



Escapement mechanisms and the conversion of disequilibria; *the engines of creation*



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ABSTRACT

Virtually every interesting natural phenomenon, not least life itself, entails physical systems being forced to flow thermodynamically up-hill, away from equilibrium rather than towards it. This requires the action of a mechanism, acting as an “engine”, which lashes the up-hill process to a more powerful one proceeding in its spontaneous, down-hill direction; in this way converting one disequilibrium into another. All organized and dynamic elements of creation, from the galactic to the atomic, can be viewed as powered by, or being the result of, engines of disequilibria conversion; each a link in a great hierarchical cascade of conversions. There is, however, widespread misunderstanding about how disequilibria conversions happen – and indeed about what physically causes them to happen – especially regarding the role of energy and of the physical meaning of free energy. We attempt here to describe and justify what we assert is the correct alternative view of how phenomena are powered in nature, focusing especially on the molecular-level conversion processes (often called “energy conserving”) that power life and that must, then acting in an entirely abiotic context, have driven it first into being.

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“...a nano-Sisyphus would not have to push the boulder, and the boulder would move itself, driven by the molecular storm. Does this mean Sisyphus does not need energy to move the boulder? No! Sisyphus still needs to move up the slope, step by step (to block downward movements, thereby kinetically trapping upward ones after they occur by chance). These steps require energy”.

[Peter Hoffmann, “Life’s Ratchets; How Molecular Machines Extract Order from Chaos”[1, p. 157]; note: “energy” in this quote is used by the author as a shorthand for “free energy”]

1. Preface

Living systems inherently depend on a host of endergonic, thermodynamically ‘up-hill’, reactions each of which must therefore be forced, or driven, by being coupled to a thermodynamically larger down-hill (exergonic) reaction [2]. Such coupling processes effect a conversion of thermodynamic disequilibria, creating one by dissipating another. They require the mediation of a particular type of “mechano-molecular” device: which does not act merely as an enzyme, whose action inherently “transcends” chemistry [3], and which does not function by the transfer or “consumption” of energy. The main purpose of the present work is to explain and justify these assertions and to clarify the universal thermodynamic and mechanistic principles involved in the devices that power life by carrying out such conversions.

However, our primary motivation is to lay fresh foundations for an experimental assault on the question of how metabolism first emerged, one grounded in the submarine alkaline vent (“AHV”) theory for the emergence of life [4–6]. A central tenet of that theory is that a core subset of life’s endergonic processes, necessarily driven initially by geophysically-supplied disequilibria and mediated by abiotic conversion devices, were the prerequisite requirement for kickstarting the evolution of metabolism and thus life’s emergence. Therefore, understanding in fundamental terms the mechano-molecular essentials involved in driving endergonic processes, whether biotically or abiotically, is arguably of value in guiding the ‘experimental assault’. The questions that arise in this connection are then: what were the necessary founding endergonic processes, what naturally-supplied exergonic processes functioned as their drivers, what were the abiotic devices that mediated the conversion couplings between them, and how did these devices operate. We return to this issue in the paper’s final section.

The main points advanced here regarding how endergonic reactions are driven, and the role of energy in the process, reflect an intellectual history that began not later than Boltzmann writing in the 1880s and, for our purposes, largely culminating in the 1970s with the development of the linear non-equilibrium thermodynamic (“LNET”) treatment of “free energy conversion” (or “transduction”). This theoretical approach had been initiated in the 1930s by Lars Onsager [7,8] but acquired its classically mature form through the work in the 1960s and thereafter of Ora Kedem, Terrell Hill, S. Roy Caplan, William Jencks, and others [9–12].

However, fairly recent progress on two major fronts has both clarified and significantly advanced this earlier theoretical work while also reifying its insights quite dramatically. First, the immense advances of the last few decades in the genetic, structural and single-molecule dynamic analysis of proteins and protein complexes has made it possible to understand, often in atomic detail, the operational workings of a substantial and rapidly growing number of the macromolecular complexes that mediate biological disequilibria conversion.¹ Second, these conversion processes involve dynamic systems operating far-from-equilibrium and at high flux rates, and, in the case of biological conversions, in an inherently stochastic (small particle number), fluctuation-driven manner. As a result their comprehension has also begun to benefit significantly from fundamental and relatively recent advances in the statistical physics of thermodynamic processes. These have produced rigorous formulations which countenance a wide range of far from equilibrium, fully dynamic, and fully stochastic processes (see, e.g., Refs. [13–28]).² In later sections we make a brief introductory foray into some basic aspect of this theory and its application to disequilibria converting processes.

To set the conceptual stage for our discussion of how endergonic processes are driven, particularly in the biological context, we note that in general chemical reactions necessarily entail both forward (exergonic) and backward (endergonic) reaction events with the ratio of the two rates given by $k_f/k_r = \exp(-\Delta G/k_B T)$ where ΔG is the “Gibbs free energy of reaction” (see discussion in Section 3.3). Thus in the context of molecular-level conversions processes the central question is this: *How can one process, whose forward reaction events are (by definition) statistically predominating over its backward ones, act to force the backward events of another process to predominate?* We expend considerable effort on justifying the claim that this is the core conceptual issue in disequilibria (free energy) conversions, in biological conversions in particular, and in answering the question posed. And we return to this particular question explicitly in Section 3.3.3.

2. Disequilibria enlivened: life’s third rail

Our discussion focuses primarily on the mechanisms that power the activities of living systems, i.e. on “bioenergetics”, though most of the principles that emerge apply to all systems that convert disequilibria—which function, that is, as engines [30]. And we begin with a review of life’s “third rail”, the ATP disequilibrium that essentially all cells generate, and then immediately consume, to power the bulk of the many processes of life that must be driven away from equilibrium. And a hot third rail it is.

¹ A representative selection of such complexes, shown in animated action, is to be seen in Drew Berry’s “Animations of unseeable biology” (<http://www.deliberation.info/drew-berry-animations-of-unseeable-biology/>); at least implicitly emphasizing the point that life’s driven processes cannot be reduced to mass action, quasi-equilibrium chemistry.

² For the discussion in this paper particular attention is drawn to: Phil Attard’s book “Non-equilibrium thermodynamics and statistical mechanics: foundations and applications” [25]; the two cited papers by Udo Seifert: “Stochastic thermodynamics of single enzymes and molecular motors” [22], and “Stochastic thermodynamics, fluctuation theorems, and molecular machines” [23]; and to “The mechanochemistry of molecular motors” by David Keller and Carlos Bustamante [29].

The essential point is that ATP is not simply synthesized (e.g. by ATPsynthase) and then consumed as a chemical reagent. It is instead, and as a categorical necessity must be, driven out of equilibrium with respect to its hydrolysis products ADP and Pi (orthophosphate); were that not so it would be useless to the cell as a carrier of free energy no matter what its concentration, since it is the disequilibrium itself, not the molecule ATP, that “carries” the free energy. In actual fact it is driven to a quite astronomical disequilibrium. The Gibb’s free energy³ of the ATP disequilibrium maintained in cells typically stands at between 20 and 24 fold above $k_B T$ [32] meaning simply that the ratio of the concentration of ATP to that of its hydrolysis products: $[ATP]/[ADP][Pi]$, is of the order of 10^9 – 10^{10} higher than it would be at equilibrium. That is (assuming unit activities) (see e.g. Stucki, 1980 [33]),

$$\begin{aligned} \frac{-\Delta G}{k_B T} &= \ln \left[\frac{[ATP]/[ADP][Pi]}{[ATP]_{eq}/[ADP]_{eq}[Pi]_{eq}} \right] \\ &= 20 - 24 \quad \text{implying that :} \\ \frac{[ATP]/[ADP][Pi]}{[ATP]_{eq}/[ADP]_{eq}[Pi]_{eq}} &= e^{20} - e^{24}, \quad \approx 5 \times 10^8 - 3 \times 10^{10}. \end{aligned} \quad (1)$$

These magnitudes and their derivation suggest several general conclusions, all of which we later make some attempt to justify:

1. In its physical content “free energy” is a somewhat misleadingly named measure of the extent of a disequilibrium (equivalently, and arguably more revealingly, a (log) measure of the ratio of a reaction’s forward to backward rates (in Section 12.2 we discuss this point, and seeming exceptions to it).⁴
2. It is not the bond energy of the terminal phosphoanhydride bond of ATP whose liberation upon hydrolysis is what powers (by supplying energy to it, it is commonly, and incorrectly, supposed) the thermodynamically “up-hill” (endergonic) reactions to which the hydrolysis is coupled (much more on this point later); that bond energy is of course liberated whether the hydrolysis occurs at equilibrium or not. But more importantly, as we will see, the liberated bond energy is itself in no sense transferred to the driven reaction. Further, in all adequately explicated cases, (1) the free energy available in the hydrolysis of ATP is realized only after, and is contingent upon, the *completion* of the driven reaction, and (2) is necessarily dissipated, not ‘conserved’, since it is the dissipation itself that ‘drives’ the conversion.
3. The magnitude of the ATP disequilibrium, and the incredible biochemical machinery and metabolic effort involved in its maintenance, is compelling testimony that life hangs by the thread of, and is in general “rate limited” by, an extreme state of dynamic disequilibrium – one which it perforce must strive quite ‘desperately’ to sustain; it is the disequilibrium itself, particularly its magnitude, that is essential – and that powers life.
4. Specificity is critical. Chemically non-specific states of disequilibria are not simply useless to life, they are implacably incompatible with it. Living systems interconvert extremely specific pairs of disequilibria, and are at immense pains to achieve that specificity. Life never employs, nor could it, non-specific means (e.g. temperature or hydration excursions) of generating the disequilibria it requires.
5. The disequilibria economies of life are inherently dynamic. That we turn over our ATP inventory in the order of a minute, and consume nearly our body’s weight of ATP per day, speaks forcefully to the point that it is not the static state of disequilibria that is the essential point, but the dynamics of its generation and dissipation—and that the race is to the swiftest.

However, implicit in life’s obligate deployment of disequilibria to power its activities lies a linked pair of deeper truths that are the main point of this piece. The first is that, contrary to general belief, it is never the “use” or “consumption” of energy that powers life, nor indeed any other powered activity in nature; it is, and in principle can only be, the dissipation of a disequilibrium. The second reflects the almost paradoxically opposed fact that virtually every relevant instance of something happening in nature, let alone in life, involves the creation of a disequilibrium, not just the dissipation of one; a feat, which if it happened by itself, would violate the inviolable 2nd law. The upshot is that the entire drama of the dynamic universe, life quintessentially, lies in activities that couple thermodynamically opposed processes in order to create one disequilibrium at the expense of dissipating another. How does this come about?

The short answer to the question of how disequilibria are created, as we have just indicated, is that (recasting, a bit, the 2nd law) “it takes one to make one”; one process creating a disequilibrium must be “coupled” to another that is dissipating a different disequilibrium with the rate of the creation being necessarily less than that of the dissipation (a good deal less, if the job is to be done with dispatch [23,35]). But as we will see, the long answer is much more interesting, fairly counterintuitive, and more than a little at odds with widely prevailing views of the matter, not least within the professions and text book renderings of biology and biochemistry.

³ We note that the still very common practice of using the qualifier “free” in “free energy” was nominally banned as of the 1988 IUPAC conference, to be replaced simply by “Gibbs energy” or “Helmholtz energy” [31]. However, this nomenclature change does not address the issue raised here about these quantities not being, in physical fact, “energies”.

⁴ Henceforth we use the term “free energy” with the understanding that it is, in physical meaning, a measure of the magnitude of a disequilibrium; that in general $\Delta G/k_B T = -\ln(W_{after}/W_{before})$ where W_{after}/W_{before} is the multiplicative factor by which the number of microstates to which the systems has access would change due to a process whose free energy change is ΔG [34].

The short answer is sufficient, however, to support one key point. As life's barn-burning ATP disequilibrium suggests must be the case, in their molecular workings living systems are almost inconceivably complex, one might fairly say 'frantic', circuses of dynamic, interwoven activities of disequilibria generation and dissipation: a myriad of high wire acts, plate jugglers, labors of assembly and disassembly etc., all of which are, furthermore, held up and enabled by extremely elaborate, tightly organized, dynamic, meta-stable structures that are themselves states of very high disequilibria. Yet, as we already noted, every out-of-equilibrium component must be (or have been) driven into that condition by being specifically coupled to the dissipation of greater – ultimately externally supplied – disequilibria. Seen in this light, the extreme to which life drives its main internal "power bus" disequilibrium, is less surprising.

It seems clear, moreover, that it could not be otherwise. It is not just that the activities of life are observed to be everywhere dependent on generating and maintaining highly specific, dynamic, far-from-equilibrium states, but rather that no configuration of matter languishing in the margins of quasi-equilibrium could conceivably display any of the essential properties and activities of life. In consequence, of which much more later, their comprehension requires an explicitly non-equilibrium thermodynamic ("NET") treatment. Relatedly, since mass action chemical reactions, no matter how catalyzed, can only dissipate disequilibria- and cannot by themselves generate them, a fundamentally different mechanism is required, one which technically functions as an engine, and this brings us to the long answer which we discuss in general terms in the next section.⁵

3. How disequilibria are created

3.1. The general requirements for disequilibria conversion

If one process, by flowing in its spontaneous downhill direction, is to force a second and potentially unrelated process to flow uphill against its thermodynamic will, the device mediating their coupling must link together the two processes such that they are:

1. thermodynamically unified but acting in opposition: as has already been suggested, the processes must be mechanistically linked in such a manner that they: (a) function as a single thermodynamic process which must be in the net exergonic, and (b) operate in thermodynamic opposition; i.e. if one is acting to dissipate a disequilibrium (i.e. is exergonic and is thereby the driving process) the other must simultaneously act to create one (is endergonic = entropy reducing, and is thereby the driven process) [10,11]. What this means in general mechanistic terms brings us to the next requirement:
2. coupled via an escapement: the mechanism coupling the processes together must make the progress of the driving process conditional on, in fact gated by, the progress of the driven process. That is, in ideal 'lossless' operation, an instance of the driving process must be prevented from completion unless a correspondingly-sized instance of the driven one does so 'coincidentally' (i.e. in the same instance of the conversion process). The coupling mechanism must, therefore, act as an *escapement*.⁶ And although the aptness of this term is less clear in couplings involving macroscopic processes operating in a continuous flow manner, such as turbines, tornadoes, ram jets, etc.⁷ in other contexts, and in the molecular-level conversions of biology in particular, the escapement action is manifest and is the essential operating principle of the engine. In particular, it dictates that the physical system mediating the conversion operates in a "tick-tock", step-wise, fundamentally cyclic, manner, by means of which it quantitatively parses the fluxes of the two linked processes into single-step amounts and enforces the driven-over-driving conditionality requirement. This is the fundamental design requirement of all engines and generators, man-made ones included.

How these requirements are met mechanistically, particularly at the molecular level, is the central question we need to address, and the turning issue of this piece. The essential general idea is that the escapement puts the driving process under

⁵ As is conventional in NET, we will consider processes taking place within a bounded open system and the effect those processes have on that system's entropy; in that context an endergonic (resp. exergonic) process, by definition, decreases (resp. increases) the entropy of the system, and, by the same amount, the entropy of the universe, since the latter is not changed by flows of either energy or matter across the system's boundary [10,36]. Relatedly, a system is in a state of equilibrium if its entropy is maximal (cannot be increased by a process respecting all governing conservation laws), and in a state of disequilibrium otherwise. Finally, the "work" output of a conversion system as here treated is the amount of entropy reduction produced in the driven flux and therefore its "power" output is the time rate of that entropy reduction (see, e.g., [23,33,37]; we argue briefly below that these same entropy-denoted definitions of work and power are applicable to disequilibria conversions in general, including those "classical" ones, in which either the input or the output processes involve mechanical motion).

⁶ A number of authors have previously invoked the metaphor of the escapement mechanism in connection with their studies of specific converting systems (e.g.: [38–42, p.92]). We are here arguing, however, that Brownian ratchet escapement mechanisms form the enabling heart of all molecular-level disequilibrium converting systems.

⁷ In this connection we note the existence of many spontaneous disequilibria conversion phenomena that in a sense self-organize their own conversion mechanisms. These include conversions between thermal and concentration disequilibria (the Soret and Dufour effects) [25, §4], thermal and electrical disequilibria (the Peltier and Seebeck effects) [25], coupled diffusive flows [36, §16.3] and 'reaction-diffusion' networks [36, §19.4]. These direct physical-chemical conversion processes stand apart, qualitatively and mechanistically, from 'engine-mediated' conversions such as those that power 'bioenergetics' and which primarily concern us here. On the other hand conversions of this type may well have been critical in enabling the emergence of life as discussed in Section 14.

the control of the driven one according to a ‘neither of us gets to finish in a given cycle unless we both do’ requirement. In all cases of which we are aware wherein the inference can be drawn, this requirement is met in the following somewhat counterintuitive manner: (1) the driven process fully completes before the driving one is permitted to do so, (2) the state in which the driven process is fully complete is what triggers, or “gates”, the completion of the driving process, and (3) the state in which both processes are fully complete functions as the necessary pre-condition ‘trigger’ for the engine to return to its starting configuration. In such engine designs, the rule is, therefore, ‘pay upon completion’, whereby a cycle’s worth of disequilibrium creation must be completed before a cycle’s worth of the driving disequilibrium is allowed to be dissipated.

We pause here to note by way of illustration a particularly compelling example, that of the nitrogenase enzyme. In each of its conversion cycles this engine reduces one molecule of molecular nitrogen N_2 to two molecules of ammonia NH_3 ; an eight-electron reduction. It performs this by sequentially executing eight subcycles each one of which performs a one-electron reduction. Impressively, each of these single-electron subcycles is driven by the quasi-coincident hydrolysis of *two* molecules of ATP (providing an ‘irreversibility bias’ of $\approx 10^{-20}$). And, tellingly for our current concerns, these paired hydrolyses occur *after* the individual electron reductions and are triggered by them, a point only recently established. The relevant paper, by Duval et al. [43], has the title “*Electron transfer precedes the ATP hydrolysis during nitrogenase catalysis*” where the term “precedes”, carries the paper’s primary, highly counter-intuitive, and highly contentious, burden. Tellingly also, the finding leaves the authors compelled to wonder where the “energy”, which, they believe, must be needed to force the electrons on to the nitrogens, could be coming from. A quest which, the present paper is largely devoted to establishing, is inherently misdirected.

However, for molecular-scale processes at least, ‘pay before completion’ designs are also possible in principle provided that cycle re-initiation is still made to be conditional on the joint completion of both driver and driven processes. In Appendix C we discuss this seemingly more reasonable design possibility, and in Section 7.3 offer some considerations that may explain why life seems to uniformly employ pay-upon-completion designs.

3.2. The special case of molecular-scale disequilibria conversion

Importantly, the above general operational requirements are differently realized depending on whether the engine is operating in the macroscopic or molecular realms. In the former case, such as convection cells, man-made heat engines, etc. the flux increments that make up individual engine cycles comprise immense numbers of particles and can be treated as continuously varying quantities obeying macroscopic laws; further, as we have noted, the cyclic nature of the process can be rendered in unapparent, continuous flow manners (as in, e.g. tornadoes or turbines).

In the molecular-scale case, which includes all engines of relevance to biology, three related and profoundly important new circumstances arise. First because the engines themselves are individual macromolecular complexes (protein or protein–RNA complexes) operating in an aqueous environment, viscous forces (kinetic “damping”) completely overwhelm inertial ones (the extreme low Reynolds number limit); to the extent that there are no significant inertial effects and molecules never ‘coast’. In consequence they are everywhere in mechanical equilibrium [24,44]; although this equilibrium often entails, as an essential enabling strategy, long-range, visco-elastic conformational changes in the complexes involved, as we will explore later [45–48]. Second, the systems are inherently buffeted by unimaginably violent Brownian impacts [32,44,49] which would inescapably defeat any conventional macroscopic-style engine design. Finally, the flux increments involved in a single engine cycle are individual molecules or very small collections thereof, giving rise to relatively large stochastic fluctuations in the engine’s operation [23,50].

In this world, in fact, fluctuations are everything, both the thermal fluctuations that define the interaction between the bath and the molecules involved in the conversion, and the statistical fluctuations that arise in the necessarily stochastic operation of the molecular engines themselves. And the central conceptual insight here relates to the essential causal role played, somewhat paradoxically, by fluctuations that ‘violate’, i.e. run counter to, the 2nd law. We address this point next.

3.3. The essential role of fluctuations, especially those that run counter to the 2nd law

The 2nd law of thermodynamics is purely statistical, proscribing only the average course of events while in no way prohibiting individual particle-level transitions that run contrary to that average [23]. Furthermore, in molecular-scale systems, individual ‘violating’ transitions (perhaps better called “contra transitions”; since their occurrence is not in fact a ‘violation’) are not just unavoidable in principle and directly observed experimentally, they are at bottom what makes ‘chemistry’ – and therefore life – happen.

And in particular, as we will explore presently, contra transitions are the *sine qua non* of disequilibria conversion.

However, to clarify the role of fluctuations in disequilibria conversions we first consider a better known and simpler case, namely the ‘transition state’ theory of chemical reactions.

3.3.1. The role of counter-entropic fluctuations in chemical reactions

van’t Hoff, writing in the late 1800s [51], is generally credited with being the first to argue in detail that a chemical reaction, e.g. $A \rightarrow B$, is necessarily accompanied by its reverse $A \leftarrow B$, with the net forward reaction flux being just the difference between the forward and reverse fluxes. Clearly however, if the forward reaction is spontaneous, i.e. exergonic,

then the individual ‘back reaction’ events ‘violate’ the 2nd law. What makes these happen? The issue becomes a bit clearer viewed in the light of transition state reaction theory.

This theory was first introduced by S. Arrhenius in 1889 to explain the otherwise inexplicably large effect increasing temperature has on most reaction rates. In the 1930s the theory was put on more rigorous quantum and statistical mechanical foundation (see, e.g. the discussion in [36, Sec. 9.2]). In general terms this theory posits that in chemical reactions there is interposed between its initial and final states a thermodynamic obstacle in the form of a high-free energy “transition state” through which both forward and backward reaction events are obliged to pass (see e.g. [52, §8]). However, the transitions to that elevated state, in either direction, necessarily entail positive free energy changes, $\Delta G > 0$. So in both forward and reverse directions, the reaction participants must first execute a transient 2nd-law ‘violating’ transition by moving to a (macro) state that is less probable than is that of either the starting or ending states of the reaction. It is well understood, however, that these contra ‘boost’ transitions are generated by inevitable, if rare, thermal (“Brownian”) fluctuations occurring in the molecular system as a result of its interaction with its heat bath. The question then is what determines the likelihood that such a boost fluctuation will occur, how that depends on the magnitude of the free energy changes involved, and what in the end determines the ratio of forward to backward events?

Following in spirit the classical approach to this problem (see e.g. [52, §19]) we consider the simple three (macro) state scheme:

$$n_r \rightleftharpoons n^\ddagger \rightleftharpoons n_p \quad (2)$$

which we imagine is being carried out by an enzyme and where the three states are: reaction reagents bound to the enzyme n_r , intermediate ‘transition’ state n^\ddagger , and products bound to the enzyme, n_p .

A core assumption of the Arrhenius theory is that the transitions to the transition state can be considered an equilibrium reaction (and thus subject to the principle of “global detailed balance”; see [22] and Appendix A) and that the rate for the ‘forward’, reactants-to-products reaction, for example, can be expressed as the concentration of the transition state complexes times a decay rate, which rate depends only on the properties of the transition state, for the step from transition state to product state. This has the implication that the equilibrium constant for the transition state is related to the net forward and reverse rates by $K = k_f/k_r$. Arrhenius in addition proposed that the forward and reverse rates obey van’t Hoff relationships with the consequence that they are each proportional to the exponent of a (dimensionless) “activation energy”: $k_{f,r} \propto \exp(-E_{f,r}/k_B T)$.

It is now understood that it is more correct to interpret these activation energies in free energy terms. That is, assigning free energies to each of the three macrostates: $G_{\text{reactants}}$, G^\ddagger , and G_{products} where $G^\ddagger > G_{\text{reactants}} > G_{\text{products}}$, the activation energies are $E_f = G^\ddagger - G_{\text{reactants}} \equiv \Delta G_f$ and $E_r = G^\ddagger - G_{\text{products}} \equiv \Delta G_r$ and $E_r > E_f$. We therefore have that

$$\begin{aligned} \frac{k_f}{k_r} &= e^{-(\Delta G_f - \Delta G_r)/k_B T} = e^{-(G_{\text{products}} - G_{\text{reactants}})/k_B T}, \\ &= e^{-\Delta G_{\text{reaction}}/k_B T}. \end{aligned} \quad (3)$$

However, it is still widely held that the changes needed to elevate either the reactants or the products to the transition state, involve increasing the state’s “energy”. Therefore, given that the Brownian fluctuations induced by the bath are Boltzmann distributed, wherein higher energy fluctuations are exponentially less probable, it has intuitive appeal to conclude that the transitions to the transition state are more likely coming from the reactant state than from the product state, for the reason that less energy is required.

As we review in Appendix A, however, the transitions from the terminal states to the transition state are not, fundamentally, changes in the energy of the state, but in the state’s probability. And the reason closeness in probability implies higher transition probabilities between states is not directly obvious and rests on the statistical physics of fluctuation transitions occurring between macrostates (or “mesostates”, when a single macro-molecule is being considered), as explicated by “stochastic thermodynamics”. We therefore briefly note here the principle concepts in stochastic thermodynamics that are relevant to ‘transition state’ theory, and refer to the discussion in Appendix A and in [22,23].

The main points to note here are the following. First, the observable states of the chemical reaction, here taken to be three, reactants, transition, and products, each represent true thermodynamic macrostates (i.e. each comprising a large number of ‘unobservable’ microstates which are at thermal equilibrium with respect to the heat bath in which the molecules are suspended). This confers on each macrostates a well-defined Boltzmann distribution function for the macrostate’s microstates, and from that well-defined thermodynamic properties of entropy, energy (expectation value), and Helmholtz free energy F (defined as minus $k_B T$ times the log of the partition function for the microstate distribution).

Second, the ratio of the ‘conditional probability rates’ (the transition probability per unit time conditional on knowing the starting state) of an allowed (fluctuation) transition between macrostates, satisfies a “local detailed balance” relation

$$\frac{w_{\rho}^{+}}{w_{\rho}^{-}} = e^{-\beta \Delta F_{\rho}} \quad (4)$$

([22, Sec. 5.5] and see Appendix A), where $\beta = 1/k_B T$, ρ labels the particular transition being considered, the $+/-$ superscripts indicate the rates for the forward and reverse transitions respectively, and ΔF_{ρ} is the change in the system’s

Helmholtz free energy during the macrostate transition (wherein, as required, $\Delta F_\rho < 0$ implies that forward reactions occur at a rate exponentially greater – in the dimensionless free energy change – than the rate for the reverse reaction).

Finally, since transition state theory demands that transitions between the reactant and product macrostates pass through the transition state, we must consider two-step transitions in the sequence $n_r \rightleftharpoons n^\ddagger \rightleftharpoons n_p$. However, for multi-step transitions the ratio of the net forward to reverse currents is the product of the constituent one-step forward to reverse transition rate ratios [23, Sec. 10.2]; in our present case this leads to

$$\begin{aligned} \frac{k_f}{k_r} &= \frac{w_{n_r \rightarrow n^\ddagger}}{w_{n^\ddagger \rightarrow n_r}} \times \frac{w_{n^\ddagger \rightarrow n_p}}{w_{n_p \rightarrow n^\ddagger}}, \\ &= e^{-\beta(\Delta F_{n_r \rightarrow n^\ddagger} + \Delta F_{n^\ddagger \rightarrow n_p})}, \\ &= e^{-\beta(\Delta F_{n_r \rightarrow n^\ddagger} - \Delta F_{n_p \rightarrow n^\ddagger})}, \\ &= e^{-\beta(\Delta F_{n_r \rightarrow n_p})}. \end{aligned} \quad (5)$$

And given that no pV work is done in these transitions, $\Delta F = \Delta G$, so we can express the above result in the more conventional terms we arrived at above based on the Arrhenius model as

$$\frac{k_f}{k_r} = e^{-\Delta G_{\text{reaction}}/k_B T} \quad (6)$$

where, as before, $\Delta G_{\text{reaction}} \equiv G_{\text{products}} - G_{\text{reactants}}$ and the reaction proceeds in the net forward direction ($k_f > k_r$) if $G_{\text{reactants}} > G_{\text{products}}$.

Of course, the ‘violation’ of the 2nd law involved in producing the transition state is necessarily transient and the offense immediately redressed whether or not the excited state relaxes abortively back to whence it came, or on to the alternative state. In either case, $\Delta G_{\text{relaxation}} \leq 0$ and the 2nd law is satisfied—as a statistical law constraining only the average run of events. It is further worth noting that the system in the transition state has no memory of how it got there, and so is not biased, by history, as to which way the state relaxes, whether to reactants or products.

3.3.2. Free energy redux; entropy, and a system's macrostate probability

The preceding discussion makes explicit that the free energy change (free energy “consumed”) in a reaction has no role at all in causing the transition state to be occupied and no effect in biasing which way the transition state, once formed, resolves, whether ‘forward’ to the products or ‘backward’ to the reactants. The reaction’s ΔG is, to restate, strictly and only a ‘probability bias factor’ as between forward vs. backward transitions—and is so simply because it captures how much more probable it is that the “molecular storm” will produce the fluctuation that ‘raises’ the reactants, rather than the products, to the transition configuration; wherein, we again emphasize, the ‘raising’ is not in energy, but in improbability (from a more to a less probable configuration, a change conventionally expressed in free energy language).

So although the quantity ΔG is quite often seen as not only the force that makes reactions happen but also as measuring a kind of mobile energy phlogiston, it is neither; and in no literal sense does energy flow from the reactants to the products in a chemical reaction. Nor is ΔG , in physical content, an energy difference, of any sort, however discounted in its “ability to perform work”; it is instead, as we hope to make convincing, a surrogate (one might say disguised) measure of an entropy change (or equivalently, and more revealingly, of a macrostate probability change).

The concept of macrostates and their probabilities invoked in the preceding discussion is conceptually fundamental to our analysis, and an essential element in modern ‘stochastic’ thermodynamics, but relatively unfamiliar in biochemical contexts. For that reason we provide an introductory summary of stochastic thermodynamics in [Appendix A](#).

Two points regarding chemical reactions deserve particular emphasis leading into the following discussion of disequilibria conversion. First, as is well understood, enzymes do not change the free energies, equivalently the chemical potentials, of either reactants or products—much less could they, in principle, turn an endergonic reaction into an exergonic one. Second, and to draw out an implication of a point made before, the free energy of a state is physically a measure of its relative probability and is in no valid physical sense a literal energy, even a discounted one. Much less is it an energy that can be passed to another reaction to ‘excite’ or ‘drive’ it. The point is, that in the conversion of disequilibria, the driven endergonic reaction is not made to happen by energy, free or otherwise, donated to it by its driving, exergonic partner. As we will see, that is just not how disequilibria conversion works.

We note that in light of the discussion to this point, that “free energy” is by convention named an energy, given energy units (via a gratuitous division by temperature), and widely considered to be a form of energy, must be viewed as the regrettable consequence of an “inconvenient quirk of history” (to lift a remark made by Dill and Bromberg, in a different – but closely related – context [52, p. 96]). In Section 12 we offer some thoughts about how this came about.

We are now in a position to begin the discussion about how disequilibria conversion actually works.

As we have noted, a chemical reaction’s ongoing back reactions, as individual events, run counter to the 2nd law, and not just transiently; for them, the free energy of its product is greater than that of its reactants. The 2nd law (noting again that in general $\Delta G = -T\Delta S_{\text{universe}}$ —see footnote 5 and also the discussion in [Appendix B](#)) merely asserts that in a given

process those of its transitions that increase the entropy of the universe will (simply as a matter of probability) outnumber the oppositely directed ones that decrease it, so that in the net, i.e. on average, the state of the universe becomes more probable (of higher entropy; see Appendix A).

Thus the stochastic, fluctuation-driven nature of chemical reactions reviewed above suggests that to drive a disequilibrium we need some legal way to reverse the natural statistical bias of forward vs. reverse reactions, so that now the latter predominate; hopefully dramatically so. How is this to be done?

It is here that we establish the most important connection between the ‘fluctuation’ reality of molecular processes and the question of how such processes can carry out the conversion of disequilibria. The key point is that molecular-scale disequilibria conversion relies entirely on the unavoidable existence of ‘violating’, and therefore disequilibrium-creating, ‘back reaction’ events, and works simply by trapping them, albeit by legal means, once they occur—just as in Hoffmann’s “nano-Sisyphus” metaphor with which we began.⁸ We turn to the details of this point next.

3.3.3. Disequilibria conversions via escapements; trapping fluctuations that run against the 2nd law

A disequilibria converter’s job is specifically to drive a reaction, or process, in its ‘backward’ (i.e. endergonic) direction; from, that is, a more to a less probable configuration. In principle, how might this be done in a molecular-scale, Brownian-fluctuation-driven world? How can the well understood requirement that the reaction to be driven up-hill must be ‘coupled’ to a larger process proceeding down-hill be met in a world in which both reactions are being driven by Brownian fluctuations from the bath as we have just reviewed in our discussion of reaction rate theory? That is, as we asked in the Preface, *how can one process, whose forward reaction events are predominating over its backward ones, act to force the backward events of another process to predominate?*

Clearly, and as the previous discussion implied, we need a daemon, albeit a thermodynamically righteous one. A device, that is, that can first set up conditions that makes it possible for the driven reaction to proceed according to the tenets of reaction rate theory as just discussed, wait until a Brownian impact occurs that produces an instance of that reaction’s ‘backward’ transition, necessarily decreasing the entropy of the universe, and then trap that event irreversibly (more or less), all in such a way that at the end of the conversion cycle, the universe’s entropy account has increased.

This can in fact be achieved in a variety of ways, some of which, for example, involve time-dependent, externally applied forces and asymmetrically sculptured potential landscapes [52, §29] [53,54]. But we are here interested in those in which the daemon operates with ‘chemical’ processes alone, a class that includes all biological examples of which we are aware.

In the abstract, here is how the direct conversion of chemical disequilibria is accomplished.

The daemon is necessarily embodied in a molecular device, in biology universally comprising a protein or protein–RNA complex, which acts as a dual enzyme mediating both chemical processes in parallel and jointly, but upon which processes it imposes two rules. First, the two processes under its control are (ideally) never allowed to complete unless both of them do so ‘coincidentally’ (before the complex is allowed to return to the state in which it could take up new reaction substrates). Second, the two chemical processes are directionally linked in the sense that the engine mediates a specific pairing of reaction directions for the two processes.

That is, if $R \rightleftharpoons P$ and $T \rightleftharpoons S$ represent the two reactions to be coupled, then the engine complex might permit, say, $R \rightarrow P$ and $T \leftarrow S$ to proceed as a joint reaction under the constraint imposed by the first rule. In this case, if the chemical potentials of all of the reagents are such that $R \rightarrow P$ is an entropy producing (“forward”) reaction and $T \leftarrow S$ is an entropy reducing (“back”) reaction, and if more entropy is gained in the first reaction than is lost in the second, the system will operate spontaneously and, with each instance of the driving first reaction allowed to proceed (downhill) only as it is logically gated by an instance of the second having made it uphill, generate a disequilibrium in the $T \rightleftharpoons S$ reaction at the expense of dissipating one in the $R \rightleftharpoons P$ reaction.

The device, in other words, has to operate as a molecular *escapement*, parsing out instances of the driving reaction preceding in its forward direction *conditional on* completed instances of the driven reaction moving in its backward direction. Within the escapement the completion of an instance of the back reaction functions mechanically as a trigger, or “gate” to permit the functionally coincident completion of an instance of the thermodynamically larger forward reaction. Metaphorically, the device holds the driving process hostage to the accidental, and relatively improbable, Brownian impact-induced occurrences of the driven process’s back reaction; in this way permitting the former, by being (sufficiently) irreversible, to have the effect of trapping the latter (preventing their reversal). This operational design amounts to making the behavior of the engine dependent on the joint probability that two inherently stochastic, thermodynamically opposed events occur coincidentally: one producing a more probable state, the other producing a less probable one.

Moreover, a general observation is that the control of each process by the other needed to enforce the above “escapement” rules is achieved, as William Jencks has argued [55,56], by having the mediating engine divide the two participating reactions into “half reactions” which are obligatorily interleaved, typically in a standard sequence. His discussion of this important point is specifically in reference to couplings involving one “vectorial” process. To quote: “*The coupling between the vectorial reaction, the transport of two Ca^{2+} ions into the sarcoplasmic reticulum, and the chemical reaction of ATP hydrolysis is brought*

⁸ See the illuminating discussion of this point in the context of molecular motors by George Oster and Hongun Yang: *How Protein Motors Convert Chemical Energy into Mechanical Work* [32]. Our contention here, however, is that these same principles apply to all molecular-scale disequilibria conversions.

about simply by dividing the vectorial reaction and the chemical reaction into two alternating half-reactions, **so that the chemical reaction can only occur if the vectorial reaction also occurs and the vectorial reaction can only occur if the chemical reaction also occurs**". (emphasis added), and "Movement in biological systems, such as muscle contraction and the active transport of ions, is generally brought about through a series of alternating chemical and vectorial steps that involve a series of changes in the specificity for catalysis of the chemical and vectorial reactions. These changes divide the overall reaction into segments so that neither the chemical nor the vectorial reaction will be completed unless the other is also completed", both from [56], and "The ordered mechanism divides the chemical reaction and the vectorial reaction into two parts, which are sandwiched between each other. Therefore, neither reaction can be completed without the other taking place". [12].

We emphasize, however, that subsequent studies, covering a large variety of converting system, have made evident a point that is a fundamental tenet of the present argument; namely that the general principles articulated by Jencks are in no way limited to conversions involving "vectorial" input or output processes, but characterize all molecular-level disequilibria conversions (see Section 11 and the discussion of a variety of example conversion processes given in a companion paper now in preparation [57]).

We also emphasize that, as in the case of simple chemical reactions, molecular-scale disequilibria conversions are necessarily driven by Brownian impact fluctuations, specifically fluctuations that produce entropy-reducing 'contra' transitions to less probable states. However, in conversions, the fluctuations act on two levels: first, as in the case of ordinary chemical reactions, to produce the necessary 'transition states' involved in all of the detailed molecular transitions of the engine, including, for example, substrate binding and product release (of which more later); second, and most centrally, to produce the 'violating' back reactions of the driven processes which the escapement mechanism then permits to be trapped by an instance of the driving process (of which a great deal more later). No energy is transferred between the systems so coupled, nor is any "consumed" in the operation of the conversion.

Therefore, molecular-scale disequilibria converting systems, whether motors or not, all operate as "Brownian Ratchets" [58], demonstrating not just the aptness but the universality of Hoffmann's nano-Sisyphus metaphor [1] with which we began, and likewise that of the equally beguiling metaphorical bicycle, imagined by Oster and Wang [32] which the rider never needs to pedal.

Further, to our knowledge, all sufficiently characterized biological cases conform to the operational principle noted above that the completion of the driving process is triggered by the full completion of the driven one (see a representative list of examples given in Section 11 and the discussion in Section 7.3 for why this might be the case).

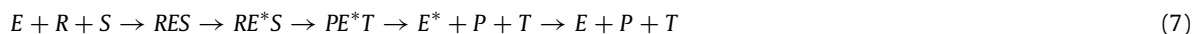
On the other hand, the above description leaves unaddressed several essential points, the lead one of which is how, in molecular-mechanistic terms, molecular escapements actually impose their 'rules'. These issues take up much of the remainder of our discussion, in preparation for which we conclude this section with a statement of a few general principles and a summary of the argument to this point.

3.4. Engineering principles underlying molecular-scale disequilibria conversion

3.4.1. Engines operate in cycles (albeit not always continuously)

The above escapement rules have the implication that the mechanism must alternate between a 'loading' phase in which it takes in the reactants for the driving reaction and, in parallel, the reactants for the driven reaction running in reverse and an 'unloading' phase in which it releases the products of the driving reaction and of the driven back reaction. As a precondition to the unloading step the reactions involved must have been allowed, generally with catalytic assist, to complete. Thus, if a conversion is ongoing the device mediating it must step through a directed cycle as we now describe.

Consider again the simple example discussed above in which the reactions $R \rightleftharpoons P$ and $T \rightleftharpoons S$ are the two processes to be coupled with the former operating in its forward direction $R \rightarrow P$ to drive the latter in its backward direction $T \leftarrow S$. Further, following convention (see e.g. Hill [11]), let E represent the engine complex in the state where it can take in, and/or "bind" both R and S , and E^* to be the form of the complex in which it can release, respectively P and T . Then the engine's forward operational sequence can be represented in its simplest form as the cycle:



where it is here presumed that the binding of the two substrates triggers the transition between the complex's two forms: $RES \rightarrow RE^*S$, that after that the reactions are allowed to complete *in situ*: $RE^*S \rightarrow PE^*T$, a precondition for product release, itself the precondition for the complex to switch from E^* back to E . Considering the catalysis and product-release steps $RE^*S \rightarrow PE^*T \rightarrow E^* + P + T$ as is more-or-less conventionally done, to comprise a single step, interprets the above scheme as a four stage cycle.

In Section 4 directly following, we present and analyze a simple statistical model of disequilibrium conversion based on a simple 'balls in a partitioned box' diffusion model which conforms to the above 'four stage' cycle. But as we note the conversion devices of life have invariably been found to embody elaborations on this design. Though these retain its essential features, in particular of dividing both processes into 'half-reactions' on which is imposed the iron rule that the cycle is prevented from returning to its start state unless the completion half-reaction of both processes has taken place, through to product release.

3.4.2. Real converters in biology invariably have more than four steps in the conversion cycle

For biological systems that have been characterized in sufficient detail, it has been invariably observed that the conversion cycle is more complex with, typically, the loading and unloading phases are further separated into individually ordered steps for the two processes, a point Jencks in particular extensively studied and emphasized as we noted above. To repeat the central point about this more complex design, the mediating engine intercedes in both of the processes by dividing them into individual binding and catalysis and release ‘half reactions’ which are, in the conversion cycle, sequenced in a more-or-less specific ‘interleaved’ (or “sandwiched”) and rigidly imposed manner. Often, however, the cycle has even more states and transitions, such as those that segment the “completion” of a driving process that involves a catalysis and product release, into two or more separate and specifically ordered steps. However, these refining complexities can be safely left aside for the moment.

One typical ordering of events (which we will here call the ‘canonical Jencks’ ordering), and assuming again the simple example in which $R \rightleftharpoons P$ and $T \rightleftharpoons S$ are the two coupled processes with the former driving the latter in reverse) is:



That is: driven reactants (i.e. that reaction’s products when running in its ‘forward’, spontaneous direction) must load first, then driving reactants can load, then, with both reaction’s reactants bound, the enzyme typically makes a major conformational/allosteric transition from E to E^* ; at this point the system becomes catalytically active to produce the driven (i.e. ‘back’ reaction) $S \rightarrow T$ *in situ*, after which T is released (into the teeth of a necessarily high probability of that release being reversed), then (that is *after* the cycle’s ‘work’ is done) the system becomes catalytically active for the driving reaction, again taking place *in situ*, $R \rightarrow P$, after which P is released (a transition necessarily having a low probability of being reversed), which event produces the pre-condition that allows the system to transition back to its starting form $E^* \rightarrow E$. Such refinements of functional design appear to be exploited in, and critical to, all biological examples known to date.

The critical functional element of this ordering of events is that the completion of the endergonic reaction precedes and is the gating trigger for the completion of its exergonic partner. An essential point about this structuring is that the free energy changes involved in the two reactions, both the driving negative change, and the driven positive one, are only, or predominantly, realized (‘harvested’) in the release of the reaction products, not in the reactions themselves taking place within a catalytic active sites—these typically take place near equilibrium [12,46]. That is, in general, and crudely, $\Delta G_{\text{product release}}^{\text{driven}} > 0$ and $\Delta G_{\text{product release}}^{\text{driver}} < 0$. The consequence is that in general the ratio of the frequencies of product release vs re-association is greater than one for the driving reaction and less than one for the driven one.

Thus in this architecture the completion of the driving reaction, through to product release, plays a three-fold role. First, to statistically overwhelm the probability of the reversal of the release of the driven reaction’s products—even though the latter is necessarily facing a high rate of re-association. It is this probability bias that drives the overall cycle forward productively. To put this in other words, the relative irreversibility of the driving reaction, due mainly to the irreversibility of its product release step, is what allows Brownian fluctuation-induced ‘back reactions’ in the driven process to be trapped and, through multiple engine cycles, accumulated. Second, to *kinetically* trap the completion of the driven reaction as soon as it occurs, thereby minimizing wasted time and the risk of futile cycles. For this kinetic effect to predominate, of course, the rate of the completion of the driven reaction must exceed that of the reversal of the release of the driven reactions products. Third, to be the event which acts as the prerequisite trigger for the return of the complex to its starting, relaxed, conformation. Such a system might be said to act as a “crossed-dual” enzyme, mediating two reactions each managed as two half reactions and forced to operate in thermodynamic opposition by the specific order in which the half reactions are sequentially interleaved (see Section 7).

3.4.3. The protein complex mediating the conversion must step through a cyclic series of alternative “enzyme” forms

Implicit in such cyclic reaction schemes as just summarized, whether simple as in Eq. (7) or more complex as in Eq. (8), is the fact that the protein complex mediating a conversion passes through a corresponding ordered series of alternate enzyme forms in each of which it takes on distinct binding and catalytic properties [12]. In operation, the completion of the task of each of these alternative enzyme forms triggers the transition to the next one in the cycle. We will explore what this means in more detail in Section 7.1. However, this operational characteristic makes explicit that these devices are not just catalysts, i.e. entities that passively accelerate a reaction that would take place on its own. Nor even are they just ‘enzymes’ in the ordinary sense denoting biological catalysts. They are true engines, in spirit and operation; and, as Nick Lane has remarked in speaking of one of the more dramatic of them, the ATPsynthase converter, they do and must “transcend chemistry” [3].

A critical question about such devices, however, is what causes and controls the conditional transitions? We address this in general terms next.

3.4.4. Substrate binding and release reactions play a key, if not dominant, role in triggering transition steps in the conversion cycle

As we have already reflected in the quote from Jencks just above the binding and unbinding of substrates and products appears to be in general the dominant mechanism responsible for the transitions between steps in a conversion cycle, not just that of what is typically its single major conformational change (here $E \rightleftharpoons E^*$). Beginning in the mid 1960s with the discoveries of the mechanisms of “induced fit” substrate binding in proteins [59,60], and the “allosteric” control of a protein’s functional properties [61,62], it had increasingly been realized that proteins, whether functioning as enzymes or in other roles such as in signal transduction [63–66] typically move through cycles in which they assume alternative “conformational” configurations with distinct properties [67]. This led Jencks and others to argue that similar allosterically-driven conformation change mechanisms were involved in inducing the functional transitions being perceived in the operation of the protein complexes mediating free energy conversions [12,55,56,63,68,69].

An important achievement of these proposals, as laid out fully in the literature just cited, was in providing a solid model of the equilibrium thermodynamics governing the binding events, the interaction between them and associated conformational changes in the mediating protein complex, and the impact of those conformational changes on the thermodynamics of the catalysis of the reactions being coupled. That is not to say that these investigators failed to appreciate the importance of kinetics in these processes. To quote Jencks discussing the ATP hydrolysis-driven vectorial pumping of calcium by Ca^{2+} -ATPase [12], “This switch cycle works because it follows an ordered kinetic mechanism; it represents one of the few instances in biochemistry in which it is essential that a particular kinetic mechanism be followed for the successful functioning of an enzyme”. and “The primary role of binding energy is kinetic. Binding energy is utilized in order to avoid high- or low-energy intermediates along the reaction path under physiological conditions”.

These conclusions have received strong support in the last decade or so which has seen an eruption of publications presenting detailed mechanisms for various biological free energy conversion systems. A general theme to which these mechanisms appear to unanimously attest is the central role played by more-or-less large-scale conformational changes, changes driven, or at least triggered by, substrate binding and unbinding events. Further, it has become clear that the conformational changes often, perhaps typically, also produce significant free energy-storing elastic “stress” distortions of the macromolecular complex which in some cases are exploited later in the cycle to favorably bias subsequent transitions [46,67].

In addition, recent work by Zocchi and colleagues quantitatively demonstrating the importance of cyclic “viscoelastic” transitions in the operation of even ordinary enzymes [47,48], makes it clear that protein complexes that function as simple, ‘single process’ catalysts and those that are true engines mediating free energy conversions—acting that is, as ‘dual process, cross-controlled enzymes’, are all “mechano-chemical” machines [46] with much in common mechanistically.

3.4.5. The “Brownian storm” is responsible for every step

To properly acknowledge the role thermal impacts play in how molecular conversion mechanisms operate, we reiterate that all of the transitions involved are stochastic and induced to move over transition barriers by thermal fluctuations. And all can take place in both their ‘forward’ and ‘reverse’ (i.e. ‘endergonic’) direction (with the forward-to-reverse ratio of rates of every allowed reaction given by a ‘fluctuation relation’, as discussed below in Section 4.1.2 and in Appendix A). In disequilibrium conversions, after the substrates for both reactions are loaded and the major conformational change that loading induces has taken place, the device then makes it possible for the “storm” to induce the completion of an instance of the ‘back reaction’ for the driven process—through to the release of its product, and then ‘waits’ until it does so. Of course, the probability of this product release is necessarily less, generally very much less, than that of that product’s re-association. However, this release triggers the system to catalyze the completion of the driving reaction through to the release of its products—a step whose reversal is improbable (in general highly); and, when the conversion cycle is proceeding forward, sufficiently improbable that the two sequential release events taken together are jointly probable (i.e. exergonic). Further, if in the state in which both product binding sites are empty, the complex is free to be induced by Brownian fluctuations to return to its starting conformation it will, in doing so, have accomplished the more-or-less irreversible trapping of a Brownian fluctuation-induced anti-2nd law event by a Brownian fluctuation-induced pro-2nd law event- and be ready to do it again. This causal sequence acts as a stochastic escapement mechanism which allows one process to function as a “ratchet” controlling another, and thereby makes it possible for Brownian events to power an engine that converts molecular disequilibria.

The main conceptual points are these: the relatively improbable ‘transition’ states through which reactions must pass are produced by Brownian impact fluctuations. The “free energies” of initial, transition, and final states merely measure (minus the log of) the probabilities of those states. Statistically, there will be (exponentially) more transitions that increase the system’s probability than those that decrease it. In “free energy transducing”, aka “energy conserving”, reaction couplings, ‘back reaction’ fluctuations in the driven process are trapped – and accumulated – by forward reaction fluctuations in the driving process. No energy passes from the driving to the driven reactions and the process is not driven by energy consumption.

3.4.6. It is the full cycle that converts free energy

It is the completed cycle that must (on average) satisfy the 2nd law. And, it is correspondingly the full cycle, all of whose steps must complete in the correct sequence, which effects the conversion of disequilibria (equivalently the

“transduction” of free energy); it is not any part thereof or step therein (see the discussion of this point in [70] and also in [12, p. 155]).

3.4.7. Dissipation is necessary to effect conversion

Of course, to function as proposed the cycle cannot be in equilibrium, but must be to some extent irreversible; that is, the overall forward rate must exceed the overall backward one. For this to be the case the probabilistic forward bias of the driving reaction (here: $R \rightarrow P$) must overwhelm the probabilistic bias against the driven (“back”) reaction (here: $T \leftarrow S$); and for this in turn to be true, the cycle must be “dissipative”, transforming the overall (negative) change in free energy into heat [23,71]. This, of course, does not require that all of the forward transitions involved in the cycle are strongly, or even at all, exergonic, and in real systems near-equilibrium, and even significantly endergonic (but kinetically driven) transitions are common [72].

Further, speed, and finite power output, comes at the necessary cost of dissipation. Whereas the 2nd law is satisfied as long as the difference between the entropy produced by the driving process and that lost in the driven one is, on average, not negative, if finite power is to be produced the net entropy production of the engine must be greater than zero; much greater if the power production is to be maximal; see later discussion of this point and also the discussions by Jeremy England [35] and Udo Seifert [23, Sec. 10].

3.4.8. Pay-on-completion designs appear to be universally adopted in biology, but are not required in principle

In the model being presented as to how real biological disequilibria conversions operate mechanistically, as summarized in the reaction scheme Eq. (8) describing the ‘canonical’ Jenck’s ordering for a simple converting system, the core functional element is that the completion of the driving reaction occurs after that of the driven reaction, and is triggered by it. To some this has a disturbing, cart-before-horse, implication. It might seem much more intuitive, and indeed is often supposed, that in such conversion processes the driving reaction must complete before, or at least simultaneously with, the driven one, thereby allowing, it is usually thought, that reaction’s free energy to be transferred either directly, or indirectly via the complex, to the driven reaction’s reagents. What is being supposed is that the driving reaction’s liberated free energy acts in some way to raise the free energy of the bound reagents for the driven reaction to a high enough value such that the free energy change for completing the driven reaction, including releasing the products into the external medium, is now negative—and the second half-reaction of the driven reaction thus spontaneous.

Our argument in this paper is largely an attempt to explain why the idea that endergonic reactions are made to happen by the transfer of energy to them from an exergonic process is fundamentally incorrect. But that is not the same thing as saying that the system cannot proceed with a different ordering of the key steps such that, for example, the driving reaction does complete before the driven one and indeed such designs are in principle possible. We defer a discussion of this question to Appendix C. But we note here that to our knowledge all biological disequilibria converting systems for which experimentally-based mechanisms have been proposed conform to the ‘driven reaction completes first’ principle. In Section 7.3 we discuss why this might be the case.

3.5. Summary to this point

For molecular-scale converters the question of what physically causes the endergonic transitions in the driven process to occur has a general, and somewhat counterintuitive answer. This is that, echoing Peter Hoffmann’s discussion of “nano-Sisyphus” molecular motors [1] from which we took our opening quote, it is not the driving process, much less “energy” taken from it; it is instead Brownian impacts delivered by the “molecular storm” (referring again to the opening quote). The driving process’s essential role is to kinetically, and quasi-irreversibly, “trap”, after the fact, completed instances of the driven one [32,58]. All molecular-scale disequilibria converters, not just molecular motors, are “nano-Sisyphuses”, or, more conventionally, Brownian ratchets, which block the unwanted effects of the “storm”, wait until a favorable event has allowed the completion of an instance of the work the engine is doing, and then rush back in to again block undoing events. This they do by initiating, as promptly as possible, a step transition in the engine to a state in which the driven products cannot reassociate and in which a quasi-irreversible further transition will occur (e.g. ‘firing’ an instance of the driving process, such as the hydrolysis of an ATP and release of its products). This irreversibility ‘latches’ the engine into a state that is then prevented from re-entering the prior state in which the driven reaction’s products could reassociate, and is also the state from which the engine can return to its conformational starting state. It is this mechanism that, at the molecular scale, embodies the escapement functionality required of all disequilibria conversions.

However, as Jencks has in particular emphasized [12], substrates typically bind to the complex by induced fit mechanisms. These in turn produce quasi-elastic, ‘free energy storing’ conformational changes in the complex, changes that are themselves endergonic (but not significantly dissipative). As these bonds are made and then later broken, the associated conformational changes trigger the sequential transitions between the alternative “specificity” forms of the complex that make up the conversion cycle. As a result, part of the chemical potential loss in the driving process is taken up in driving these conformational changes.

To restate a central point, disequilibria conversion processes do not transfer energy from the driving process to the driven one, nor in any valid sense do they transfer “free energy”, if that term is taken to denote a discounted quantity of energy

rather than just the probability of a macrostate. Free energy, we noted, is simply a measure of “improbability”—i.e. reflecting how relatively improbable a particular condition of disequilibrium is. Free energy transduction involves nothing more than trading one improbability for another.

We note that chemical conversion reactions in which the endergonic and exergonic reaction pairs are coupled by means of shared reagents plausibly stand as exceptions to the “escapement mechanism” requirements just stated. We consider this case briefly below (Section 8) and there argue that such coupling processes are not in fact exceptions.

3.6. Turning from generalities to concrete cases, conceptual and real

We now turn from these introductory generalities to an attempt to clarify in more concrete terms how Brownian-escapement devices actually operate to convert – and thereby create – disequilibria. Many of the points introduced as in general terms above will be encountered again in the context of specific models and examples.

We begin by analyzing the simplest explicit model of disequilibria conversion we can conceive, namely one based on the classical statistical thermodynamics paradigm of particles distributed unequally between the sides of a partitioned box. In this toy system we ask what thermodynamically legal and biologically feasible mechanistic arrangements are required to cause the dissipation of one such disequilibrium to automatically generate another disequilibrium of the same type. This maximally simple model system is considered first to display the minimal essential elements of molecular-level conversion processes. We later consider somewhat more sophisticated and ‘biological’ versions of the model to bring forward some additional points.

For clarity we analyze the thermodynamics of this system from several perspectives, beginning with a static statistical analysis based on the conventional ‘particles in a box’ assumption that the system’s microstates are the distinguishably different possible specifications of particle locations within the partitioned box—and thus occur with equal probability (see [52, §5&6] [34, Prologue]), and that the macrostates of interest are distinguished by the number of particles in one side of the partitioned box.

4. Particles diffusing in a partitioned box; the “coupled diffusion chambers” (“CDC”) model of disequilibria conversion

4.1. A partitioned box; causation and the “force” that powers change: the self-maximization of the count of microstates

Consider first a collection of N identical non-interacting particles of finite size distributed in a volume in which the particles can occupy any one of M positions. The particles are implicitly assumed to form a ‘gas’ in thermal equilibrium. All possible “configurations” or “microstates” – i.e. specifications of which positions are occupied by particles within the volume – are taken to be equally likely (the “micro-canonical ensemble” assumption). Under this assumption, the Boltzmann multiplicity (equivalently “configuration” or “microstate”) count, or in more modern nomenclature, the system’s total “weight”, “ W ” is given by the standard combinatorial formula:

$$W(M, N) = \frac{M!}{(M - N)!N!}, \quad (9)$$

where the system’s entropy, in dimensionless units (i.e. taking $k_B = 1$), is therefore $S = \ln W$.

We note here that an appropriate form of Stirling’s approximation for our purposes is $n! \approx (n/e)^n$ [52, Appendix B] in terms of which, given sufficiently large values for M and N , we may convert the above combinatorial expression for the system’s microstate count into a more convenient one for its entropy:

$$W(M, N) = \frac{M!}{(M - N)!N!} \approx \frac{M^M}{(M - N)^{(M - N)} N^N}, \quad \text{equiv.} \quad (10)$$

$$S = \ln W(M, N) \approx M \ln M - (M - N) \ln (M - N) - N \ln N.$$

Next, and in order to introduce a system of this type capable of supporting a disequilibrium, we consider the classic diffusion chamber comprised of two such volumes separated by a partition with a portal through which particles can pass one at a time. We depict this in Fig. 1 in which it is assumed to be in a state of disequilibrium, that is, with more particles on one side than the other:

Assuming there are N total particles in the two compartments depicted in Fig. 1 the system can exist in $N + 1$ different macroscopically specifiable (that is to say, externally observable and controllable) states distinguished by how many of the N particles are in one compartment, say the left one: “ N_L ”. These states, assuming the total number of particles is held constant, comprise the system’s ‘macrostates’. Since Boltzmann we understand that a key property of thermodynamic systems is the probability that the system would be found to be in any one of its possible macrostates and further that this probability is proportional to the number of microstates of the system to which it has ‘effective’ access when in that macrostate [34, Prologue]:

$$\begin{aligned} p(N_L) &\propto W(N_L) = e^{-S(N_L)}, \\ &= W(N_L) / Z_{\text{macrostates}} \end{aligned} \quad (11)$$

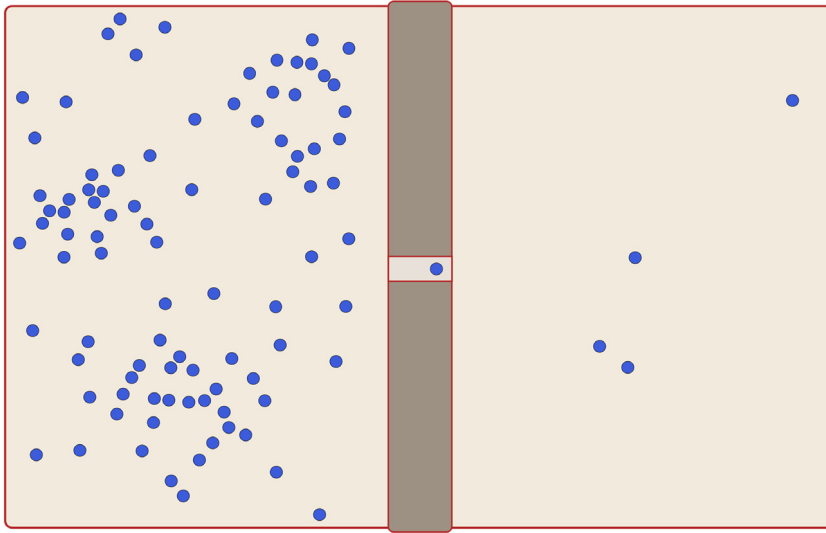


Fig. 1. Disequilibria relaxation and macrostate probability.

Given a disequilibrated distribution of particles between two connected compartments as depicted in the figure it is obvious that the net flow of particles through the portal in the partition dividing them will be from the more to the less densely occupied compartment and will proceed until the forward and reverse single particle transit probabilities are equal—reflecting that then, apart from statistical fluctuations, the particle densities in the two partitions are the same. During this relaxation the probability of the system’s ‘macrostate’ increases quasi-continuously. This increase, since it inherently implies a degree of irreversibility, is arguably the physical cause of the relaxation. In the accompanying text we illustrate how macrostates are defined in the present system and how their probabilities relate to the system’s microstate count W (equivalently its entropy) when in a particular macrostate. And we also discuss the circumstances, more generally, in which it is legitimate to consider these quantities as well defined, and as increasing quasi-continuously, during a spontaneous, non-equilibrium, thermodynamics process.

where $Z_{\text{macrostates}} = \sum_{N_L=0}^N W(N_L)$ is the partition function for the macrostate distribution, and $S(N_L) = \ln(W(N_L))$ is the entropy of the system when it is in its “ N_L ” macrostate.

Given our assumptions about this “balls in a box” diffusion model, in which microstates are simply alternative choices as to which of the occupiable locations hold particles, and using that the diffusion chamber’s microstate count is, to a good approximation, the product of the counts of its individual chambers:

$$W(N; N_L) = W_{\text{left}}(N_L) \times W_{\text{right}}(N_R) \quad (12)$$

where $N = N_L + N_R$. Clearly the macrostate probability of the diffusion chamber is highest when $W(N; N_L)$ is maximal and that, as we explicitly show below, if the two compartments are equal in volume this happens when $N_L = N/2$. Clearly also, the system is in a disequilibrium if the total microstate count is less than this value (equivalently the system’s macrostate probability is less than its maximal possible value), and spontaneously executes a transition from a non-equilibrium starting state to the equilibrium one in which the number of accessible microstates, and with it the macrostate’s probability and its entropy, increases (apart from fluctuations) from their starting to their maximal values. In this model system, the probability of the macrostate in which N_L particles are on in the left hand compartment is the fraction of times in placing N particles randomly in two boxes each having room for M particles, exactly N_L of them end up in one of them. In other words, the macrostate probability of the distribution shown in Fig. 1 is extremely small since a correspondingly small fraction of all equally probable microstates have that degree of numerical imbalance. The overwhelming majority – a rapidly increasing majority as N grows in size – are within ‘fluctuation’ range of those that correspond to the most probable macrostate.

Moreover a highly important result of modern thermodynamics has been the development of a class of relationships called “fluctuation theorems” (see [13–18,20,23,24,28,73]) which in general (and oversimplified) terms state that the ratio of the probability of an entropy increasing fluctuation to that of its reverse, entropy decreasing mirror event is equal to the exponential of the magnitude of the (dimensionless) entropy change. Given the core importance of fluctuation transitions to disequilibria conversion as was discussed above in Section 3.3, we derive the applicable fluctuation relationship for single particle transitions in our toy diffusion chamber model in Section 4.1.2.

Further, in recognition of the fact that our treatment of the ‘thermodynamics’ of a model based on a (potentially small) number of particles in a box violates the conceptual tenets of classical thermodynamics, still to be found in most textbook renderings of the subject (by way of contrast see [52]), we offer a digression on the transition from classical to non-equilibrium thermodynamics in Appendix B.

We turn next to extending the combinatorial configurations analysis given above for a simple particles-in-a-box model to the case of a partitioned box as pictured in Fig. 1.

4.1.1. The configurations analysis of the partitioned box

Since the number of configurations of a system comprised of statistically independent subsystems is the product of the subsystem numbers, in the partitioned box model, we have:

$$\begin{aligned} W_{\text{total}} &= W(M_L, N_L; M_d, N_d) = W(M_L, N_L) \times W(M_d, N_d), \quad \text{or} \\ \ln W_{\text{total}} &= \ln W(M_L, N_L) + \ln W(M_d, N_d) \end{aligned} \quad (13)$$

where L and R denote the left and right partitions respectively and where N_L and N_R must sum to $N_{\text{total}} = N_L + N_R \doteq N$.

Net particle flow between the sides of the box will necessarily proceed in the direction that increases W_{total} (equivalently the system's total entropy $S_{\text{total}} \sim \ln W_{\text{total}}$). We explore what this implies in terms of the system's macrostate parameters in two ways. First, by considering the effect on W_{total} of single particle transfers; this we take up next. Second by considering the large N limit and computing the rate of change of the system's entropy with the number of particles in the left partition $\partial (\ln W_{\text{total}}) / \partial N_L$.

Single particle transfers. We first just calculate how a single box's microstate count changes when we increment and decrement the number of particles in the box N by 1; assuming, as we have above, that the box has M possible particle positions. Given that:

$$W(M; N) = \frac{M!}{(M-N)!N!}. \quad (14)$$

If increase N by one:

$$\begin{aligned} W(M; N+1) &= \frac{M!}{(M-N-1)!(N+1)!}, \\ &= \frac{M!(M-N)}{(M-N)!(N+1)N!}, \\ &= W(M; N) \frac{(M-N)}{(N+1)}. \end{aligned} \quad (15)$$

And similarly, if decrease N by one:

$$\begin{aligned} W(M; N-1) &= \frac{M!}{(M-N+1)!(N-1)!}, \\ &= \frac{M!N}{(M-N)!(M-N+1)N!}, \\ &= W(M; N) \frac{N}{(M-N+1)}. \end{aligned} \quad (16)$$

Now consider the diffusion box example, in which the particles are divided between the left and right compartments: $N = L + R$, and in which the total microstate count is $W_D = W_L \times W_R$. We ask how W_D changes when a single particle moves from left to right.

$$\begin{aligned} W_D(L-1; R+1) &= W_L(M; L-1) W_R(M; R+1), \\ &= W_L(M; L) \frac{L}{(M-L+1)} W_R(M; R) \frac{(M-R)}{(R+1)}, \\ &= W_D(L; R) \frac{L}{(M-L+1)} \frac{(M-R)}{(R+1)}, \\ W_D(L-1; N-L+1) &= W_D(L; N-L) \frac{L}{(M-L+1)} \frac{(M-N+L)}{(N-L+1)}, \end{aligned} \quad (17)$$

where in the final equation we have used that $R = N - L$.

From the above we see that there is no change in microstate count upon the exchange of a particle from left to right if

$$\begin{aligned} \frac{L}{M-L+1} &= \frac{N-L+1}{M-N+L}, \\ LM - NL + L^2 &= MN - LM + M - LN + L^2 - L + N - L + 1, \\ 2LM &= MN - 2L + N + 1, \\ 2L &= N + \frac{1}{M+1}. \end{aligned} \quad (18)$$

So if assume that $M \gg 1$, a particle transfer leaves the microstate number unchanged if

$$L = N/2. \quad (19)$$

This unsurprising result serves mainly to emphasize that the physical meaning of the fact that the ‘force’ causing the dissipation of a disequilibrium. It is simply a quantitative bias in favor of stochastic transfer events which increase, rather than decrease, the number of microstates, and in so doing move the system quasi-continuously through macrostate space (here parametrized by L , with M and N being taken as fixed) to macrostates of increased (on average) intrinsic probability; that probability being maximal when $L = N/2$ and the particles are distributed equally between the two compartments.

To make this point a bit more concrete, we ask, under the same $M \gg 1$ condition, for what values of the system’s macrostate parameters does the system’s microstate count increase upon a transfer of a single particle from left to right. The answer clearly is:

$$\begin{aligned} W_D(L-1; R+1) > W_D(L; R) &\iff \\ \frac{L}{M-L} > \frac{R}{M-R}, & \\ \frac{L}{M-L} > \frac{N-L}{M-(N-L)}. & \end{aligned} \quad (20)$$

Defining ‘fullness’ (full/empty) factors for the two sides of the diffusion chamber: $\varepsilon_L = L/(M-L)$ and $\varepsilon_R = R/(M-R)$, the above result can be economically expressed as:

$$\varepsilon_L > \varepsilon_R \quad (21)$$

where again we are assuming $M \gg 1$.

In words, such a transfer increases the system’s microstate count if and only if the ratio of full-to-empty locations in the donating chamber is greater than the same ratio in the receiving chamber. Physically, this just corresponds to the fact that under those conditions, a transfer from the more full to the less full chamber is more probable than the reverse.

Large N limit. Using the Stirling approximation expression given above in (10), we can, for sufficiently large particle numbers, write

$$\begin{aligned} \ln W_{\text{total}} = \ln \left(M_L^{M_L} M_R^{M_R} \right) - (M_L - N_L) \ln (M_L - N_L) - (M_R - N + N_L) \ln (M_R - N + N_L), \\ - N_L \ln (N_L) - (N - N_L) \ln (N - N_L) \end{aligned} \quad (22)$$

and can further treat the particle numbers as continuous variables in order to compute the partial derivative of W_{total} with respect to N_L (chosen arbitrarily). This is:

$$\begin{aligned} \frac{\partial (\ln W_{\text{total}})}{\partial N_L} &= \ln \left(\frac{M_L - N_L}{N_L} \right) - \ln \left(\frac{M_R - N_R}{N_R} \right) \quad \text{equiv.}, \\ &= \ln \left(\frac{\text{empty}}{\text{full}} \right)_L - \ln \left(\frac{\text{empty}}{\text{full}} \right)_R, \\ &= \ln (1/\varepsilon_L) - \ln (1/\varepsilon_R) \quad \text{so that,} \\ \frac{\partial (\ln W_{\text{total}})}{\partial N_L} &= \ln \left(\frac{\varepsilon_R}{\varepsilon_L} \right) \end{aligned} \quad (23)$$

where, the “ ε ” quantities, ε_L and ε_R are the “fullness factors” defined above; in which, e.g., $\varepsilon_L \equiv (\text{full/empty})_L = \text{ratio of full to empty sites of the } L \text{ chamber}$. In the dilute limit, where the number of filled sites is very much less than the total number of sites, $N \ll M$, then $\varepsilon \approx N/M$, i.e. the ‘concentration’ of particles in the chamber.

Thus, requiring this derivative to be positive (for the entropy of the system to increase as N_L is increased):

$$\begin{aligned} \frac{\partial (\ln W_{\text{total}})}{\partial N_L} > 0 &\text{ if and only if,} \\ \ln \left(\frac{\varepsilon_R}{\varepsilon_L} \right) > 0 &\text{ equivalently,} \\ \varepsilon_R > \varepsilon_L. & \end{aligned} \quad (24)$$

That is as we expect, and recapitulating the result obtained above for single-particle transfers, the entropy of the system increases only when particles move from the more completely filled to the less completely filled side, and equilibrium is reached when both sides have the same ratio of full to empty locations. We note that the analysis to this point of the partitioned box diffusion chamber is essentially that given in [34, Prologue].

Our core argument in this paper is erected on the quite trivial conceptual foundation laid out using the diffusion model in this section. The key next step is to make concrete what is required, as a generality, to have the relaxation of one

disequilibrium of the above type generate another of the same type. For this we need to consider two such diffusion process equipped with linked gating mechanisms. How we will represent the gating mechanisms necessary to effect a conversion of disequilibria will be introduced next, first as merely passive, thermally-driven ‘do-nothing’ toggles acting in a single diffusion chamber whose only effect is to break particle movement through the barrier into separate loading and unloading steps.

However, because of its general conceptual importance to our discussion we insert an analysis of the fluctuation dynamics of the diffusion model (see [Appendix A](#) for a discussion of the non-equilibrium thermodynamic concepts on which this argument is based).

4.1.2. Macroscopic reversibility and a fluctuation relationship for the diffusion chamber

A fluctuation relationship for the diffusion chamber model can be derived by assuming: (a) that the probability of a macrostate is proportional to its microstate count (termed its “weight” in the conventions of stochastic thermodynamics [23,34]): i.e., for macrostate “A”, $P^A \propto W(A)$, and (b) that the macrostate weights may be taken as unchanging in time and thus that the system is operating in steady state. In general, of course, steady state operation in the CDC model requires that the converter is connected to a “chemostat”, in this case ‘source’ and ‘load’ circuits, as imagined in [Fig. 11](#), which maintain constant particle densities in the compartments.

Under these assumptions the principle of “macroscopic reversibility” applies [25, Eq. 1.35] (alternatively that of “global detailed balance” [22, Sec. 5.4]); see discussion in [Appendix A](#). In the CDC model, where states are even under time reversal, macroscopic reversibility requires that the unconditional probability of a transition between macrostates A and B in time t, namely $p(B, A | t)$ must equal that of the reverse transition: $p(A, B | t) = p(B, A | t)$. Given further that the unconditional probability of the $A \xrightarrow{t} B$ transition, is equal to the product of its conditional probability: $p(B | A, t)$ (i.e., the probability of that transition given that the system is in macrostate A), and the probability P^A of the system being in macrostate A: $p(B, A | t) = p(B | A, t) \times P^A$, and adopting the shorthand notation for the conditional transition probabilities: $p(B | A, t) = p_{A \rightarrow B}$, (and retaining the convention of this section that entropies are dimensionless) we have

$$\begin{aligned} P^A \times p_{A \rightarrow B} &= P^B \times p_{B \rightarrow A}, \\ \frac{p_{A \rightarrow B}}{p_{B \rightarrow A}} &= \frac{P^B}{P^A}, \\ &= \frac{W(B)}{W(A)}, \\ &= e^{\Delta S(A \rightarrow B)} \end{aligned} \quad (25)$$

where the final ‘canonical’ form is simply the result of inserting $S = \ln W$ in the 3rd eqn. in the stack. Conceptually, the key points in this relationship are that the probability of a transition between macrostates is proportional to the probability of the target macrostate, and that, in consequence, forward transitions are exponentially more probable – in the entropy increase produced by that transition – than their reverse. Noting finally that the entropy produced in the forward transition must equal the negative of that produced in the reverse transition, we note that the above result may be written

$$\frac{p(\Delta S)}{p(-\Delta S)} = e^{\Delta S} \quad (26)$$

which is the so-called “detailed fluctuation theorem” [22, Eqn. 70], and since there is no restriction of the magnitude of ΔS nor on the size of the system under study, this may also be regarded as a “macroscopic” fluctuation theorem (see also the alternative approaches to the derivation of “macroscopic” fluctuation relations by Ambaum [73] and by Bertini et al. [74]).

If in the CDC model we consider only single particle transfers, the ratio of the probabilities for forward and reverse transfers is equal to the exponential of the entropy produced in the ‘forward’ transfer

$$\begin{aligned} \frac{p_{L \rightarrow L-1}}{p_{L-1 \rightarrow L}} &= \frac{W_D(L-1)}{W_D(L)}, \\ &= \frac{L}{(M-L+1)} \frac{(M-N+L)}{(N-L+1)}, \\ &= e^{\Delta S_D(L \rightarrow L-1)} \end{aligned} \quad (27)$$

where in the first equation we have used Eq. (25), and the middle equation in this stack was as taken from Eq. (17). This is manifestly an instance of a ‘detailed fluctuation theorem’.

Notice that if $M \gg N \gg 1$ then the ratio of the conditional transition probabilities becomes:

$$\begin{aligned} \frac{p_{L \rightarrow L-1}}{p_{L-1 \rightarrow L}} &\approx \frac{L}{N-L}, \\ &= \frac{L/N}{1-L/N}. \end{aligned} \quad (28)$$

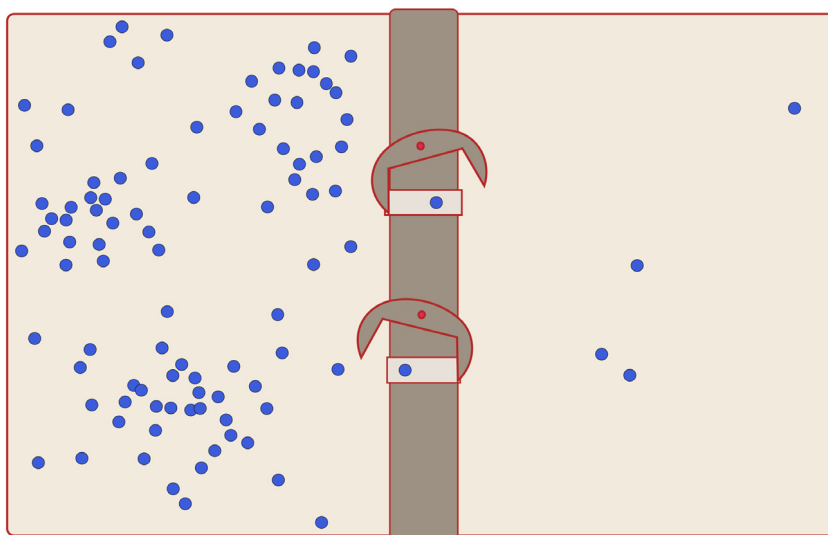


Fig. 2. Diffusion through passive, thermally-driven gates.

It is assumed that the passive gating devices shown oscillate in a stochastic, binary manner (i.e. they ‘flip’), driven by thermal fluctuations, between the two orientations: open right and open left. Their presence divides the transport of particles through the partition into ‘half reactions’ involving the loading and unloading of the portal chamber; clearly a portal must become loaded from one side before a flip of the gate makes it possible for the particle to leave the portal to the other side; this action affects the rate of equilibration, not the outcome.

In the next section we introduce a mechanistic modification of the portal in the box’s partition that we will require in order to achieve disequilibria conversion.

4.2. Adding a passive, ‘Brownian’ gate

Fig. 2 depicts the introduction of thermally driven, directionally unbiased, gating mechanisms into the transit of particles through the portal in the diffusion chamber’s partition. These mechanisms have the effect of breaking the transit into separate, thermodynamically independent, and in principle separately controllable, ‘half-transit’ events of loading and unloading. Doing so is an essential design element of all free energy converting devices.

With these notions in hand we can address the question of how the dissipation of one such disequilibrium could force the creation of another of the same type. For this we set up two diffusion chambers each with a gate of the above type, but with the two gates linked together mechanically so that they are forced to move, i.e. to flip, together, and can thereby function as the sought-after Brownian escapement. This is shown in **Fig. 3**.

4.3. The CDC toy model: two diffusion chambers with coupled gates forming a “Brownian escapement”

Fig. 3, depicts the situation in which two gated diffusion chambers are coupled by having their gate mechanisms linked so that they must flip orientation together. If in addition it is assumed that thermal fluctuations are prevented from flipping the linked gate’s orientation (in either direction) unless both portals are in the same state of being either full or empty, then the linked gates function as an escapement and the entire system as an engine by which the dissipation of a disequilibrium in one chamber can drive the formation of a disequilibrium in the other. We discuss later how this kind of ‘flipping’ rule is achieved in real disequilibrium-converting engines, biological ones in particular.

In any case, the key condition is that the linked gating mechanisms can flip (in either direction) only if both portals are either loaded or empty (that is, the state of only one portal being empty prevents thermal fluctuations from flipping the linked gates). This imposes a directionally unbiased coupling of the processes that functions as a stochastic escapement mechanism that can cause the dissipation of one disequilibrium to create another as is shown in **Fig. 4**.

It is to be emphasized that this disequilibria converting mechanism is blind as to direction, and also as to which chamber’s process, if either, is driving the other; it is therefore inherently reversible, and “Maxwell daemon” free (that is, it does not filter events based on their probability in order to permit only improbable ones—in violation of the 2nd law; it merely enforces that it is the joint probability of events in the two chambers that controls both). To be useful, of course, any device of this type has to be sustained in a dynamic and approximately steady state by being embedded in a hierarchical cascading network of such devices, wherein many of the product disequilibria have critical extra-network uses (and are depleted in those uses). We expand a bit on this point below in Section 10.

Note also that the assumed linked-gates flipping rule makes the escapement mechanism function as a logical “XNOR” gate; emitting a ‘yes’, i.e. a flip, only if both inputs are in the same state, and a ‘no’ otherwise. We return to this point later in discussing more realistic models of disequilibria conversion.

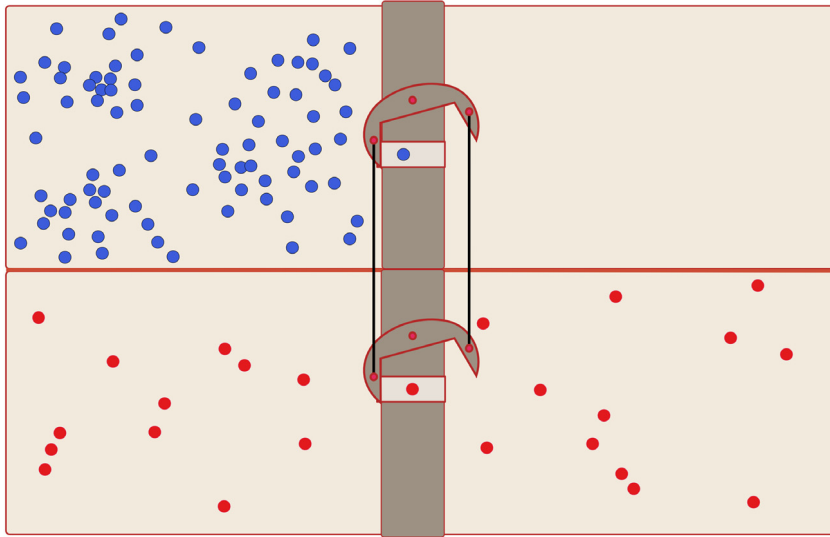


Fig. 3. Diffusion chambers coupled by linked gates forming an escapement; an engine that can convert concentration disequilibrium.

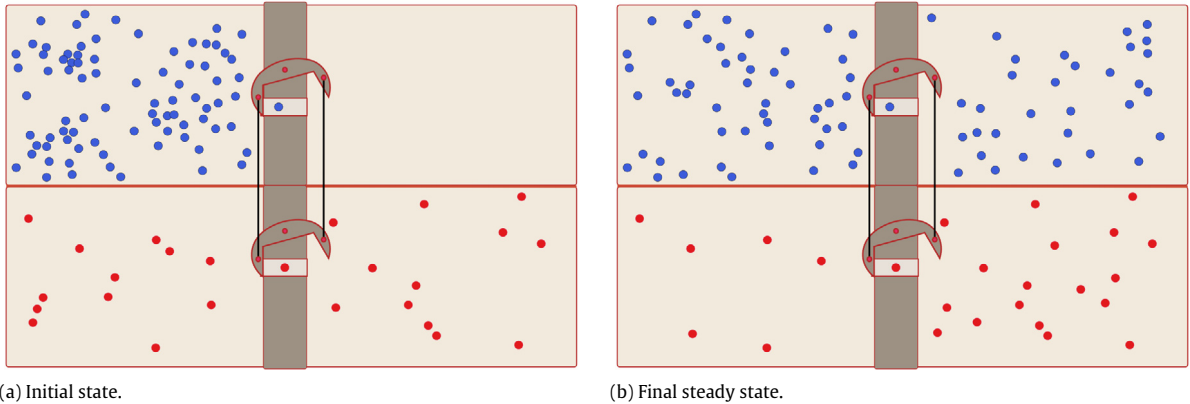


Fig. 4. Converting diffusion disequilibria.

A pair of gated diffusion chambers coupled via a mechanical linkage between their gating mechanisms which forces the two gates to flip orientation together thereby potentially driving the conversion of disequilibria between the top and bottom diffusion chambers; panel (a) depicts a potential starting state for the system of complete disequilibrium in the top (blue) system, and approximate equilibrium in the bottom one (red). This state would then evolve, purely by stochastic diffusion, into a steady state represented in panel (b), in which the initial disequilibrium in the blue ‘driving’ chamber is partially dissipated while a partial disequilibrium is created in the red ‘driven’ chamber; that is, in steady state neither of the two processes are at equilibrium.

In Section 5 we present a dynamic non-equilibrium analysis of this simple model engine in the large particle number limit. However, we first analyze the steady state properties of the toy engine’s operation based on the simple ‘configuration count’ analysis begun above in describing the partitioned diffusion chamber.

4.4. Microstate count analysis of the CDC model engine

The number of configurations for the CDC engine is the product of the numbers for its two constituent diffusion chambers: $W_{engine} = W_{total}^D \times W_{total}^d$ where D and d denote the two coupled diffusion processes. We will examine how the total microstates count of the engine changes as particle transitions occur under the same two limit conditions we employed above; single particle transitions, and the large-particle number approximation.

4.4.1. Single particle transits

We can directly express the change in the microstate count in our coupled diffusion chambers model for disequilibrium conversion by noting that the system’s microstate count is the product of the count for the two coupled diffusion systems:

$$W_C = W_D \times W_d \quad (29)$$

where, W_D and W_d are the microstate count for the two coupled diffusion chambers. As was noted in the introduction to the CDC model just above (Section 4.3) we assume that the coupling requires that single particle exchanges must occur together in the same direction in both chambers (but in either direction).

Using lower case symbols for the 2nd chamber, and using the result for a single particle transfer from left to right (in both chambers) given in Eq. (17) and Eq. (21), we can write the above product as

$$\begin{aligned} W_C(L-1; l-1) &= W_D(L-1) \times W_d(l-1), \\ &= W_D(L-1; N-L+1) \times W_d(l-1; n-l+1), \\ &= W_D(L; N-L) \frac{L}{(M-L+1)} \frac{(M-N+L)}{(N-L+1)} \times W_d(l; n-l) \frac{l}{(m-l+1)} \frac{(m-n+l)}{(n-l+1)}, \\ &= W_C(L; l) \frac{L}{(M-L+1)} \frac{(M-N+L)}{(N-L+1)} \times \frac{l}{(m-l+1)} \frac{(m-n+l)}{(n-l+1)}, \end{aligned} \quad (30)$$

from which it is evident that a single dual transfer of a particle from L to R in the two coupled chambers will increase the system's total microstate count if and only if

$$\begin{aligned} \frac{L}{(M-L+1)} \frac{(M-N+L)}{(N-L+1)} \frac{l}{(m-l+1)} \frac{(m-n+l)}{(n-l+1)} &> 1, \\ \frac{\epsilon_L}{\epsilon_R} \frac{\epsilon_l}{\epsilon_r} &> 1. \end{aligned} \quad (31)$$

Where in the last line we have again imposed the assumptions that $M \gg 1$, and $m \gg 1$ and used the above ‘fullness factors’ notation.

Consider the case in which $\epsilon_l/\epsilon_r = 0.5$, implying that the $L \rightarrow R$ transit of a particle in the 2nd chamber reduces that chamber's microstate count; that is, the transit in this chamber increases that chamber's disequilibrium and is thus a “driven” one. But this then implies that $\epsilon_L/\epsilon_R > 2$ for the dual transit action required by the coupling rule to be one that increases the system's total microstate count (and thus would thus be the statistically predominant transit direction).

This illustrates that a conversion of a disequilibrium in the first (upper case) chamber to one in the second (lower case) chamber will occur when:

$$\begin{aligned} \frac{\epsilon_l}{\epsilon_r} &< 1 \quad \text{and also,} \\ \frac{\epsilon_L}{\epsilon_R} &> \frac{\epsilon_r}{\epsilon_l}. \end{aligned} \quad (32)$$

And in a general sense it also illustrates, at the “microstate count” level, the thermodynamic underpinnings of disequilibria conversion.

4.4.2. Large-particle-number approximation

Referring to Eq. (23), and denoting the driving and driven processes with, respectively, the superscripts D and d , we observe that the toy engine's escapement rules imply that $\Delta N_L^D = \Delta N_L^d = -\Delta N_R^D = -\Delta N_R^d$, so that $\partial N_L^d / \partial N_L^D = 1$, it follows that the partial rate of change of the entropy of the engine with respect to changes in the number of particles in the (arbitrarily chosen) left chamber of the “D” process is:

$$\begin{aligned} \frac{\partial (\ln(W_{\text{engine}}))}{\partial N_L^D} &= \frac{\partial (\ln(W_{\text{total}}^D))}{\partial N_L^D} + \frac{\partial (\ln(W_{\text{total}}^d))}{\partial N_L^D}, \\ &= \frac{\partial (\ln(W_{\text{total}}^D))}{\partial N_L^D} + \frac{\partial N_L^d}{\partial N_L^D} \frac{\partial (\ln(W_{\text{total}}^d))}{\partial N_L^d}, \\ &= \ln\left(\frac{\epsilon_L^D}{\epsilon_R^D}\right) + \ln\left(\frac{\epsilon_L^d}{\epsilon_R^d}\right), \\ &= \ln\left(\frac{\epsilon_L^D \epsilon_L^d}{\epsilon_R^D \epsilon_R^d}\right) \end{aligned} \quad (33)$$

which says that entropy goes up as particles move (together) from R to L if the product of the two empty/full ratios is greater on L than on R . And conversely for the opposite direction. So, the system achieves steady state when the product empty/full ratios for L and R are equal:

$$\begin{aligned} \frac{\epsilon_L^D \epsilon_L^d}{\epsilon_R^D \epsilon_R^d} &= 1 \quad \text{equivalently,} \\ \epsilon_L^D \epsilon_L^d &= \epsilon_R^D \epsilon_R^d, \end{aligned} \quad (34)$$

that is, the product of the ratios of empty to full sites in the two left partitions must equal the same product for the two right partitions.

Alternatively, if the product of the empty/full ratios is greater on the right than on the left (as in our assumed starting condition):

$$\frac{\varepsilon_L^D \varepsilon_L^d}{\varepsilon_R^D \varepsilon_R^d} < 1 \quad \text{equivalently,} \quad (35)$$

$$\varepsilon_L^D \varepsilon_L^d < \varepsilon_R^D \varepsilon_R^d$$

then $\partial (\ln(W_{\text{engine}})) / \partial N_L^D < 0$, which implies that if R is (in the product) more empty than L then a flow from R to L decreases the engine's total entropy, and, conversely, that, in this state, the entropy of the system is increased by a flow from L to R . This corresponds to the assumptions made above for the CDC model, in which d is the driven process and starts out at (or near) equilibrium" namely with $\varepsilon_L^d = \varepsilon_R^d$), whereas D is the driving process and starts out compressed into the left hand compartment so that ($\varepsilon_L^D < \varepsilon_R^D$), thereby satisfying the engine conditions $\varepsilon_L^D \varepsilon_L^d < \varepsilon_R^D \varepsilon_R^d$ for creating a disequilibrium in the d (driven) process.

Kinetically these linking conditions just mean that within the model net particle flow, equal in direction and magnitude for both processes, proceeds from the side in which the joint probability of both gates being simultaneously loaded exceeds that for the other side.

This maximally simple model captures, we claim, every essential mechanistic element of disequilibria (free energy) conversion—save one: how, in the molecular domain, does the escapement mechanism actually impose the essential requirement that neither process is permitted to complete unless both do. In our model this requirement is embodied in the rule that the gating device is permitted to flip sides only if both portals are in the same filled or empty state. But how do molecular machines impose such rules? We take up this topic beginning with Section 7 but in the next section augment the preceding statistical-thermodynamic, 'microstate counting' static analysis of the CDC model with an explicitly dynamical "equations of motion" analysis. This involves moving to the limit of large particle numbers and treating the system as comprised of idealized 'gases'. In this, we adopt a notational change to reflect the fundamental switch in underlying concept structure; wherein, e.g., 'static' particle counts are replaced by explicitly time-dependent particle densities.

5. The large-particle-number dynamic analysis of the CDC model

Our goal in this section is to derive and solve a set of differential equations that describe, in the large particle number limit, the dynamics of the CDC model as defined in the preceding Section 4. We could approach this in terms of discrete particle dynamics, i.e via a Markov model/master equation approach [16,75], however this would both take us quite far afield and leave us with analytically intractable results. On the other hand, as we now show, some useful insights can be obtained by framing and solving the model in large particle number limit where the continuum approximation can be employed.

5.1. Model definition: the CDC equations

Two gases, whose molecules are labeled respectively X (blue) and Y (red), are distributed among four chambers, two on the left (L) and two on the right (R). We denoted the molecules of the X species in the left chamber by X_L , and similarly the others. As shown in Fig. 3, the two gases are not in contact, however, the gating mechanism allow a X molecule and a Y molecule to simultaneously flip from the left chambers to the right chambers, or vice versa. The main insight is that these processes are formally equivalent to the two chemical reactions:



The above processes do not imply that changes occur in chemical structure (although this is certainly what happens in real engines, as described later on), but rather that the kinetics of the CDC model are tantamount to those of the chemical reactions (36). In this way, we can resort to the law of mass action to obtain a set of dynamical equations from reactions (36). Note that this approach is widely used in statistical physics for modeling population dynamics in varied fields [75].

Reactions (36) conserve the total number of particles of the two gases, and we denote by N_X (respectively, N_Y) the total number of X (respectively, Y) molecules, and their ratio by $\beta = N_X/N_Y$. The reaction constant α quantifies the reaction rate and depends on the volume of the chambers and the kinetic energy of the single particles. To simplify our analysis we consider α a free parameter. We also indicate the molar concentrations in the chambers using square brackets, e.g. $[X_L]$ denotes the number of X molecules in the corresponding left chamber, divided by N_X . To model the system dynamics, we invoke mass action on reactions (36) (see e.g. [75]) which yields the following system of ordinary differential equations, which we call the CDC equations:

$$\begin{aligned} \dot{[X_L]} &= \alpha[X_R][Y_R] - \alpha[X_L][Y_L], & \dot{[X_R]} &= \alpha[X_L][Y_L] - \alpha[X_R][Y_R] \\ \dot{[Y_L]} &= \alpha[X_R][Y_R] - \alpha[X_L][Y_L], & \dot{[Y_R]} &= \alpha[X_L][Y_L] - \alpha[X_R][Y_R]. \end{aligned} \quad (37)$$

These four equations possess a high degree of symmetry, in that, $[\dot{X}_R] = [\dot{Y}_R] = -[\dot{X}_L] = -[\dot{Y}_L]$. System (37) has been obtained under the approximation (operated implicitly by the law of mass action) that the two gases are macroscopic so

that the whole system can be thought of two fluids ($N_X, N_Y \gg 0$). In this limit, intrinsic fluctuations, related to the fact that gases are composed of discrete entities, are neglected although they can be easily accounted for by turning System (37) into a stochastic process, as commonly done [75].

The purpose of System (37) is to provide us with the simplest model that shows quantitatively how a disequilibrium in a chemical species (e.g. X) can be used to drive a disequilibrium in another species (Y), the latter supposed to be initially at equilibrium. These conditions are realized by choosing proper initial conditions, which complete the definition of the dynamical CDC model. We assume that at time zero, $t = 0$, every X molecule is located in the left chamber, whereas the Y molecules are equally spread among the two chambers. In formulae, this means that

$$\begin{aligned} [X_L](t = 0) &= 1, & [X_R](t = 0) &= 0, \\ [Y_L](t = 0) &= \frac{1}{2}, & [Y_R](t = 0) &= \frac{1}{2}. \end{aligned} \quad (38)$$

The notions of equilibrium and disequilibrium are given with respect to the single gases. Each gas, in the absence of mechanical coupling, reaches equilibrium by uniformly occupying the left and right chambers. However, since the two gases are coupled, the final state is different and revealed by the analysis of the model which we carry out in the next section.

5.2. Mathematical analysis

System (37) consists of four equations, which can be reduced to one by applying three constraints. Two of them are given by the conservation laws $[X_L] + [X_R] = 1$ and $[Y_L] + [Y_R] = 1$, whereas the third constraint follows from the observation that to a creation of a X_R molecule must correspond a creation of a Y_R molecule. Together with the initial conditions (38), this translates into the following equality:

$$[X_R](t) = \beta[Y_R](t) - \beta[Y_R](0) = \beta[Y_R] - \frac{\beta}{2}. \quad (39)$$

Using the two conservation laws and Eq. (39) in System (37), we can make the (arbitrary) choice of expressing each concentration as a function of $[X_L] \equiv x$. After some tedious but simple algebraic manipulations, we arrive at the following equation:

$$\dot{x} = \frac{1}{2}\alpha(2\beta - 2(\beta + 1)x + 1), \quad (40)$$

the initial condition now being simply $x(0) = 1$ (from Eq. (38)). Eq. (40) is a linear, first-order, ordinary differential equation, whose solution is given in textbooks [76] and, for our case, reads:

$$x(t) = \frac{1 + 2\beta + e^{-t/\tau}}{2 + 2\beta}, \quad \text{where the timescale is } \tau = \frac{1}{\alpha(\beta + 1)}. \quad (41)$$

As shown in Fig. 5, the concentration of X molecules in the left chamber diminishes progressively, following an exponential decay with characteristic timescale τ . After a time $t \sim \tau$, the system reaches its final state where the two reactions (36) have identical occurrence probability. The concentration of X_L in this final state results

$$x^* \equiv x(\infty) = \frac{1 + 2\beta}{2 + 2\beta} \quad (42)$$

meaning, for instance, that if the gases have equal size (i.e. $\beta = 1$) then $x^* = 3/4$. Thus, only a fourth of the X molecules can be employed to produce “useful work”—moving Y molecules to the right chamber. Eq. (39) further shows $[Y_d](\infty) = 3/4$, and indeed confirms, that the number of Y molecules in the right chamber has incremented by a fourth, with respect to the initial condition (38). Instead, if the X gas possesses a much larger size than the Y gas, i.e. $\beta = \infty$, then $x^* = 1/2$ meaning that the X gas is not hindered by the presence of Y molecules and can thus spread uniformly through the two chambers.

Eq. (42) is shown in the inset of Fig. 5. Finally, note that the time taken by the system to reach the final state depends on both α , the rate at which both molecules are loaded and transferred to the other chamber, and the system size ratio β . A larger α makes the system reach equilibrium faster but does not affect the final system state. A larger β speeds up reaching equilibrium as well, in that a large number of X molecules facilitates driving the Y molecules towards a disequilibrium, and also affects the final state in a way described by Eq. (42).

5.3. Thermodynamics of the CDC dynamical model based on the above mechanistic analysis

Analyses of coupled chemical reactions do not usually adopt a mechanistic perspective, like the one in the previous section, but instead rely upon the formalism of thermodynamics which provides general principles of wide applicability. However, in the past essentially nothing could be said about the out-of-equilibrium behavior of these reactions, as classical thermodynamics can only analyze equilibrium states or systems at most linearly perturbed from their equilibrium state,

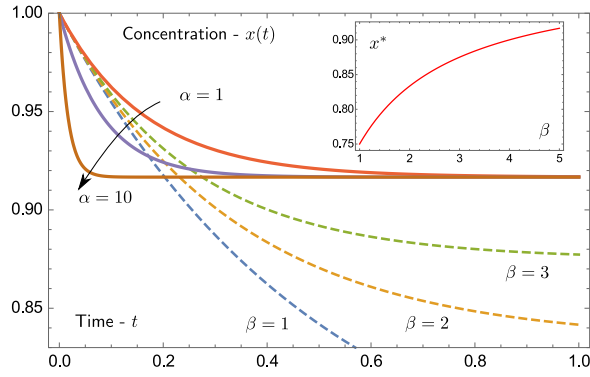


Fig. 5. Dynamics of the concentration of X molecules in the left chamber, $x(t)$, as predicted by Eq. (41). Dashed lines are obtained for $\alpha = 1$ for various β (see figure). Solid lines are obtained for $\beta = 1$ for various α (orange, $\alpha = 1$; purple, $\alpha = 2$; brown, $\alpha = 10$). (Inset) Concentration of X molecules in the left chamber at the steady state, x^* , (i.e., when the engine stops converting disequilibria), as a function of β [see Eq. (42)].

this latter case being called the Onsager's regime. However, we here show that once the system dynamics have been fully obtained, as we did in Eq. (41), the thermodynamics of the model can be inferred without any restriction, and the Onsager's regime recovered as a specific limiting regime. The approach described below follows the classic book by Hill [71].

Before initiating the analytical treatment, let us recall that the two gases, in the absence of mechanical coupling, spread uniformly among the two chambers. Thus, the CDC model *without* mechanical coupling is equivalent to the chemical reactions



As we already remarked, in our model these reactions describe molecule displacements between the two chambers, but we can easily imagine that in real engines they signify processes that also involve chemical change. The crucial points are that the two reactions are mechanically coupled which makes their kinetics related, and that the first reaction is prepared in a non-equilibrium state whereas the second reaction is not. Therefore, the second reaction is driven “up-hill” due to mechanical coupling and the equilibrium relaxation of the first reaction.

To each of the (43), we associate the chemical potentials:

$$\chi_1 = -k_B T \log \left(\frac{[X_d]}{[X_L]} \right) + \chi_1^{eq}, \quad \chi_2 = -k_B T \log \left(\frac{[Y_d]}{[Y_L]} \right) + \chi_2^{eq}, \quad (44)$$

where k_B is the Boltzmann constant, T the absolute temperature and the constants χ_i^* ($i = 1, 2$) are chosen such as when the (uncoupled) reactions are respectively in equilibrium, their chemical potentials read respectively zero. For our model, it is convenient to omit the temperature ($k_B T \equiv 1$) and set $\chi_i^{eq} = 0$ ($i = 1, 2$) which imposes the equilibrium states of the uncoupled reactions by having the molecules equally spread among the two chambers.

We now consider the system in presence of mechanical coupling. We have seen that in this case the model reduces to a single-variable model, thus, considering the first chemical potential, we introduce the notation $\chi \equiv \chi_1$, and express every concentrations in the first of Eqs. (44) in terms of the variable x . This leads to

$$\chi = -\log \left(\frac{1-x}{x} \right) \quad (45)$$

which can be inverted to give a relation between the concentration of X molecules in the left chamber and the chemical potential of the corresponding reaction, in the absence of mechanical coupling:

$$x = \frac{e^\chi}{e^\chi + 1}. \quad (46)$$

These two relations can be used to obtain the thermodynamics of the model. In presence of mechanical coupling, the initial disequilibrium in X drives a disequilibrium in Y . The driving occurs until $x = x^*$, as shown in Eq. (42). Using Eq. (45), we find that this condition corresponds to a chemical potential difference between the top chambers:

$$\chi^* = -\log \left(\frac{1-x^*}{x^*} \right) = \log(1 + 2\beta). \quad (47)$$

In the absence of mechanical coupling the system would relax to equilibrium, i.e. $\chi = 0$, because the first of reactions (43) would occur without being affected by the state of the Y gas. Therefore, Eq. (47) measures the displacement from the equilibrium state, due to the fact the system is coupled.

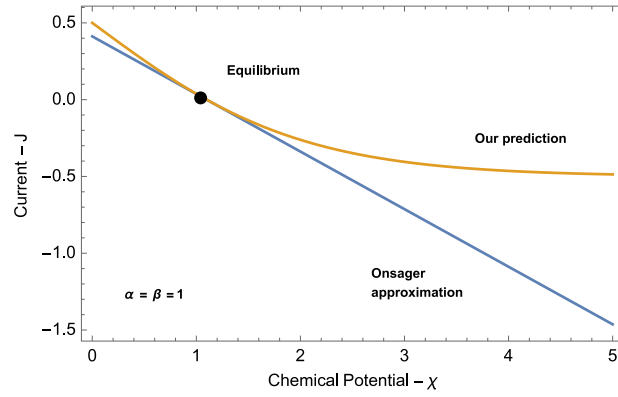


Fig. 6. Current vs. chemical potential. Yellow line: the flux of particles J is shown as a function of the driving chemical potential χ , as predicted by Eq. (48). The blue line corresponds to the Onsager approximation $J = L\chi$, where L is given by Eq. (49). The Onsager's approximation yields an accurate approximation around the steady state χ^* (black dot).

The system dynamics can thus be seen as a change in chemical potentials from the initial $\chi(0)$ to the final χ^* . The chemical potential gradient causes a flux of particles, represented by a current J . Since the rate of change of x molecules is given by its time derivative, \dot{x} , it is natural to make the identification $J = \dot{x}$. The expression of the current in terms of the chemical potential can be obtained by applying Eq. (46) into Eq. (40):

$$J(\chi) = \frac{1}{2}\alpha \left(-\frac{2(\beta + 1)e^\chi}{e^\chi + 1} + 2\beta + 1 \right). \quad (48)$$

This equation gives the non-linear thermodynamics of the system. Recall that non-equilibrium thermodynamics is characterized by fluxes of physical quantities, caused by entropy–density gradients called forces [10,11,33]. In our chemical system, the chemical potential χ is the force that drives the flux J . The product force time flux, $\dot{S} \equiv \chi J(\chi)$, defines the rate of change of entropy in the system and thus yields the thermodynamic description of the system's dynamics. Note moreover that $J(\chi^*) = 0$, consistent with what we expect. The Onsager's regime is recovered by considering the system close to equilibrium, that is, $\chi - \chi^* \sim 0$. Under that approximation we have:

$$J(\chi) \approx -L(\chi - \chi^*), \quad \text{where } L = \frac{\alpha}{4} \frac{2\beta + 1}{\beta + 1} \quad (49)$$

is the coupling Onsager coefficient. The comparison between Onsager approximation and non-linear thermodynamics is shown in Fig. 6.

6. Summary discussion of the CDC toy model

6.1. Key points made by the model

1. Changes take place in nature if and only if the process dissipates a condition of disequilibrium; that is, increases the configuration/microstate count, equivalently the entropy, of the 'universe'; which in this model case is the physical system bounding the process, but in general is that of the system plus its surroundings. That is so merely because in a system not in equilibrium – that is, whose microstate count is less than it could be – the particle-level events which lead to that increase are more probable than those which act in reverse. As a result, it is reasonable to say that it is the increase in the microstate count itself that causes the process to happen.
2. In any such process, however, events at the particle level are inherently stochastic and 'fluctuating', with the result that those which move the system further from, rather than towards, equilibrium, i.e. in its 'backward', entropy decreasing, direction, inherently occur along with the 'forward' events moving the system towards equilibrium. In a spontaneous stand-alone process, of course, the latter events must, on average, outweigh the former.
3. Nature, life most spectacularly, is nonetheless preoccupied with creating states of disequilibrium, necessitating that specific processes be made to run predominantly in their backward direction. Yet individual backward events cannot be forced to occur; they can only be selected once they happen – produced by random 'thermal' fluctuations – by chance. Furthermore, the required selection can only take place in the converse; that is by blocking the effects of fluctuations that would move the system in its more probable, entropy increasing, forward direction thereby reversing the desired backward events. That is, there is no way to selectively cause the thermal fluctuations you want; all that can be done is to block the effects of ones you do not want and trap the one's you do (though the 'trap' only works if the overall probability of the final state is greater than the starting one).

4. But the system must not just preserve individual backward fluctuations by blocking fluctuations that would reverse them, it must accumulate them; that is, it must follow such events with a (more-or-less) irreversible transition to the state in which the system can initiate a fresh conversion cycle.
5. The core requirement therefore is for a subsequent transition, after the completion of a “backward” event in the driven process, to a state: (1) that is itself closed to reversing that completion (in the model, with the portal for the driven process no longer open to the output side), (2) whose own probability of being reversed – per unit time – is less than the probability of the reversing reaction in the driven process, and (3) which either is, or permits the system to transition to, the state in which it can initiate a new conversion cycle.
6. It is here, of course, that the irreversible aspect of the driving process (of, e.g. ATP hydrolysis) plays its essential role. By having the system’s escapement mechanism require, as in our toy model, that it can only make a ‘flip’ transition from the ‘catalysis-and-product-release’ configuration back to the ‘load reagents’ configuration, if both driver and driven reactions are complete (in the model, if both portals are empty facing in the product release direction), and if, in addition, the probability of reversing the driving reaction from that side is less than the probability of reversing the driven reaction, then, on average, the cycle will complete in the direction that converts the driver’s disequilibrium (in part) into a disequilibrium in the driven process.
7. It is tautological to say that the sequence of events in this engine cycle will, on average, spontaneously proceed in the direction that has the greater probability of occurring; and likewise that, in doing so, it will perforce satisfy the 2nd law—creating, in each cycle (on average), a joint ‘macrostate’ that is more probable (equivalently has a larger entropy, i.e. comprising a larger number of microstates). In this evident fact, however, whether in describing a joint process carrying out a conversion of disequilibria as we have just done, or a single, merely dissipative process, is the inference that the 2nd law is both ‘trivial’ and at the same time the generative cause of all change—including “creative”, order-producing change.

6.2. *Main inadequacies of the model*

We next note the principle respects in which the CDC model is unsatisfactory as a representative of all disequilibria converting engines, molecular-level ones especially. The more consequential of these are addressed briefly in subsequent sections.

1. The model leaves unexplained how the escapement mechanism is actually embodied in real converters. Within the model’s own frame of reference it is unexplained, in particular, how the ‘flip’ action is made to depend on the loading status of the portals; in more realistic converters, which have more states in their operational cycle, this issue extends to the need to understand what causes and controls each of the transitions of the cycle.
The most important missing aspect of this question is how the escapement enforces the gating requirement whereby the completion of the driving process is made to be contingent on the completion of the driven one. In the toy, in which the two completions must never take place separately if the engine is to return to the input side of its biphasic cycle this rule is simply posited and its mechanistic underpinnings are neglected. In subsequent sections we attempt in various ways to clarify this question, particularly in the context of somewhat more realistic generalizations of the CDC model.
2. Real converters are both less perfect and more sophisticated – including more articulated – in their operation than is our toy model, and some of these differences are important even at the abstract level. First, as to ‘imperfections’, most importantly real engines do not completely suppress all undesirable transitions, and have various kinds of ‘leaks’. This issue we will not treat further and refer to the classical ‘NET’ analysis of these points [10–12,33]. The issue of sophistication of design, however, is one we cannot neglect. In the next section we extend the CDC model to a more biologically realistic version in which, for example, a minimum of six transitions take place within the conversion cycle. This brings our analysis into closer alignment with the classical linear NET analysis of free energy converters just cited and allows us to discuss an apparently universal, and evidently highly important feature of biological conversion not captured in the CDC model; namely the use of “interleaved” half reactions and on that basis of “kinetic trapping” (a feature invoked in the opening Hoffmann quote). This latter, highly important point, we introduce next.
3. Kinetic trapping: molecular-level free energy converters face the inherent problem that the greater the disequilibrium created in the driven process, the greater the chemical potential against which they are required to expel the products of the driven reaction, and therefore the greater the frequency with which those products rebind to the converter before it has progressed on to a form in which that rebinding is prevented.
In the simple biphasic CDC model, the escapement device simply samples the equilibrium fraction of states in which both the driven and driving reaction’s product ‘binding’ sites (portals, in our model) happen to be coincidentally empty. Biology, on the other hand, seems to everywhere improve on this design by using a system that can ‘kinetically trap’ the completion of the driven reaction thereby reducing the “rebinding” problem. This appears to be generally achieved by separating the completion half of the two reactions into distinct states or stages in the converter and having one, that of the driven reaction, precede, and be what triggers, the other.
4. The toy engine is missing the (disequilibria) sources and sinks it would need to keep running and to be useful. As presented, our model is a stand-alone system which has to be hand-started in a useful disequilibrium state after which

it merely runs down to its steady state stable point. In contrast, of course, useful engines must be embedded in a hierarchical cascade of engines in which it stands between a superordinate 'power source' engine that maintains the engine's driving disequilibrium (as e.g., the electron transport chain maintains the trans-membrane proton concentration disequilibrium that can then be used to drive the production of the ATP disequilibrium), and one or more "loads", often subordinate engines, which are driven by, and thus act to deplete, the driven disequilibrium (e.g. all of the conversions in metabolism that are driven by the ATP disequilibrium). We consider this issue very briefly, and only diagrammatically, in Section 10.

5. In the model as so far presented, in which the events involved are merely single particle transits through a barrier, the effects of reaction kinetics are neglected. If, as mentioned briefly above (Section 3.3.3) however, one or both of the processes being coupled are chemical reactions, say $R \rightleftharpoons P$ and/or $S \rightleftharpoons T$, a 'transit' event may or may not involve a literal trans-membrane transit into a chemically distinct environment. But in either case the event probabilities are not just proportional to 'reactant' concentrations, but that probability times the probability that the reactant(s) will be activated (by thermal fluctuation interactions with the bath) after they are loaded. In the conversion context, this introduces kinetic constraints in addition to (equilibrium) thermodynamic ones. Such effects become particularly material if, e.g., some consumption process is acting to remove the products of the driven reaction yet the concentration of those products is functionally important.

7. The architecture of real bio-molecular disequilibrium converters; moving beyond the assumptions of the CDC toy model

The CDC model involves a cycle consisting of four states: two conformations (left or right-facing) each of which can exist in two states (portals loaded or empty). As we have noted above, real converter engines in biology appear to invariably involve more articulated cycles, comprising transitions between a minimum of six states. This reflects in part that in such engines the loading of reactants and unloading of products for the individual reactions is in general carried out by distinct steps in the cycle that are sequenced in a specific order. This modification breaks both the loading and unloading sides of the cycle as depicted in the CDC model into two separate steps yielding six steps in the cycle rather than four. In this section we discuss these more complex designs and why they appear to be dictated by considerations of operational efficiency.

Of course, these more complicated designs, in having more distinct and strictly ordered transitions, greatly increase the challenge in understanding the mechanisms that enforce the sequential conditionality of these transitions. In other words, the centrality of 'transition control' now becomes much more salient. And transition management is not a simple problem; neither in molecular disequilibria conversion nor in life in general; complex and important enough, as the classical Greeks understood, to have its own God, namely Janus.

7.1. Janusian enzymes; Hill diagrams, and the Jencks "interleaved half-reactions" analysis



Janus: the God of Transitions

We invoke Janus here to emphasize a point made before, namely that the macromolecular devices mediating the conversion of disequilibria are not just 'enzyme catalysts', but instead true engines which must step through a specific ordered cyclic sequence of different enzyme alternatives each having its own distinct binding and catalytic specificities. The transitions between these alternative forms, and how those transitions are controlled, necessarily fall within the remit of Janus, and they comprise the beating heart of the conversion process.

To describe the simplest possible six-state conversion cycle, consider (following Hill [77]) that the task is to convert disequilibria between two simple reactions $R \rightleftharpoons P$, and $S \rightleftharpoons T$ where we arbitrarily assume that the second reaction is the one being driven in its 'back' direction $S \leftarrow T$, thereby creating a disequilibrium, whereas the first reaction is the driving reaction and therefore proceeding in its disequilibrium-dissipating direction, taken to be $R \rightarrow P$. A simplified "Hill diagram"[11] of the six steps in the conversion cycle is presented in Fig. 7 wherein, following convention, we represent the assumed forward direction as a counter-clockwise transit.

Implicit in the Hill diagram is that step #3, the conformational flip from E to E^* , enables the completion half-reactions for both processes but only in a particular sequence: first the completion of the driven reaction $T_{bound} \rightarrow S_{bound} \rightarrow S_{free}$ then that of the driving reaction $R_{bound} \rightarrow P_{bound} \rightarrow P_{free}$. We note here that even more complex designs, having more separated steps in the cycle, are commonplace; we briefly discuss two examples in Section 8. However, what appears to be invariant is that the final product release step for the driving reaction, serving as the transition that introduces the dominant element of irreversibility in the cycle, is invariably the last step prior to, and acting as the trigger for, the conformational transition back to the starting state. When, for example, ATP hydrolysis is the driving reaction, the product release step often involves the

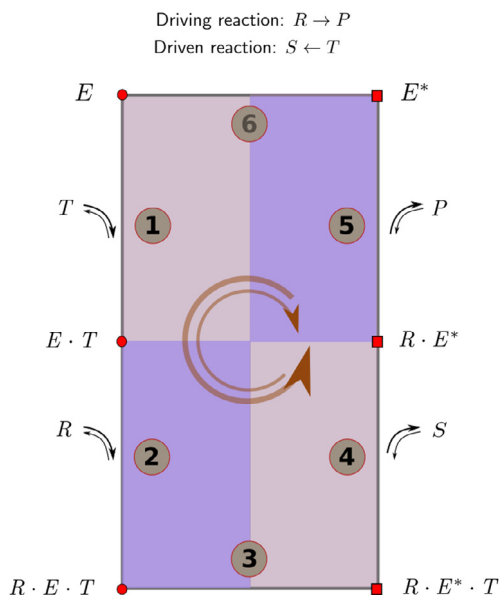


Fig. 7. Hill diagram for a simple, 'leakless' conversion.

In this diagram (here maximally idealized in that all 'crossing' reactions connecting the nodes in the diagram are suppressed), the exergonic reaction $R \rightarrow P$ is driving the endergonic 'back' reaction $S \leftarrow T$; the sequence starts in the upper left-hand corner and proceeds counter-clockwise; E and E^* represent the complex in its two major "allosteric" conformations: mediating 'loading/binding' (E) and 'catalysis and product release' (E^*) respectively. The transition between these forms corresponds to the left–right orientation flip transition in our CDC toy model. As drawn, with the loading phases beginning with the driven reactant (T), followed by the driving reactant (R), and with the release of the driven reaction product (S) occurring before the release of the driving reaction product (P), the process reflects the Jencks interleaved half-reactions cannon mentioned above; both in having the two processes segmented into interleaved half reactions, and in the particular ordering of the half reactions (the colors of the diagram's quadrants are meant to reflect the 'crossing', 'interleaved' flow of the two reactions: steps 1–4 for the driven reaction, 2–5 for the driving reaction). In the toy model, by way of contrast, in neither of the two engine phases (open left and open right) was there any distinction made or required as between which process, driver or driver, went first—since they were on both sides obliged to act together. On the other hand, the "flipping" rules here are exactly what they were in the toy model; that is, the engine can flip between its E and E^* conformations only if both processes are either "loaded" (step #3) or "empty" (step #6). It is just that now the cycle has separated the loading/binding and catalysis and product release steps for the two processes and imposed ordering rules on these separated steps.

'simultaneous' release of both ADP and Pi, but not always. The release of the last one of the products to be released appears to always be the final, and triggering, step before conformational reset, and in that the step in the full driving reaction that makes the major contribution to the thermodynamic force driving the cycle forward. Note that whereas it is not necessary that all steps in the cycle be exergonic, and they are in general not, the whole cycle must of course be if it is to proceed forward (i.e. $\Delta G_{\text{cycle}}/k_B T < 0$). These points emphasize, we note in passing, that conversion cycles are not, and could not be, equilibrium chemistry.

Reflecting this fact, if satisfying the 2nd law were the only thermodynamic design criteria effecting the engine it would be of little interest to life. This for two reasons, both having to do with kinetic aspects of the cycle. First, the cycle's net forward speed is proportional to $e^{-\Delta G_{\text{cycle}}/k_B T}$, (the ratio of the cycle's overall forward to reverse reaction rates) [11,23,35]; that is, the more each cycle increases the entropy of the universe, the faster it goes. Second, and more consequentially so far as mechanism goes, the release of the products of the driven reaction presents an intrinsic kinetic challenge, since the system must reject the products directly into the wind of a high back reaction rate—over which it has no control (and which, moreover, it is working to worsen). Yet the system must have successfully rid itself of the products of both reactions as a precondition for the return of the engine to its starting state, and with as little waste of time and driving free energy as possible. Therefore kinetics matters, and the faster and more decisively the system can respond to having an 'empty' binding site for the driven reaction's products by transitioning 'irreversibly' to a state in which it can to 'flip' back to its starting conformation, the better.

How can the above requirements be met in a 2nd-law-consistent manner, and how are they met in real biological converters? One qualitative aspect of the answer to this question is that the complex is obliged to change its enzymatic "specificities" at each step in the cycle, not just in the alternation between the two major forms that are conventionally indicated—here E and E^* . Fig. 8 is meant to bring this aspect forward.

These two diagrams provide a useful picture of the sequence of events that make up an operational cycle of a simple but representative molecular-level disequilibrium conversion engine. But they beg the question of what makes the engine change its enzymatic properties, its binding and catalytic "specificities" (in Jenck's terminology), in the specific sequence required, as it steps through each of the stations of its cycle. We take up this subject next.

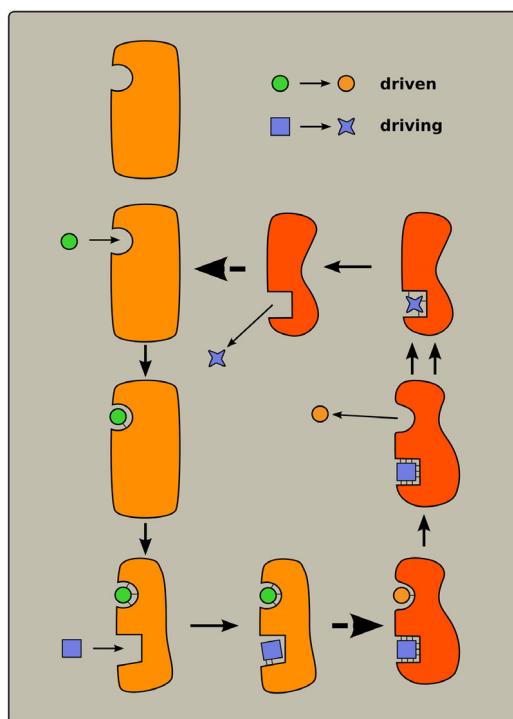


Fig. 8. Specificity transitions in Janusian Enzymes.

In its starting state (upper left) the complex can only bind the substrate for the driven reaction (green sphere); subsequently it can only bind the substrate for the driving reaction (blue square), both of which reactions can involve ‘induced fit’ mechanisms; these binding reactions, taken together, drive the major conformational change in the complex (orange to red body color), completed in the right bottom state. In this state the complex is enzymatically active to complete the driven reaction (sphere going from green to orange) and to permit the release of that product—whose release is in general taking place against the back pressure of a high chemical potential. Subsequently, the system becomes enzymatically active to complete the driving reaction through to product release; these steps, the product release predominantly, constitute the ‘irreversibility’ (free energy change) driver responsible for the forward motion of the cycle, but also for providing a ‘latch’ acting to kinetically block the reversal of the driven process.

7.2. Substrate binding and the control of the conditional transitions in the conversion cycle

In Section 3.4.4, we summarized in general terms the main points regarding the role played by events of substrate binding and unbinding in controlling the transitions in a conversion cycle. We here reframe the main points as they apply in the explicit context of realistic, multi-step conversion cycles.

In the simple six-step conversion engine pictured in Figs. 7 and 8, the complex becomes, in a specific order, six distinct enzymes. Notably, each of these transitions is preceded by a distinct ‘substrate’ binding or unbinding event. This is the decisive clue as to how these system’s work. All of these binding-change events involve ‘induced fit’ interactions between the substrates and the protein to which the substrates are binding; in consequence the conformational and other properties of the protein change at each step, as we have tried to suggest in Fig. 8. A highly relevant and illustrative example of this kind of process, namely the binding and unbinding events in the active site of the F1 motor of ATPsynthase, has been quite thoroughly characterized by Oster and colleagues [46,78]. There, the binding of ATP (alternatively of ADP and Pi), involves forming a dozen or so weak bonds through a ‘zipper-like’ mechanism which is progressive, ordered, and cooperative. This binding sequence drives a correspondingly progressive, and ultimately substantial, elastic deformation of the protein partner, and involves a significant conversion of the free energy of the bond formation to the (more or less) reversibly stored elastic free energy of conformational ‘distortion’ in the protein (which to say that these ‘induced fit’ binding processes are conversion engines themselves). On the other hand, while similarly dramatic conformational responses on the complex’s part have very regularly been observed and shown to be mediating transitions in conversion cycles, drama is not required. As Koshland has noted in his essay *Conformational changes: how small is big enough?* [66], very minor changes in a protein’s conformational state can have very large, and even very distant, effects on its binding and catalytic specificities [65].

What is essential is the point given particular emphasis by Jencks [12,55]. This is that the quasi-reversible inter-conversions of some part of the *intrinsic* binding free energies of substrates, as bonds are either formed or broken, to ‘distortion’ free energies stored in changes in the complex’s conformation, both impose the transition rules of the cycle, and enable the catalytic acceleration of the conversion process [54]. Usefully, molecular level understandings of these interactions are now emerging at a brisk pace (see examples given in the literature cited in Section 11).

7.3. Apparent advantages of the Jencks canonical ordering

What explains the apparent preference in biology for the Jencks ordering, especially the apparently obligate preference for its most counterintuitive element, namely having the driving reaction only take place, or at least only complete, after the driven process has completed? We first consider the ‘product release’ side of the cycle.

An essential requirement of a conversion cycle, as Jencks argued, is that neither process be allowed to complete within a cycle unless both do. This means, as our CDC toy model posited, that the ‘flip’ in the system’s conformational state back to its starting configuration must be conditional on both reaction’s having completed through to product release. In other words, the state of both product binding sites being empty must be the gating condition for the ‘return to go’ conformational resetting. However, the two product release reactions typically face dramatically different reassociation rates (over which the system itself has no control): very high for the driven process and very low for the driving one. This difference has two practical implications. First, if the driving reaction’s products are released without the driven reaction’s product binding site also being empty then with high probability that cycle will have been a futile one, and the precious resource of an instance of the driving disequilibrium will have been wasted. This by itself argues for making the completion of the driving reaction only become possible once the driven reaction has completed to the point of having emptied its product binding site. Second, every instance of product release from the driven reaction that is reversed before the system has moved to a state in which that reversal is no longer possible wastes time and increases the risk of a wasted cycle. The premium is therefore high on not allowing that reversal to happen, on having, that is, a way to kinetically trap such a release as soon as it happens. But that is exactly what the completion of the driving reaction, because of its low probability of being reversed, is able to do. Moreover, absent this kinetic trapping effect the completion of the driven reaction would be governed by equilibrium chemistry and thus not able to drive a disequilibrium greater than (in practice even approaching) the K_m for the release of the driven reaction’s products. Therefore, the optimal design on the ‘product release’ side of the cycle is to not allow the driving reaction to complete until after the driven reaction has done so, and have that final step, when triggered by the release of the driven reaction’s product, occur as rapidly as possible.

As to the input half-reactions side of the cycle two considerations seem likely to be in play in dictating a preference for the Jencks ordering. First, having the driven reagents load first as a precondition for the loading of the costly driving reagents spares them from being removed from the pool and from being placed in ‘harm’s way’ until there is certifiably something for them to do. Second the full ‘canonical’ Jencks interleaved half reaction sequencing pattern has the property that it is functionally invariant to a reversal in the cycle’s direction. That is, upon reversal, whereby the driver and driven processes exchange roles, it preserves the property just discussed, namely that the completion half reaction of the driving reaction should directly follow, and be triggered by, the completion half reaction of the driven reaction. This observation suggests that even in cases in which the sequencing of the input loading/binding steps is not known to be, or is thought not to be, canonical Jencks (i.e. that the driver loads before the driven is permitted to), that it likely is nevertheless.

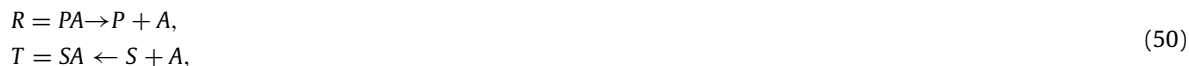
We need to emphasize, however, that many biological converting systems have mechanisms that are a good deal more complex than is reflected in the simple six-step model considered here. Some of these involve multiple driving reactions and also multiple and separated ‘internal’ steps (e.g. in-situ catalysis and/or moiety “parking” reactions), as well as multi-step product release reactions in both driven and driving processes, all carried out by distinct “specificity” forms of the ‘Janusian enzyme’ (see the discussion of a variety of different types in [57]). However, what appears to be an invariant is the interleaving of partial reactions in such a way that none of the processes involved can complete until all do and specifically that the completion of any of the driven reactions serves as the immediate trigger for the completion of at least one of the driving reactions. As a result one of the latter is invariably the final step preceding, and triggering, conformational reset, and the benefits of kinetic trapping are made available to each driven product release step.

8. Disequilibria conversions involving molecular exchange

The toy CDC model, and essentially all of the preceding discussions, have more-or-less implicitly assumed that when two chemical reactions are partners in a disequilibrium conversion process, they are chemically independent. However, disequilibrium conversions involving the exchange of a molecular moiety between driving and driven chemical reactions are ubiquitous in biology and indeed often presented as the only way “free energy conversions” take place (e.g. [79, p. 117]). How do such processes fit into the present discussion of disequilibria conversion?

Consider again two processes, represented as $R \rightarrow P$ and $S \rightarrow T$, under conditions in which the first is exergonic, the second endergonic (i.e. $T \rightarrow S$ is exergonic).

Further, consider that the two processes are chemical reactions sharing a common molecular moiety “A”, in particular that R is P bound to A : $R = PA$, and T is S bound to A : $T = SA$, and that the exergonic reaction is the dissociation of A from R to form P , its endergonic partner the association of A with S to form $T = SA$:

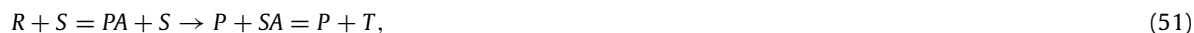


where here the right (left) facing arrow indicates an exergonic (endergonic) process direction.

As noted, biology is replete with instances in which two such reactions are “coupled” whereby the first both drives, and also donates a molecular moiety to, the second. Many of the so-called “energy conserving” reactions of biochemistry are

of this type. Note, however, that in such conversions the driving reactant $R = PA$ serves two conceptually and physically independent functions: a ‘chemical’ function in which it merely acts to supply the exchanged moiety A , in which service only the concentration of PA is relevant, and a ‘thermodynamic driving’ function in which the disequilibrium of PA vs. that of P , namely the ratio $([PA]/[P]) / ([PA]_{\text{eq}}/[P]_{\text{eq}})$, is essential in that without it the overall reaction would not go—no matter what the ‘standard conditions’ free energies have to say on the matter. That these are physically distinct functions is easily overlooked, particular when it is incorrectly imagined that what is needed to endergonically produce $T = SA$ is energy, and that this energy is supplied by the exergonic breakup of PA .

In our present notation, the coupled disequilibrium-converting exchange reaction is:



which, if it is overall exergonic in the indicated direction, would proceed spontaneously.

But how in fact can this happen? By what mechanism does the exergonic occurrence of the dissociation $R \rightarrow P + A$ force the endergonic association reaction $S + A \rightarrow T$ to take place? And why is it that the moiety ‘ A ’ liberated in the first reaction does not take what in solution would be the path of highest probability and escape into the bath? We trust it will now be accepted that this does not take place because “energy released by” the first reaction (nor even the “free” part thereof) is somehow transferred to the second.

Clearly, if the conversion reaction takes place within an enzyme’s embrace the escaping moiety problem can be avoided. But to accomplish the thermodynamic conversion more than an enzyme catalyst is required just as in the cases earlier considered in which the two processes being converted are independent and involve no shared reagents. In particular we need a conversion engine that will catalyze the dissociation reaction but prevent either of that reaction’s products from dissociation from the engine until (and unless) the driven “association” reaction, and the release of that reactions product “ T ” has completed. Critically, it is the “irreversible” release of P into the medium, triggered by the release of T , that serves to statistically trap the endergonic “back-fluctuation” event that created the T product of the driven reaction.

The following sequence indicates a ‘Hill/Jencks’ cycle that has the desired properties.



The fourth reaction is the dissociation of R to $P + A$ with both parts remaining bound to the enzyme; the fifth is the driven, ‘backward fluctuation’ reaction, drawn right-to-left to indicate that fact. The highly unstable product of that transition must then be quickly trapped by the release of its product T and finally, as triggered by that release, by the ‘irreversible’ release of the driving reaction’s product P (indicated with the double arrow) and the completion the conversion cycle.

We note that it the above analysis does not require that in any literal sense the coupling reaction proceed through two physically separable half reactions. In any case the reaction must pass through a thermal fluctuation-induced transition state in which A is dissociated from the donor but not yet bound to the acceptor.



where $(R \cdot A \cdot S)^\ddagger$ is the excited state from which the system can relax in either direction, i.e. either back to $RA + S$ or forward to $R + SA$ (see the discussion of the specific case of 2-electron redox reactions presented in [72]). The requirement that the engine mediating this conversion must satisfy is that the inherently much more likely (and unless blocked much more frequent) dissociation reaction must not be allowed to complete until the up-hill dissociation reaction has completed and escaped the complex.

We conclude this discussion by considering the explicit example of hexokinase along with a brief mention of a common mechanism of intermediate type; that in which the hydrolysis of ATP, while complete within the cycle, takes place in two internal, separated steps involving the transient phosphorylation of the enzyme. For this we use the example by calcium-pumping ATPases.

8.1. Hexokinase

Perhaps the most intensely studied example of moiety-exchange disequilibrium conversion entails the endergonic formation of glucose-6-phosphate ($G6P$) by hexokinase enzymes, in which ATP plays the two functionally separate roles we noted just above: a chemical role as the phosphate donor in which only the concentration of ATP is important, and the exergonic driver role in a disequilibrium conversion in which ATP’s (free energy of) disequilibrium is the essential property.

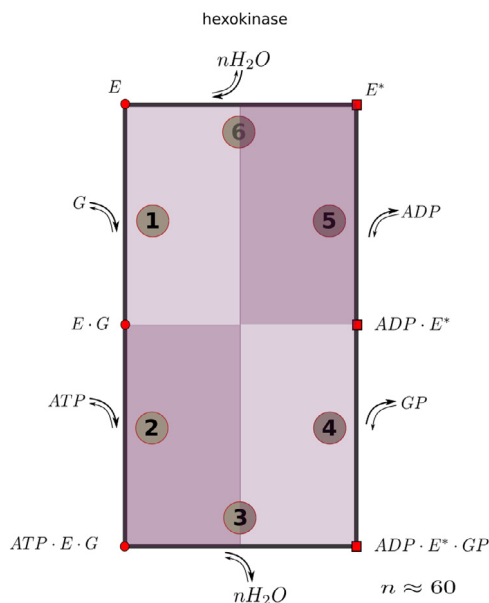


Fig. 9. Hexokinase conversion cycle.

The ATP-driven formation of G6P appears to conform to a canonical Jencks design in all important respects including that the completion in the driving reaction, namely the net free-energy-yielding (irreversibility providing) release of ADP, is triggered by, and therefore only allowed to take place subsequent to, the completion of the driven formation of G6P through the release of that product.

Source: Adapted from [79, p. 117].

The driver, driven, and net reactions, along with their standard conditions ΔG values are:



where we note that under physiological conditions, the ΔG for the hydrolysis of ATP is much stronger; approximately -55 kJ/mol . However, since the phosphate is not released in the above kinase reaction the relevant driving disequilibrium is that reflected simply in the physiological chemical potential difference between free ATP and ADP.

What is known about how the mechanism by which hexokinases mediate the above conversion? It is first of all well established that in carrying out this conversion the enzyme undergoes a number of specificity transitions, dominated by a major allosteric conformational transition. This transition is driven by the induced fit free energy of binding of the substrates and involves a clam shell-like closing of the protein around the bound substrates in which upwards of a hundred waters dissociate from the protein [80–84]. Further, it now appears to be resolved that the binding of glucose proceeds, and triggers, the binding of ATP [82]. Finally, and most tellingly for the current discussion, it also seems now resolved that in the reaction cycle, ADP is released from the complex (only) after the driven product, G6P, is released, and is in turn the trigger for the conformational relaxation back to its starting/resting state [85]. This is noteworthy because in this case, it is predominantly through the release of ADP that the driving reaction contributes free energy (in our terms provides a ‘latching’, quasi-irreversible transition) to drive the conversion cycle forward.

Thus, this mechanism appears to conform to the conceptions advanced here as to how disequilibria are converted, and more specifically to the ‘canonical Jencks’ ordering of the half reactions for the two processes, as is pictured in Fig. 9. It is not, in other words, “just chemistry” and would never take place as a solution reaction or if otherwise deprived of the ‘bespoke’ engine functions that hexokinase provides for this specific conversion reaction.

8.2. Plasma membrane calcium-pumping ATPases

In this conversion, two Ca^{2+} ions are pumped up-hill across the plasma membrane driven by the hydrolysis of one ATP. However, the cycle involves the transient, and reversible, phosphorylation of the protein – temporarily ‘parking’ the phosphate – in a step which triggers the protein’s major conformational ‘flip’. Though some uncertainty regarding the ordering of the binding steps apparently remains, a mechanism published by MacLennan et al. [86] is overall consistent with most alternatives and posits the following steps (see Figure 1A of that paper): (1) facing ‘inward’ the protein has high affinity for, and binds, two Ca^{2+} ions, displacing two protons; it then binds ATP; it then transfers ATP’s terminal phosphate to a binding site on the protein and releases the ADP into the medium; this induces, and serves to drive, the protein’s

major conformation flip to be now facing 'outward' in which state it has very low affinity for both calcium ions, and in this state the (endergonic) release of the calciums (replaced by two protons) to the outside "blowing against the wind" of the high external Ca^{2+} concentration can take place; when that release has been completed and the Ca sites are empty, the protein dephosphorylates, releasing the phosphate into the medium in a strongly exergonic step. We note that in its essential features, this mechanism is orthodox Jencks. Indeed somewhat earlier Jencks presented an analysis of the same conversion process and propounded essentially the same model [12]. In any case, there appears to a consensus that the strongly exergonic release of the phosphate occurs after both transported calcium ions have been released and their binding sites are empty. This fully empty state (of the phosphate and both calciums) is, as in the toy model, what triggers the return of the protein to its starting conformation. And again we have an instance of the driving reaction not completing, and its major (free energy) driving irreversibility not being deployed, until after the work is done.

9. A summary of the main qualitative conclusions regarding disequilibria conversion

- All disequilibria (aka free energy) conversions require the mediation of an "engine" whose essential function is to act as an *escapement* mechanism; this mechanism makes the completed "down hill" passage of a metered amount of the driving flux dependent on (requires it to be gated by) the completion of a metered amount of the driven flux. The fundamental and essential operation principle of a disequilibria converting device is the contingency rule that the driving process is only allowed to complete in a given conversion cycle if the driven process has also completed in the same cycle.
- In general, conversion engines execute an ordered cyclic sequence of distinct steps, the completion of each triggering the transition to the next. The protein complex-based engines powering life by interconverting chemical disequilibria achieve their escapement functions by moving through a corresponding series of enzyme forms, or "alters", each having its own set of binding and catalytic specificities.
- An essential operational feature of this contingent series of enzyme alters is that the two coupled processes are each segmented into "half reactions", with the halves of the two processes generally interleaved in a specific pattern, the canonical form of which is "driven first half, driver first half, driven second half, driver second half"—as first specifically emphasized by Jencks [12]. This particular sequencing allows the essential contingency rule noted in point 1 above to be mechanistically enforced in an efficient and economical manner—one which is also functionally invariant under the reversal of the coupling cycle.
- In molecular-scale conversions the escapement necessarily operates as a Brownian ratchet acting to "rectify" the thermal fluctuations impacting the driven process. In particular, the ratchet blocks the effect of thermal fluctuations that would induce an instance of the driven reaction proceeding in its 'natural', 'forward', exergonic direction, while permitting those that could induce an instances of the reaction's 'back', endergonic direction to have that effect, while at the same time ensuring that it is the event of completing the driven half-reaction that triggers the completing half-reaction of the exergonic driving process—the irreversibility of which half-reaction captures, and entropically 'pays for', the driven, endergonic one. That is, Brownian impacts from the medium produce the 2nd-law "violating", i.e. "back", transitions in the driven reaction whose preferential accumulation is the work output of the conversion process. The driving process's only *essential* role is to prevent the inherently much more probable "forward" transitions in the driven process that would undo these desired up-hill transitions, from taking place. And the engine's escapement mechanism achieves this by making the completion of an instance of the driving reaction contingent on the completion of an instance of the driven reaction.
- It is the entire conversion cycle which effects the conversion and which, in consequence, must on average (only on average) satisfy the 2nd law; the net free energy change in the cycle (as a multiple of $k_B T$) is merely minus the log of the ratio of forward to backward (2nd law "violating") transits through the cycle.
- Steps involving the binding of reactants and unbinding of products modify the properties and behavior of the engine complex including both its binding and catalytic specificities and what responses to Brownian impacts it suppresses or permits. These effects appear to typically reflect that many of the binding reactions involve 'induced fit' mechanisms which necessarily entail quasi-reversible, "elastic", stress-inducing conformational changes of the complex; 'distorting' changes which have the effect of storing potentially recoverable free energy (i.e. disequilibria). It is through these effects that binding and unbinding events:
 - Induce the transitions between the complex's specificity alters; often including a major 'flip' transition between two 'half-reaction' phases of the cycle: 'loading' and 'catalysis and product release'.
 - Accelerate catalysis, increase the rate of product release, and convey mechanical force to neighboring complex members.
 - Through product release step(s), deliver, in the case of the driven reaction, the point at which the primary work of the engine is done, i.e. forcing its product out into the teeth of a more-or-less fierce back-reaction wind, and for the driven reaction the 'irreversible' transition which provides the statistical force that moves the engine forward, and also, potentially, allows the engine to kinetically trap completions of the driven reaction.

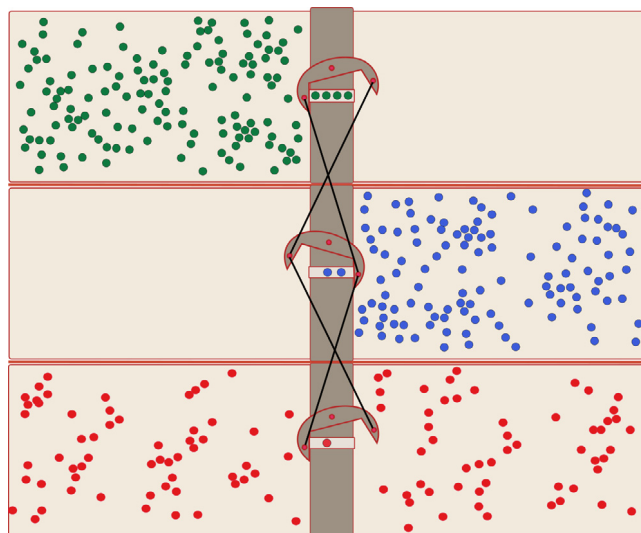


Fig. 10. CDC model with two driving processes.

The model in which two diffusion chambers operate in parallel to drive one disequilibrium generation. The figure presents a potential starting configuration in which the two driving chambers are taken to be in complete disequilibrium, and the driven one in approximate equilibrium. The two driving processes are drawn operating in opposing directions merely to make the point that the choice of direction in the diagram is irrelevant.

10. Functional architecture extensions to the toy CDC model

We here present in diagrammatic form a few elaborations on the functional architecture of the CDC model to illustrate points that arise in real-world examples. In particular we: (1) indicate how more than one process can be harnessed in parallel to drive a third, and how requiring multiple coincident events within a process can be used as “free energy amplifying” devices in conversions, (2) illustrate how steady state conversion is established through attaching the CDC model to external source and sink circuits, (3) show how engines can be arranged in tandem to amplify the effective power of a given driving disequilibrium, and (4) show a notional representation of the coupling of a diffusion chamber to mechanical work.

Fig. 10 presents a version of the CDC model in which two, potentially unrelated driving disequilibria are used in (anti-) parallel configuration (the two top compartments with, respectively, green and blue particles) to drive a single disequilibrium generating process (as before the bottom compartment). In this figure we also introduce the possibility that portal loading/unloading rules can require coincident individual particle events (here taken to be that 4 green particles, 2 blue particles, and 1 red particle must be loaded, or that all three portals are empty, before the gate can flip orientation).

Fig. 11 extends the simple CDC model to one in which the driving and driven disequilibria are connected respectively to supply and load circuits—which act to establish a steady state dynamical conversion. For example, in the OxPhos system, the proton gradient driving the ATPsynthase converter is sustained by the electron transport change while the ATP disequilibrium produced by the synthase is maintained in steady state by being dissipated through all of the endergonic loads driven by it.

Fig. 12 shows the situation in which a single externally maintained driving disequilibrium (green particles) is used in tandem to drive a two-stage serial disequilibrium conversion process, in which the output of the first stage is directly fed as input to the second.

Fig. 13 pictures a notional representation of the coupling of a diffusion driver to the production of molecular-level mechanical work. The scheme shown in this figure echoes the concepts invoked in our opening “nano-sisyphus” quote by Peter Hoffmann [1, p. 157], and illustrates the basic operating principles underlying the manner in which chemical disequilibria are converted into mechanical work by the “molecular motors” of life—most closely the cargo-hauling ones such as kinesin, wherein relatively large objects are moved through a medium in which viscous forces completely dominate.

Here, the driving disequilibrium is, as in our earlier examples, a diffusion gradient spanning a gated portal. In this case, however, the escapement’s gating mechanism is represented as a gear whose teeth when downward-facing engage a toothed rack and when upward-facing act as the gating mechanism that conducts particles through the partition with at most one particle – held between adjacent teeth – passing for every single-tooth rotational motion of the gear. The gear rotation is tied rigidly to linear movement of the rack and with that the movement of the cargo “load” (the large sphere attached to the rack’s left end), which movement, when predominantly in one direction (here to the left) is, of course, the work performed by the engine. It is further assumed that the gear is only allowed to rotate in either direction if in so doing it moves a diffusion chamber particle into the portal penetrating the chamber’s barrier (this is fairly closely analogous to the rule imposed on proton translocation by the FO motor of ATPsynthase, see [46]).

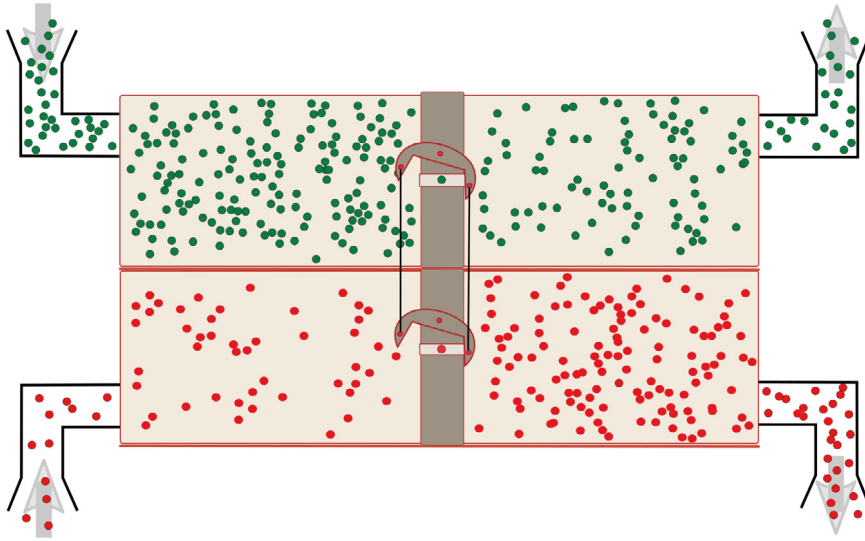


Fig. 11. CDC model with supply and load circuits. Steady state configuration of a CDC model connected to externally maintained supply and load circuits.

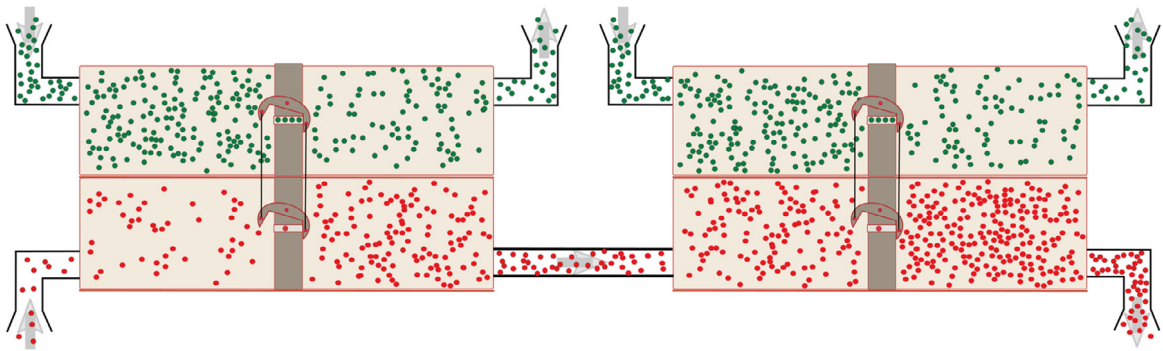


Fig. 12. Tandem escapement CDC. The same externally maintained disequilibrium, embodied in the green particles, is used to drive a two-stage tandem disequilibrium converter (the red particles), in which the output of the first stage (lower left chamber) forms the input of the second (lower right chamber).

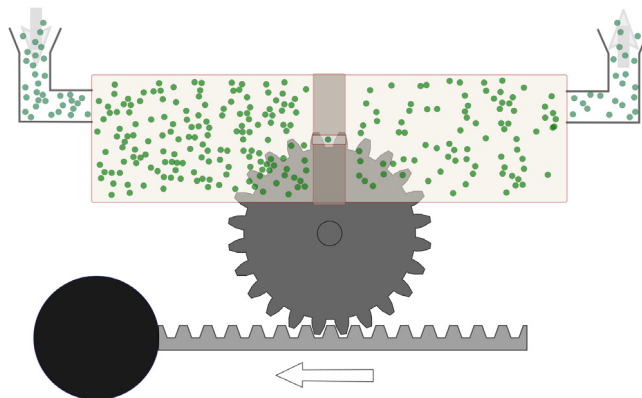


Fig. 13. Molecular-level mechanical work driven by a diffusion gradient. Particles are conducted into the portal by the teeth in the round gear, with the rule being that the gear can rotate only in single-tooth steps and only, in either direction, if in so doing it conveys a particle into the portal. Again, all actions are driven by random Brownian impacts, those acting on the gear and those acting on the load, in both cases doing so without directional bias.

Of course, the cargo load is moved by Brownian impacts. But only those impacts are allowed to actually move the load if the motion thereby induced in the rack (in either direction) – together with a matching motion of the gear – are permitted by the escapement mechanism. However, the escapement rule dictates that only motions of the gear that move a particle into the portal are permitted—and this can only happen if the inter-tooth space next to the barrier on the side which the pending motion would move into the barrier is holding a particle.

Therefore if the particle concentration disequilibrium is as shown in the figure, clockwise rotational steps will happen more often than counter-clockwise ones, so single-step motions of the load leftward will happen more often than those in the reverse. As a result, the dissipation of the diffusion gradient, being in this manner gated by the occurrence of Brownian impacts that move the load in the desired direction, allows thermal fluctuations to be filtered and perform mechanical work. And once again, the only role of the driving disequilibrium (free energy) is to provide a statistical, after the fact, “rectification” bias favoring desirable fluctuations.

This conversion engine is reversible in two senses. First, if the particle gradient had opposite left–right polarity, then the dominant motion of the load would be left-to-right. Second, if a sufficiently strong additional force, opposing the motion which the engine on its own would produce, were applied to the load then the engine would be driven backwards to the effect of increasing the particle concentration gradient, rather than dissipating it.

It is not immediately obvious, however, that this conversion mechanism is consistent with the view advanced here that all engines, even those that produce mechanical work, are creating a disequilibrium at the expense of dissipating a greater one. What disequilibrium is being created by a molecular-scale body being forced to move through a medium against overdamped viscous forces? While the full answer takes us afield of our present purposes, the essential point is that each step moving the load particle in a particular specific direction reduces the spatial entropy (equivalently the positional “ignorance”) of the system from what it would be if the load were simply allowed to proceed on the Brownian motion random walk to which it would otherwise be inescapably subject. Idealizing the motion as taking place on a three-dimensional discrete lattice for example, in which a particle can move in 6 directions in any one step, the engine, functioning ideally, limits the motions to a specific one at each step and to a high correlation between the directions taken in successive steps. This situation is somewhat analogous to so-called “entropic springs” in which stretching the elastic material of the spring reduces the spatial randomness of the paths traced out by the linear molecules making up the material—rendering these paths more predictable and correlated; this change is thereby necessarily opposed by an entirely “entropic” force acting to restore the more random, higher probability, molecular configurations of equilibrium [52, §33].

11. Do real engines conform to the principles advanced in this paper?

Proposed mechanistic models for a large, varied, and rapidly growing array of free energy converting systems are now available in the literature. To our knowledge, none of these appear to contradict the tenets of disequilibria conversion advanced here. At the base level, all of the proposed mechanisms envisage devices that are multi-step “Janusian” engines cycling through an ordered series of alternate binding and catalytic “specificities” [12] and in which transitions conformational changes, driven by, or at least triggered by, substrate and product binding and unbinding events play an essential role. At the next level, all appear to conform to the Jenck’s dictum that a necessary functional requirement of the conversion engine is that it not allow either the driver or the driven process to complete within a cycle unless both do. Furthermore, in instances in which the relevant sequencing question is considered resolved, as is true of many, the mechanistic strategy for ensuring this dictum is that the completion of the driving reaction (through to full product release) is made to be mechanistically triggered by, and thus contingent upon, the full (to product release) completion of the driven reaction, and it therefore occurs subsequent to it. This, ‘pay after completion’ design, as we have noted, serves not merely to ‘ensure’ the joint completion requirement, and with that the non-wasteful satisfaction of the 2nd law. It also enables the kinetic trapping of the driven reaction’s product release step by first having that release trigger the initiation of the corresponding, but in that case quasi-irreversible, completion step in the driving reaction and then further having that event trigger the conformational reversal that takes the system back to its starting configuration. That is, all proposed mechanisms of which we are aware conform, at least in its essential elements, to the Jenck’s ‘interleaved half reactions’ model.

Thus, the proposed mechanisms appears to be consistent with the escapement-mediated “Brownian ratchet” conceptual model of the process of disequilibria conversion here advocated. Of particular note is the general observation that the driving reaction is not allowed to take place, or at least to complete to full product release, until after (or only coincidental with) the full completion of the reaction which that hydrolysis is driving. This supports our general claim that the driving process, e.g. the hydrolysis of ATP, acts as a driver not by supplying energy to the driven process but by supplying irreversibility to the conversion cycle, and, moreover, by doing so at the one point in the cycle when that ‘expensive’ irreversibility is least likely be wasted and further can function kinetically to ‘trap’ instances of the driven reaction by blocking the reversal of completion phase of the driven reaction.

As was noted above, a companion paper is in preparation [57] which aims to make the above claims concrete and credible by examining in some detail a representative collection of biological free energy conversion processes whose experimental study has given rise to functional mechanistic models. Of these we focus primarily on models that propose an ordering of the completion of the driven reaction with respect to that of the driving one. The main questions in each case are whether that ordering is as predicted here: driven completes first, driver second, and further is there evidence as to how this sequential conditionality requirement is mechanistically imposed. Of additional interest is whether there is evidence

for kinetic trapping of the driven reaction and whether the loading half reactions are also ordered in the canonical “Jencks” fashion. We have, of course, sought to consider as diverse a collection of conversion types as possible. Some of the cases we consider there, along with relevant literature citations, are listed next.

- PPI-ase driven proton pumps [87–90].
- Tryptophan synthase [41,91].
- Nitrogenase [43,92–98].
- Co–Fe Reductases [99].
- Gyrases and recombinases [100,101].
- Flippases [102].
- ABC exporters [103,103–109].
- SNARES [110–112].
- AAA+ ATPases [113–115].
- Antiporters [116–119].
- NiFe Hydrogenases [120–122].
- Kinesin [123].
- ATP-synthase [46].
- Electron bifurcation and Complex III/ bc_1 [42,124].

However, of course, these models and their supporting data, by themselves at least, cannot be construed as having proved, much less as universal ‘laws’, everything here claimed about disequilibria conversion, those in particular the role of energy in disequilibria conversion, and the physical meaning of “free energy”.

12. What happened to “energy” (and to “high energy bonds”)?

We hope that the preceding discussion has at least made it plausible that:

- Neither life nor anything else is powered by “energy” – neither its “use” nor its “consumption” – but instead, and necessarily, by disequilibria dissipation.
- This in practice requires a hierarchy of processes that, acting as engines, convert disequilibria. Most often the conversions of life are between manifest thermodynamic disequilibria (i.e. states with many degrees of freedom), consuming one to generate another. However, disequilibria conversions between thermodynamic and mechanical (i.e. single degree of freedom) systems are also common, though these too conform to the general ‘statistical’ analysis outlined here (see discussion below in Section 12.2).
- Conversion engines, those that power life included, do not “do work” by transferring energy from the driving to driven process—even energy discounted as nominally required by the 2nd law, but instead operate by means of an escapement mechanism which makes the progress of the driving dissipative process incrementally conditional on, i.e. gated by, the progress of the driven, thermodynamically uphill process.⁹
- Molecular-scale conversions are mediated by “Brownian escapement” mechanisms, according to which, once a conversion cycle is begun with the loading of both driven and driving reactants, neither of the reactions are allowed to complete, and the conversion cycle return to its starting state, unless both do. In all known instances this requirement is met by the engine acting to prevent the predominantly irreversible step in an instance of the driving reaction, and in fact to be gated by, the completion of an instance of the uphill driven process through to product release.
- Such conversions are only “trading probabilities”: producing a reduction in the probability of one body of energy and matter, at the expense of increasing the probability of another such body, and doing so by locking the two changes together into fused actions which inescapably proceed in the direction which increases the joint probability of the two bodies of energy/matter taken together. But since the probability of a system being in one of its macrostates is proportional to the number of microstates underlying that macrostate, “trading probabilities” is physically just “trading numbers of microstates”.
- Whereas energy is necessarily and absolutely conserved in such conversion processes, changes in the microstate count of that energy – in either direction – is typically a large, potentially exclusive, part of the overall change in microstate count driving the evolution of the system’s macrostate. That is, energy is a medium, often the dominant one, in which is wrought the change in microstate count (equivalently the state’s probability) that is responsible for the system’s dynamics.

⁹ Note, for example, that if the earth were illuminated by a radiant body at ~ 300 K, it could receive the same amount of energy per day, and have approximately the same surface temperature (namely 300 K), as it now does. But this energy flux could not drive, nor could it possibly have spawned, photosynthetic life. The utility of the photons provided by our sun is that they come in with an average temperature of ~ 6000 K. As a result, our ~ 300 K planet exports approximately 20 photons for every one it receives. It is this disequilibrium, this $\sim 20\times$ gain in “ W ”, that makes photosynthetic life possible and profitable. That is, it is, once again, disequilibria alone, not energy, that can make things happen.

Admittedly, the points advanced above, specifically those regarding the role of energy and the proper physical meaning of free energy, are at more-or-less jarring odds with both history and common understanding. We therefore think it worthwhile to take a step back and consider this conceptual conflict with a bit more care. First, we attempt to provide some historical perspective on the ideas underlying the conflict. Second, we undertake to deal more explicitly with some technical issues that seem pivotal to understanding how the conflict arose. One of these issues relates to the concept of work in free energy conversion and specifically to the class of free energy conversions in which either the driver or driven process is one of mechanical work. Biology is, of course, rife with such “motor” conversions (ATP synthase involving two, fighting in opposition over which way the shaft connecting them will turn). And, of course, thermodynamics was born in the ‘fire’ of the effort to understand such processes (heat engines and canon boring). Another is the role of “high energy bonds” in the processes of chemical free energy conversion.

12.1. History: enter Boltzmann, Schrödinger, Penrose

Interestingly, the quite fundamental point that life is not powered by energy consumption was well understood by Ludwig Boltzmann himself. In 1886, he wrote (in translation from the original German) [125]:

“The general struggle for existence of animate beings is therefore not a struggle for raw materials – these, for organisms, are air, water and soil, all abundantly available – nor for energy which exists in plenty in any body in the form of heat (albeit unfortunately not transformable), but a struggle for entropy, which becomes available through the transition of energy from the hot sun to the cold earth”.

At least superficially the assertion about not struggling for energy (and by implication not even “using” it) is at fairly jarring odds with essentially all subsequent teaching and discussions on the relationship between life and energy—and with nearly all professional writing on bioenergetics. Everyone knows – and has been taught – that living systems need an unflagging input of energy to keep them running. Conventional biochemistry is everywhere preoccupied with the “use” of energy: by the particular metabolic processes that are said to “conserve energy”, by the energy that can be extracted from certain so-called “high energy” chemical bonds (most famously and centrally the terminal phosphoanhydride bond in ATP; Fritz Lippman’s iconic “squiggle”), by the energy “consumed” to make certain up-hill reactions go, or to power physical movement, trans-membrane pumping, structural assembly and disassembly, and the like.

In most contexts, of course, it is more or less implicitly understood that “energy” is often being used as a shorthand for “free energy”, albeit almost always with the tacit understanding that the difference between the two is not a *categorical* one, but merely one of amount: i.e. the difference between the energy present and the fraction of that which is “available to perform work” (see e.g., https://en.wikipedia.org/wiki/Gibbs_free_energy and literature cited therein).

In this conceptual world, that is, “entropy” is just a tax that must be paid when using energy, one which discounts energy’s ability to “perform work”, leaving only the remainder “free” for that employment; or alternatively, entropy is the peculiar property of matter whose ineluctable increase over time is what dictates that, left to their own devices, all things active and organized run down, fall apart, and stop. Entropy, in the conventional view, is certainly not what keeps life running, nor what it is struggling for. Much less can the ways of entropy be in any way given credit for the existence of organized states of matter and dynamically persistent processes.

Is it possible that there is really only a “semantic” disagreement here? If not was Boltzmann just wrong? And if he was right, why has the error he calls out so persisted, and in fact so dominated thinking, including that in many of the scientific disciplines most closely concerned with the issues involved?

We want to first emphasize that what Boltzmann very clearly meant in his statement is that life struggles to obtain a source of *low* entropy – necessarily embodied in some quantity of matter and/or energy – lower specifically than the entropy of the forms into which life can convert, and then exhaust, that same matter and energy. In other words, how he would likely phrase the point today, we propose, is that life struggles to find exploitable thermodynamic gradients, i.e. disequilibria. And this because, as we have belabored above, it is the entropy increase which the dissipation of these disequilibria would produce that is ultimately the driving ‘force’ for life (as it is in fact for all macroscopic dynamical processes) [52], and, indeed, the only one that could in principle exist. Admittedly, energy is usually the dominant (though not the only) medium in which the relevant entropy changes are wrought, but it itself causes nothing and is never used or consumed. So the distinction between energy and free energy is in fact a categorical one.

In other words, there seems no avoiding the conclusion that either Boltzmann’s remark (and along with it the argument presented here) is just wrong, or that the prevailing view, at least within the chemistry/biochemistry community, is.

Indeed it would perhaps be difficult to cite a more conceptually consequential claim than the one we quote by Boltzmann, yet even harder to cite a more ignored one. A state of affairs notwithstanding that this was by no means the last to be heard of Boltzmann’s insight.

Notably, for example, half a century after Boltzmann penned the above remark, the theoretical physicist Erwin Schrödinger published a small monograph titled: “What is Life?” [126]. This celebrated gem of insight and exposition has been widely noted as one of the most influential such efforts in the history of science. In it, Schrödinger, along with discussing a number of other deep matters, deals at length with the issue alluded to by Boltzmann, and in so doing emphatically affirms his point. Schrödinger frames his exposition of the same insight by introducing the notion that “*life feeds on negative entropy*”—a concept meant simply to convey the need for the intake of entropy which is less than, is ‘negative’ with respect

to, the entropy that must be continuously exhausted by the organism. In his chapter on “Order, Disorder, and Entropy”, in which Schrödinger discusses why it is that living systems can maintain themselves in a very highly organized (i.e. low entropy) state without violating the 2nd law, he remarks:

“How does the living system avoid decay? The obvious answer is: By eating, drinking and (in the case of plants) assimilating. The technical term is metabolism. The Greek word μεταβάλλειν means change or exchange. Exchange of what? Originally, the underlying idea is that, no doubt, exchange of material (e.g. the German for metabolism is *Stoffwechsel*). That the exchange of material should be the essential thing is absurd. Any atom of nitrogen, oxygen, sulfur, etc., is as good as any other of its kind; what could be gained by exchanging them? *For a while in the past our curiosity was silenced by being told that we feed upon energy. ... Needless to say, taken literally, this is just as absurd. ...*

What then is that precious something contained in our food which keeps us from death? That is easily answered. Every process, event, happening—call it what you will; in a word, everything that is going on in Nature means an increase in the entropy of the part of the world where it is going on. Thus a living organism continually increases its entropy—and thus tends to approach the dangerous state of maximum entropy, which is death. It can only keep aloof from it, i.e. alive, by continually drawing from its environment negative entropy—which is something very positive as we will see. What an organisms feeds on is negative entropy. (emphasis added)

In the reprint published in 1967, the theoretical physicist Roger Penrose provides a forward, the closing paragraph of which reads:

“Like so many works that have had a great impact on human thinking, it makes points that, once they are grasped, have a ring of almost self-evident truth; yet they are still blindly ignored by a disconcertingly large proportion of people who should know better. How often do we still hear that quantum effects can have little relevance in the study of biology, *or even that we eat food in order to gain energy?* This serves to emphasize the continuing relevance that Schrödinger’s *What is Life?* has for us today. It is amply worth rereading!” (emphasis added)

Nor was that by any means the last. As we earlier noted for example, in 1994 the physical chemist P.W. Atkins published (the second edition of) a general-audience book titled “*The Second Law; energy, chaos, and form*” [127] (see particularly chapters 5, 6, and especially chapter 8, “Transformations of Chaos”). This unique and elegant work of conceptual exposition is arguably the best general discussion available bearing on the basic points advanced here on energy, entropy, disequilibria and disequilibria conversion, the probability of physical states, and the “forces” of change and creation. And the entire work is an affirmation of, and expansion on, the insights underlying the quoted remarks of Boltzmann, Schrödinger and Penrose. Yet it too, perhaps in part because of its nominally ‘introductory’ level, has been, in the main at least, “ignored”.

Doubtless many factors account for why these basic insights have so little penetrated conventional thought, especially in the biological sciences. But the accidents of history through which the science of thermodynamics was born are certainly much to blame. Thermodynamics’ core and distinguishing concept of entropy emerged just as the concept of energy was itself crystallizing, and was first glimpsed, i.e. by Clausius, as a property of heat energy—one that reduced how much of that energy could be converted to mechanical work. Thermodynamics thus arose as a drama about energy, but one in which the utility of certain forms, initially just heat, had to be discounted by an undeniably strange factor of the form Q/T ; and entropy was thus seen as just an unwelcome, and not a little mysterious, tax on the “use” of energy. And cemented in from the beginning, in thinking both learned and popular, was the profound misconception that it is “energy consumption” that makes things happen.

To consider in a bit more detail both how this came to pass and how we can reconcile the counter view advocated here with the classical one, we briefly discuss the three technical issues noted above which seem central to the development of this energy-based misreading of thermodynamics: (i) the specific subset of disequilibria conversions in which one of the coupled processes either begins or terminates in mechanical motion, and relatedly, (ii) the numerical equivalence in such processes between changes in the system’s Helmholtz free energy and the mechanical work done, and (iii) the role of the energy stored in “high energy” chemical bonds.

12.2. Converting from and to mechanical work

We have argued that it is a consequential conceptual mistake to regard energy as the physical quantity whose consumption or use is what drives endergonic processes. The conventional rebuttal, namely that if one everywhere substitutes “free energy” for “energy” in such discussions they are then not just legal, but conceptually accurate—is also mistaken. This view is based on the belief that it is still energy consumption, in particular the transfer of energy from driver to driven process, that makes a driven endergonic process proceed, while acknowledging that one must, upon each transfer, pay the 2nd law tax with the consequence that the amount of energy transferred is necessarily less than the amount taken from the driving process. But this is incorrect, and the root error, as we argued above, is in part that “free energies” are not physically measuring an energy—history, units, and convention notwithstanding. Physically, they are measuring an entropy difference—equivalently a “probability” difference. And that therefore disequilibria conversion must be understood, and analyzed in terms of, the reduction of the entropy of one system as driven by the (greater) increase in the entropy of another. And indeed, in the classical NET analysis of the conversions in which “chemical work” is being done, work and power are defined in entropy terms [10,11,33,37].

However, there is one conversion context in which “free energy” and energy in the strict, unqualified sense appear to be unambiguously and directly interconverted; justifying, and in historical fact giving rise to, the classical interpretation of free energy as a discounted form of energy. This arises in the context of conversions from and to mechanical work. And it raises the possibility that in these cases, the general theory of disequilibria conversion we have advanced here, in which both input and output are understood to be entropy changes (as in the discussion of LNET given in B), does not apply to conversions involving mechanical work.

Indeed, the paradigmatic problems that birthed thermodynamics: heat engines and cannon boring, and did so in the triumphal flowering of classical mechanics, are of course conversions of this type. That it was concluded that in these conversions the driving process’s “free energy” was being literally converted to, and is a form of, energy in the strict mechanical meaning of the term, is not just understandable, it seems virtually inescapable.

However, we here argue that conversions involving mechanical work are not exceptions to the entropically-based model advanced here.

The central point is that mechanical-thermodynamic conversions can be viewed as ‘limit-cases’ in the general scheme presented here. In the case, for example, of the conversion of a physical–chemical disequilibrium having a well defined thermodynamic characterization, into mechanical work, the driven process is still one of entropy reduction, but one in which the entropy of the driven flux is not just reduced, but maximally reduced, that is to zero – corresponding to the single degree of freedom embodied in mechanical motion in one dimension – i.e. the “work” (see the analysis of heat engines given in Appendix E). And in that limit case there is a numerical equivalence between the product of (minus) the entropy reduction achieved by the conversion and the temperature at which the conversion takes place, to the maximal amount of mechanical work done (denoted in literal and correct energy units) by the engine. We discuss this point briefly in the next sub section which considers the connection between disequilibria conversions involving mechanical work and changes in the Helmholtz free energy of the process that is either producing, or being driven by, that work.

Furthermore, as we noted briefly in our discussion of the ‘extended CDC model’ in which a diffusion disequilibrium is converted to, or from, mechanical work, that work (e.g. transporting a load) does in fact entail a reduction in the entropy of the system being driven (see the discussion of Fig. 13 in Section 10).

12.2.1. Helmholtz free energy and the “limit cases” of conversion to and from mechanical work

Consider a system in thermal contact with a heat bath, or reservoir, large enough to insure that the system’s temperature is both well-defined and held constant. The 2nd law requirement that any spontaneous change not decrease the entropy of the universe has the form $dS_{\text{total}} = dS_{\text{sys}} + dS_{\text{bath}} \geq 0$, and the conservation of energy requires that $dU_{\text{sys}} + dU_{\text{bath}} = 0$.

For the bath, the fundamental equation for entropy is: $dS_{\text{bath}} = (1/T)dU_{\text{bath}} + (p/T)dV_{\text{bath}} - (1/T)\sum_j \mu_j dN_j = (1/T)dU_{\text{bath}} = -(1/T)dU_{\text{sys}}$ given that for the bath $dV = 0$ and $dN_j = 0, \forall j$ and using the conservation of energy as between bath and system.

Thus the 2nd law requirement becomes is $dS_{\text{sys}} - dU_{\text{sys}}/T \geq 0$ or equivalently $dU_{\text{sys}} - TdS_{\text{sys}} = d(U_{\text{sys}} - TS_{\text{sys}}) = dF_{\text{sys}} \leq 0$ where by definition $F \equiv U - TS$ is the Helmholtz free energy. That is, in a thermostated system in which the system’s volume is also held constant, the 2nd law’s requirement that the entropy of the system plus bath not decrease is equivalent to requiring that the system’s Helmholtz free energy not increase.

The first law dictates that in a transition in which work is done on, or by, a system, $dU_{\text{sys}} = \delta w + \delta q$, where δw is the incremental work done on the system and δq is the increment of heat transferred from the bath to the system during the transition. The 2nd law of Clausius asserts that for reversible transitions δw is maximal and $\delta q_{\text{rev}} = T dS_{\text{sys}}$. Therefore $\delta w_{\text{max}} = dU - \delta q_{\text{rev}} = dU_{\text{sys}} - T dS_{\text{sys}} = dF = \delta w_{\text{max}}$. That is, the maximum mechanical work that an isothermal system can perform on its surroundings is achieved if the process is carried out ‘reversibly’ (i.e. slowly enough that the system can stay in effective equilibrium throughout), and in this case the work performed is equal to minus the system’s Helmholtz free energy change [52, §7].

We note, however, that when mechanical work is done by (on) a thermodynamic system, the process involves a mechanical force acting in opposition to a thermodynamic one in a ‘contest’ over which way motion will take place along a spatial dimension. In this contest the thermodynamic force (e.g. pressure) is the rate at which changes in that dimension change the entropy of the thermodynamic system (e.g. $p = T(\partial S/\partial V)_{U,N}$). That is, the process is fundamentally one in which the system is progressively changing macrostates by moving to ones comprising greater (fewer) numbers of microstates. For the expanding gas the gain is primarily (for an ideal gas, entirely) due to increasing the number of ‘positions’ the molecules of the gas can occupy (the quantity we called M in the ‘diffusion model’ analysis in Section 4). A detailed discussion of this point, based on the kind of ‘particles in a box’ microstate-counting approach used here, is given in Dill and Bromberg [52, §6]; see in particular page 104.

Further, in such conversions, the work done by the system must be exactly balanced by a reduction in the systems internal energy, with the implication that the entropy of that converted energy has been, by the conversion, reduced to zero (i.e. to that of a single degree of freedom, namely movement on the ‘work’ dimension). For this reason, this entropy-reducing energy flux can be viewed as the ‘driven’ process in a disequilibrium conversion, wherein the necessarily equal or greater increase in the entropy of the energy that is not converted is the ‘driving’ process. This point is illustrated in our discussion of heat engines given in Appendix E.

12.3. High energy bonds; Lipmann's squiggle

To discuss the role in free energy conversion of making and breaking “high energy bonds” we consider the prototype example: the terminal phosphoanhydride bond in ATP.

To quote from Oster and Wang “*At physiological conditions, the free energy of hydrolysis of one ATP is $\sim 20\text{--}24 k_B T$, of this, about $8\text{--}9 k_B T$ is enthalpic, the balance being entropic*”. [32]. That is, $\Delta H/k_B T \sim -(8\text{--}9)$ meaning that the hydrolysis of a single terminal phosphoanhydride bond of ATP in the $\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{Pi}$ reaction increases the thermal degrees of freedom of the bath by a factor of about $\exp(8.5) \sim 5000$. If the hydrolysis takes place under a “physiological” degree of disequilibrium, the accompanying change in the system's entropy of $\Delta S/k_B \sim 13.5$ increases the system's degrees of freedom by an additional multiplicative factor of $\sim \exp(13.5) = 7.3 \times 10^5$. Taking together, the “physiological” hydrolysis of a single molecule of ATP increases the microstate count of the universe by a multiplicative factor of $5 \times 10^3 \times 7.3 \times 10^5 \sim 3.6 \times 10^{10}$ which is also then the ratio of forward to reverse reactions.

Thus, in the case of ATP hydrolysis the net conversion of bond energy to heat energy, yielding a one-to-many numerical ratio in energy quanta of about 8.5, provides an important part of the statistical ‘probability’ force that makes the reaction irreversible and thereby makes it useful as a driving process in chemical disequilibria conversions. However, it is only that: an “irreversibility” bias that can be used to trap useful Brownian fluctuations. The bond energy thus liberated is not transferred to the driven process; it is dissipated, and needs to be, to generate the needed statistical irreversibility. It is the same for all molecular-scale conversion processes.

But the above does bring forward the important role of literal energy in conversion processes: not to be consumed, nor to be transferred to the driven process to make that process happen, but simply to be ‘divided’ – into a larger number of quanta (or coalesced into a smaller number) and to thereby be part – often the dominant or even exclusive part, of the medium in which is wrought a change in the number of microstates—and with that the probability of the system's macrostate.

Dynamics, to summarize, is the manifestation of systems moving to macrostates of increased probability (increased number of microstates); and the greater the increase in probability produced by an instance of the underlying process, the faster the process runs [127].

We close this discussion of ‘high energy bonds’ by restating a central contention of this piece: *It's not energy, or energy transfer, or energy consumption that makes things happen, it's disequilibria; primarily through their ‘interconversion’; interconversions that operate by mechanistically tying events that dissipate one to events that create another. But since all ‘events’ in this sense are induced by thermal fluctuations what this requires is a molecular escapement that uses the relative improbability of individual ‘backward’ fluctuation events in a statistically dominant process acting to relax a disequilibrium to probabilistically capture ‘backward’ fluctuations in another, statistically subordinate, process.*

13. What is settled and what isn't?

We take as established the main general points advanced above regarding disequilibria and how and why they are created: namely that (i) all ‘organized’ structures and processes, those of life most dramatically, are ‘artifacts’ of disequilibria, (ii) all change is driven by, in fact caused by, the dissipation of a disequilibrium (a process increasing the number of microstates to which a body of energy/matter has access), never by the “consumption” of energy, (iii) a process can only create a disequilibrium if it, and a process dissipating a disequilibrium, are mechanistically linked so that they function as a single thermodynamic process which is in the net dissipative, (iv) the linkage mechanism necessarily functions as an escapement which parses the two processes into individual increments controlled according to the rule that a driving increment can complete only if in the same cycle the driven increment has also done so, (v) in molecular-level conversions, all events in both the driving and driven processes are caused by thermal (“Brownian”) ‘impact’ fluctuations from the medium, inducing both “forward” (2nd law compliant) and reverse (2nd law “violating”) transitions, (vi) the only essential role of the driving process (e.g. ATP hydrolysis) in such a conversion is to provide the statistical irreversibility needed to trap “2nd law violating” instances of the driven process, (vii) energy is never passed from the driving to the driven process, (viii) “free energy” is a measure of the strength of a disequilibrium (equivalently a measure of relative improbability), specifically that $\Delta G/k_B T = -\ln(W_{\text{after}}/W_{\text{before}}) = -\ln(p_{\text{after}}/p_{\text{before}})$ where the argument to the log function on the right hand side is the ratio of the number of microstates to which the system (plus surroundings) has access after a change in a system's macrostate to that before which is in turn equal to the ratio of the probabilities of the two macrostates.

Arguably less established are at least some of the proposed generalities about how molecular disequilibria converting mechanisms work. Perhaps most importantly, (i) that in a conversion process it is always the completion of an increment of the driven process that the controlling escapement mechanism uses as the trigger, or the “gate”, to allow (an appropriately sized) increment of the driving process to complete (i.e. to ‘escape’), and also perhaps (ii) that conversions between mechanical and thermodynamic processes can be validly interpreted as ‘limit case’ instances of the general ‘disequilibrium-to-disequilibrium’ paradigm advanced here.

14. Connection to the emergence of life; the story within the story

It is our position that the emergence of life and the emergence of “bioenergetics”, as here understood, are an indivisible continuity and that life's fraught emergence is a story within the story just told. One, that is, in which the essential

enabling events in the transition from inanimate to animate organizations of matter involved the abiotic conversion of geophysically and geochemically-supplied disequilibria into the prerequisite founding disequilibria of life. These were necessarily particular conversions; specific as to the driver and driven process pairs being inter-converted, and specific as to the chemical, mineralogical, and physical properties of the environment in which the conversions were taking place. Conversions that were also, and necessarily, ongoing and dynamic—forming an active mechanistic text of constant dissipation and regeneration upon which all the like processes and devices of extant life are, we further contend, overprinted in a faithful, yet significantly transparent, palimpsest. Furthermore, not only can we decrypt what must have been life's very first devices, but we can also descry its first motive. The scene is well documented. As the early Earth cooled, a rapid rain-out from the carbon dioxide atmosphere produced a carbonic ocean very far from equilibrium from the hydrogen leaking out of submarine alkaline springs. The thermodynamic imperative was to reduce the CO_2 with this hydrothermal H_2 in order "to produce a small but ever-renewed stock of organic molecules" [128, p. 34]. And life's main task for the planet ever since has been dedicated to the same process—the hydrogenation of carbon dioxide.

We emphasize that from this perspective, life's essential launching requirements were not "building blocks", i.e. not particular molecules, or classes of them, at any concentration or in any mix. Such a "soup" however produced, cycled, or concentrated; no matter what its constitution, and regardless of what chemically non-specific "energy" inputs were supplied to it, stably or otherwise—is in no meaningful way 'lifelike', nor capable, we assert, of engendering it [129, p. 453]. It would be as exactly useless to emergent life, and in part for the same fundamental chemical and thermodynamic reasons, as is ATP for extant life when not dynamically maintained in a high state of disequilibrium, as we argued in our introductory Section 2.

As a categorical necessity, we maintain, life's actual transition-enabling requirements were dynamic *processes*—specific processes acting to generate and maintain specific states of physical and chemical disequilibrium, and, necessarily, doing so in a quite specific type of physical environment. Extant life everywhere has just these ineluctable requirements: specific types of externally supplied chemical disequilibria to act as 'drivers', and correspondingly specific engines to carry out the particular conversions needed to produce, from those drivers, an essential and universal and specific set of 'internal' disequilibria; and all supported within confinements with specific properties, including those of selective chemical exchange with the environment [130]. As it is now in this regard, we contend, so it had to have been at the beginning.

Of course, attitudes are changing. Yet the origin-of-life community, even when disengaging from 'organic soup' to embrace the relevance of geochemistry in defining initial conditions and providing the necessary thermodynamic driving forces, still largely remains wedded to the view that just finding the right chemicals, 'energy' sources, and catalysts will lead us to life's origins. However, biology is not a kind of speedier chemistry or even geochemistry—it really is, as we have emphasized, fundamentally different. Recapitulations of the acetyl coenzyme-A pathway, for example, reveal essential endergonic steps in the ascent from an inorganic and autogenic proto-metabolism through to a ligand-accelerated autocatalysis [131–134]. Of course, it is these endergonic reactions that must be specifically and selectively driven. Yet, as we have argued above, how an endergonic reaction is made to happen is complicated and counter-intuitive, requiring a case-specific 'bespoke' engine, whose operation is not just that of an enzyme-catalyst, and indeed, must "transcend chemistry". Yet we must understand them and their thermodynamic drivers, both in their general *modus operandi* and in the specifics that suit each to its conversion task, if we are to attempt to reverse-engineer their mechanisms towards comparable operations in the inorganic hydrothermal world. In this way we expect experimental reconstructions of conversion engine prototypes out of the 'minéral trouvé' present at the alkaline vent to show that life, or at least metabolism, probably arose to relieve these particular tensions (cf. [135]).

Thus the pursuit of the unicorn of life's emergence must begin with a hunt for those conversions; what drove them, what they produced, how the conversion engines could have arisen and functioned abiotically; and how the physical environment could not only have enabled this to happen, but have kept the tenuous flickerings of first ignition nurtured to the point that it could capture the wind of autocatalysis and explode into the great organic disequilibria-converting 'conflagration' which now so enlivens, and impacts, the planet.

What must the "founding disequilibria of life" have been? Fixed carbon (e.g. formyl phosphate and thioacetate) held out of equilibrium with respect to $\text{CO}_2 + \text{H}_2 \pm \text{CH}_4$ is clearly primary, as we have argued [5,130,131]. Also likely is an early requirement for a primitive analogue of ATP to provide the free energy supply function now largely delegated to ATP. Pyrophosphate, "PPI", seems a likely candidate, as has long been advocated by the Baltscheffsky and colleagues [136,137], though acetyl-phosphate has been suggested as a possible alternative—presuming the prior existence of a carbon fixation path producing thioacetate which could convert to acetyl-phosphate via methyl thioacetate [5]. Again, the requirement is not for the maintenance of these molecules at sufficiently high concentrations, but instead that they be held sufficiently out of equilibrium with respect to their hydrolysis products (and thus be able to 'carry' free energy). Concomitantly there would have been the requirement for a high potential electron acceptor to partially oxidize the hydrogen to protons. Nitrate and/or nitrite in the ocean could have played such a role while at the same time using the protons to hydrogenate the nitrogen oxides to produce ammonia, so driving NH_3 out of equilibrium with respect to $\text{H}_2 + \text{NO}_3$ [138,139]. In these circumstances ammonia/ammonium has been shown to exergonically aminate carboxylic acids to amino acids [140].

These choices for the founding, driven disequilibria can plausibly be defended on general, and presumably universal, biochemical and biophysical grounds [141]. However, addressing the remaining questions about driving disequilibria, abiotic conversion engines, and physical environment on similar *a priori* grounds present a different and more daunting challenge. Fortunately, it is likely not one we will have to take on in that manner.

While there are a number of ideas as to how life first emerged [142] the ‘serpentinization-driven, alkaline hydrothermal vent’ hypothesis (“AHV”) provides in general terms an integrated and testable package of candidate answers to these questions. The AHV proposal, originally advanced in 1989 by one of us (MR) [143] (see [6,141,143–145] and references cited therein), began to attract serious attention after 2000 when the first instance of an active AHV, the so-called “Lost City” formation in the North Atlantic, was discovered and subsequently shown to have the key properties and behaviors the AHV proposal had predicted [146–150]. Further, the AHV proposal is now attracting increased interest and research effort [151–162], and dispositive experimental tests of its key predictions seem in prospect [163].

Importantly also, the geophysically-based AHV model directly implies a marked and multifactorial congruence between the devices of protolife and those of extant life. As we will lay out, this embraces key structural, chemical and thermodynamic aspects, the ones responsible for the maintenance of life’s essential disequilibria in particular. To a striking degree, the vent’s geochemistry appears not merely to set the table, for free, with everything life requires, chemically, structurally and thermodynamically, but to do so with the most ancient and ‘universal’ of the devices and components that it still uses—albeit that it now has to struggle to recreate them on its own. The extent and detailed character of this continuity implies strongly that in strategic and mechanistic terms biochemistry is a direct, and indeed conservative, descendant of – is in fact merely an elaboration upon – the dynamic, far-from equilibrium geochemistry produced by serpentinization-driven alkaline hydrothermal springs venting into the primal ocean of the Hadean.

We next summarize the key elements and predictions of the AHV theory and discuss the relevance of understanding the ‘mechano-thermodynamics’ of disequilibria conversion to the issue of putting the AHV theory to decisive experimental tests.

14.1. The AHV theory and the abiotic conversion of disequilibria

A distinguishing feature of the alkaline vent theory is the *ab-initio* manner in which it addresses the defining thermodynamic distinction between inanimate and animate organizations of matter: that living systems are inherently maintained in highly specific, dynamic, very far from equilibrium states; without which they would have neither structure nor coherent activity. The AHV theory argues in particular that not only must this have been also true of proto life but further that the need to stably maintain and appropriately confine a specific minimal set of chemical disequilibria constituted the initial and quintessential barrier to life’s emergence. And it provides a geophysically-based hypothesis as to how this barrier was surmounted, one based on the upper mantle convective processes that serve to cool the planet. It is these that in specific ocean floor contexts give rise to the process of serpentinization which in turn generates long-lasting alkaline ‘effluent’ springs.

In the comparable Hadean systems hot, highly reduced and alkaline effluents, rising from sites of active serpentinization beneath the ocean floor, vent into the acidulous, oxidized and cool ocean. At the mixing interface mineral precipitate structures develop comprised of complexly patterned, labyrinthine, micro-compartments bounded by membranous mineral films, through which the fluids percolate [141,143,146,164–166]. These precipitates develop as a growing edifice with a permeable core whose ‘walls’ function as nano-porous and nano-permeable membranes acting to selectively separate the alkaline hydrothermal vent solution from the ocean. In the Hadean, the ocean would have been 4–5 pH units more acidic than the effluents, and bearing substantially higher CO₂ concentrations than do earth’s current oceans [161,167,168].

Across this semi-permeable barrier separating effluent and ocean, the serpentinization engine maintains several highly ‘biological’ disequilibria in the form of proton and redox gradients. Suggestively, if not arrestingly [3,153,161,168], the proton gradients have the same directional orientation (alkaline inside, acidic outside) and effective strength (≥ 200 mV) as gradients produced in the OxPhos/ATPsynthase systems of all extant life¹⁰ Similarly, the redox gradients are present at comparable strengths (~ 500 mV) and involve the same donors and acceptors (H₂ + CH₄ versus NO₃⁻, Fe^{III}, Mn^{IV} + CO₂) as can be found powering many extant life forms [167,171,172]. Therefore of course, these disequilibria are attractive candidates to be the sought-after geochemically-provided drivers. The system looks crudely like an immense ‘mineral mitochondria hive’, but one spared the trouble and cost of maintaining its own proton gradients (much more importantly, spared the trouble of figuring out how to do it); or at least seems primed to function as one [173,174]. But that is by no means all the AHV system has to offer aspirant life.

The vent precipitates also provide selectively permeable ‘cell-sized’ micro-chambers: that are held at appropriate temperatures and pressures (30–116 °C and ~ 600 bars) and at an appropriate pH (~ 10.5) for organic chemistry; that supply

¹⁰ “Effective strength” here refers to the proton electrochemical potential difference across the membrane: $\Delta\mu' = \Delta\mu + e\Delta\psi$, where $\Delta\mu = k_B T \ln(c_{\text{out}}/c_{\text{in}})$, “e” is the charge of the proton, and the c terms are concentrations (often a proton gradient’s strength is expressed in units of volts as $\Delta\psi' \equiv \Delta\mu'/e$ whether or not there is an electrostatic contribution). In cases where the proton gradient is “charge compensated” as it is, more or less completely, in chloroplasts [169], $\Delta\psi = 0$ and gradient strength is due simply to concentration differences. In that case if $\Delta\mu'/e = \Delta\psi' = 200$ mV then $c_{\text{out}}/c_{\text{in}} \approx 2 \times 10^4$. However in mitochondria for example, there is little or no charge compensation and the electrostatic contribution is significant. In that case, typically, $c_{\text{out}}/c_{\text{in}} \approx 25$ (i.e. a ΔpH of about 1.4) equivalent to a voltage difference of ≈ 80 mV. However, this proton concentration ratio, in the absence of charge compensation, generates an electric field strength difference of roughly 140 mV, thus the total electrochemical potential difference, in voltage units, is again ≈ 200 mV [170]. The meaning of this is that the ratio of the probability of a proton entering the F0 motor from the ‘outside’ vs from the inside is roughly the same in both “compensated” and “uncompensated” cases, namely $\approx 10^4:1$. In the AHV case, there is also no charge compensation and a concentration gradient of ≈ 4 pH units and an electrostatic gradient of ≥ 200 mV exist together, implying a total electrochemical potential of ≥ 400 mV.

the inorganic trace elements required for life's functioning (e.g., Fe, Mg, Ni, Zn, Co, Mo or W and S, Se, P); that sustain relatively gentle fluid flows; and that are stable for times measured in tens of thousands of years = $\sim 10^{20}$ nanoseconds (see [6,149,175] and references therein). No less critically, the particular minerals that comprise the matrix of the precipitates include those, perhaps most notably “green rust”, supported by Fe–Ni sulfides, that on independent grounds appear to be most promising for incubating life—for providing the needed disequilibria conversion engines in particular [153,155,174,176].

These considerations invite the speculation that in some way “engines” arose within the AHV mineral membranes that, for example, could couple the relaxation of the proton and redox disequilibria produced in the AHV system to the formation of a P_{pi} vs P_i disequilibrium, and the endergonic reduction of CO₂ to CO or formate, of NO₃[−] to NH₃, the oxidation and sulfidation of methane to methane thiol, and the formation of thioacetate [148,177,178]. But exactly how might they have done that?

We recall that what such couplings require is ‘merely’ that the driver-driven processes be functionally lashed together via an escapement mechanism whose defining behavior is that it frustrates the ‘natural’ down-gradient flow of both processes, allowing instances of each to take place only if they happen ‘simultaneously’ and, if there is to be a conversion, only if they happen in thermodynamic opposition: one exergonically (down-gradient) while the other (as the result of a thermal fluctuation, has been thrust) endergonically (up-gradient) [130].

In a companion paper currently in preparation [179] we discuss our present proposals as to how these critical disequilibria conversions may have operated in the AHV precipitates of the Hadean and review the status of efforts to test these and related ideas bearing on the problem. Here we note the main elements of the argument presented in that paper that relate directly to the present general discussion of disequilibria conversion.

Our focus in that paper is on so-called “double-layer hydroxides” (“DLH”), particularly those, such as the brucite-like mineral green rust, a metal hydroxide semiconductor, likely precipitated at early submarine alkaline vents, which display variable valence [4,146,167,180–183]. There is now quite compelling reason to think that these minerals, their interlayer galleries in particular, can naturally function as the specific disequilibria converting engines that we claim are needed.

The last decade in particular has seen a growing realization that the galleries between the layers of DLH minerals display a number of exceptionally promising physical–chemical properties and activities. This was first detailed in a 2003 paper by Gustaf Arrhenius. Quoting from that paper: “*Surface-active DLH minerals ... serve as compartmental systems with flexible membranes and what may be called primitive cellular metabolic function. Like cells, they retain phosphate-charged reactants against high concentration gradients and exchange matter with the surroundings by controlled diffusion through the ‘pores’ provided by the opening of the interlayers at the crystal edges. Here, the exposed negative charge on the interrupted metal hydroxide ‘membrane’ leads to sorption of cations as ‘gatekeepers’*” [184].

In addition, the bilateral internal surfaces of DLH minerals can be redox active [185] and active in a manner that allows the galleries to function as disequilibria converters. In fact, green rust, driven by redox disequilibria, can rapidly reduce nitrate to the ammonium ion as well as the redox sensitive metals such as MoVI to MoIV [186–195]. Note that these are 2, or 2 × 3 electron reductions that correspond to the preferred redox states of green rust (GR), viz., GR[Fe₄²⁺Fe₂³⁺], GR[Fe₂²⁺Fe₄³⁺], GR[Fe₆³⁺] [196,197].

Also, the interlayer galleries in such minerals have been shown to form localized, particle-like dynamic structures called “small polarons” in response to local redox changes. These phonon-stabilized distortion structures are persistent, can propagate vectorially under imposed gradients, and can transport molecular species (see [182,198,199] and the discussion and references cited in [6]).

Finally, in the Hadean, molecular species migrating through these galleries would have encountered trace metal cations such as Ni, Zn, and Mo, all renowned for their highly critical and conserved ‘catalytic’ activities in bioenergetics [133,171,173,177].

A central tenet in our effort to address the above questions—as laid out at length in the mentioned companion paper [179], is that the DLH minerals of interest are essentially insulating and impermeable orthogonal to their layered structure while being, in partial degrees, conducting and permeable in the parallel direction. In particular it is posited that it is through the interlayer galleries of these minerals that the fluids propagating through the AHV precipitate mounds flow. Critically, however, and not withstanding the leakage ‘workarounds’, these gallery channels not only impose chemically selective restrictions on the passage of solute molecules, but are themselves chemically, electronically, and physically active—e.g. changing redox state and also local structure and geometry, supporting polaron dynamics, and conducting charge.

These facts set the framework in which testable answers to our disequilibria conversion questions can be posed. For example, given the observation cited above that in single-shot ‘batch mode’ reactions a sample of reduced green rust can drive the strongly endergonic, 8-electron reduction of nitrate to ammonium, it seems quite possible that in the right setting green rust might drive this same disequilibrium conversion in steady state. How might that come about? We need to understand in general how, in the forced multi-step reduction involved, each individual reduction could operate as a gating mechanism controlling the step-wise relaxation of the driving disequilibria. We need also to see how such a system can be continuously recharged and the product ammonia continuously removed. And we need to consider the possible effects of reaction–diffusion mechanisms in this context [200]; how the supporting, chemically active, and confining matrix of green rust could make for competition between the diffusion distances and reaction rates involving the several reagent-sharing reactions and potentially thereby optimize the self-organization of disequilibria conversions and the self-assembly of products.

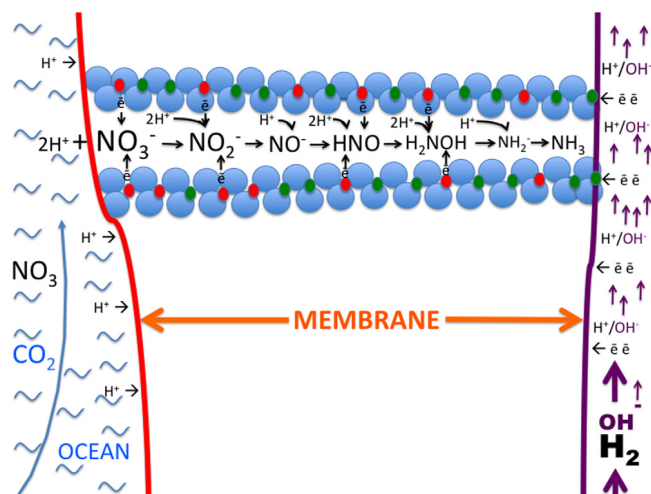


Fig. 14. A green rust ramjet reducing nitrate to ammonia?

Steep redox, pH and nitrate gradients imposed across the green rust membranes of the alkaline hydrothermal mound from left (ocean) to right (vent), along with a continuous supply of hydrothermal hydrogen in the vent effluents, drive nitrate reduction. Hydrogen in the vent effluent is presumed to be oxidized by a ‘protohydrogenase’ which we might assume to be an iron-nickel sulfide precipitate (on the vent-side margin, not shown) [154]; the resultant electrons would be conducted ocean-ward along the iron atoms comprising the green rust (brucite-type) layers drawn by the strong (probably ≥ 400 mV; see footnote 10) voltage gradient while the released protons would be driven into the vent interior by that same gradient. There they are spent on the abundant hydroxyl molecules of the alkaline vent fluids to form water and carried off as (an imperceptible) waste. Thus it is the Coulomb force generated by the proton gradient that drives the essential endergonic step of charge separation. Proton flow into the interlayer from the Hadean Ocean (left), presumably mediated by a Grotthuss-type mechanism [201], is driven by the transmembrane difference in proton chemical potential between the acidulous exterior and the alkaline interior. Nitrate in the Ocean, entering the margins of the gallery from the ocean side oxidizes the green rust causing a repulsion of the opposing ferric iron atoms, thereby giving access to the nitrate anion. This nitrate oxidizes adjacent ferrous iron atoms and is thereby concomitantly reduced to nitrite [188]. Being of the same charge, the nitrite too, is partially immobilized. The negatively charged nitrogen-bearing anions are charge-trapped at the green rust ‘inlets’. It is only when they are reduced and hydrogenated can the grip of the positively-charged interlayers be relaxed and the ammonia or ammonium ion may then be driven in the interior by the nitrate gradient. The ammonium ion generated from nitrate is assumed to reductively aminate pyruvic acid to alanine [140]. The alanines tend to polymerize in this low entropy trap and are forced out of the green rust by the nitrate and proton gradient. Compare this bilateral green rust model of nitrate reduction to how the dimers constituting the nitrate and nitrite reductases function as discussed in [190,202,203]. Note that in the proposed mechanism the proton gradient performs three essential functions: providing the Coulomb force that achieves charge separation once a catalytic site on the vent side has made separate charges available by “splitting” molecular hydrogen, driving the just-liberated electrons oceanward from iron to iron down the gallery walls in a redox cascade, and driving protons into the gallery from the ocean side to provide for charge-neutral reductions of nitrate.

In Fig. 14, we present a notional proposal as to how in the AHV mound context a green rust membrane across which is maintained stable proton, redox, and nitrate gradients would continuously drive the reduction of nitrate to ammonia. In the proposed mechanism, the interlayer galleries, under the influence of the imposed disequilibria, function in rough analogy to a ramjet, supporting a linear, progressive, ‘assembly line’ sequence of conversion escapements the cyclic operation of each of which is local.

What of the other two internal disequilibria we have claimed must also be stably generated: fixed carbon and an ATP surrogate? We present extended discussions of these questions in [179]. Here we merely note two ‘encouraging’ points: the stunning mechanistic simplicity of the ‘rocking dimers’ proton-pumping pyrophosphatases discussed in [87–90], and the experimental demonstration of the electrochemical reduction of HCO_3^- to methanol, and to formic, acetic, and pyruvic acids [155].

We close this discussion with an observation: arguably the key virtue of the AHV model as a scientific hypothesis regarding the initial steps in the emergence of life is its essentially unique vulnerability to disproof. It places all of its chips on the claim that certain naturally arising, but experimentally reproducible, geochemical circumstances do produce castles of mineral ‘cells’ in which three key, undeniably life-like chemical disequilibria are ‘abiotically’ generated and maintained. If it proves not to be possible to experimentally substantiate these conjectures, then we may expect interest in the theory to wane.

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Appendix A. Stochastic thermodynamics

Contemporary non-equilibrium thermodynamics departs from its classical precursor most fundamentally in generalizing the set of all possible ‘microstates’ of a system from time-invariant points in a “phase space” to include also transitions or trajectories taking place in time between such points. Implicit in this advance is a casting off of the twin chains of classical thermodynamics. First, and obviously so, that one could only treat systems at equilibrium, or only incrementally displaced from it. Second, and much less obviously, that thermodynamic concepts could only be applied to systems comprised of immense numbers of particles. In consequence of this deliverance there has emerged (is still vigorously emerging), a theory that is truly dynamic, applicable to far-from-equilibrium contexts, and also to fluctuation-dominated systems comprised of small numbers of particles (including single molecules); see [13–25,28,34,204,205].

Our purpose here is to present a cursory introduction to the conceptual foundations of this theory, particularly as they relate to the “stochastic thermodynamic” treatment of molecular ‘machines’ operating in an aqueous medium. We will summarize the basic ideas first—following primarily Phil Attard [25,34]). We then discuss how dynamics – that is, time-dependent trajectories in the space of macrostates – is represented in the theory and focus on the context most relevant to our present discussion, namely single macro-molecules being driven through work-performing trajectories by chemical disequilibria. For this we follow the approach taken by Seifert [22,23].

We note first, however, that this quite recent and profound revolution on the path to a theory of non-equilibrium thermodynamic phenomena was preceded by an early stage which, while it did not generalize the classical state space concept, developed the highly important advance to a theory termed ‘linear non-equilibrium’ thermodynamics. The essential elements of this approach are summarized in [Appendix B](#).

In this connection we should also note the work of Onsager and Machlup, published in 1953 [206,207], which interprets transitions between nonequilibrium states as diffusion processes and analyzes trajectories composed of a sequence of such transitions in terms of a Markov model. On this basis they determine “The probability of a given succession of (nonequilibrium) states, of a spontaneously fluctuating thermodynamic system ...”. And from that the “microscopic reversibility” condition as the ratio of the forward and reverse probabilities of a stochastic trajectory between specified starting and ending states. At the end of [Appendix A.2](#) we briefly note the relevance of their results to the analysis of biological engines.

A.1. Microstates, macrostates, probabilities, entropy

The present paper’s discussion of disequilibria conversion is developed around the analysis of a maximally simple ‘statistical thermodynamics’ conceptual model (see Section 4), that of particles of finite size distributed in a ‘box’ comprised of two chambers of finite volume separated by a partition. Models of this type are to be found in a variety introductory explanations of contemporary thermodynamics [25,34,52,206]. A virtue of such models is that in them the defining dichotomy of statistical thermodynamics, that between macrostates and microstates, has a simple and intuitively obvious realization: microstates are specific choices for which of the available positions within the box are occupied by particles; macrostates are the fraction of the particles that are in one of the box’s two partitions (arbitrarily chosen). In general macrostates are defined by ‘macroscopic’ parameters that can be measured and/or fixed, and microstates are defined by the particle-level details of the system (e.g. individual particle positions and velocities) which are, typically, “inaccessible” or “unknowable”.¹¹

Of course, multiple microstates underlie any of a system’s macrostates. In particular, a system’s macrostates form a disjoint and exhaustive partitioning of the set of all microstates implying that each microstate is in one and only one macrostate. In a fair sense, moreover, all of thermodynamics blossoms out of the simple matter of how many of a system’s microstates lie within any particular macrostate (or, in the more common and physical case in which all microstates do not have the same probability, what is the total statistical weight of those microstates).

Systems abstracted in this way become ‘thermodynamic’ through the further assumption that transitions between microstates are incessantly ongoing—some within the same macrostate, some taking the system from one macrostate to another. This implies that the system is ‘sampling’ all of its possible microstates and, correspondingly, that the probability that at any instant the system is in any one of these states is well defined. We next sketch briefly how these general concepts are formulated, and follow that with an equally summary sketch of their particular application the thermodynamics of single macro-molecular engines operation in an aqueous medium.

Following Attard [34, §1] a non-negative numerical “weight” is assigned to each of a system’s microstates (taken here to form a discrete set) which is proportional to the probability that the system is in that microstate: i.e. if w_i is the weight of the “ith” microstate (by convention given the value “1” if all microstates are equally likely), then $p_i = w_i/W$ is the probability of

¹¹ For a general and detailed discussion of the matters cursorily summarized here see [25, Prologue].

the i th microstate where $W \equiv \sum_i w_i$ is the sum of the weights of all microstates in the system (i.e. the total ‘weight’ of the system, and the count of the system’s total number of microstates if all are equally likely). This implies that the ‘weight’ and the ‘probability’ of each *macrostate* are inferentially defined by the obvious sums: $w_\alpha \equiv \sum_{i \in \alpha} w_i$ where w_α is the weight of the α th macrostate and is just the sum of the weights of all of the microstates it contains. Similarly, $p_\alpha = w_\alpha/W$ where $W = \sum_\alpha w_\alpha = \sum_i w_i$, is the probability that the system will be in macrostate α , and the last expression in the preceding equation makes it explicit that the sum of all macrostate weights equals the sum of all microstate weights.

It is important here to note that implicit in the above definitions is the simplifying assumption that the weights and probabilities of both micro- and macrostates are invariant in time, and apply therefore only to systems that are in this sense in equilibrium. However, for systems embedded in a “chemostat” reservoir which maintains input and output flows at constant levels, this can include non-equilibrium steady state processes.

In terms of these weights, the (dimensionless) entropy of the system, and of macrostates, and microstates within it, have the forms:

$$S \equiv \ln W = \ln \left(\sum_i w_i \right) = \ln \left(\sum_\alpha w_\alpha \right), \quad (55)$$

$$S_\alpha \equiv \ln w_\alpha, \quad S_i \equiv \ln w_i$$

and the probability of a macrostate in terms of its entropy is therefore $p_\alpha = e^{S_\alpha}/W$.

As Attard points out however, the general relationship between the entropy of the system and that of its macrostates (or microstates) is somewhat subtle [25, pp. 18]. For example, the system’s entropy can be expressed in terms of the following sum over macrostates:

$$S = \sum_\alpha p_\alpha S_\alpha - \sum_\alpha p_\alpha \ln p_\alpha. \quad (56)$$

This qualitatively expresses that if we do not know which macrostate a system is in, the probability of guessing which microstate it is in at any instant, can be factored into the probability of guessing which macrostate it is in (in entropy terms the expectation value of the log of the macrostate probabilities: $-\sum_\alpha p_\alpha \ln p_\alpha$) and the average over all macrostates of the conditional probability of guessing the microstate given that we know the macrostate: the expression, again in entropy terms, $-\sum_\alpha p_\alpha S_\alpha$.

A.2. Transitions between macrostates in the Attard formulation

We next develop the statistical properties of transitions between microstates, and inferentially between macrostates, but emphasize again that, for simplicity, the present discussion is confined to systems that are time invariant in two senses. First, as was assumed in the preceding segment, microstate weights (and inferentially macrostate weights) are constant in time. Second, we will assume that the probability of a given microstate transition (inferentially a given macrostate transition), while dependent on the time *interval* during which the transition is being considered, has no other time dependence; i.e. that the transition probabilities are homogeneous in time.

The fraction of all microstate transitions (taking place in time τ) that are between the specific microstates i and j is by definition the ‘unconditional’ probability of that transition: $p(j, i | \tau)$. This probability is in turn associated with a “transition weight” written: $w(j, i | \tau)$ for the microstate transition $i \xrightarrow{\tau} j$: $p(j, i | \tau) = w(j, i | \tau)/W$, where, as we will show presently, the sum over all microstate transition weights is equal to the sum of all microstate weights; that is: $\sum_{i,j} w(j, i | \tau) = W$; $\forall \tau$ and is the correct normalizing factor for the transition probabilities.

As noted, in general most microstate transitions are between different macrostates and thus induce macrostate transitions. These too, reflecting that macrostates are disjoint and complete sets of microstates, are associated with transition weights which are the sums of those of their constituent microstate transitions. That is:

$$w(\beta, \alpha | \tau) = \sum_{i \in \alpha} \sum_{j \in \beta} w(j, i | \tau) \quad (57)$$

is the weight of the macrostate transition $\alpha \xrightarrow{\tau} \beta$. Correspondingly, the unconditional probability of this macrostate transition is:

$$p(\beta, \alpha | \tau) = \frac{w(\beta, \alpha | \tau)}{W} \quad (58)$$

which follows from the fact that sum of all macrostate weights is equal to the system weight W (see directly below).

The physics of any particular system enters in part via the form of the connection for that system between the weights of microstates and the weights of the transitions between them. In general however, two ‘reduction rules’ between these

two classes of weights must be satisfied:

$$\begin{aligned} \sum_j w(j, i | \tau) &= w(i) = w_i \quad \text{and,} \\ \sum_i w(j, i | \tau) &= w(j) = w_j \end{aligned} \quad (59)$$

(for the derivation of these rules see [25, 1.4.4]). The meaning of these conditions is that the total weight of all transitions from, respectively to, a given microstate is equal to the weight of the microstate that is the source, respectively destination, of the transitions. That is to say, the collective impact of the set of all transitions conserves microstate weights, and thus microstate (and macrostate) probabilities. From either of these relations it follows that the sum of all microstate transition weights and also the sum of all macrostate transition weights is equal to the weight of the system: $\sum_{i,j} w(j, i | \tau) = \sum_{\alpha,\beta} w(\beta, \alpha | \tau) = W$, which is what we asserted above in order to argue that W is the appropriate normalization factor to convert both microstate and macrostate transition weights to their corresponding (unconditional) transition probabilities.

A central element in the calculus of transitions is that for every microstate i there is a unique “conjugate” state, conventionally labeled i^\dagger , here obtained from the former by supposing that time is reversed (see [23, Sec. 4] for a more general discussion of conjugate states). If the microstate is characterized by properties, such as velocities, which change under time reversal (e.g. change sign) then the microstate and its conjugate are distinct, whereas in the opposite case they are identical. Clearly the ‘conjugate’ mapping is 1:1 and the conjugate of a conjugate is the original microstate.

In terms of this notion, and keeping in mind the assumption of equilibrium, the key principle of ‘microscopic reversibility’ can be stated:

$$w(j, i | \tau) = w(i^\dagger, j^\dagger | \tau) \quad (60)$$

that is, “...the forward transition between the original states has the same weight as the reverse transition between the conjugate states”. (to quote Attard [25, pp. 20]. Further, since there is one and only one conjugate i^\dagger for each i , we can, for example, write that $\sum_{j^\dagger} w(i^\dagger, j^\dagger | \tau) = \sum_j w(i^\dagger, j^\dagger | \tau)$. Therefore summing both sides of the microscopic reversibility equation over j , and invoking the reduction rules given in Eq. (59) yields:

$$\begin{aligned} \sum_j w(j, i | \tau) &= \sum_{j^\dagger} w(i^\dagger, j^\dagger | \tau), \\ w_i &= w_{i^\dagger}. \end{aligned} \quad (61)$$

That is, under the assumption noted above that microstate weights are time invariant, a microstate and its conjugate have the same weight.

The conjugates of all microstates in a macrostate, say “ α ” form a potentially distinct conjugate macrostate α^\dagger . Importantly, the condition of microscopic reversibility implies the analogous reversibility condition for macrostate transitions:

$$w(\alpha^\dagger, \beta^\dagger | \tau) = w(\beta, \alpha | \tau) \quad (62)$$

(see derivation in [25, p. 21]). And, given that microstates and their conjugates have the same weight, so too do macrostates and their conjugates:

$$\begin{aligned} w_\alpha &= w_{\alpha^\dagger}, \quad \text{and therefore also their probabilities:} \\ p_\alpha &= p_{\alpha^\dagger}. \end{aligned} \quad (63)$$

The last relations of importance to us here involve the conversion between unconditional and conditional macrostate transition probabilities. By definition, the fraction of all of the macrostate transitions originating in a specific macrostate, say α , which terminate in another specific macrostate β is the ‘conditional probability’ of that macrostate transition, here written: $p(\beta | \alpha, \tau)$. Of course, the ‘unconditional’ probability can be expressed in terms of the corresponding ‘conditional’ probability according to:

$$p(\beta, \alpha | \tau) = p(\beta | \alpha, \tau) p(\alpha). \quad (64)$$

In words: the fraction of all macrostate transitions that are from macrostate α to macrostate β is equal to the fraction of all macrostate transitions originating in α that terminate in β times the probability of being in the macrostate α . Combining Eqs. (58) and (62) we have the macroscopic variant of the “principle of microscopic reversibility.

$$\begin{aligned} p(\alpha^\dagger | \beta^\dagger, \tau) p(\beta^\dagger) &= p(\beta | \alpha, \tau) p(\alpha), \quad \text{hence} \\ \frac{p(\beta | \alpha, \tau)}{p(\alpha^\dagger | \beta^\dagger, \tau)} &= \frac{p(\beta^\dagger)}{p(\alpha)} = \frac{p(\beta)}{p(\alpha)}, \\ &= e^{S(\beta) - S(\alpha)} = e^{\Delta S(\alpha \rightarrow \beta)} \end{aligned} \quad (65)$$

where we have here used dimensionless entropies and the fact that a macrostate and its conjugate have the same weight and therefore the same probability (Eq. (63)). That is, the exponential of the entropy difference between macrostates β and α is equal to the ratio of the conditional transition probability for $\alpha \xrightarrow{\tau} \beta$ to that for its ‘conjugate’ reverse transition $\beta \xrightarrow{\tau} \alpha$.

In the model thermodynamic system considered in this paper, states and their conjugates are identical; in that case the above equations reduce to:

$$\frac{p(\beta | \alpha, \tau)}{p(\alpha | \beta, \tau)} = \frac{p_\beta}{p_\alpha} = e^{\Delta S(\alpha \xrightarrow{\tau} \beta)}. \quad (66)$$

We may regard the above equations as expressing a generalized, ‘macroscopic’ fluctuation relation [73]. They assert that a transition which increases the entropy of a system by ΔS is more likely than its (time) reverse by the exponent of that entropy change.

In closing this section, we emphasize that its purpose and that of the preceding Appendix A.1 is to introduce the general basic concepts of modern stochastic thermodynamics, whereas that of the following Appendix A.3 is to present an outline sketch of full stochastic thermodynamics theory in a form suitable for the analysis of the kinds of macromolecular systems that are the subject of this piece, namely those used by life to convert disequilibria. This calls for a significant shift in conceptual gears and the abandonment of several simplifying assumptions. Now macrostates will have explicit time dependence and systems are not assumed to be in equilibrium. We must also now explicitly allow interactions: between and within the molecules in the system, and between those molecules and the aqueous medium in which it embedded.

At the conceptual level, we must now see individual macromolecules in water as true thermodynamic systems – systems which are, moreover, explicitly dynamic: executing stochastic trajectories in the space of the macromolecule’s macrostates. Now, trajectories, not states, are the grist of the analytic mill. Where classical thermodynamics is erected on the probability distribution of equilibrium states, stochastic thermodynamics is erected on the probability distribution defined over the set of such trajectories. Further, as we mentioned earlier 3.2 these systems are not just inherently stochastic and fluctuation driven, they operate at the extreme low Reynolds number limit. This latter point is important in part because it defines one of the major alternatives in how such systems are to be treated theoretically, as we indicate briefly next.

Our discussion below of stochastic thermodynamics is based largely on the approach of Udo Seifert, in particular that described in his 2011 article “*Stochastic thermodynamics of single enzymes and molecular motors*” [22]. But before launching into it we should emphasize that stochastic thermodynamics is far from a settled theory, and several different approaches are in active contention. That of Attard, for example [25] differs significantly from that of Seifert. But we here take particular note of the approach advanced by Dean Astumian and his colleagues, in part because its conceptual basis is quite distinct from that of other contemporary contenders.

This approach springs from two insights. First that all molecular-scale disequilibria conversions work by ‘rectifying’ processes of thermal diffusion (adding a note of aptness to the simple diffusion model with which this paper began); second that such conversions taking place in aqueous media operate at the low Reynolds number limit (see e.g. [208]). In that context, Astumian and colleagues have pointed out, such systems are in near perfect mechanical and thermal equilibrium with the bath even while potentially being in arbitrarily strong thermodynamic disequilibrium. In consequence, they argue, diffusive fluctuations of observable thermodynamic variables (such as the extent of a chemical reaction) result from a Gaussian distributed thermal noise force and that in this and other important respects such systems conform to the prerequisite assumptions in the work of Onsager and Machlup mentioned above: “*Fluctuations and Irreversible Processes*” [206]. See in particular [209–211] but also [53,54,212,213]. The Onsager–Machlup paper, we note, analyzes system trajectories as a Markovian series of sequentially “gated” diffusion processes.

A.3. The dynamics of systems consisting of single macro-molecular machines in a thermostated aqueous solution

As just noted, our discussion here will largely follow the approach and notation used in a 2011 Seifert article [22] (though we also refer the reader to Seifert’s 2012 review [23], and in particular to the general introduction to the problem presented in section 9.1 of that paper). In this approach the macro-molecule (“enzyme” for short) is itself a thermodynamic system which can exist in a discrete set of observable macrostates, in this context termed ‘mesostates’ (and here labeled with n and m), each of which is comprised of many ‘internal’ microstates. Microstate transitions, both within and between mesostates, are assumed to be very much faster than transitions between enzyme mesostates each of which may therefore be taken to be in thermal equilibrium with the bath. Further, mesostate transitions are treated as discontinuous ‘jumps’ occurring at specific, but probabilistically determined, times (though variants of the theory have been developed in which mesostate transitions are modeled as continuous ‘diffusive’ processes. See, e.g., [26]).

In this context, the system is the bath plus the enzyme so that an individual system microstate, here labeled ξ , is comprised of a bath microstate ξ^{sol} together with an enzyme microstate ξ^{enz} , i.e. $\{\xi\} = \{\xi^{\text{sol}}, \xi^{\text{enz}}\}$. The system is assumed to have one distinct macrostate for each of the enzyme’s mesostates, and thus the system’s microstates are partitioned into disjoint subsets $\{C_n\}$ one for each enzyme mesostate; that is, each system microstate ξ is in one and only one mesostate subset C_n .

The assumption that the microstates of the bath together with those of any enzyme mesostate are in thermal equilibrium implies that the total system’s microstates are Boltzmann distributed. This involves, of course, assuming the existence of a function $V^{\text{tot}}(\xi)$ which assigns an energy to each system microstate, although for present purposes only the general form of

how this energy depends on the separate components of that microstate, i.e. on those microstates of the solution and of the enzyme, need be specified. This, as given in [22, Eq. 6], and quoting from Seifert's text, is

$$V^{\text{tot}}(\xi^{\text{enz}}, \xi^{\text{sol}}) \equiv V^{\text{sol}}(\xi^{\text{sol}}) + V(\xi^{\text{enz}}, \xi^{\text{sol}}) \equiv V^{\text{tot}}(\xi). \quad (67)$$

...where $V(\xi^{\text{enz}}, \xi^{\text{sol}})$ contains both the interaction within the enzyme and the interaction between enzyme and solution.

That is, the total energy of the system's microstate is taken to be the sum of energy of the solution, which depends only on the solution microstate ξ^{sol} , plus that of the enzyme, which is taken to depend on the microstates of both solution and enzyme. Note that no assumption is made or needed about the strength of these interactions.

Given the above (and continuing to quote),

For any specific state n , the probability $p(\xi | n)$ of finding an allowed microstate of the combined system consisting of enzyme and solution then follows from the assumption of fast equilibrium as [22, Eq. 7]

$$p(\xi | n) = \exp[-\beta (V^{\text{tot}}(\xi) - F_n)], \quad (68)$$

with $\beta \equiv 1/k_B T$ and the constrained free energy in state n

$$F_n \equiv -k_B T \sum_{\xi \in \mathcal{C}_n} \exp[-\beta V^{\text{tot}}(\xi)], \quad (69)$$

ensuring proper normalization $\sum_{\xi \in \mathcal{C}_n} p(\xi | n) = 1$. The (mean) internal energy in state n is

$$E_n \equiv \sum_{\xi \in \mathcal{C}_n} p(\xi | n) V^{\text{tot}}(\xi), \quad (70)$$

and the (intrinsic) entropy becomes as usually

$$\begin{aligned} S_n &= -k_B \sum_{\xi \in \mathcal{C}_n} p(\xi | n) \ln p(\xi | n), \\ &= (E_n - F_n) / T. \end{aligned} \quad (71)$$

Dynamics enters as the result of transitions occurring stochastically between discrete system macrostates (enzyme mesostates). An individual mesostate transition is here notationally represented as being from state n_ρ^- to state n_ρ^+ , where the symbol ρ labels the pair of mesostates involved and subsumes both forward and reverse transitions between them: $n_\rho^- \rightarrow n_\rho^+$ and $n_\rho^+ \rightarrow n_\rho^-$; which direction is intended will be denoted, when required, by a $\sigma_\rho = \pm$ superscript (i.e. $\rho_j^{\sigma_j}$ to indicate the j th transition in a series making up a trajectory) with the convention that “+” labels the “forward” transition: $n_\rho^- \rightarrow n_\rho^+$.

Trajectories in the space of mesostates are treated as being specific sequences of single-step transitions, taking place at specific times, and produced by a Markov process. This inherently stochastic dynamic generates, from any starting mesostate, a statistical ensemble of trajectories. Thus the general task is to derive the probability distribution of trajectories within that ensemble and from that the thermodynamic laws governing them, fluctuation relations in particular.

The key quantity in this analysis is the ratio between the probability of a transition and that of its “reverse”—where a few different definitions of the reverse process can be usefully considered (see [23, Sec. 4]). Here we will only be concerned with the literal meaning of the term, namely that the reverse of the transition $n \rightarrow m$ is $m \rightarrow n$.

However, the conventional approach to this problem has been based on taking the reverse process to be that generated by the underlying equations of motion under time reversal. The forward-to-reverse probability ratios are then constrained by assuming the so-called principle of micro-reversibility' (for a review see [23]).

The 2011 Seifert paper under consideration, in contrast, presents a novel approach (see [22, Sec. 5.3]) based on noting that in the stochastic thermodynamics context the average rate of (total system) entropy production taken over all possible single-step macrostate transitions must be non-negative in order that the macroscopic behavior of the system agree with the 2nd law. The upshot of imposing this condition is that the total entropy production in a single step must equal the log ratio of the unconditional probability for the forward transition in time t from n_ρ^- to n_ρ^+ to that for the (literal) reverse transition (from n_ρ^+ to n_ρ^-). That is

$$\begin{aligned} \Delta S_\rho^{\text{tot}}(t) / k_B &= \ln \frac{p(n_\rho^+, n_\rho^- | t)}{p(n_\rho^-, n_\rho^+ | t)}, \\ &= \ln \frac{p_{n_\rho^-}(t) w_\rho^+}{p_{n_\rho^+}(t) w_\rho^-}, \\ &= \ln \frac{p_{n_\rho^-}(t)}{p_{n_\rho^+}(t)} + \ln \frac{w_\rho^+}{w_\rho^-}, \end{aligned} \quad (72)$$

where in the second line the unconditional transition probabilities have been expressed as the product of the conditional probability of the transition given the transition's starting mesostate (the terms w_ρ^+ and w_ρ^- , which are conventionally called transition "rates") times the (potentially time-dependent) probability of the system being in the starting mesostate.

Seifert then shows that the terms on the right hand side of the above expression for the total entropy change in a single mesostate transition have the following meanings:

$$\begin{aligned} \ln \frac{p_{n_\rho^-}(t)}{p_{n_\rho^+}(t)} &= \frac{\Delta S_\rho(t)}{k_B}, \quad \text{and} \\ \ln \frac{w_\rho^+}{w_\rho^-} &= \frac{1}{k_B} (\Delta S_\rho^{\text{med}} + \Delta S_\rho), \\ &= -\beta \Delta F_\rho, \end{aligned} \quad (73)$$

where (i) $\Delta S_\rho(t) = s_{n_\rho^+}(t) - s_{n_\rho^-}(t)$, $s_{n_\rho^-}(t)/k_B \equiv -\ln p_{n_\rho^-}(t)$, and $s_{n_\rho^+}(t)/k_B \equiv -\ln p_{n_\rho^+}(t)$, are "stochastic entropies", (ii) $\Delta S_\rho^{\text{med}} = q_\rho/T$, where q_ρ is the heat released into the bath, is the change in the bath's entropy resulting from the transition, and (iii) ΔS_ρ is the change in the system's intrinsic entropy (71): $\Delta S_\rho = S_{n_\rho^+} - S_{n_\rho^-}$, and the final line reflects that $\Delta F_\rho = \Delta E_\rho - T \Delta S_\rho = -q_\rho - T \Delta S_\rho = -T (\Delta S_\rho^{\text{med}} + \Delta S_\rho)$ so that $-\beta \Delta F_\rho = (\Delta S_\rho^{\text{med}} + \Delta S_\rho)/k_B$.

The relationships above involving the ratio of the one-step forward and reverse transition rates w_ρ^+/w_ρ^- "... are well known under the notion of 'local detailed balance'". In contrast, in an "equilibrium ensemble", that is for which for $p_n(t) = p_n^{\text{eq}}$ so-called "global detailed balance" applies: $p_{n_\rho^-}^{\text{eq}}(t) w_\rho^+ = p_{n_\rho^+}^{\text{eq}}(t) w_\rho^-$. In this situation "...for each jump the contributions to system entropy and medium entropy exactly compensate each other so that the total entropy remains strictly constant along any individual trajectory". (See also [23, Sec. 6].)

A specific trajectory starting from state $n(t_i)$ and taking place in a definite time interval $t_i \rightarrow t_f$ consists of a sequence of single step mesostate transitions $\rho_j^{\sigma_j}$, in either direction, between neighboring states in a specific sequence of mesostates $\{n_0 = n(t_i), n_1, n_2, \dots, n_j \dots n(t_f)\}$. Note that, due to 'backward' transitions, there will in general be more transitions than there are mesostates in the sequence. The entropy produced in such a sequence is the sum of the entropies produced in each transition in such a trajectory, which can be written as:

$$\Delta S^{\text{tot}}/k_B = \sum_j \ln \frac{w_{\rho_j^{\sigma_j}}}{w_{\rho_j^{-\sigma_j}}} + s(t_f) - s(t_i). \quad (74)$$

(See [22, Eqn. 67].) Continuing to quote:

This quantity obeys a relation called the integral fluctuation theorem for entropy production [9]

$$\langle \exp[-\Delta S^{\text{tot}}/k_B] \rangle = 1, \quad (75)$$

where the average $\langle \dots \rangle$ is over many trajectories taken from any well-defined initial ensemble characterized by $p_n(t_i)$ and running for an arbitrary but fixed time interval $t_f - t_i$. From this integral relation one gets easily the second-law like statement on the mean total entropy production

$$\langle \Delta S^{\text{tot}} \rangle \geq 0. \quad (76)$$

(... skip ...)

For a non-equilibrium steady state where $p_n(t) = p_n$ is independent of time, one has the detailed fluctuation theorem

$$p(-\Delta S^{\text{tot}})/p(\Delta S^{\text{tot}}) = \exp(-\Delta S^{\text{tot}}/k_B), \quad (77)$$

for the probability distribution $p(\Delta S^{\text{tot}})$ to observe a certain total entropy production valid for any time interval in this non-equilibrium steady state [9].

Note that averaging "over many trajectories" as called for in the above quote requires that one know the probability distribution of the set of all trajectories. A discussion of how probabilities are assigned to specific trajectories is given in [23, Sec. 6.1.3].

A.4. Fluctuations and the dynamics implicit in the 2nd law

As Attard has noted [25, Prologue], history has given us the 2nd law in two significantly distinct forms. That by Clausius says that systems spontaneously evolve to states of higher entropy, resting only at the state of the highest possible entropy ("equilibrium"). That by Boltzmann focuses on the number of microstates underlying any particular macrostate and says that the macrostate has a probability proportional to that number and an entropy equal to the log of that number. The former entropy concept is implicitly dynamic, the latter static. However, these two conceptions unite (and in that union confer a

theoretical legitimacy on time's manifest irreversibility) on the fruits of the previous discussion, the 'detailed fluctuation relation' (77) in particular. According to this relation, to repeat, microstate transitions that move a system's macrostate to one of higher entropy (larger number of microstates) occur more frequently than the reverse thereof, and in the ratio of the exponent of the entropy change.

On the qualitative level, the conceptual upshot is that all transformations in nature¹² – chemical reactions included – are simply a manifestation of (and 'caused' by) a statistical bias in favor of systems undergoing changes which increase, rather than decrease, the number of microstates to which a system plus surroundings has access (equivalently, increases the state's probability); while respecting, that is, all conservation laws—the absolute conservation of energy in particular. All change is therefore an instance of a “humpty dumpty” falling apart, going from fewer to more 'pieces', in a transformation of state that is inherently more probable than having those pieces fall back together again (we here again note the exceptionally illuminating and detailed discussion in P.W Atkins “*The 2nd law; Energy, Chaos, and Form*” [127]; and the opening chapters of Phil Attard's two books “*Thermodynamics and Statistical Mechanics; Equilibrium by Entropy Maximization*” [34], and “*Non-Equilibrium Thermodynamics and Statistical Mechanics*” [25]).

Appendix B. On the leap from equilibrium to 'linear' non-equilibrium thermodynamic concepts “LNET”

In classical “equilibrium” thermodynamics, thermodynamic quantities, such as S are only defined for the equilibrium states (or states incrementally close to them) of true “thermodynamic” systems, i.e. continua or discrete systems having infinitely many particles. Thus these quantities could not be said to even exist, much less to change in any continuous sense during a manifestly non-equilibrium transformation; or to be defined in systems comprised of a small number of particles. This classical point of view puts into question the above arguments which invoke a continuous change in such quantities during a non-equilibrium relaxation of a diffusion disequilibrium in a small, discrete system—and with that the physical inference that throughout the transformation it is the increase in W , the 'effective' number of microstates, in that is, the probability of the system's macrostate, that is physically what 'drives' the process.

This would imply that it is not really correct, or not 'rigorously' correct, to define, as we have done, an entropy for states of the balls-in-a-box model, much less in the small numbers limit and seen implicitly as a 'dynamic', time-dependent quantity. Or it might be taken to imply that the 'microstate-counting' analytic approach taken here is in some way valid only in special model cases like the one considered and cannot be taken to represent non-equilibrium thermodynamic processes more generally. However, as we have argued rather heavily elsewhere in this piece, thermodynamic theory is not where it was a half century ago and neither of these possibilities is correct. See, for example, the general textbook discussion by Dill and Bromberg: “*Molecular Driving Forces; Statistical Thermodynamics in Biology, Chemistry, Physics, and Nanoscience*” [52] which we have cited many times; it is founded on the 'statistical', microstate-counting, reading of thermodynamics and makes heavy use of finite-particle models and arguments of the kind we have employed here. And see also [19,21,23,25,34,219].

These developments, among other things, introduced true time dependence, true dynamics, into the theory. Now, in a wide variety of relevant systems, including far-from-equilibrium systems, one can think about, and compute $S(t)$ (equiv. $W(t)$) during transformations proceeding at finite rates and even in systems comprised of small numbers of particles (including only one) as we review in Appendix A.

Notably, one of the principle fruits of the first major phase of the development of non-equilibrium thermodynamics, leading to so-called 'linear non-equilibrium thermodynamics' “LNET” was providing a basic understanding of free energy conversion (see e.g. Hill [11], Caplan [10]). However, this progress rested on some fundamental conceptual shifts which remain somewhat under-appreciated. For this reason we next summarize briefly the elements of LNET that are relevant to the points raised in the present discussion. But before doing so note that these earlier LNET efforts have recently been recast and fundamentally extended using the modern machinery of full non-linear, non-equilibrium, stochastic thermodynamics; see in particular [23, Sec. 10].

An essential and early step in moving beyond classical equilibrium thermodynamics was the development of a variant of it in which only “local equilibrium” was required, a condition that could often be realized in practice to an adequate approximation. This made it formally possible to assign to each point in the domain of interest a temperature and local energy and entropy densities (see [220]). And critically, this then allowed entropy gradients, and the finite fluxes to which they give rise, to be embraced within the boundaries of a thermodynamic system. The other key element, closely related to the first, involved the explicit recognition that entropy gradients, i.e. disequilibria, are the underlying cause and driving force responsible for thermodynamic transformations; a point the CDC model discussed in the main text humbly illustrates.

¹² Although gravitational dynamics are a possible exception, there is strong reason to believe it too will ultimately be understood in such thermodynamics terms; wherein increased space-time curvature involves increased numbers of gravitational microstates [214–218]. Black holes are the limiting case in this regard and one of the few circumstances in which the conversion between curvature and entropy can at present be stated with certainty.

As noted briefly in Section 2, the core relationship of first generation non-equilibrium thermodynamics characterizes the time rate of change of the entropy of a spatially bounded but open system. In particular it specifies that:

$$\begin{aligned} \frac{dS}{dt} &= \frac{d_e S}{dt} + \frac{d_i S}{dt}, \quad \text{where} \\ \frac{d_i S}{dt} &= \sum_j J_j X_j \geq 0, \quad \text{and further that} \\ J_j &= J_j(X_k; \forall k), \end{aligned} \quad (78)$$

where S is the system's entropy, $d_e S/dt$ is the rate at which the system's entropy is changing due to the movement of mass/energy across the system's boundary (which has no effect on the entropy of the universe), and $d_i S/dt$ is the rate at which that entropy is changing due to the irreversible (i.e. entropy changing) processes operating in the system—and is necessarily, by the 2nd law, non negative. Also, as the second equation indicates, this latter rate can be expressed as the sum (indexed by “ j ”) over the individual irreversible processes of the rate at which each is changing the system's entropy, and that each of these terms can in turn be expressed as the product of the flux rate of the process J_j times the rate of entropy change per unit flux of that process X_j —generally called the force for that process. And while the 2nd law requires that $d_i S/dt \geq 0$, individual processes can have negative force terms reflecting that those processes are decreasing the system's entropy. Finally, the third equation states the critical point that in general the fluxes depend on the forces, and potentially all forces present, not just on an individual flux's cognate force.

These latter two points are in fact the essential elements allowing LNET to develop a principled theoretical treatment of the inherently non-equilibrium thermodynamic processes involved in the conversion of disequilibria. In the simplest case for such conversions, the sum term in Eq. (78) has just two terms, in one (the ‘driver’ process) the force factor is positive while it is negative in the other (the ‘driven’ process). In addition, both fluxes must be functions of both forces to effect conversion (see [10,11,33]).

Furthermore, taking processes number 1 and 2 to be, respectively, the driver and driven processes in a conversion, the work output rate (resp. input) is given by $J_2 (-X_2)$ (resp. $J_1 X_1$), that is, as time rates of entropy change. Correspondingly, the conversion efficiency is naturally, and commonly, defined as the output-over-input ratio of these two rates of entropy change [33,37]. Of course these rates can be given energy-denoted power units simply by multiplying by temperature. But what is happening physically, as we saw illustrated in the CDC model, is changing entropies.

In addition, as Onsager was the first to realize [7,8], the principle of microreversibility implies a strong symmetry constraint on the coupling of driver and driven processes. Staying with the above choice that process 1 is driving process 2 ($X_1 > 0$; $X_2 < 0$), and expanding the dependence of fluxes on forces (the third equation in Eq. (78) to linear order (the zeroth order term is necessarily zero) we can write

$$\begin{aligned} J_1 &= L_{1,1}X_1 - L_{1,2}|X_2|, \\ J_2 &= L_{2,1}X_1 - L_{2,2}|X_2| \end{aligned} \quad (79)$$

where the coefficients are positive and have the units of ‘conductances’. The ‘irreversible’ time rate of the change of the system's entropy (the 2nd expression in Eq. (78)) then has the form:

$$\begin{aligned} \frac{d_i S}{dt} &= J_1 X_1 - J_2 |X_2|, \\ &= (L_{1,1}X_1 - L_{1,2}|X_2|)X_1 - (L_{2,1}X_1 - L_{2,2}|X_2|)|X_2|, \\ &= L_{1,1}X_1^2 - L_{1,2}|X_2|X_1 - L_{2,1}X_1|X_2| + L_{2,2}|X_2|^2, \\ &= L_{1,1}X_1^2 + L_{2,2}|X_2|^2 - (L_{1,2} + L_{2,1})X_1|X_2|. \end{aligned} \quad (80)$$

Onsager's reciprocal relations are, in this case, that the two ‘cross term’, ‘coupling’ coefficients, must be equal: $L_{1,2} = L_{2,1} \equiv L_c$.

How should these constraints be interpreted physically? Referring to the 2nd and 3rd expressions in the above Eq. (80) it is clear that $L_{1,1}X_1^2$ is the rate at which the driving disequilibrium would be dissipated in the absence of coupling ($L_c = 0$), whereas $L_{1,2}|X_2|X_1$ is the amount by which that rate is reduced in the presence of coupling; and also that $L_{2,1}X_1|X_2|$ is the rate at which the entropy of the driven flux is being reduced—which is the rate at which the disequilibrium in that flux is being created and work is being done. And it is further clear that the reduction in the rate of entropy production by the driving flux reflects that the coupling gives rise to a thermodynamic force acting on the driving flux that opposes its cognate force (the effect long noted, for example, in connection with electromagnetic conversions, called ‘back EMF’ [221]).

Thus the physical implication of the reciprocal relation is this: for every unit of entropy reduction achieved in the driven process, the driving process must forgo an exactly equal amount of the entropy production it would enjoy were there no coupling. Equivalently, if $r(\Delta t)$ is the factor by which the probability of the driven flux is decreased in time Δt and $R^0(\Delta t)$ is the factor by which the probability of the driving flux would be increased in the same time interval in the absence of coupling, then $R^c(\Delta t) = R^0(\Delta t)/r(\Delta t)$ is the (smaller) factor by which it is increased in the presence of coupling.

We note that this relationship is symmetrical under the reversal of driver and driven roles.

It was with the development outlined above that thermodynamic theory made its first enabling step to becoming a truly dynamic theory, able to deal with the strong disequilibria of the real world and the swift flows caused by them. At the core of that profound step lay the epiphany that the thermodynamic's central and defining concept is not energy, but entropy: seen as a dynamic variable: $S(t)$, understood to be a measure of the probability of a physical system being in one of its possible macrostates, and, through the statistical bias of systems to transition to states of higher rather than lower probability, the driving force of all change. None too soon we might conclude for the effort to come to grips with 'bioenergetics'; a system surely without equal as a complex and specifically structured web of interwoven, far-from-equilibrium, high speed, disequilibria-manipulating flows.

Appendix C. Putting the horse before the cart

All formal models of free energy conversion in biology (see Hill [11] and literature cited therein), have the feature that the coupling device segments each of the two processes being coupled into 'half reactions', where the initiating halves of both are carried out by one form of the complex and the concluding halves by another, respectively " E " and " E^* " in the Hill diagram of Fig. 7. Further, as we have noted repeatedly, Jencks argued that this design was not only essential but that it was also important that the initiating and concluding half reaction's be themselves sequenced in a particular "interleaved" 'driven-before-driver' pattern. This, he argued, allowed molecular devices to reliably and efficiently satisfy the essential operational criteria of converters, namely that neither process be allowed to complete within one cycle unless both do. Nominally, the most important aspect of this particular ordering is that the driven reaction goes to full completion before the driving reaction does (and, secondarily, that this operational property be preserved upon the reversal of the conversion). In the simple case, for example, when ATP hydrolysis is driving an exergonic reduction (as in Nitrogenases [94]), the reduction's completion precedes, and triggers, the hydrolysis.

To many if not most, this design has a disturbing, cart-before-horse character, sufficiently counterintuitive to have been the source of considerable controversy and to cause experimental observations of the fact be called out declaratively in paper titles [43,94]. And indeed, it is more-or-less flatly inexplicable on the idea that coupling processes work because (literal) energy is donated to the driven reaction by the driving one.

For these reasons it is important to note that the Jencks ordering is not required in principle. Assuming a simple system as illustrated in the Hill diagram Fig. 7, in which we take the full hydrolysis of ATP to be the driving reaction ($R \rightarrow P$), then simply exchanging the two 'catalysis and release' steps on the E^* side of the cycle, so that ATP hydrolysis precedes the completion half of the driven reaction, yields a potentially valid cycle. This of course, does not, and could not, rescue the fundamentally incorrect idea that energy released in the ATP hydrolysis is passed to, and drives, the driven reaction. On the other hand, neither does it prevent the driving process from doing more than just providing a forward statistical bias for the cycle, and in particular from facilitating more or less strongly the driven process. As we discussed in the text, in general the binding of substrates on the E side of the cycle, and unbinding of products on the E^* side induces conformational distortions of the complex in ways that can store, or release, 'free energy' and also strongly influence the binding and catalytic specificities of the complex. One such commonly observed influence is raising the effective K_m of the driven reaction's product release step; potentially to the point that under physiological conditions (of, that is, the chemical potentials of the products) the release step is itself exergonic.

This raises the interesting question of why Nature and Jencks together seemed committed to the pay-after-completion, cart-before-horse, scheme.

In Section 7.3, we offered some considerations that may explain why this is the case. We note here, however, that the horse-before-cart scheme we have just imagined puts the irreversibility step (primarily the release of ADP and Pi into the medium) just after the flip from E to E^* —which then serves to prevent the reversal of that flip step rather than to prevent the reversal of the release of the products of the driven reaction. Also, it is now the driven reaction's highly *reversible* product release step which must function as the obligate, 'must not fail' trigger for the $E^* \rightarrow E$ conformational reset. Therefore, depriving the driven product's release step of the advantages of kinetic trapping, potentially leaves that process open to the wasteful and risky mercies of a quasi-equilibrium battle between the off rates of the driven reaction's products and their inevitably high rebinding rates.

Appendix D. Free energy and the entropy of the universe

In bioenergetics, we are invariably dealing with systems embedded in a reservoir, or 'bath', of effectively infinite extent, which acts to hold one or more of the system's properties constant (e.g. temperature and volume or temperature and pressure). For such systems, the 2nd law requirement for an isolated system, namely that $dS^{\text{universe}} = dS_{\text{bath}} + dS_{\text{system}} \geq 0$ (with equality at equilibrium—at which point S is maximal), can be more conveniently expressed as a constraint on the properties of the system itself. This involves the use of so-called thermodynamic potentials. In particular, if a system's temperature and volume are held constant the Helmholtz free energy $F \equiv U - TS$ (where U is the system's energy), is the useful potential. This is because the 2nd law constraint on how the entropy of the universe changes is equivalent to the condition $dF_{\text{system}} \leq 0$, implying that F has a *minimum* value at equilibrium. Corresponding assertions apply for the Gibbs free energy $G \equiv H - TS = U + pV - TS = F + pV$ (where $H \equiv U + pV$ is the system's "enthalpy") if both temperature

and pressure are held constant [52, §8]. In other words, in the Helmholtz case, $dF = -dS^{\text{universe}}/T$ and in the Gibbs case $dG = -dS^{\text{universe}}/T$; i.e. the free energies function as useful stand-ins for the entropy of the “universe”.

In Appendix B, it is noted that the differential change in a bounded, open system’s entropy can also be expressed as: $dS_{\text{system}} = d_e S + d_i S$ where the first term is the change in the entropy of the system due to the exchange of energy or material between the system and its surroundings, i.e. the bath, and the second term is the change in the system’s entropy due to “irreversible” processes taking place within it. And noted further that the ‘exchange’ term has no effect on the entropy of the universe so that $dS^{\text{universe}} = d_i S$, and the 2nd law is satisfied if and only if $d_i S \geq 0$.

As a result, we can re-express the two free energy differentials just above, somewhat more revealingly, in terms of the entropy changes in an open system due to ‘irreversible’ (i.e. entropy changing) processes taking place within it. That is, $d_i S/T$ is equal to $-dF$ in the Helmholtz condition and to $-dG$ in the Gibbs condition.

Appendix E. The entropy flux analysis and the maximum power efficiency of heat engines

We wish here to use the case of heat engines to make a few basic conceptual points about disequilibria conversions with as little in the way in input assumptions and analytic complexity as possible. For a rigorous formal analysis of conversion efficiency, including in the context of heat engines, and based on the principles of stochastic thermodynamics so that it is applicable to ‘single molecule’ heat engines, see Seifert’s 2012 paper, Sec. 10.3 [23]. Our discussion here is placed in the classical, macroscopic domain.

As is very well understood, a heat engine operating between hot and cold reservoirs ($T_{\text{hot}}, T_{\text{cold}}$) produces zero power if the conversion to mechanical work takes place at T_{cold} (the choice that would maximize its “Carnot efficiency”) and that temperature is taken to be that of the environment (more on this presently).

Therefore in engineering practice the conversion is arranged to take place at a temperature T_{conv} intermediate between T_{hot} and T_{cold} . Suppose, for example, a heat engine converts an amount of heat energy Q_{conv} at temperature T_{conv} to mechanical work, $Q_{\text{conv}} = E_{\text{mechwork}}$ (in energy units, of course). Then the entropy of that amount of heat energy at the point of conversion will have been reduced to zero (mechanical work having a single degree of freedom: $W_{\text{mechwork}} = 1$, and $\ln(1) = 0$). Therefore, the entropy lost from the input stream in the conversion to work is $\Delta S_{\text{work}} = -Q_{\text{conv}}/T_{\text{conv}} < 0$. That is to say, the fraction of the input heat flow that is converted to mechanical work as the engine’s output is equal to minus the product of the temperature at which the conversion takes place times the amount by which the entropy of that quantity of heat is reduced in the process of being converted.

The point this makes about classical heat engines is that they too can be regarded as coupling a process acting to increase the entropy of one system (the fraction of the input heat energy destined to be exhausted) to a process acting to decrease the entropy of another (the fraction of input heat energy converted to mechanical work). And, we emphasize, these conversions also operate via escapement mechanisms that make the completion of an increment of the driving process conditional on the (prior) completion of an increment of the driven one (e.g. only when the steam engine’s piston has reached the end of its work-producing stroke is a mechanism activated that allows the “spent heat” to exit (driven by the thermal gradient between T_{conv} and T_{cold}) and the engine to recycle).

However, to provide some clarity and closure on this claim, and on the point raised just above that for a heat engine to produce finite power the conversion must take place at an intermediate temperature, it is of some interest to derive the maximum conversion efficiency, defined in power terms, of an idealized heat engine. This proceeds as follows.

At the point of conversion, the entropy then remaining in the heat flux that entered the engine but is now destined to be exhausted $Q_{\text{exh}} = Q_{\text{in}} - Q_{\text{conv}}$ must not be less than the entropy of the total input heat as it entered, i.e. $Q_{\text{in}}/T_{\text{hot}} \leq Q_{\text{exh}}/T_{\text{conv}}$, equivalently $Q_{\text{in}}/T_{\text{hot}} \leq (Q_{\text{in}} - Q_{\text{conv}})/T_{\text{conv}}$ (which constraint also ensures that the change in the entropy of the universe due to the engine’s operation is not negative, i.e. $Q_{\text{exh}}/T_{\text{cold}} \geq Q_{\text{in}}/T_{\text{hot}}$).

Rewriting this inequality we obtain:

$$\begin{aligned} Q_{\text{in}}/T_{\text{hot}} &\leq (Q_{\text{in}} - Q_{\text{conv}})/T_{\text{conv}}, \\ \eta^{\text{energy}} &\equiv \frac{Q_{\text{conv}}}{Q_{\text{in}}} \leq 1 - \frac{T_{\text{conv}}}{T_{\text{hot}}}, \\ \eta^{\text{energy}} &\leq 1 - \frac{T_{\text{conv}}}{T_{\text{hot}}} \end{aligned} \quad (81)$$

where η^{energy} is by definition the efficiency of the conversion defined in energy, not power, terms and is seen to be given by the Carnot formula in which the conversion temperature T_{conv} has the role of the Carnot engine’s cold temperature. Note that at the $\eta^{\text{energy}} = 1$ limit, the entropy lost due to the conversion of Q_{conv} to work is $\Delta S_{\text{work}} = -Q_{\text{conv}}/T_{\text{conv}} = -Q_{\text{in}}(1/T_{\text{conv}} - 1/T_{\text{hot}}) = -\Delta S_{\text{in} \rightarrow \text{conv}}$ that is, that the entropy lost to work is exactly equal (at the “Carnot limit”) to the entropy gain as the incoming heat falls in temperature from T_{hot} to T_{conv} .

Clearly, η^{energy} is maximal if the conversion takes place at the engine’s exhaust temperature: $T_{\text{conv}} = T_{\text{cold}}$ (and that is the coldest temperature available, namely that of the environment into which the engine must exhaust its spent heat). It is of course this ‘exhaust’ step which allows the engine to return to its starting condition and hence recycle. However, if the conversion temperature is the exhaust (environment) temperature, the exhaust step requires infinite time, or more

accurately, does not happen – reflecting the fact that there is then no thermodynamic driving force, i.e. no ‘irreversibility’, to cause the exhaust heat to exit the engine in finite time) – and the power delivered is zero.

James Clerk Maxwell himself gives the game away rather touchingly in his 1871 foundational treatise “*Theory of Heat*” [222, p. 141], wherein, in explaining the Carnot cycle, he says “*Fourth Operation—The cylinder is placed on the cold body B. It has the same temperature as B, so that there is no transfer of heat. But as soon as we begin to **press down the piston** heat flows from the working substance into B, so that the temperature remains sensibly equal to T during the operation. The piston must be **forced down** till it has reached the point at which it was at the beginning of the first operation ...*” (emphasis added). And, as Maxwell makes explicit, the forcing we are obliged to do must be done so slowly that everything stays “sensibly” in equilibrium. Quite naughty of the esteemed Doctor, one might be excused for feeling, to be doing all this forcing and pressing; but commendable that he is both willing to be so patient in doing it, and also content not to require that his “engine” actually deliver finite power, much less do so on its own.

On the other hand it has long been known (at least in engineering circles; see the discussion by Bejan [223]) that, given standard simplifying assumptions, a heat engine achieves maximum power if the conversion temperature is the geometric mean of the hot and cold limit temperatures: $T_{\text{conv}} = \sqrt{T_{\text{cold}}T_{\text{hot}}}$.

This pleasantly surprising result is because (1) when the conversion mechanism is converting the heat entering it at the Carnot limit, the rate at which entropy must be withdrawn from it in the exhaust flow, that is at T_{conv} , is exactly equal to the rate at which entropy entered the gradient at T_{hot} , and (2) the rate at which entropy is drawn into a thermal gradient having conductance L is $L(1 - T_{\text{cold-temp}}/T_{\text{hot-temp}})$. Therefore, assuming equal thermal conductances, the two half gradients must have the same cold-to-hot temperature ratios. That is we must have: $T_{\text{hot}}/T_{\text{conv}} = T_{\text{conv}}/T_{\text{cold}}$ which implies the above geometric mean expression for T_{conv} .

Taking that value of the conversion temperature, the 2nd law constraint on the conversion given above implies an upper limit on the efficiency of the engine defined in power terms:

$$\begin{aligned} Q_{\text{in}}/T_{\text{hot}} &\leq (Q_{\text{in}} - Q_{\text{conv}})/T_{\text{conv}}, \\ Q_{\text{in}}/T_{\text{hot}} &\leq (Q_{\text{in}} - Q_{\text{conv}})/\sqrt{T_{\text{cold}}T_{\text{hot}}}, \\ \eta^{\text{max-power}} &= \frac{Q_{\text{conv}}}{Q_{\text{in}}} \leq 1 - \sqrt{\frac{T_{\text{cold}}}{T_{\text{hot}}}}. \end{aligned} \quad (82)$$

That this is not just the upper limit on the efficiency at maximum power, but is the upper limit on the engine’s efficiency defined in power terms, reflects first that at the extraction point the maximum amount of heat is converted to work that the 2nd law allows (i.e., that given by the Carnot expression) and second that this conversion point provides a lower gradient which exactly produces the rate at which exhaust entropy must be withdrawn to allow the upper gradient to feed a maximally ‘efficient’ conversion step. One cannot do better in conversion than Carnot, and cannot move Carnot waste entropy out of the engine exactly as fast as it is being generated except by splitting the total gradient at its geometric mean temperature.¹³

In any case, the above expression for $\eta^{\text{max-power}}$, is necessarily a lower bound than given by the Carnot efficiency between the same two temperatures (and is the proper upper limit on “The Motive Power of Fire”).

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¹³ A rigorous formal discussion in modern, stochastic thermodynamics terms of molecular-level driven processes, heat engines in particular, is presented in [27].

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