STOCHASTIC DYNAMICS OF PROTEINS AND THE ACTION OF BIOLOGICAL MOLECULAR MACHINES

Michal Kurzynski

Faculty of Physics, Adam Mickiewicz University, Umultowska 85, 61-614 Poznan, Poland, E-mail: kurzphys@amu.edu.pl

ABSTRACT

Biological molecular machines are proteins that operate under isothermal conditions hence are referred to as free energy transducers. They can be formally considered as enzymes that simultaneously catalyze two chemical reactions: the free energy-donating (input) reaction and the free energy-accepting (output) one. It is now well established that most if not all enzymatic proteins display a slow stochastic dynamics of transitions between a variety of conformational substates composing their native state. A hypothesis is stated that, like higher level biological networks: the protein interaction network and the metabolic network, the protein conformational transition networks have evolved in a process of self-organized criticality. All three classes of networks are scale-free and, probably, display a transition from the fractal organization in a small length scale to the small-world organization in the large length scale. Good mathematical models of such networks are stochastic critical branching trees extended by long-range shortcuts. The degree of coupling between the output and the input reaction fluxes have been studied both theoretically and by means of the Monte Carlo simulations on model networks. For single input and output gates the degree of coupling values cannot exceed unity. Study simulations of random walks on several model networks involving more extended gates indicate that the case of the degree of coupling with the value higher than one is realized on the mentioned above critical branching trees extended by long-range shortcuts.

ENZYMATIC PROTEINS – CHANGE OF THE FUNDAMENTAL PARADIGM

Proteins are linear polymers of amino acids arranged in a sequence determined by genes. Since the origin of molecular biology in the 1950s, a paradigm has been commonly accepted, expressed shortly in two successive implications:

$$\text{sequence} \rightarrow \text{structure} \rightarrow \text{function}.$$

It assumes implicitly that the dynamics of native proteins reduces to simple normal vibrations about a single well defined conformational state referred to as the 'tertiary structure' of the protein. For et least two decades, however, it becomes more and more clear that not only structure but also more complex dynamics determine the function of proteins thus the paradigm has to be changed onto [1]

$$\text{sequence} \rightarrow \text{structure \& dynamics} \rightarrow \text{function}.$$

Two classes of experiments imply directly that besides fast vibrations enzymatic proteins display also a much slower stochastic dynamics of transitions between a variety of conformational substates composing their native state. The first class includes observations of the non-exponential initial stages of reactions after special preparation of an initial microscopic state in a statistical ensemble of biomolecules by, e.g., the laser pulse [2; 3]. The second class concerns statistics of the dichotomous noise generated by single biomolecules in various processes, which often displays a non-exponential time course [4; 5]. The even more convincing proof if the conformational transition dynamics of simple native proteins has been afforded by early molecular dynamics simulations [6; 7]. Research of biomolecular dynamics is being developed faster and faster and today, even in the case of small, water-soluble proteins, one speaks about the 'native state ensemble' rather than a single native state, and for very small proteins or protein fragments trials to reconstruct the actual networks of conformational transitions are realized [8].

Because of the slow character of the conformational dynamics, both chemical and conformational transitions in an enzymatic protein have to be treated on an equal footing [9] and jointly described by a system of coupled master equations

$$\dot{p}_l(t) = \sum_{l'} [w_{ll'} p_{l'}(t) - w_{l'l} p_l(t)], \qquad (1)$$

determining time variation of the occupation probabilities $p_l(t)$ of the individual protein's substates (Fig. 1). In Eq. (1), $w_{l'l}$ is the transition probability per unit time from the substate l to l' and the dot denotes the time derivative. The conformational transition probabilities satisfy the detailed balance condition which, however, can be broken for the chemical transition probabilities controlled by concentrations of the enzyme substrates. Eqs. (1) are to be treated as a model of microscopic dynamics in the stochastic theory of reaction rates [10; 11] the origins of which go back to the Smoluchowski theory of diffusion-controlled coagulation and the Kramers one-dimensional theory of reactions in the overdamped limit. It is the stochastic theory of reaction rates and not the conventional transition state theory that has to be applied in the description and interpretation of biochemical processes [9; 12].

Contrary to the transition state theory the stochastic theory of reaction rates takes seriously into account the very process of reaching the partial thermodynamic equilibrium in non-chemical degrees of freedom of the system described. In the closed reactor, a possibility of a subsequent chemical transformation of an enzyme to proceed before the conformational equilibrium have been reached in the actual chemical state re-

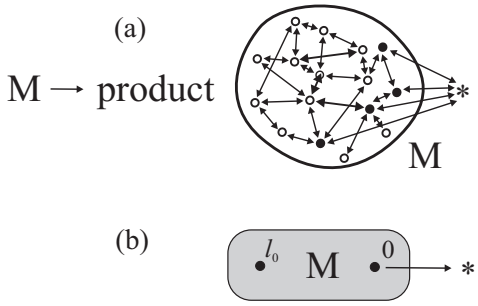

Figure 1. (a) Exemplifying realization of the model intramolecular dynamics underlying the irreversible reaction $\mathrm{M} \rightarrow$ product. Chemical state M is composed of many substates (the white and black circles) and the dynamics involves purely stochastic transitions between these states (the arrows). Chemical state product is represented by a single, totally absorbing 'limbo' state $*$. The reaction is realized through transitions between distinguished substates in M, jointly forming what is called the transition state $\mathrm{R}^{\ddagger}$ (the black circles) and the limbo $*$. (b) Particular case of the irreversible reaction when the transition state is reduced to a single 'gate' substate 0. The shaded box represents a network of an arbitrary number of sites and the direct transitions between them.

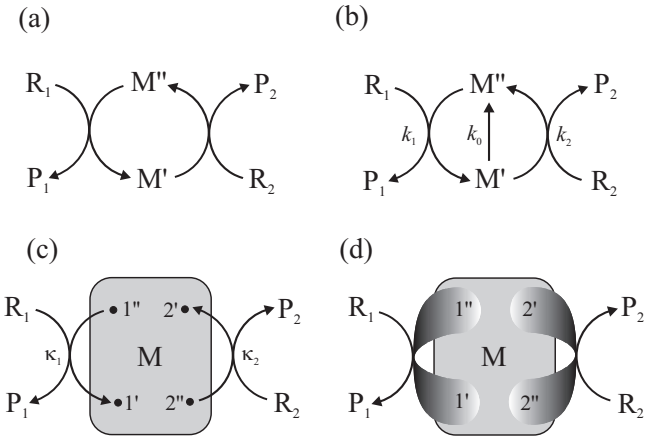

Figure 2. Development of kinetic schemes of the chemo-chemical machine. (a) Principle of the chemo-chemical free energy transduction. Due to proceeding on the same enzyme, reaction $\mathrm{R}_1 \leftrightarrow \mathrm{P}_1$ drives reaction $\mathrm{R}_2 \leftrightarrow \mathrm{P}_2$ against its conjugate force determined by steady state concentrations of the reactant and the product. (b) Assumption of a possible short circuit or slippage of the input vs. output reaction. (c) Assumption of both the free energy-donating and the free energy-accepting reaction to participate in a kinetic scheme like the one shown in Fig. 1(b). (d) Further generalization of the kinetic scheme to involve many input and output of gates.

sults in the presence of a transient non-exponential stage of the process and in an essential dynamical correction to the reaction rate constant describing the following exponential stage. In the open reactor under stationary conditions (the concentrations of reactants and products of the reaction kept constant), the general situation is more complex but for reactions gated by single transition conformational substates (Fig. 1(b)) a simple analytical theory was proposed [9; 13]. A consequence of the slow conformational transition dynamics is that the steady-state kinetics, like the transient stage kinetics, cannot be described in terms of the usual rate constants. This possibility was suggested forty years ago by Blumenfeld [14]. More later on, we have shown that adequate physical quantities that should be used are the mean first-passage times between distinguished transition substates [9; 13]. The subject of the present paper is an application of this formalism to elucidate the action of biological molecular machines.

BIOLOGICAL MACHINES AS CHEMO-CHEMICAL FREE ENERGY TRANSDUCERS

The primary purpose of thermodynamics, born in the first half of the 19th century, was to explain the action of heat engines. The processes they are involved in are practically reversible and proceed in varying temperatures. As a consequence, thermodynamics being the subject of the school and academic teaching, still deals mainly with equilibrium processes and changes of temperature. Meanwhile, biological machines as well as many other contemporary machines act irreversibly, with considerable dissipation, but at constant temperature. Machines that operate under the condition $T = \text{const.}$ are free energy transducers [12]. A good example are enzymes kinases that catalyze simultaneously two reactions, the ATP hydrolysis and a substrate phosphorylation.

From a theoretical point of view, it is convenient to treat all biomolecular machines, also pumps and motors, as chemo-chemical machines [12], enzymes that simultaneously catalyze two chemical reactions: the free energy-donating reaction and the free energy-accepting one. Under isothermal conditions, all chemical reactions proceed due to thermal fluctuations: a free energy needed for their realization is borrowed from the environment and then returned to it. In fact, the biological molecular machines are biased Maxwell's demons: their mechanical or electrical elements are 'soft' and perform work at the expense of thermal fluctuations [15; 16; 17]. Of course, Maxwell's demon can operate only out of equilibrium and it is a task of the free energy-donating reaction to secure such conditions.

The principle of action of the chemo-chemical machine is simple [18]. It is a protein enzyme that catalyzes simultaneously two chemical reactions (Fig. 2(a)). Separately, each reaction takes place in the direction determined by the second law of thermodynamics, i.e., the condition that energy dissipated, determined by the product of flux and force, is positive. However, if both reactions take place simultaneously in a common cycle, they must proceed in the same direction and the direction of the first reaction can force a change of direction of the second. As a consequence, the first reaction transfers a part of its free energy recovered from dissipation performing work on the second reaction.

In formal terms, the chemo-chemical machine couples two unimolecular reactions: the free energy-donating reaction $\mathrm{R}_1 \leftrightarrow \mathrm{P}_1$ and the free energy-accepting reaction $\mathrm{R}_2 \leftrightarrow \mathrm{P}_2$. Bimolecular reactions can be considered as effective unimolecular reactions on assuming a constant concentration of one of the reagents, e.g. ADP in the case of ATP hydrolysis. The input and output fluxes J_i (i = 1 and 2, respectively) and the conjugate thermodynamic forces A_i are defined as [18]

$$J_i = \frac{d[\mathrm{P}_i]/dt}{[\mathrm{E}]_0} \tag{2}$$

and

$$\beta A_i = \ln K_i \frac{[\mathrm{R}_i]}{[\mathrm{P}_i]}, \quad K_i \equiv \frac{[\mathrm{P}_i]^{\mathrm{eq}}}{[\mathrm{R}_i]^{\mathrm{eq}}}. \tag{3}$$

Here, symbols of the chemical compounds in the square brackets denote the molar concentrations in the steady state (no superscript) or in the equilibrium (the superscript eq). $[E]_0$ is the total concentration of the enzyme and β is proportional to the reciprocal temperature, $\beta \equiv (k_B T)^{-1}$, where k_B is the Boltzmann constant. The flux-force dependence is one-to-one only if some constraints are put on the concentrations $[R_i]$ and $[P_i]$ for each i. There are two possibilities. Either the concentration of one species, say R_i, in the open reactor under consideration is kept constant: $[R_i] = \text{const.}$, or is such the total concentration of the enzyme substrate: $[R_i] + [P_i] = \text{const}$.

The free energy transduction is realized if the product $J_2 A_2$, representing the output power, is negative. The efficiency of the machine is the ratio

$$\eta = -J_2 A_2 / J_1 A_1 \tag{4}$$

of the output power to the input power. In general, the degree of coupling

$$\varepsilon = J_2 / J_1 \,, \tag{5}$$

being itself a function of the forces A_1 and A_2, can be both positive and negative.

Usually, the assumption of tight coupling between the both reactions is made (Fig. 2(a)). It states that the flux of the first reaction equals the flux of the second, $J_1 = J_2$ thus $\varepsilon = 1$. However, an additional reaction can take place between the two states M' and M'' of the enzyme-substrates complex (Fig. 2(b)). The latter reaction can be considered either as a short circuit, the non-productive realization of the first reaction not driving the second reaction, or a slippage, the realization of the second reaction in the direction dictated by its conjugate force.

The multiconformational counterpart of the scheme in Fig. 2(b) is shown in Fig. 2(c). Here, like in the scheme in Fig. 1(b), a network of conformational transitions within the enzyme-substrates complex is represented by the gray box and the assumption of gating by single pairs of transition conformational substates is made. In Ref. [13], using a technique of summing up the directional diagrams proposed by Terell L. Hill [18] who formalized an old idea of Gustav Kirchhoff, we shown how the input and the output reaction fluxes are related to the mean first-passage times between the distinguished substates.

For all the schemes shown in Figs. 2(a-c), the flux-force dependence for the two coupled reactions has a general functional form [13]:

$$J_i = \frac{1 - e^{-\beta(A_i - A_i^{\rm st})}}{J_{+i}^{-1} + J_{-i}^{-1} e^{-\beta(A_i - A_i^{\rm st})} + J_{0i}^{-1} (K_i + e^{\beta A_i})^{-1}} \,. \tag{6}$$

The parameters J_{+i}, J_{-i}, J_{0i} and $A_i^{\rm st}$ depend on the other force and are determined by a particular kinetic scheme. $A_i^{\rm st}$ have the meaning of stalling forces for which the fluxes J_i vanish: $J_i(A_i^{\rm st}) = 0$. The dependence $J_i(A_i)$ is strictly increasing with an inflection point, determined by J_{0i}, and two asymptotes, J_{+i} and J_{-i} (Fig. 3). The asymptotic fluxes J_{+i} and J_{-i} display the Michaelis-Menten dependence on the substrate concentrations. Because of high complexity, we refrained from giving any formulas for the turnover numbers and the apparent dissociation constants, but simpler formulas for the degree of coupling ε and the stalling forces $A_i^{\rm st}$ are given and discussed in Ref. [8].

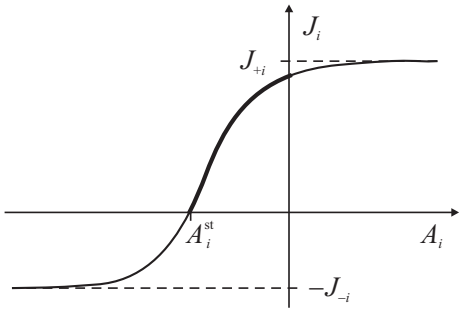

Figure 3. Character of the functional dependence of the output flux J_i versus force A_i determined by Eq. (6). Only when the stalling force $A_i^{\rm st}$ is negative does free energy transduction take place. The $J_i(A_i)$ dependence in this range is marked with a bold line.

In Ref. [8], we have compared theoretical results with Monte Carlo simulations on several model networks. Fig. 4 shows an example for 5-dimensional hypercube. It is seen that even for such simple and small network of 32 nodes large fluctuations make determination of the input and the output fluxes in 10^4 iteration steps impossible. Only the increase of the number of the iteration steps to 10^9 enables one to determine the fluxes with the error lower than 0.3%. Preliminary estimations indicate that the result is in a good agreement with the Gallavotti-Cohen

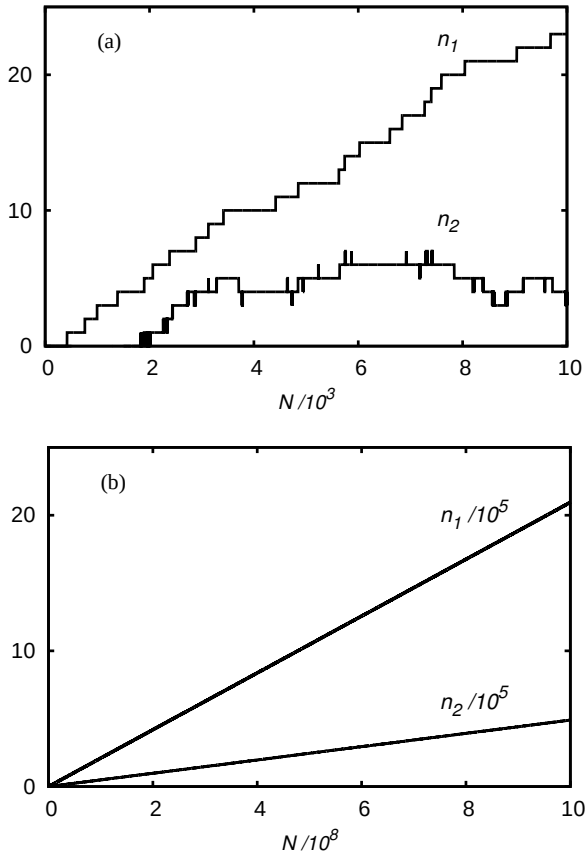

Figure 4. Simulated time course of the net number of the input ($R_1 \leftrightarrow P_1$) and the output ($R_2 \leftrightarrow P_2$) external transitions for the 5-dimensional hypercube with gates and parameters described in text. (a) Snapshots made every step. (b) Snapshots made every 10^5 steps.

fluctuation theorem [19]

$$\frac{p(\sum_i \beta A_i j_i^+)}{p(-\sum_i \beta A_i j_i^-)} = \exp(-\sum_i \beta A_i j_i t)\,, \qquad (7)$$

which can be equivalently rewritten as

$$\langle \exp(-\sum_i \beta A_i \mathcal{J}_i t) \rangle = 1\,. \qquad (8)$$

Above, $\mathcal{J}_i = \mathcal{J}_i^+ - \mathcal{J}_i^-$ denotes the random variable of the i-th net flux being the difference of the forward and backward components $\mathcal{J}_i^+$ and $\mathcal{J}_i^-$, respectively, and $j_i = j_i^+ - j_i^-$ is the value of that flux.

NETWORKS OF CONFORMATIONAL TRANSITIONS AND CRITICAL BRANCHING TREES

The essential motive of our studies is a trial to answer the intriguing question whether is it possible for the degree of coupling to have a value higher than unity. A dogma in the physical theory of, e.g., biological molecular motors is the assumption that for making a single step along its track the motor molecule has to hydrolyze at least one molecule of ATP [20]. Several years ago this assumption has been questioned by a group of Japanese biophysicists from the Yanagida laboratory who, joining a specific nanometry technique with the microscopy fluorescence spectroscopy, shown that the myosin II head can make several steps along the actin filament per ATP molecule hydrolyzed [21; 22]. The structure of myosin II is similar to that of small G proteins, e.g., protein Ras (rat sarcoma) p21, both proteins having a common ancestor [23]. After the bounded nucleotide triphosphate hydrolysis, both in the G proteins [24] and in the myosin II [25; 26] one of the α helices unwinds in part what makes the neighboring region partly disordered, highly flexible, thus fluctuating. Also for the transcription factor p53 a DNA binding core domain is partly disordered [27]. The commonly assumed model of facilitated, alternating 3- and 1-dimensional passive diffusion, does not explain all the known facts concerning the search for a proper biding site on DNA [28], so a hypothesis that this search can be active, using multiply the free energy of a single ATP molecule hydrolysis seems reasonable.

No conventional chemical kinetics approach is able to explain such behaviors. In Refs. [13] and [12], basing on approximations carried too far, we suggested that the degree of coupling can exceed unity already for reactions proceeding through single pairs of transition substates. In Ref. [8] we proved the theorem that the value of the degree of coupling should be lower or at the most equal to unity, but only in the case when the input and output reactions proceed through single pairs of transition conformational substates. It is reasonable to suppose that a possibility of higher degree of coupling is realized if the output gate is extended to two or more pairs of the transition substates. In fact, it is obvious that replacing the single output gate in the scheme in Fig. 2(a) by n gates succeeding each other, we get the degree of coupling $\varepsilon = n$. Such reasoning has been proposed in order to explain multiple stepping of the myosin molecule along the actin filament [22]. One can also imagine an incorporation of a system of additional nonreactive transitions what was for the first time considered by Terada and coworkers [29]. In

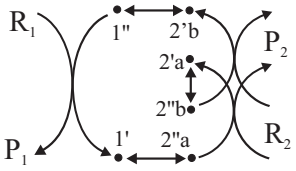

Figure 5. Extension of the kinetic scheme in Fig. 2 (c) to one input and two output gates. Obligatory transitions are drawn by arrows. If no other transitions are realized, the degree of coupling between second and first reaction equals two. Otherwise, it is lower than two but possibly higher than one.

Fig. 5 a scheme is shown with one input and two output gates, being an extension of the kinetic scheme in Fig. 2(c). Unfortunately, even in the case of only two output gates the analytical formulas are so complex and not transparent that serious approximations are needed to be made from the very beginning. Being not able to formulate presently such approximations, we decided to apply computer experiment for a preliminary study of the problem.

Since the formulation by Bak and Sneppen a cellular automaton model of the Eldredge and Gould punctuated equilibriums [30], the biological evolution is more and more often considered as a self-organized criticality phenomenon [31; 32]. There are grounds to suppose that the conformational transition networks, like two networks of the systems biology: the protein interaction network and the metabolic network, have evolved to reach a scale-free structure [8]. A controversy emerges if this structure is simultaneously small-world or fractal. The former feature is suggested by results of molecular dynamics simulations for small atomic clusters [33] and by a specific spatial organization of proteins [34]. The latter has been shown already in the pioneer papers from the Hans Frauenfelder laboratory [3] and confirmed in early molecular dynamics simulations for the very proteins [6; 7]. Only recently, an apparent contradiction between fractality and small-worldness have been explained by application of the renormalization group technique [35]. It appears that on adding to an original fractal network shortcuts with the distance r distribution following the power law $r^{-\alpha}$, a transition to the small world network occurs below some critical value of the exponent α. Close to this critical value the network can be fractal in a small length-scale, simultaneously having the small-world features in the large length-scale and this is the case of the protein interaction network, the metabolic network and, probably, the protein conformational transition network as well.

The topological structure of the flow (of probability, metabolites, energy or information) through a network is characterized by a spatial spanning tree composed of the most conducting links not involved in cycles. It is referred to as the skeleton [36] or the backbone [37] of the network, all the rejected links being considered as shortcuts. The skeleton of the scale-free and fractal network is also scale-free and fractal. For the scale-free fractal trees a criticality feature appears important that denotes the presence of a plateau equal to unity in the mean branching number dependence on the distance from the skeleton root. The critical trees can be completed to self-similar scale-free networks and such is the case of the protein interaction and metabolic networks [36; 38].

Fig. 6(a) shows a scale-free fractal tree with $N = 200$ nodes constructed following the algorithm described in Ref. [36], and Fig. 6(b) shows an extension of this tree by 200 shortcuts with

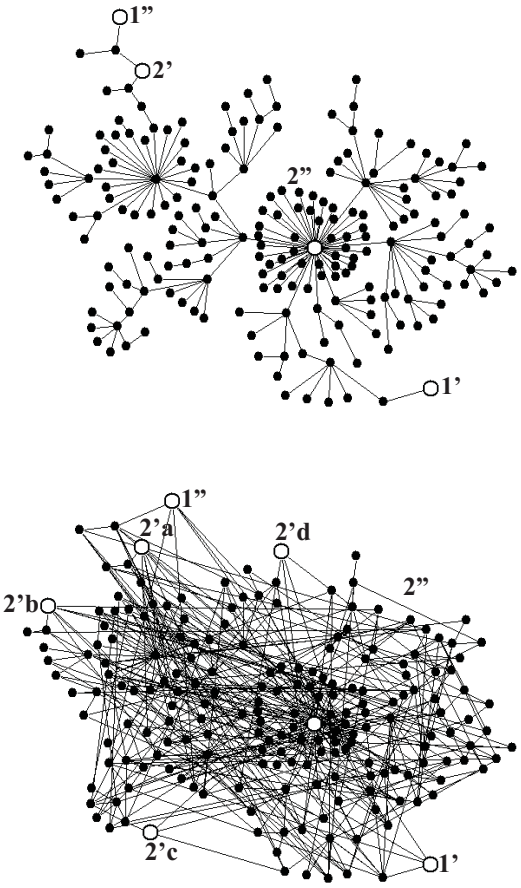

Figure 6. (a) Exemplifying realization of a scale-free fractal tree with $N = 200$ nodes constructed following the algorithm described in Ref. [36]. The single input and output gates are distinguished, chosen for the Monte Carlo simulations. (b) Tree from the upper figure extended by 200 shortcuts with the distance distribution following the power law r^2 what makes the network a scale free small world. Four output gates are distinguished, chosen for the Monte Carlo simulations; the unlabeled largest hub is the fourfold degenerated complement gate $2''$.

the distance distribution following the power law r^2, with negative α, what makes the network a scale free small world. To provide the network with a stochastic dynamics described by Eq. (1), we assume the probability of changing a node to any of its neighbors to be the same in each random walk step. Consequently, the transition probability from the node l to the neighboring node l' per computer step

$$w_{l'l} = 1/k_l\,, \tag{9}$$

where k_l is the number of links (the degree) of the node l. The network with such a dynamics cannot be isoenergetic and following the detailed balance principle the equilibrium occupation probability of the node l,

$$p_l^{\rm eq} = k_l / \sum_{l'} k_{l'}\,. \tag{10}$$

To complete Ref. [8], for the system of gates shown in Fig. 6(a) we performed a series of Monte Carlo simulations and found $\varepsilon = 0.99$ for mean times of external transitions $\tau_1 = \tau_2 = 40$, those times being the order of magnitude shorter than the internal relaxation time $\tau_{\rm rx} = 400$, and $\varepsilon = 0.88$ for $\tau_1 = \tau_2 = 400$. In the latter case of the comparable external and internal transition rates, there is some little slippage, but the output reaction proceeds backward relatively rarely. The case of multiple output gates needs more systematic studies. For the system of gates shown in Fig. 6(b) and $\tau_1 = \tau_2 = 40$ we found $\varepsilon = 1.40$, larger then unity. Random search for more optimal configuration of gates indicates a possibility of obtaining much higher value of the degree of coupling.

SUMMARY

It is now well established that most if not all enzymatic proteins display a slow stochastic dynamics of transitions between a variety of conformational substates composing their native state. This makes a possibility of chemical transformations to proceed before the conformational equilibrium has been reached in the actual chemical state. In the closed reactor, it results in the presence of transient, non-exponential stages of the reactions. In the open reactor, a consequence is the necessity of determining the steady-state reaction fluxes by mean first-passage times between transition conformational substates of the reactions rather than by conventional reaction rate constants. A hypothesis is stated that, like higher level biological networks: the protein interaction network and the metabolic network, the protein conformational transition networks have evolved in a process of self-organized criticality. All three classes of networks are scale-free and, probably, display a transition from the fractal organization in a small length scale to the small-world organization in the large length scale. Good mathematical models of such networks are stochastic critical branching trees extended by long-range shortcuts.

Biological molecular machines are proteins that operate under isothermal conditions hence are referred to as free energy transducers. They can be formally considered as enzymes that simultaneously catalyze two chemical reactions: the free energy-donating (input) reaction and the free energy-accepting (output) one. The degree of coupling between the output and the input reaction fluxes have been studied both theoretically and by means of the Monte Carlo simulations on model networks. In the steady state, on taking advantage of the assumption that each reaction proceeds through a single pair (the gate) of transition conformational substates of the enzyme-substrates complex, the degree of coupling between the output and the input reaction fluxes has been expressed in terms of the mean first-passage times between the distinguished substates. The theory has been confronted with the results of random walk simulations on various model networks.

For single input and output gates the degree of coupling values cannot exceed unity. As some experiments for the myosin II and the dynein motors suggest such exceeding, looking for conditions of increasing the degree of coupling value over unity (realization of a 'molecular gear') challenges the theory. Probably it holds also for the G-proteins and transcription factors, mutations of which can result in the cancerogenesis. Study simulations of random walks on several model networks involving more extended gates indicate that the case of the degree of coupling with the value higher than one is realized in a natural way on the mentioned above critical branching trees extended by long-range shortcuts. For short-range shortcuts, the networks are scale-free and fractal, and represent an ideal model for the

biomolecular machines with the tight coupling, i.e., with the degree of coupling value equal exactly to unity.

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