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Thermodynamic performance of coupled enzymatic reactions: A chemical kinetics model for analyzing cotransporters, ion pumps, and ATP synthases

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ABSTRACT

Previous research has suggested that molecular energy converters such as ATP synthases, ion pumps, and cotransporters operate via spatially separate pathways for free energy donor and acceptor reactions linked by a protein molecule. We present a chemical kinetics model based on these works, with the basic assumption that all molecular energy converters can be thought of as linked enzymatic reactions, one running downhill the chemical potential gradient and driving the other uphill. To develop the model we first look at how an enzyme process can be forced to go backwards using a basic kinetic model. We then use these findings to suggest a thermodynamically consistent method of linking two enzymatic reactions. Finally, in the context of the aforementioned energy converters, the thermodynamic performance of the resulting model is thoroughly investigated and the obtained results are contrasted with experimental data.

1. Introduction

The theory of evolution through natural selection, the unity of biochemistry, and cell theory, according to Luria [1], are the three essential generalizations of biology. The unity of biological processes acknowledges that all living species share certain basic biochemical reactions because the chemical building blocks are the same: nucleic acids, proteins, and protein-producing mechanisms. Cell theory recognizes that all creatures are made up of cells, and that cells may be thought of as closed domains in which the chemical reactions required for life are carried out. One of the major duties of cells, from this perspective, is to keep the concentration of vital components high enough so that the chemical processes required for life can occur at functionally sufficient rates.

Despite the fact that cell membranes ensure high concentrations of key chemical species, many biochemical reactions would not be rapid enough without particular enzymes to speed them up. According to Berg et al. [2], enzyme catalysis is required for practically all metabolic activities in the cell to occur at rates rapid enough to support life. Enzymes, like all catalysts, can speed up naturally occurring processes, but they can't drive them up a chemical potential gradient.

In some circumstances, cells must carry out chemical processes that are not thermodynamically spontaneous. This is accomplished by the use of specific proteins (molecular energy converters) that link a favorable chemical process to an unfavorable one, allowing the first's chemical potential to propel the second up its corresponding energy gradient. Cotransporters, ion pumps, and ATP synthases are some examples.

Cotransporters are membrane transport proteins that couple one molecule's favorable movement (down its concentration gradient) with the unfavorable movement (against its concentration gradient) of another molecule [3]. Active transporters, also known as ion pumps, are ion transporters that use energy from a variety of sources, including adenosine triphosphate (ATP), sunlight, and other redox reactions, to pump an ion against an electrochemical potential gradient [4]. Finally, ATP synthases are proteins that catalyze the production of ATP. Since ATP production is generally energetically unfavorable [5,6], ATP synthases couple ATP synthesis to an electrochemical gradient, caused by a trans-membrane proton concentration differential.

Because translocation of a chemical substance can be thought of as a chemical reaction in which a molecule disappears from one compartment and reappears in another, cotransporters, ion pumps, and ATP synthases can be thought of as proteins that link two different chemical reactions and transfer energy from the thermodynamically favorable one to the unfavorable one. Previous works—see [7–17]—have proposed that molecular energy converters may work by means of spatially

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separate pathways for free energy donor and acceptor reactions, linked through a protein molecule. Inspired on these works, we introduce a chemical kinetics model whose basic assumption is that all molecular energy converters can be conceived as linked enzymatic reactions, one running downhill the chemical potential gradient and driving the other uphill.

The feasibility of the present model is evident in ATP synthases. They are made up of two linked subunits called F_O and F_1, which are connected by a rotor [5]. When F_O and F_1 are separated, F_O acts as an enzyme that catalyzes proton translocation across a membrane, whilst F_1 acts as an enzyme that catalyzes ATP hydrolysis. The F_O-F_1 complex, however, can use the energy stored in the proton concentration gradient to synthesize ATP against its chemical energy gradient. To the best of our knowledge, it is unclear whether our model is applicable to cotransporters and ion pumps, though there are some evidences that support this assumption [10].

To develop the previously referred model we first look at how an enzyme process can be forced to go backwards using a basic kinetic model. We then use these findings to suggest a thermodynamically consistent method of linking two enzymatic reactions. Finally, in the context of the aforementioned molecular energy converters, the thermodynamic performance of the resulting model is thoroughly investigated and the obtained results are contrasted with experimental data.

The key changes between our model and previous ones are that it takes into account the nonlinear behavior of enzymatic reactions and explicitly considers the coupling mechanism between driver and driven reactions (the occurrence of one alters the rate constants of the other).

2. A simple model for a reversible enzymatic reaction

Following Beard and Qian [18], consider the simple enzymatic-reaction model shown in Fig. 1. An enzyme can be in two states in this model: E and E_S. When a free enzyme (E) is bound by a substrate molecule S, it moves from state E to state E_S, and when the substrate molecule is released, it shifts back to state E. Alternatively, the substrate (S) can be converted into the product (P), releasing this molecule at once. The enzyme switches from state E_S to state E when this happens. Finally, the product molecule may bind to the enzyme, where it is converted back into substrate as the enzyme transitions from E to E_S. The four stated reactions are shown in Fig. 1. In it, k_S^+ is the rate constant for reaction $E + S \rightharpoonup E_S$, k_S^- is the rate constant for reaction $E_S \rightharpoonup E + S$, k_P^+ is the rate constant for reaction $E + P \rightharpoonup E_P$, and k_P^- is the rate constant for reaction $E_P \rightharpoonup E + P$. Because the substrate and product concentrations are considered to be time independent, they are included into the corresponding pseudo-reaction rate constants. That is, $k_S^+[S]$ is regarded as the rate constant for reaction $E \rightharpoonup E_S$, while $k_P^+[P]$ is regarded as the rate constant for reaction $E \rightharpoonup E_P$.

According to Beard and Qian [18], the stationary rate of product synthesis is:

$$J = \frac{V^f_{max}[S]/K_{M,S} - V^r_{max}[P]/K_{M,P}}{1 + [S]/K_{M,S} + [P]/K_{M,P}}, \tag{1}$$

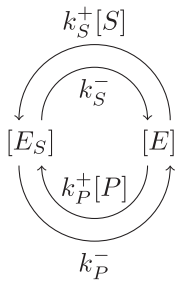

Fig. 1. Simple chemical-kinetic model for an enzymatic reaction.

where

$$V^f_{max} = k_P^-[E_T], \quad V^r_{max} = k_S^-[E_T], \quad K_{M,S} = \frac{k_S^- + k_P^-}{k_S^+}, \quad K_{M,P} = \frac{k_S^- + k_P^-}{k_P^+}. \tag{2}$$

V^f_{max} and V^f_{max} are the maximum possible reaction velocities in the forward (product synthesis) and reverse (substrate) synthesis for the model in Fig. 1.

Enzymatic reactions are usually considered to be irreversible because $V^r_{max} \ll V^f_{max}$, whereas product levels are kept low since they are constantly eliminated; i.e. $[P] \ll K_{M,\,P}$. Given these assumptions, the following Michaelis-Menten equation can be used to approximate Eq. (1):

$$J \approx \frac{V^f_{max}[S]/K_{M,S}}{1 + [S]/K_{M,S}}.$$

For the sake of thermodynamic coherence, this approximation is not used in the current work, although the fact that commonly $V^r_{max} \ll V^f_{max}$ will be taken into consideration in further discussions.

Let us now pay attention to chemical equilibrium: $J = 0$. In this situation,

$$\frac{V^f_{max}[S^*]}{K_{M,S}} = \frac{V^r_{max}[P^*]}{K_{M,P}}, \tag{3}$$

where $[S^*]$ and $[P^*]$ are the substrate and product equilibrium concentrations. Upon substitution of the relations in Eq. (2), the above equation yields:

$$exp\left(-\frac{\Delta\mu^o}{RT}\right) = \frac{[P^*]}{[S^*]} = \frac{k_S^+ k_P^-}{k_P^+ k_S^-}, \tag{4}$$

in which $\Delta\mu^o$ is the change in chemical potential associated to the reaction $S \rightharpoonup P$. Since an enzyme can speed up a reaction but cannot modify the corresponding $\Delta\mu^o$, Eq. (4) implies that not all reaction rates are independent.

Without loss of generality, assume that the product concentration remains fixed at $[P^*]$, ($[P] = [P^*]$). Then, solving for $[P^*]$ in Eq. (3) and substituting into Eq. (1) leads to the following expression for the rate of product synthesis:

$$J = V^f_{max}\frac{[S] - [S^*]}{[S] + K_{M,S} + \left(V^f_{max}/V^r_{max}\right)[S^*]}. \tag{5}$$

The expression given by Eq. (5) is a monotonic increasing function of $[S]$. It is negative for $[S] < [S^*]$ ($[S^*]$ is the substrate equilibrium concentration given the value of $[P^*]$), asymptotically approaches V^f_{max} as $[S] \to \infty$, and reaches the half saturation value at

$$\left[S_{1/2}\right] = K_{M,S} + \left(2 + \frac{V^f_{max}}{V^r_{max}}\right)[S^*].$$

In summary, the preceding results show that, given the concentration of product molecules, there is an equilibrium concentration of substrate molecules, $[S^*]$. When $[S] < [S^*]$, the enzymatic process runs backwards, and product molecules are converted to substrate molecules. If $[S] > [S^*]$, on the other hand, the rate of product molecule synthesis is positive. Such a rate is, in fact, a rising function of $[S]$, which saturates at V^f_{max} as $[S] \to \infty$.

3. Forcing an enzymatic reaction to run backwards

As discussed above, an enzymatic reaction can run backwards when $[S] < [S^*]$. Is it possible to achieve this goal at high substrate concentrations? To tackle this question, assume that the reaction rate for substrate binding is decreased by a factor $\alpha > 1$, i.e. consider a new parameter value

$$k_S^{+'} = \frac{k_S^+}{\alpha}.$$

Notice from Eq. (2) that this change leaves V^f_{max}, V^r_{max}, and $K_{M,\,P}$ unmodified, but changes $K_{M,\,S}$ to

$$K'_{M,S} = \alpha K_{M,S}. \tag{6}$$

Further observe from Eq. (4) that the shift in parameter k_S^+ to $k_S^{+'}$ implies changes in $\Delta\mu^o$ and the equilibrium substrate concentration; the last one being:

$$[S^{*'}] = \alpha[S^*]. \tag{7}$$

Finally, it follows from Eqs. (5)–(7) that, with the new $k_S^{+'}$ value, the rate of product synthesis becomes:

$$J' = V^f_{max}\frac{[S] - \alpha[S^*]}{[S] + \alpha\left\{K_{M,S} + \left(V^f_{max}/V^r_{max}\right)[S^*]\right\}}. \tag{8}$$

When comparing Eqs. (5) and (8), it can be observed that both J and J' are monotonic increasing functions of $[S]$, that they asymptotically approach V^f_{max} as $[S] \to \infty$, and that they take the value $-[S^*]/\{K_{M,\,S} + (V^f_{max}/V^r_{max})[S^*]\}$ at $[S] = 0$. However, if $[S^*] < [S] < \alpha[S^*]$, $J > 0$ while $J' < 0$. This means that the direction of an enzymatic reaction can be reversed with a substrate concentration that would normally drive it forward. However, in order to achieve this purpose, an energetic cost must be paid because, as previously stated, the reaction $\Delta\mu^o$ value must be modified.

To simplify the energetic analysis, define the following normalized variables and parameters:

$$j = \frac{J'}{V^f_{max}}, \quad s = \frac{[S]}{[S^*]}, \quad k = \frac{K_{M,S}}{[S^*]} + \frac{V^f_{max}}{V^r_{max}}.$$

With these definitions, Eq. (8) becomes:

$$j = \frac{s - \alpha}{s + \alpha k}. \tag{9}$$

Recall [19] that the change in potential energy associated to the reaction $S \rightharpoonup P$ is given by

$$\Delta\mu = \Delta\mu^o + RTln\frac{[P]}{[S]} = RTln\frac{[S^*][P]}{[P^*][S]}.$$

In the derivation of the second equality we have made use of the relation between $\Delta\mu^o$, S^* and P^* given by Eq. (4). Further taking into consideration that $[P]$ has been assumed to be equal to $[P^*]$ at all times ($[P] = [P^*]$) implies that

$$\Delta\mu = RTln\frac{[S^*]}{[S]} = -RTlns.$$

Notice from (9), that $j = 0$ when $s = \alpha$. Then, if $\alpha = 1$, the above relation means that thermodynamic equilibrium is reached at $\Delta\mu = 0$. However, when $\alpha > 1$, the no flux condition is $\Delta\mu = -RT\ln\alpha$. That is, parameter α (the reduction factor of the reaction rate for substrate binding in this example) determines the energy shift required to compel the enzyme process to run backwards.

We point out that an enzymatic reaction can be forced to run backwards by modifying parameters other than k_S^+, leading to similar results. These other possibilities will not be addressed in the present work for the sake of simplicity.

4. Coupling two enzymatic reactions

We have seen how providing chemical energy can cause an enzyme process to proceed backwards, even when the associated substrate concentration is high. Next, we look into how a forward-running second enzymatic reaction can provide the required energy.

Consider two enzymatic reactions, termed 1 and 2, such that reaction 1 runs forwards while reaction 2 runs backwards. Let j_1 and j_2 denote their respective normalized fluxes, which according to Eq. (9) are given by:

$$j_1 = \frac{s_1 - \alpha_1}{s_1 + \alpha_1 k_1}, \quad \text{and} \quad j_2 = \frac{s_2 - \alpha_2}{s_2 + \alpha_2 k_2}. \tag{10}$$

Further assume that both enzymatic reactions are coupled in such a way that $j_1 = -\rho j_2$. That is, ρ individual type 1 reactions must occur in the forward direction before one type 2 reaction can occur in the reverse direction.

An energy flux can be computed for each enzymatic reaction as follows $-\Delta\mu_i V^f_{max,\,i} j_i = RTV^f_{max,\,i}\ln(s_i)j_i$, in which $i = 1, 2$, $\Delta\mu_i$ is the change in chemical potential associated to the reaction $S_i \rightharpoonup P_i$, and $V^f_{max,\,i}$ is the corresponding maximum forward reaction velocity. Assume that both reactions are spontaneous: $\Delta\mu_i < 0$. Since enzymatic reaction 1 runs forwards while reaction 2 runs backwards, $j_1 > 0$ and $j_2 < 0$. Then, the energy flux associated to reaction 1 is positive (meaning that it delivers energy), while that of reaction 2 is negative (it consumes energy). Taking this into consideration, together with the first and second laws of thermodynamics, implies that

$$V^f_{max,1}ln(s_1)j_1 + V^f_{max,2}ln(s_2)j_2 \le 0,$$

in which the inequality accounts for energy dissipation. Further considering that both fluxes are coupled in such a way that $j_1 = -\rho j_2$, with $\rho \ge 1$, means that

$$\rho V^f_{max,1}ln(s_1) - V^f_{max,2}ln(s_2) \le 0.$$

Finally, in thermodynamic equilibrium $s_1 = \alpha_1$, $s_2 = \alpha_2$, and energy dissipation is null. Hence,

$$\rho V^f_{max,1}ln(\alpha_1) = V^f_{max,2}ln(\alpha_2).$$

Let us make the following simplifying assumption: $V^f_{max,\,1} = V^f_{max,\,2} = V^f_{max}$. Then, parameters α_1 and α_2 ought to satisfy the following relation

$$\alpha_1^\rho = \alpha_2$$

to comply with the assumed relation between fluxes ($j_1 = -\rho j_2$) and the principles of thermodynamics.

From the above considerations, the stationary condition for the two coupled enzymatic reactions reads as

$$\frac{s_1 - \alpha_1}{s_1 + \alpha_1 k_1} + \rho\frac{s_2 - \alpha_1^\rho}{s_2 + \alpha_1^\rho k_2} = 0. \tag{11}$$

Define function

$$F(\alpha_1) = \frac{s_1 - \alpha_1}{s_1 + \alpha_1 k_1} + \rho\frac{s_2 - \alpha_1^\rho}{s_2 + \alpha_1^\rho k_2} = 0. \tag{12}$$

By finding the positive root of $F(\alpha_1)$ one can compute the value of the coupling parameter α_1 (which determines the energy shift in both enzymatic reactions), and then reckon fluxes j_1 and j_2 by means of Eq. (10).

5. Maximal driven-reaction flux

To simplify the analysis of the roots of $F(\alpha_1)$, define the efficiency of energy conversion for the coupled enzymatic reactions as:

$$\eta = \frac{-\Delta\mu_2 V^f_{max,2} j_2}{-\Delta\mu_1 V^f_{max,1} j_1} = \frac{1}{\rho}\frac{lns_2}{lns_1}.$$

This allows us to write $s_2 = s_1^{\rho\eta}$, and rewrite $F(\alpha_1)$ as

$$F(\alpha_1) = \frac{s_1 - \alpha_1}{s_1 + \alpha_1 k_1} + \rho\frac{s_1^{\rho\eta} - \alpha_1^\rho}{s_1^{\rho\eta} + \alpha_1^\rho k_2}.$$

Since $\eta \le 1$ and $s_1 > 0$, it follows that $s_1^\eta < s_1$. Taking this into consideration, it is straightforward to verify that $F(s_1^\eta) > 0$, whereas $F(s_1)$

< 0. Then, given that $F(\alpha_1)$ is continuous, the positive root $\alpha_1{}^*$ of $F(\alpha_1)$ lies in the interval $\alpha_1{}^* \in (s_1^{\eta}, s_1)$. This means that $\alpha_1{}^*$ is a monotonically growing function of s_1. As a matter of fact, it can be straightforwardly verified that, in the range of very large s_1 values, $\alpha_1{}^* \propto s_1$ when $k_2 > \rho$, while $\alpha_1{}^* \propto s_1^{\eta}$ when $k_2 < \rho$. Of these two choices, the first is the one relevant to us; recall that we defined

$$k = \frac{K_{M,S}}{[S^*]} + \frac{V^f_{max}}{V^r_{max}},$$

and usually $V^f_{max} \gg V^r_{max}$.

Define the backward velocity of enzymatic reaction 2 as

$$v = -j_2 = \frac{\alpha_1^{*\rho} - s_1^{\eta\rho}}{\alpha_1^{*\rho} k_2 + s_1^{\eta\rho}}.$$

It follows from this and the fact that $\alpha_1{}^* \propto s_1$ at large s_1 that

$$v_{lim} = -\lim_{s_1 \to \infty} j_2 = \frac{1}{k_2}.$$

Interestingly, this limit velocity is solely determined by parameter k_2. This finding is noteworthy because it implies that reaching the limit velocity is always feasible, even at maximal efficiency $\eta = 1$. However, it can be verified that s_1 and $s_2 = s_1^{\eta\rho}$ need to attain extremely large values to approach v_{lim} when $\eta \approx 1$. Certainly, this is not always possible in real life. In what follows, we look at how velocity v behaves when s_1 and/or s_2 are upper bounded.

The following inequality follows directly from the definition of v:

$$v = \frac{\alpha_1^{*\rho} - s_1^{\rho\eta}}{s_1^{\rho\eta} + k_2 \alpha_1^{*\rho}} < \frac{1}{k_2}\left(1 - \frac{s_1^{\rho\eta}}{\alpha_1^{*\rho}}\right).$$

Taking into account that $s_1^{\eta} < \alpha_1{}^* < s_1$, and $s_2 = s_1^{\rho\eta}$, this further implies that

$$v < \frac{1}{k_2}\left(1 - \frac{s_1^{\rho\eta}}{s_1^{\rho}}\right) = \frac{1}{k_2}\left(1 - s_1^{\rho(\eta-1)}\right) = \frac{1}{k_2}\left(1 - s_2^{1-1/\eta}\right).$$

This last result yields the following upper bounds for the backward velocity of enzymatic reaction 2:

$$v_{max} < \frac{1}{k_2}\left(1 - s_{1,max}^{\rho(\eta-1)}\right) = \frac{1}{k_2}\left(1 - s_{2,max}^{1-1/\eta}\right).$$

Since $\eta \leq 1$, $\lim_{s_{1,\,max}\to\infty} v_{max} = \lim_{s_{2,\,max}\to\infty} v_{max} = v_{lim}$. The behavior of v_{max} as a function of $s_{1,\,max}$, η and ρ is illustrated in Fig. 2, whereas Fig. 3 shows the behavior of v_{max} in terms of $s_{2,\,max}$ and η.

Observe in Fig. 2 that, when s_1 is constrained (in this case s_2 is also upper bounded because $s_2 < s_1^{\rho}$), v_{max} is a decreasing function of η for all values of ρ. Moreover, as $s_{1,\,max}$ increases, v_{max} remains close to its maximal value for larger η values before beginning to decline. A similar effect can be observed with ρ increments. Thus, increasing ρ and $s_{1,\,max}$ helps run the driven process close to its maximum speed, specially at higher efficiencies. This is an interesting and intriguing finding, because higher s_1 and s_2 values indicate that enzyme reactions 1 and 2 would be further from equilibrium if they were uncoupled. It is thus conceivable to make the system operate faster without reducing the efficiency of energy conversion by pulling its components further from equilibrium.

When s_2 is constrained (while s_1 has no upper bound), v_{max} also happens to be a decreasing function of η (see Fig. 3). The rationale for this is because in order to achieve higher efficiencies, the chemical potential changes associated with both enzymatic reactions must be very similar. Since

$$\Delta\mu_1 = -RTlns_1, \quad \Delta\mu_2 = -RTlns_2,$$

this means that at higher efficiencies, smaller s_1 values are allowed, limiting the value of v_{max}. At low efficiencies, however, $|\Delta\mu_1|$ can be much greater than $|\Delta\mu_2|$, allowing s_1 to take extremely large values with a corresponding rise in v_{max}. It can also be seen in Fig. 3 that, when the

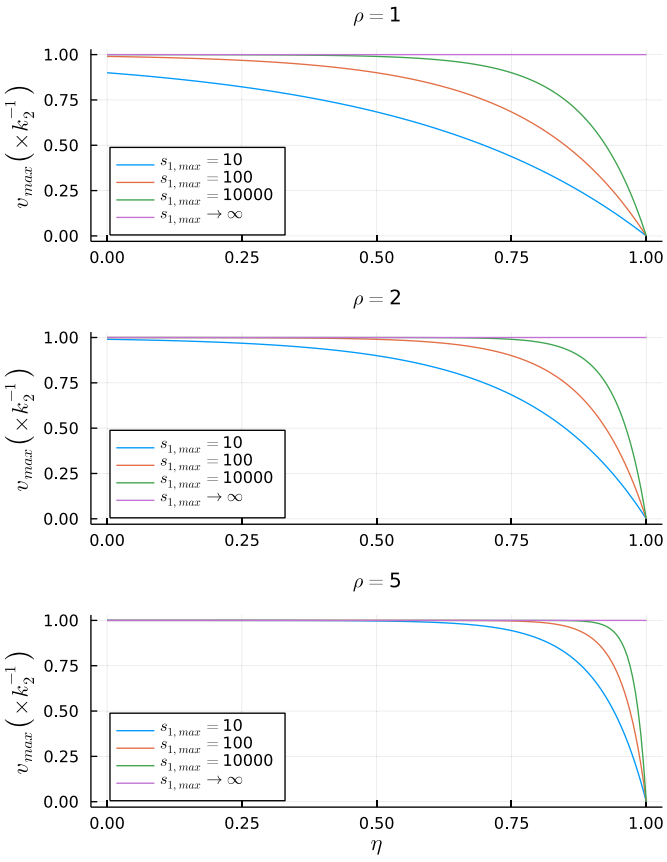

Fig. 2. Plots of maximum velocity of the driven reaction vs. efficiency, for different values of parameter ρ, when parameter s_1 (which sets the change in chemical potential of the driver reaction) is upper bounded.

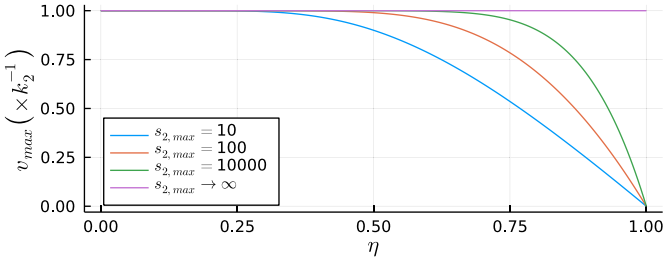

Fig. 3. Plots of maximum velocity of the driven reaction vs. efficiency when parameter s_2 (which sets the change in chemical potential of the driven reaction) is upper bounded.

efficiency value is fixed, v_{max} rises as $s_{2,\,max}$ increases.

We have seen how the maximum velocity of the driven reaction v_{max} determines the system thermodynamic performance. Let's take a closer look at the limit value of this quantity ($v_{lim} = 1/k_2$). Notice from the definition that

$$k_2 = \frac{V^f_{max,2}}{V^r_{max,2}} + \frac{K_{M,S,2}}{[S_2]^*}.$$

Then,

$$v_{lim} = \frac{1}{k_2} = \frac{V^r_{max,2}}{V^f_{max,2}} \frac{1}{1 + \frac{V^r_{max,2}}{V^f_{max,2}} \frac{K_{M,S,2}}{[S_2]^*}}.$$

By further taking into account that j_2 is a normalized flux ($j_2 = J_2/V^f_{max,\,2}$), the limit driven-reaction non-normalized velocity becomes

$$V_{lim} = V^r_{max,2}\,\frac{1}{1+\frac{V^r_{max,2}}{V^f_{max,2}}\frac{K_{M,S2}}{[S_2]^*}} < V^r_{max,2}. \tag{13}$$

This means that the value of $V^r_{max,\,2}$ determines the greatest velocity at which enzymatic reaction 2 can be forced backwards. This is not a simple result, despite its appearance. Making $[S_2] = 0$ and $[P_2] \to \infty$ in an uncoupled enzymatic reaction is the only way to reach $V^r_{max,\,2}$. On the other hand, when linked to enzymatic reaction 1, reaction 2 can be compelled to run backwards at velocity V_{lim} regardless of the values of $[S_2]$ and $[P_2]$ by raising $[S_1]$ and/or lowering $[P_1]$.

6. Thermodynamic performance

By regarding the coupled enzymatic reaction as an energy converting system, in which reaction 1 occurs spontaneously and drives reaction 2 against a chemical potential gradient, it is possible to define energy input and output fluxes as follows

$$E_{in} = -\Delta\mu_1 J_1 = RTV^f_{max}\rho ln s_1 v, \quad E_{out} = \Delta\mu_2 J_2 = RTV^f_{max} ln s_2 v.$$

Following Wagoner and Dill [16], we also define the following objective function, which represents a compromise between speed and efficiency:

$$\Xi = -J_2\left(\frac{\Delta\mu_2}{\Delta\mu_1}\right)^{\lambda} = V^f_{max}\eta^{\lambda} v,$$

in which parameter λ determines the weight of the efficiency in the objective function. This parameter value is to be determined when contrasting with experimental results.

In order to eliminate system-size effects in the present analysis, let us normalize Ξ by V_{max}, and E_{in} and E_{out} by factor

$$-\Delta\mu_2 V^f_{lim} v_{lim} = RTV^f_{max} ln s_2/k_2 = RTV^f_{max}\rho\eta ln s_1/k_2,$$

which sets the system maximum output energy flux. With this, the normalized thermodynamic performance variables become:

$$\varepsilon_{in} = \frac{k_2}{\eta} v, \quad \varepsilon_{out} = k_2 v, \quad \xi = k_2\eta^{\lambda} v. \tag{14}$$

Notice that all quantities are proportional to v. Then, the maximal values for ε_{in}, ε_{out} and ξ are given by

$$\begin{aligned}
\varepsilon^{max}_{in} &< \frac{1}{\eta}\left(1 - s^{\rho(\eta-1)}_{1,max}\right) = \frac{1}{\eta}\left(1 - s^{1-1/\eta}_{2,max}\right),\\
\varepsilon^{max}_{out} &< 1 - s^{\rho(\eta-1)}_{1,max} = 1 - s^{1-1/\eta}_{2,max},\\
\xi^{max} &< \eta^{\lambda}\left(1 - s^{\rho(\eta-1)}_{1,max}\right) = \eta^{\lambda}\left(1 - s^{1-1/\eta}_{2,max}\right).
\end{aligned} \tag{15}$$

In the limits $s_1, s_2 \to \infty$, the expressions above yield the following limit values for ε_{in}, ε_{out} and ξ:

$$\varepsilon^{lim}_{in} = \frac{1}{\eta}, \quad \varepsilon^{lim}_{out} = 1, \quad \xi^{lim} = \eta^{\lambda}. \tag{16}$$

These findings are important because they imply that reaching a maximum power output is always feasible, even when $\eta = 1$ (recall that higher power outputs usually mean lesser efficiency). However, the price to pay is that s_1 and $s_2 = s_1^{\eta\rho}$ need to attain extremely large values as η gets close to one.

It is straightforward to prove from Eq. (15) that ξ^{max} is a concave function of η with a single maximum in the interval $[0, 1]$, when s_1 and s_2 attain finite values. Denote the value of η that maximizes ξ^{max} for given values of $s_{1,\,max}$, ρ and λ as $\eta_{opt,\,\xi}(s_{1,\,max}, \rho, \lambda)$, and the value of η that maximizes ξ^{max} for given values of $s_{2,\,max}$ and λ as $\eta_{opt,\,\xi}(s_{2,\,max}, \lambda)$.

Wagoner and Dill [16] reported experimental data regarding the chemical potential changes and stoichiometry of pumps, cotransporters and ATP synthase proteins from different species. The values of parameters s_1, s_2, η and ρ can be computed from such data. The results are tabulated in Table 1. Such experimental values were used to estimate the value of parameter α as follows:

- Given the value of λ, the optimal $\eta_{opt,\,\xi}(s_{1,\,max}, \rho, \lambda)$ value was numerically computed (by means of algorithm `optimize`, introduced in Julia's package `Optim`) for each one of the data-points corresponding to the rows of Table 1.
- With this, an error was calculated as $\mathrm{Err}_i = (\eta_{exp} - \eta_{opt,\,\xi})^2$, with η_{exp} the experimental efficiency reported in Table 1.
- The total error was then computed as $\mathrm{Err}_T = \sum_i \mathrm{Err}_i$.
- Finally, parameter λ was estimated as the value that minimizes the total error Err_T. This was done via algorithm `optimize`, introduced in Julia's package `Optim`.
- After implementing the above described procedure, it yielded the value

$$\lambda = 0.18.$$

To test the suitability of the estimated λ value, we computed with it $\eta_{opt,\,\xi}$ vs. η curves for all the reported ρ and $s_{1,\,max}$ values, as well as for all reported $s_{2,\,max}$ values, and plotted them in Fig. 4, along with the experimental points in Table 1. Observe that the optimal efficiency is a growing function of either $s_{1,\,max}$ or $s_{2,\,max}$, and that raising ρ also improves the efficiency when s_1 is upper bounded. Further note that the $\eta_{opt,\,\xi}$ vs. $s_{2,\,max}$ plot qualitatively reproduces the trend exhibited by the experimental points. Interestingly, the majority of experimental points are close to the $\eta_{opt,\,\xi}$ vs. $s_{1,\,max}$ curves with matching ρ values, and that the experimental points corresponding to larger ρ values have in general higher efficiencies, which is consistent with the arrangement of theoretical curves. All of this suggests that the proteins in Table 1 function in a regime close to that of maximal ξ^{max}.

The behavior of ε^{max}_{in}, ε^{max}_{out}, and ξ^{max} vs. η is illustrated in Fig. 5 when s_1 is upper bounded, considering different $s_{1,\,max}$ values and $\rho = 2$. Equivalent plots are presented in the appendix for $\rho = 1, 5$. The behavior of performance variables ε^{max}_{in}, ε^{max}_{out}, and ξ^{max} in the case that s_2 is upper bounded, taking into account various values of $s_{2,\,max}$, is shown in Fig. 6.

Note in the plots in Figs. 5, 6, A.7 and A.8 that the maximum power output (ε^{max}_{out}) behaves similarly to the velocity v_{max}, as both are proportional. The value of ξ^{max} rises in tandem with $s_{1,\,max}$ and $s_{2,\,max}$, meaning that a more favorable balance between speed and efficiency is reached as the system components deviate from thermodynamic equilibrium. The ξ^{max} vs η curves have a single maximum point at efficiency values between 0 and 1. As $s_{1,\,max}$ or $s_{2,\,max}$ increase, not only does the value of these maximum ξ^{max} function points increases, but they also migrate to greater efficiency values. That is, the better the thermodynamical performance (higher ξ^{max}, and efficiency values) of the optimal ξ regime, the larger the values of $s_{1,\,max}$ or $s_{2,\,max}$.

When s_1 is upper bounded, ρ increments raise the maximum ξ^{max} value and shift it to higher η values. Thus, increasing the minimum number of driver enzymatic reactions required for a single driven reaction has a positive effect on all aspects of the system thermodynamic performance: higher efficiency and speed.

Table 1
Performance and design parameters for various molecular energy converters (pumps, cotransporters and ATP syntheses, computed from the experimental values reported in [16]).

Protein	$\ln s_1$	$\ln s_2$	η	ρ
E coli F_OF_1	6.93	19.40	0.84	3.33
Animal F_OF_1	7.83	16.70	0.8	2.67
S cereviseae F_OF_1	6.44	17.4	0.81	3.33
PMCA	22.99	15.40	0.67	1
SERCA	11.5	18.40	0.8	2
Na-K ATPase	4.61	20.30	0.88	5
PPi Pump	8.78	6.50	0.74	1
PM H+ Pump	19.83	11.70	0.59	1
Chloroplast V-ATPase	9.92	13.1	0.66	2

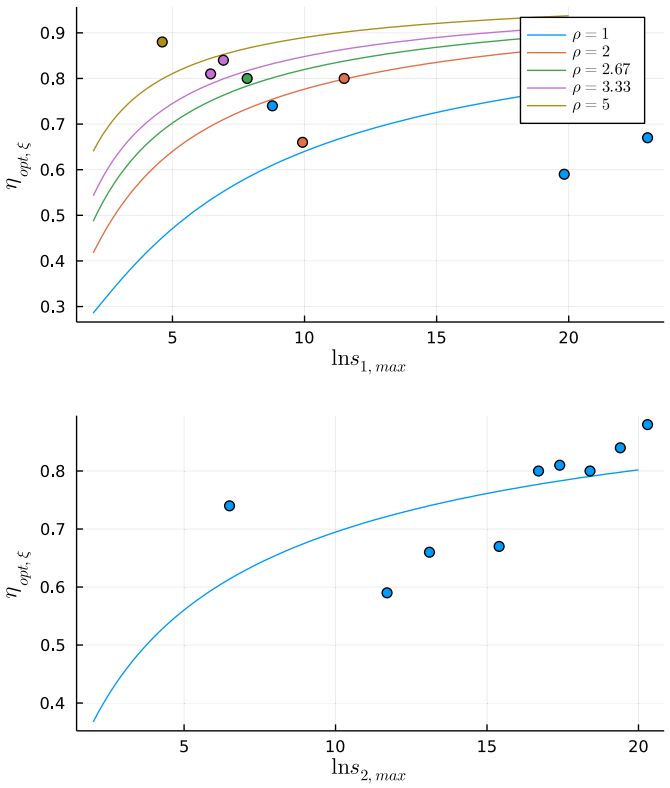

Fig. 4. Plots of optimal ξ^{max} vs. $\ln s_{1,\ max}$ and $\ln s_{2,\ max}$ (solid curves) and experimental data points from various pumps, cotransporters, and ATP synthase proteins from various species.

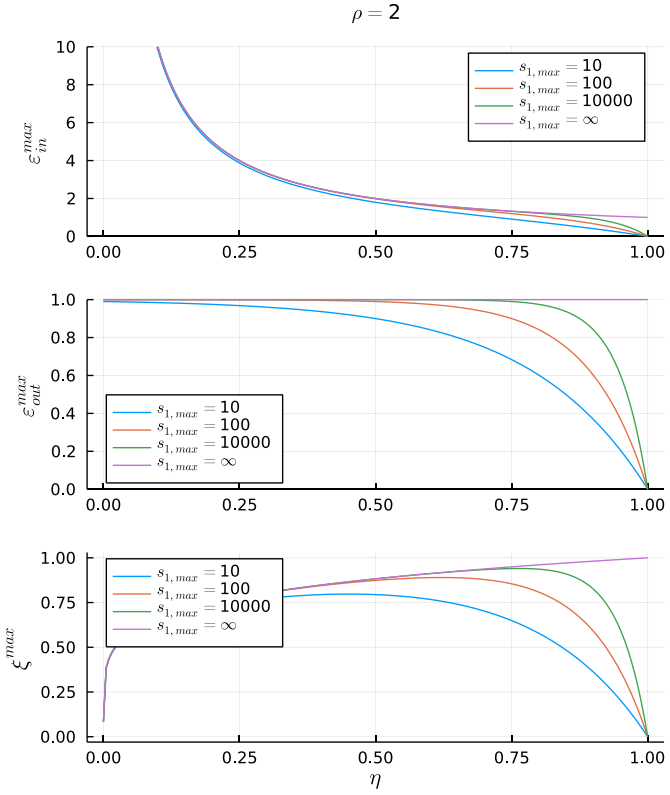

Fig. 5. Plots of maximum power input ε_{in}^{max}, maximum power output ε_{out}^{max} and maximum function ξ, for $\rho = 2$, when parameter s_1 (which sets the change in chemical potential of the driver reaction) is upper bounded.

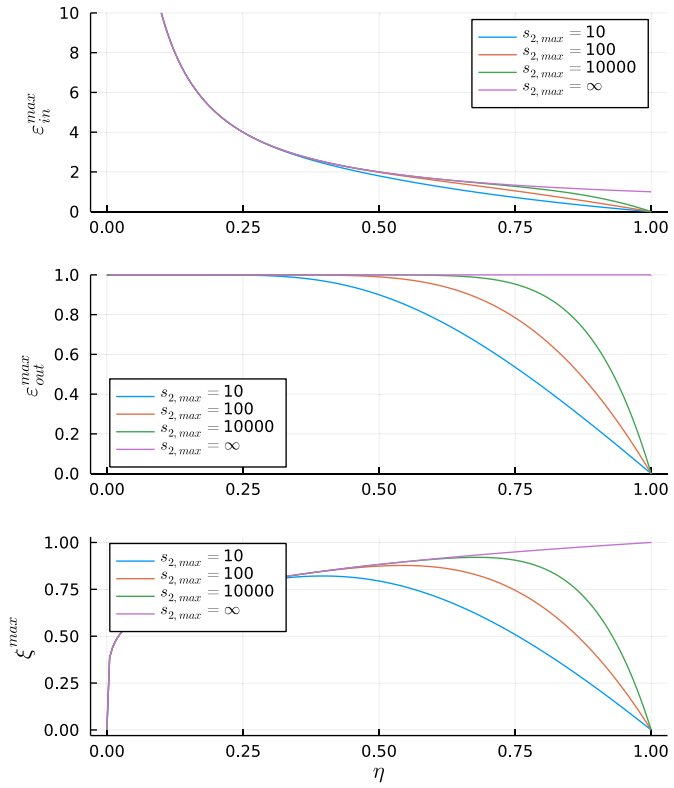

Fig. 6. Plots of maximum power input ε_{in}^{max}, maximum power output ε_{out}^{max} and maximum function ξ^{max}, when parameter s_2 (which sets the change in chemical potential of the driven reaction) is upper bounded.

7. Concluding remarks

Previous research has suggested that molecular energy converters operate through spatially separate pathways for free energy donor and acceptor reactions, and has developed numerous kinetic models. Among the existing literature, two types of models can be identified. On the one hand, there are detailed models for specific energy converters [10,13–15,20]. On the plus side, these models can explain how the modeled systems work and even make quantitative predictions about their dynamics. However, drawing conclusions applicable to molecular energy converters in general is not easy. On the other hand, some simple models result in general conclusions that hold true for all molecular energy converters [7,9,16,17]. The linear approximation that relates reaction velocities and chemical potential changes via a symmetrical matrix of phenomenological coefficients is of particular interest [7,8]. Because linear approximation validity is limited to near equilibrium systems [21], it fails to capture nonlinear phenomena associated with enzymatic reactions, such as saturation. The present work addresses this limitation.

We developed a chemical kinetics model for coupled enzymatic reactions, claiming that it can be used to investigate the thermodynamic performance of molecular energy converters like cotransporters, ion pumps, and ATP synthases. The conditions under which an enzymatic reaction can drive another up its corresponding chemical energy gradient were thoroughly investigated using this model. These results were then used to study the effect of model parameters on the system thermodynamic performance. Our findings are summarized below.

- The maximal reverse velocity of the uncoupled reaction limits the velocity at which the driven reaction can be run backwards. This quantity is solely determined by the rate constants of the enzymatic reaction, which can be thought of as design parameters. This coincidence is interesting because it states that the maximum velocity of

the driven process is independent of whether it is achieved by increasing product concentration much more than substrate concentration in an uncoupled reaction, or by manipulating the chemical potential differences of the driven and driver reactions in the coupled system.

- In addition to the upper bound imposed by the design parameters, the actual velocity of the driven reaction is determined by the chemical potential differences of the driver and driver processes (which can be regarded as operation parameters). Both changes in chemical potential are related by the energy conversion efficiency (η) and parameter ρ. When an upper bound is implemented in one or both chemical potential changes, the velocity of the driven reaction becomes a decreasing function of η. This is the behavior that one would expect. The maximum velocity is attained when the change in chemical potential of the driven reaction is zero and/or that of the driver reaction tends to infinity. Maximal efficiency, on the other hand, is synonymous with thermodynamic equilibrium (thus null velocity). Surprisingly, by enhancing both chemical potential differences, the driven process velocity can be raised without affecting the system efficiency. Recall that increasing the chemical potential difference pushes an uncoupled enzymatic reaction further away from equilibrium. This result may explain how, using coupling mechanisms such as the one presented here, numerous molecular engines can enhance speed without compromising efficiency, or vice versa [16].
- When the chemical potential change of the driver reaction is upper limited, raising the value or parameter ρ (the number of individual driver reactions required for one driven reaction to occur) increases the driven process speed without sacrificing system efficiency.
- As a result of the preceding discussion, augmenting both chemical potential differences can improve the system thermodynamic performance (i.e. increase speed without sacrificing efficiency). A similar behavior is observed when the change in chemical potential of the driven process is upper bounded and the value of ρ is increased.
- A comparison of theoretical and experimental data on pumps, cotransporters, and ATP synthase proteins from various species suggests that the operation regime of such molecular energy converters is near to that of maximal ξ^{max}.

CRediT authorship contribution statement

Julián González-Ayala: Methodology, Validation, Resources, Writing – review & editing. **Antonio Calvo-Hernández:** Methodology, Validation, Resources, Writing – review & editing, Funding acquisition. **Moisés Santillán:** Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2022.106932.

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