**Abstract:** In the intention of its creator [1], Maxwell’s demon was thought to be an intelligent being able to perform work at the expense of the entropy reduction of a closed operating system. The perplexing notion of the demon’s intelligence was formalized in terms of the information processing by Szilard [2] and followers [3], who pointed out that, in order for the total system to obey the second law of thermodynamics, the entropy reduction should be compensated for by, at least, the same entropy increase related to the demon’s information gain on the operating system state. A non-informational formulation of the problem was proposed by Smoluchowski [4] and popularized by Feynman [5] as the ratchet and pawl machine, which can operate only in agreement with the second law. A. F. Huxley [6,7] and followers [8,9] adopted this way of thinking to propose numerous flashing ratchet mechanisms of the protein molecular machines action. Here we show that, because of the necessary energy dissipation, no entropy reduction takes place for these models. More general models of protein dynamics have been proposed [10–12] with a number of conformational states organized in a network of transitions. Here, a computer realization is investigated of the conformational network displaying, like networks of the systems biology [13], a transition from the fractal organization on a small length-scale to the small-world organization on the large length-scale. This model, when allowing the performance of work in a variety of ways [12], obeys the generalized fluctuation theorem [14] with negative total entropy production and is able to explain a surprising observation to Yanagida and co-workers [15,16] that the myosin II head can take several steps along the actin filament per ATP molecule hydrolysed. From a broader perspective, of especial importance could be the supposition that a similar mechanism of action is characteristic for most intrinsically disordered proteins.
Keywords: protein dynamics; conformational transition networks; fractal-small world transition; fluctuation theorem; biological molecular machines; free energy transduction

PACS classifications: 05.70.Ln, 87.15.hp, 87.15.Ya, 89.75.Hc

The biological molecular machines are proteins that operate under isothermal conditions, thus are referred to as free energy transducers [17]. We understand the word "machine" generally as denoting any physical system that enables two other systems to perform work one on each other. The pathways of energy processing in any stationary machine operating under isothermal conditions [18] are shown in Figure 1, where also notation of the physical quantities being on in use is explained.

**Figure 1.** Energy processing in any stationary (cyclic) isothermal machine. $X_i$ denote the input ($i = 1$) and the output ($i = 2$) thermodynamic variables (mechanical, electrical or chemical), $A_i$ are the conjugate thermodynamic forces, and $J_i$ are the fluxes (the time derivatives, $J_i = \dot{X}_i$). $T$ is the temperature of the system and $S$ is the entropy. By convention, we assume the thermodynamic fluxes $J_2$ and $J_2$ to be of the same sign. Then, one system performs work on the other when the forces $A_1$ and $A_2$ are of the opposed sign. The directions of the energy fluxes shown are for $J_1, J_2, A_1 > 0$ and $A_2 < 0$. The direction of the flux denoted by the forward-reverse arrow is discussed in the text.

According to the second law of thermodynamics, the net dissipation flux (the internal entropy production rate multiplied by temperature) $A_1J_1 + A_2J_2$ must be nonnegative. However, it consists two components. The first component $(A_1 + A_2)J_1$, realized when the input and output fluxes are tightly coupled, $J_1 = J_2$, also must be, according to the same law, nonnegative. For the present considerations, essential is the sign of the complement $A_2(J_2 - J_1)$. In the macroscopic systems, the entropy $S$ is additive and always can be divided into two parts $S_1$ and $S_2$, related to the input and output thermodynamic variables $X_1$ and $X_2$, respectively. As a consequence, the flux $A_2(J_2 - J_1)$, which corresponds to $S_2$, also must be nonnegative. This means that, for negative $A_2$, the output flux $J_2$ should not surpass the input.
flux $J_1$. Macroscopically, the second component of the dissipation flux has an obvious interpretation of a slippage in the case of mechanical machines, or a leakage in the case of electrical machines or pumps.

However, in the mesoscopic systems like the protein molecular machines, the entropy is not additive [14], and the output flux $J_2$ can surpass the input flux $J_1$ [12], what changes the sign of the flux $A_2(J_2 - J_1)$ to be positive. The relation between the output and input fluxes in biological molecular machines was the topic of our previous studies [11,12]. From the theoretical point of view, it is convenient to treat all biological machines, also molecular motors, as chemo-chemical machines. In fact, the external load influences the free energy involved in binding the motor to its track, which can be expressed as a change of an effective concentration of this track [18]. The chemo-chemical machines are enzymes that simultaneously catalyze two chemical reactions: the free energy-donating input reaction $R_1 \leftrightarrow P_1$ and the free energy-accepting output reaction $R_2 \leftrightarrow P_2$ (Figure 2a). The system considered consists of a single enzyme macromolecule surrounded by a solution of its substrates involving, in the case of the molecular motors, also the track. We assume that bimolecular reactions can be considered as effective monomolecular reactions (the concentration of one of the reagents is kept constant) and that there are some relations between the concentrations of the reagents and the products [12]. Then, two independent stationary concentrations of the products are to be treated as the input and output thermodynamic variables considered in Figure 1:

$$[P]_1 = X_1, \quad [P]_2 = X_2,$$

with the conjugate thermodynamic forces [17]

$$\beta A_i = \ln \frac{[P_i]^{eq}}{[R_i]^{eq} [P_i]}.$$

Above, $\beta = 1/k_B T$ and the superscript eq denotes the corresponding equilibrium concentrations.

The dynamics of a particular biological chemo-chemical machine is determined by a network of conformational transitions satisfying the detailed balance condition, and a system of distinguished states (the "gates") between which the input and output chemical reactions force transitions that break the detailed balance (Figure 2a). Recently, we proved analytically [12], that for single output gates, when the enzyme has no possibility of any choice, the degree of coupling $J_2/J_1$ cannot exceed unity. This case also includes the various thermal ratchet models for which, consequently, the flux $A_2(J_2 - J_1)$ is positive, hence resulting in the entropy increase. The output flux $J_2$ can prevail the input flux $J_1$ only in the case of many output gates.

As yet, very poor experimental knowledge is available on actual conformational transition networks in native proteins. That is why here we restrict our attention to model networks. We shown [12], that the case of many output gates realizes in a natural way on critical branching trees extended by long-range shortcuts. Such networks are scale-free and display a transition from the fractal organization on a small length-scale to the small-world organization on the large length-scale [13]. An hypothesis seems reasonable that the protein conformational transition networks, like higher level biological networks, the protein interaction network and the metabolic network, have evolved in the process of self-organized criticality. In Figure 2b, a network of 100 nodes with such properties is shown. It was constructed following the algorithm described in the Methods section. There, also the dynamics is determined. We
have chosen the gates tendentiously replacing the Darwinian evolution optimizing the value of \( J_2/J_1 \) close to 1 (the tight coupling) for the single gate, and possibly the highest for many gates.

**Figure 2.** Kinetics of the enzymatic chemo-chemical machine. (a) General scheme. The grey box represents an arbitrary network of transitions between conformational states composing either the enzyme, the enzyme-substrate, or the enzyme-substrates native state. All these transitions satisfy the detailed balance condition. Distinguished is a single pair (the "gate") of conformational states \( 1'' \) and \( 1' \) between which the input cyclic reaction \( R_1 \rightleftharpoons P_1 \) brakes the detailed balance. Also, distinguished is a single or a variety (the heavy line) of pairs of conformational states \( 2'' \) and \( 2' \) between which the output cyclic reaction \( R_2 \rightleftharpoons P_2 \) makes the same. All the reactions are reversible; the arrows indicate the directions assumed to be forward. (b) Exemplifying realization of the network constructed stochastically following the algorithm described in the Methods section. This one is studied in more details in the present paper. Note two hubs, the states of the lowest free energy, that can be identified with two main conformations of the protein machine, e.g., "open" and "closed". The single pair of the output transition states chosen for simulations is \((2''a, 2'd)\). The alternative four output pairs \((2''a, 2'a)\), \((2''b, 2'b)\), \((2''c, 2'c)\), and \((2''d, 2'd)\) are chosen tendentiously to lie one after the other.

In the mesoscopic machines, the work, dissipation and heat are not well determined but fluctuating variables [19–21]. The probability distribution function for the input and output fluxes, in general depending on the time \( t \) of determination, satisfies the stationary fluctuation theorem in the Andrieux-Gaspard form [22]:

\[
p(j_1(t), j_2(t))/p(-j_1(t), -j_2(t)) = \exp \beta [A_1 j_1(t) + A_2 j_2(t)] t ,
\]
which can be equivalently rewritten as the Jarzynski equality \[19\]
\[
\langle \exp(-\sum_i \beta A_i J_i(t) t) \rangle = \langle \exp(-\sigma) \rangle = 1.
\] (4)

Above, \(p\) is the joint probability distribution function for the statistical ensemble of the fluxes, and \(\langle \ldots \rangle\) is the average over that ensemble. \(J_i(t)\) denote the random variables of the mean net fluxes over time \(t\), and \(j_i(t)\) denote the particular values of those fluxes (\(J_i\) in Figure 1 are the corresponding averages for \(t \to \infty\)). \(\sigma\) has the meaning of a dimensionless entropy production.

From the point of view of the force \(A_2\), subsystem 1 performs work on subsystem 2 while subsystem 2 performs work on the environment. Jointly, the work \(A_2(J_2 - J_1)\) is performed on or by system 2. From the fluctuation theorem (3) for the whole system, the partial fluctuation theorem for subsystem 2 follows:

\[
\ln p(j_2 - j_1)/p(-j_2 + j_1) = \beta A_2(j_2 - j_1)t
\]
\[+
\int d j_1 p(j_1) \ln p(-j_1| - j_2 + j_1)/p(j_1|j_2 - j_1) + \beta(A_1 + A_2) J_1 t,
\] (5)

where we introduced conditional probabilities. The corresponding Jarzynski equality can be written in the form \[14\]
\[
\langle \exp(-\sigma + \iota) \rangle = 1,
\] (6)

where \(\iota\) has the meaning of some information gain in nats.

We performed Monte Carlo simulations of random walk in \(10^{11}\) computer steps for the network shown in Figure 2b with both the single and the fourfold output gate and found that the probability distribution functions \(p(j_1, j_2)\) satisfy the Andrieux-Gaspard fluctuation theorem (3) for \(t\) longer than 200 computer steps (the mean equibration time between the nearest neighbors). One pair of such distribution functions is presented in Figures 3a and 3d. In Figures 3b and 3e the corresponding marginals \(p(j_2 - j_1)\) are shown, from which the fluctuation theorem dependences (5), presented in Figures 3c and 3f, were calculated. We threw off the rare results for the higher values of \(j_2 - j_1\) as burdened with too large statistical error.

The broken lines in Figures 3c and 3f correspond to the first term to the right hand side of Eq. (5). We see that the corrections from the second and third terms are very large indeed. In the limit \(j_2 - j_1 \to \infty\), the total dependences are approximated by the full straight lines corresponding to the first two terms, shifted by a constant originated mainly in the third term. Important is that the corrections are positive for the single gate, when \(J_2/J_1 = 0.93 < 1\), and negative for the multiple gate, when \(J_2/J_1 = 1.11 > 1\).

To summarize, we studied fluctuations of the flux \(J_2 - J_1\) which is the time derivative of the variable \(X_2 - X_1\). This variable characterizes not energy but organization of the system (in the case of the macroscopic mechanical machine, it could be a relative position of two wheels). The main conclusion of these studies is that the energy processing has to be distinguished from the organization processing (Figure 4). Both energy \(E\) and organization (complexity?) \(C\) are functions of the state of the system whereas information \(I\), like work \(W\) and heat \(Q\), are functions of the process. The quantity \(I\) is the product of the dimensionless quantity \(\iota\) occurring in Eq. (6) and the Boltzmann constant \(k_B\). In the macroscopic systems, information is negligible when compared with work and heat. It becomes
Figure 3. (a - c) The probability distribution function $p(j_1, j_2)$, the marginal $p(j_2 - j_1)$, and the fluctuation theorem equality (5) found in Monte Carlo simulation for the network shown in Figure 2b with the single output gate $(2''a, 2'd)$. (a - c) Same for the fourfold output gate $(2''a, 2'a), (2''b, 2'b), (2''c, 2'c),$ and $(2''d, 2'd)$. We assumed $\beta A_1 = 1.0$ and $\beta A_2 = -0.1$, for which $J_2/J_1 = 0.93$ in the case of the single output gate and $J_2/J_1 = 1.11$ in the case of the fourfold output gate. We chose the optimal time of averaging $t = 10^5$ steps for the single gate and $t = 10^3$ for the fourfold gate.
important for mesoscopic (and quantum) systems. For the systems operating under stationary isothermal conditions, the second law of thermodynamics

\[ W = -Q = D \geq 0 \]  

holds for the energy processing \( (D \) denotes dissipation), whereas the generalized second law of thermodynamics [14]

\[ W = -I - Q = -I + D \geq 0 \]  

holds for the organization processing. Note, that we do not distinguish the system and the memory as Sagawa and Ueda do. For the macroscopic machines as well as microscopic enzymatic machines with a single output gate, \( I \) is negative thus \( D \) is always positive. However, for some enzymatic machines with multiple output gates, we shown, that \( I \) can be positive thus \( D \) can be negative. And only such biological molecular machines may be said to act as Maxwell’s demons.

**Figure 4.** Energy (a) and organization (b) processing in any physical system.

As mentioned in Reference [12], our model is able to explain a surprising observation to Yanagida and co-workers that the myosin II head can take several steps along the actin filament per ATP molecule hydrolysed [15,16]. Probably, also the mechanism of the action of small G-proteins, having a common ancestor with the myosin II [23] and alike partly disodered structure [24], is, after a malignant transformation, similar. Presumably, also, transcription factors look actively and not passively for their target on the genome [25].

**Methods**

The algorithm of constructing the stochastic scale-free fractal trees was adopted after Ref. [26]. Shortcuts, although more widely distributed, were considered in Ref. [13]. The network in Figure 2b is too small to determine its scaling properties, but a similar procedure of construction applied to \( 10^5 \) nodes results in a scale-free network that is fractal in a small length-scale and a small world in a large length-scale. To provide both networks with stochastic dynamics, we assumed the probability of changing a node to any of its neighbours to be the same in each random walk step. Consequently, the transition probability to the neighbouring node is inversely proportional to the number of links (the degree). Following the detailed balance condition, the free energy of a given node is proportional to the
node’s degree. The larger the number of links, the higher the equilibrium occupation probability of the node, thus, the lower its free energy. The most stable conformational substates are the hubs.

Acknowledgements

MK thanks Yaşar Demirel and Hervé Cailleau for discussing the problem at early stages of the investigation.

Author Contributions

The general conception and the theory is due to MK, who also wrote the manuscript. The application of the critical branching tree model and numerical simulations are due to PC.

Conflicts of Interest

The authors declare no conflicts of interest.

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