during chemical transitions and which is subsequently dissipated in a power stroke.

Mutual information quantifies the correlation between the two parts of a bipartite system\(^{40,55}\), here the chemical and mechanical states. For example, when \([1H^1]\) (the concentration of species 1H) and \([1H^1]\) are larger than \([1H^1]\) and \([1H^1]\), respectively, a correlation is present between the mechanical and chemical states: when the motor's...
Fig. 2 | Rotary motor as an open and bipartite chemical reaction network. The state of the motor can be represented as a combination of two mechanical states (subscript D or H, expressing whether the macrocycle is bound to the deuterated or non-deuterated site on the track) and three chemical states (1D, 2 or 1H). The number in the chemical state shows the number of the Fmoc groups attached to the track, and the superscript H or D denotes the binding site close to the attached Fmoc group. Note that chemical states without any Fmoc group on the track are neglected as these are present in negligible concentration during machine operation due to the faster barrier formation than removal. The mechanical transitions involve displacement of the macrocycle is proximal (p) or distal (d) to the reacting hydroxy group. The rate constants of the waste-forming reaction and its reverse are denoted
chemical state is 1H, its mechanical state is more likely to be D than H. Similarly, when the motor’s chemical state is 1H, its mechanical state is more likely to be H than D. A concentration distribution with this kind of correlation has smaller (Shannon-like) entropy than one without ([1H]1 and [1H]1 equal to [1H]1 and [1H]1, respectively). Therefore, correlation between the mechanical and chemical states (mutual information) generates an entropic driving force for a directional current (from [1H]1 to [1H]1 and from [1H]1 to [1H]1). From the thermodynamic viewpoint, mutual information constitutes the entropic contribution of the free energy that comes from the fuel. As mutual information is constant in the stationary state, changes in mutual information are due to the non-negativity of $\Sigma^{\text{chem}}$. From an information thermodynamics perspective, the molecular motor operates by using chemical processes to transduce the free energy supplied by the fuel into the free energy supplied to the mechanical processes. The efficiency of this transduction is the ratio of the latter to the former and is bounded between zero and one due to the non-negativity of $\Sigma^{\text{chem}}$.

$$0 \leq \eta = \frac{RT\dot{Z} + \dot{\mathcal{E}}}{I_p(\mu_p - \mu_W)} = 1 - \frac{T\dot{\Sigma}^{\text{chem}}}{I_p(\mu_p - \mu_W)} \leq 1.$$  

The efficiency of the transduction of the free energy of fuelling to the mechanical processes ($\eta$) is not directly comparable with the efficiencies usually reported for biological motors, which are often defined with respect to the work performed by the motor against an external force or as the fraction of fuel molecules that are productively consumed, on average, over an operational cycle (the latter measure of performance is computed in Supplementary Section VI for the rotary motor). In this set-up, the energy and information flows are entirely dissipated by the shuttling of the macrocycle as $T\dot{\Sigma}^{\text{mech}}$. If the mechanical steps of the motor were...
to work against a force (for example, if a load were attached to the macrocycle), \( T \Sigma_{\text{mech}} \) would incorporate a negative work term in addition to \( RT \dot{\Sigma} + \dot{E} \), which could serve to define the efficiency of the energy and information flows being converted into output work, instead of just being dissipated. The energy and information flows would thus constitute the maximum work output that can be delivered by the motor. A traditional thermodynamic analysis of such a motor\(^{25,26,60}\) would exclusively focus on the efficiency with which the input free energy supplied by the fuel-to-waste chemical potential gradient is converted into output work, thus over-estimating the maximum work output as the overall free energy input \( I_k(\mu_f - \mu_w) \).

The present approach refines this analysis by showing how the input-to-output transduction is mediated by the free energy transfer within the motor, whose efficiency \( \eta \) (equation (5)) limits the maximum work output potentially deliverable by the motor. It also formally defines a thermodynamic efficiency that can be applied to motors while they perform no appreciable output work, as is the case for most of the synthetic molecular motors made so far, and can serve to compare the efficiencies of their operation.

The framework we have outlined can also be used to re-derive previous results obtained using kinetic arguments as a consequence of the second law of thermodynamics in bipartite systems. In Supplementary Section V-B, we show that the condition \( T \Sigma_{\text{mech}} = 0 \) implies \( K_s = 1 \), whereas the condition \( T \Sigma_{\text{mech}} > 0 \) implies \( K_s > 1 \), with forward movement when \( K_s > 1 \). This shows how the nonequilibrium thermodynamic framework, which focuses on energetic aspects quantified by the dissipation \( T \Sigma_{\text{mech}} \), is consistent with previous analysis\(^{14,15-17,44}\) focusing on kinetic aspects quantified by \( K_s \), which determines the sign of the current \( J \) according to equation (4). This reiterates the effectiveness of this information thermodynamics-based approach and, again, demonstrates the usefulness of this minimalist molecular motor as a Rosetta Stone for the translation of meaning and understanding between different frameworks for describing phenomena.

Previously\(^4\), the ratcheting constant has also been related to the ability of a dissipative self-assembly system to store free energy, but this connection is only valid in an operating regime where chemical transitions are substantially faster than mechanical ones. The information thermodynamics framework offers a general understanding of dissipative chemical systems and establishes limits to the maximum work deliverable by them that are valid in any operating regime, thanks to concepts such as the efficiency \( \eta \) introduced in equation (5).

Design principles for molecular motors. To demonstrate the use of our framework as a design tool, we explored the effects of altering the design features of the rotary motor on its current and efficiency (Supplementary Section VI). Basing the simulations on experimentally derived parameters\(^{14}\) (Supplementary Section VI-A), we established that, under the experimental conditions employed, the rotary motor is driven purely by information flow as long as the macrocycle distribution, rather than binding site affinity (within the detection limits of \( ^1H \) NMR measurements), is altered during operation. Under experimental conditions (\([\text{motor}] = 10 \text{ mM}, [\text{Fmoc-Cl}] = 30 \text{ mM}, [\text{Et,NI}] = 15 \text{ mM}, [\text{KHCNO}] = 200 \text{ mM}, \text{CH}_2\text{Cl}_2, \text{room temperature}]\)), the current was estimated to be \( 2.1 \times 10^{-8} \text{ mol dm}^{-3} \text{s}^{-1} \), requiring an average of seven fuel molecules per cycle per motor, although only 10% of the free energy provided by the fuel is used to sustain the current (efficiency, \( \eta = 10^{-8} \); equation (5)).

Varying the model parameters allows consideration of the effects of potential structural and chemical changes on the rotary motor (Supplementary Section VI-B1). Greater chemical gating for either the fuelling (Fig. 3a,b) or waste-forming reaction (Supplementary Section VI-B2) increases the current and efficiency by increasing information flow. The former has been achieved by increasing the steric bulk of the barrier-formation catalyst\(^{14,43,62}\) (Fig. 4a) and the latter by catalysis of barrier removal by a proximal macrocycle (Fig. 4b)\(^{24}\). Gating of both fuelling and waste-forming reactions (Supplementary Section VI-B3) was recently demonstrated in a rotaxane information ratchet\(^{44}\). Inverting chemical gating is predicted to reverse the direction of the motor and could be achieved if the macrocycle activates, rather than hinders, proximal barrier formation (Fig. 4c). In the absence of a kinetic preference, or when gating from the fuelling and waste-forming reactions cancel out, the motor stops working, as this precludes information flow and hence mechanical dissipation (\( \Sigma_{\text{mech}} = 0 \)). These results are consistent with kinetic models where kinetic asymmetry predicts the direction of the current\(^{15-17}\).

The relevance of power strokes in molecular machinery is contentious, as power strokes are often observed experimentally in biological molecular motors\(^{21-40}\), but, according to analysis based on kinetic asymmetry, the magnitude of the free energy released by such conformational changes does not affect the properties of chemically driven molecular machines, such as directionality and stopping force, and cannot improve the efficiency of a motor to work against an external force\(^6\). To reconcile differing viewpoints as to the importance of power strokes in molecular machines, we attempted to use our framework to understand the ways in which a power stroke can affect a molecular motor while staying kinetically and thermodynamically consistent. Power strokes can be used to induce energy flow (equation (2)) and, in principle, could be added to the rotary motor by introducing attractive interactions between the barrier and the macrocycle (Fig. 4d), stabilizing distal co-conformers \( 1_0^H \) and \( 1_1^H \) or, by adding repulsive interactions between the free barrier site and the macrocycle (Fig. 4e), destabilizing proximal co-conformers \( 1_0^H \) and \( 1_1^H \). Our simulations show that power strokes can change the magnitude of the current and efficiency of internal energy transduction (as defined in equations (4) and (5), respectively), despite kinetic asymmetry remaining unaltered (Methods and Supplementary Section VI-B4). This is because, while not altering \( K_s \), power strokes can still increase the value of \( I \) in equation (4), reflecting their ability to favour forward cycles by inducing energy flow out of equilibrium (Fig. 3c,d). However, power strokes cannot drive directional motion in the absence of kinetic asymmetry (\( RT \dot{\Sigma} \approx -\dot{E} \) when \( K_s = 1 \)), nor can they invert directionality while the kinetic asymmetry remains constant. In these simulations, altering the power strokes and kinetic asymmetry together (Supplementary Section VI-B5) gave the greatest simulated efficiency (up to ~1%), suggesting that modifying both aspects may be important for optimizing the design of synthetic molecular motors. We note that improvements that occur through power strokes arise from the induced energy flow rather than from any special role for the energetically downhill nature of the power stroke in determining the motor’s behaviour. As our analysis shows, a prerequisite for the motor’s operation is the availability of free energy to dissipate through mechanical motion. Therefore, any design feature that enhances free energy transduction from the chemical to the mechanical transitions could equally well foster improvements in performance.

If power strokes cancel out over the motor cycle, then no net energy flow is introduced: free energy gained from one mechanical transition is lost in the other (Methods and Supplementary Section VI-B6). This could be realized in molecular form by using non-degenerate binding sites, for example, changing one fumaramide site to a more weakly binding succinamide unit (Figs. 3e,f and 4f), with a typical difference in binding energy of 23 kJ mol\(^{-1}\) (pink + symbol, Fig. 3e,f, equilibrium distribution > 99:1)\(^{43}\) under experimental conditions similar to those used for rotary motor operation. Kinetic asymmetry remains unaltered in this scenario and, correspondingly, the direction of the motor could not be inverted in the simulations\(^{15-17}\). However, our analysis suggests that such a
change would be sufficient to effectively stall the motor, if operated under the original experimental conditions, despite the unchanged kinetic asymmetry. The simulations predict that, with power strokes cancelling out, any change from degenerate binding sites lowers the current and efficiency, although a smaller difference would leave the motor functional, albeit less effective. A 3:1 bias (orange × symbol, Fig. 3e,f) is predicted to reduce the current by ~20%, rendering a design with non-degenerate binding sites plausible but less effective than a motor with binding sites of equal affinities.

In all of the cases considered, the highest efficiencies are predicted for when the rates of all of the forward processes are approximately equal, leading to a cycle with no single rate-limiting step. Rate-limiting mechanical steps promote futile cycles, in which fuel is consumed without taking a forward step, as the unfavourable fueling reaction is kinetically favoured over shuttling, decreasing both the current and efficiency. Rate-limiting chemical reactions result in lower thermodynamic efficiency but do not reduce the current or substantially change the fuel consumption per cycle. This is because relatively fast shuttling hinders the generation of a concentration bias (relative to mechanical equilibrium), which decreases the information flow in the steady state. The strong dependence of efficiency on shuttling rate indicates that, like macroscopic engines, the efficiency of a motor will be dependent on the load against which it is working. As a consequence, instead of discussing a generic efficiency of a molecular motor, it is more proper to discuss the efficiency to work in a specific range of force/attached load. To use molecular machines most efficiently, they must either be tailored to the job they perform—such as using diesel engines for heavy loads—or they must use the equivalent of gears for macroscopic engines, to ensure they are working under optimal conditions.

Conclusions

Information thermodynamics-based analysis of a minimalist autonomous chemically driven molecular motor shows how information and energy flow, the two components of free energy transfer from chemical to mechanical transitions, enable the generation of directional motion from free energy supplied by a chemical fuel. The experimental rotary motor is a pure ‘chemical Maxwell’s demon’, as information flow is the sole driving force. However, energy flow could potentially be introduced using power strokes, one of several design variations explored using our model. The predicted effect of energy flow is in line with observations made in biological motors and contributes to the ongoing debate regarding the role of power strokes in molecular motors. Information thermodynamics confirms that, in line with kinetic analysis, power strokes do not affect some key properties of chemically driven molecular machines, such as directionality. However, the magnitude of power strokes is able to affect the magnitude of the current (how fast the motor components rotate), the efficiency in terms of how free energy is dissipated, and the number of fuel molecules consumed per cycle. However,
these results should not be misinterpreted as supporting a special importance of power strokes compared to other processes in the chemomechanical cycle. The information thermodynamics framework used in this article should be generally applicable to other types of synthetic molecular machine, such as non-autonomous and light-driven motors, providing a quantitative basis through which to compare molecular machine designs. Additionally, it could, in principle, be extended to other types of (supra)molecular system (such as dissipative self-assembly) powered by chemical engines. We have uncovered substantial roles for ‘energy flow’ and ‘information flow’ in the mechanism of the transduction of free energy from chemical reactions by molecular machinery, although the exact nature of the connection of energy and information flow to energy and information ratcheting remains to be clarified. The minimalist autonomous chemically driven molecular motor acts as a Rosetta Stone for relating energy, information, kinetics and molecular structure by aiding the translation of concepts and relationships between the ‘languages’ (that is, frameworks) of chemical kinetics, thermodynamics and chemical reactions.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41557-022-00899-z.

Fig. 4 | Potential ways of achieving different molecular motor design modifications. a, Increasing the steric bulk of the catalyst could be used to increase the fuelling chemical gating by slowing the undesired proximal reaction. b, Addition of a catalyst for the waste-forming reaction to the macrocycle could increase waste-formation chemical gating by increasing the rate of the proximal waste-forming reaction. c, Addition of a catalyst to the macrocycle that accelerates rather than hinders proximal barrier formation could negate chemical gating arising from steric hindrance, leading to an inversion of the directionality of the motor. d, Allowing the formation of a complex that binds the barrier and the macrocycle could allow the introduction of a power stroke by stabilizing the co-conformer in which the macrocycle is adjacent to the barrier. e, Conversely, a power stroke might be introduced by destabilizing the co-conformer with the macrocycle adjacent to the site with no barrier, for example, by the introduction of coulombic repulsion. f, Swapping one fumaramide binding site for a succinamide should, in principle, be sufficient to stall the rotary motor under the previous experimental operating conditions.

Received: 4 July 2021; Accepted: 28 January 2022; Published online: 17 March 2022

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Methods

Wegscheider's conditions. To ensure that the system reaches thermodynamic equilibrium (detailed balance), when there is no chemical potential gradient between fuel and waste species ($\mu_\text{f} = \mu_\text{w}$), the rate constants must satisfy Wegscheider's conditions. These are equivalent to the constraints on the rate constants imposed in previous kinetic analyses and dictate that the product of the forward rate constants along each independent cyclic pathway of reactions in the network, with neither net consumption nor net production of fuel or waste species, must equal the product of the corresponding backward rate constants:

$$k^a_{\text{f}+} k^d_{\text{f}+} k^e_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+} = k^a_{\text{f}+} k^d_{\text{f}+} k^e_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}$$

(6)

$$k^a_{\text{f}+} k^d_{\text{f}+} k^e_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+} = k^a_{\text{f}+} k^d_{\text{f}+} k^e_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}$$

(7)

See Supplementary Section III for the derivation. These conditions were always imposed in numerical simulations to guarantee thermodynamic consistency.

Local detailed balance. In Fig. 3, variations in the power stroke magnitude have been related to changes in the ratio of shutting rate constants by virtue of the so-called 'principle of local detailed balance' (see below), which relates the log-ratio of forward and backward rate constants of a single chemical reaction to the difference in standard chemical potentials between its reagents and products (Supplementary equation (33)). For example, it implies the relation

$$\frac{k_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}}{k^a_{\text{f}+} k^d_{\text{f}+} k^e_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}} = \frac{\mu_\text{f}^0 - \mu_\text{w}^0 + \mu_\text{f}^0 - \mu_\text{w}^0}{\mu_\text{f}^0 - \mu_\text{w}^0}$$

(8)

which was employed in the numerical simulations.

In addition, Wegscheider's conditions (equations (6) and (7)) imply that a variation in the power stroke magnitude must always be compensated by a variation in the fueling and waste-forming reactions, with the following constraint (equation (9)) must always hold for thermodynamic consistency:

$$\frac{k_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}}{k^a_{\text{f}+} k^d_{\text{f}+} k^e_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}} = \frac{k_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}}{k^a_{\text{f}+} k^d_{\text{f}+} k^e_{\text{f}+} k^d_{\text{f}+} k^a_{\text{w}+} k^d_{\text{w}+}}$$

(9)

When energy flow-inducing power strokes were introduced in Fig. 3c,d, the constraint in equation (9) was imposed by changing rate constants $k^a_{\text{f}+}$ and $k^d_{\text{f}+}$ according to variations in the shuttling rate constants. By doing so, kinetic asymmetry ($K_\text{a}$) is not altered during the simulation, but the magnitude of the current in equation (1) can still change by virtue of alterations in the value of the positive factor $I'$ (for its mathematical expression, see Supplementary equation (69)). Instead, in Supplementary Fig. 5, rate constants $k^a_{\text{f}+}$ and $k^d_{\text{f}+}$ were changed to vary the energy flow and $K_\text{a}$ together. Experimentally, this could correspond to introducing an interaction between the macrocycle and the Fmoc group that affects (Supplementary Fig. 5) or not (Fig. 3c,d) the transition state of the proximal fueling reaction, without affecting the transition state of the proximal waste-forming reaction. Note that, when binding affinities are modified as in Fig. 3e,f, the left-hand side of equation (9) stays constant and the constraint is automatically satisfied.

We end by noting that the terminology 'local detailed balance' comes from statistical physics, where it has become the central concept to formulate thermodynamically consistent dynamics. Its chemical counterpart (Supplementary equation (33)) is fully equivalent to the usual conditions imposed on the rate constants to ensure that microscopic reversibility holds (see, for example, equation (5) in ref. [16]). We note that use of the term 'local detailed balance' in this context is considered contentious by some. For a more detailed discussion see Supplementary Section III.

Data availability

All data needed to reproduce the numerical results are reported in the Supplementary Information.

Code availability

The code that generated the plots is available at the following link: gitlab.com/emanuele.penocchio/infothermotnot

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Acknowledgements

We acknowledge support from the European Research Council (ERC Consolidator grant no. 681456 to M.E. and funding to E.P.; ERC Advanced grant no. 786630 to D.A.L.), the FQXi Foundation, project 'Information as a fuel in colloids and superconducting quantum circuits' (grant no. FQXi-IAF19-05 to M.E.), the Engineering and Physical Sciences Research Council (EPSRC; grant no. EP/P027067/1 to D.A.L.), the Deutsche Forschungsgemeinschaft (a postdoctoral fellowship to E.K.) and the University of Manchester and EPSRC for PhD studentships to S.A. and B.M.W. D.A.L. is a Royal Society Research Professor. We thank R. D. Astumian for valuable discussions regarding the science in this study as well as robust debate regarding the use of the term 'local detailed balance' within the stochastic thermodynamics community.

Author contributions

S.A., B.M.W. and E.K. proposed the collaboration. E.P. developed the theoretical model. S.A., B.M.W., E.P. and E.K. carried out the theoretical analysis and simulations. D.A.L. and M.E. directed the research. All authors contributed to the analysis of the results and the writing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41557-022-00899-z.

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Peer review information Nature Chemistry thanks the anonymous reviewers for their contribution to the peer review of this work.

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