Functional Roles of Slow Enzyme Conformational Changes in Network Dynamics

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ABSTRACT Extensive studies from different fields reveal that many macromolecules, especially enzymes, show slow transitions among different conformations. This phenomenon is named such things as dynamic disorder, heterogeneity, hysteretic or mnemonic enzymes across these different fields, and has been directly demonstrated by single molecule enzymology and NMR studies recently. We analyzed enzyme slow conformational changes in the context of regulatory networks. A single enzymatic reaction with slow conformational changes can filter upstream network noises, and can either resonantly respond to the system stimulus at certain frequencies or respond adaptively for sustained input signals of the network fluctuations. It thus can serve as a basic functional motif with properties that are normally for larger intermolecular networks in the field of systems biology. We further analyzed examples including enzymes functioning against pH fluctuations, metabolic state change of *Artemia* embryos, and kinetic insulation of fluctuations in metabolic networks. The study also suggests that hysteretic enzymes may be building blocks of synthetic networks with various properties such as narrow-banded filtering. The work fills the missing gap between studies on enzyme biophysics and network level dynamics, and reveals that the coupling between the two is functionally important; it also suggests that the conformational dynamics of some enzymes may be evolutionally selected.

INTRODUCTION

Macromolecules like proteins move through high-dimensional energy landscapes with many potential minima corresponding to different stable conformations (1). A molecule typically spends most of the time within a stable potential well, with thermal fluctuations driving the system transiting from one conformation to another one. The landscape, thus transition rates, can also be affected by many environmental factors such as a change in temperature, pH, voltage, ion concentration, phosphorylation, or the binding of a ligand. At a higher level, molecules in a cell interact with each other to form an interconnected complex network regulating every aspect of the cell dynamics. Understanding the design principles of biological regulatory networks becomes an active research area. Several structural motifs have been proposed to possess certain dynamic properties and corresponding physiological functions (2–6). For example, it has been widely discussed on how a network functions robustly despite all the stochastic processes in a biological system. In these existing studies, focuses have been on network topology. Traditionally, one assumes that the intramolecular dynamics is fast compared to dynamics involving intermolecular interactions, and thus is unnecessary for explicit considerations (2). That is, molecules are treated as structureless vortices or edges within a network (e.g., the one shown in the *left panel* of Fig. 1).

However, numerous examples from experimental evidence challenge this view. Dynamic disorder, or dynamic heterogeneity, has long been discussed extensively in the physical chemistry and biophysics communities. The dynamic disorder refers to the phenomena that the rate constant of a process is actually not a constant but fluctuating with time while the enzyme molecule slowly changes its conformation. Since the pioneering work of Austin et al. (8) on ligand binding to myoglobin, extensive experimental and theoretical studies have been performed on this subject (see, for example, Zwanzig (9) for further references). Recently, single molecule enzymology and NMR measurements demonstrated convincingly that the catalytic activity of several enzymes at single-molecule level is slowly fluctuating (10–16). For instance, the activity change due to the fluctuation of pH value for alkaline phosphatase can last hours (17). In contrast, for β-galactosidase, an essential enzyme in the human body, the timescale for conformational changes measured by single molecule enzymology experiments ranges from milliseconds to seconds.

Moreover, even the measured rate constant for conformational changes under the same environment has a continuous broad distribution (11,13). These studies suggest that the existence of dynamic disorder in macromolecules is a rule rather than exception (20). Consistently, biochemistry studies reveal that a large number of enzymes involve conformational changes comparable to or even slower than the actual chemical bond forming and breaking processes, and show non-MM behavior. Hysteretic enzymes refer to "those enzymes which respond slowly (in terms of some kinetic characteristic) to a rapid change in ligand, either substrate or modifier, concentration" (21). Mnemonic behavior refers to the following model for enzymes with memory (22,23), in which an enzyme molecule has (at least) two conformers with different stability and catalytic activity: Conformer 1

Submitted February 6, 2012, and accepted for publication August 2, 2012.
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Editor: Daniel Beard.

0006-3495/12/09/1052/8 $2.00
http://dx.doi.org/10.1016/j.bpj.2012.08.008

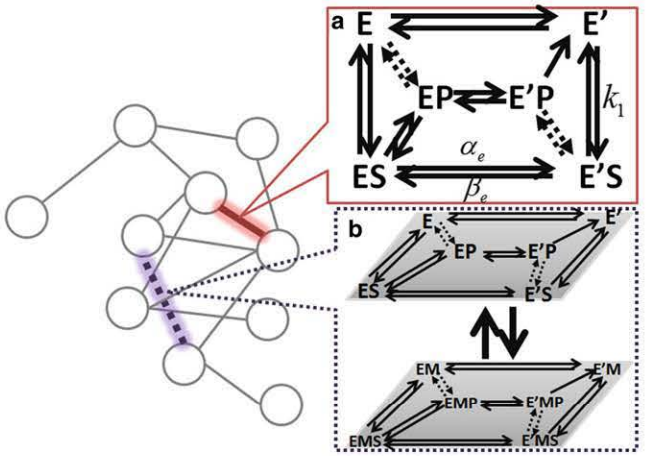

FIGURE 1 Edges in biological regulatory networks representing enzymatic reactions may have complex dynamics for substrate-to-product transition (*upper-right panel*) and regulation by modulating molecules M (*lower-right panel*). E and E' refer to different conformations of the same enzyme. S, P, and M are substrate, product, and regulator, respectively. These two models are adapted from Frieden (21) and Ricard and Cornish-Bowden (54).

is more stable without the substrate, but substrate binding changes the relative stability, and at the end of an enzymatic cycle, a product release generates free conformer 2 more readily. Given sufficient waiting time before new substrate binding, this then converts to conformer 1. Increasing substrate concentration decreases the waiting time for substrate binding, and thus more enzymes are locked in conformer 2.

This type of behavior has recently being called "conformational adaptive teamwork" (24). The catalytic activity of a mnemonic enzyme varies with substrate concentration. The phenomena discussed in these different fields all originate from the fact that proteins are not rigid bodies, but are ever-fluctuating entities with broad timescale distribution from picoseconds to hours (19).

The slow end of the timescale is comparable to many network level processes. Therefore, it is natural to ask the consequences of molecular level fluctuations on network dynamics. Furthermore, can the intramolecular dynamic property of a macromolecule be a consequence of natural selection? Actually, Frieden (25) noted that "it is of interest that the majority of enzymes exhibiting this type of (hysteretic) behavior can be classed as regulatory enzymes".

As schematically shown in the right panels of Fig. 1, a single enzymatic reaction (a single edge of the network in the *left panel* of Fig. 1) within a protein interaction network may itself have complicated dynamics involving slow enzyme conformational changes and activity fluctuations. This single edge in the network could also be a complicated embedded network. In fact, it has been argued that there are analogs between feedback in network dynamics and cooperativity in cellular biochemical processes (26). This work is to fill the gap between decades of accumulations of molecular level experimental data and observations, and analysis in the context of network dynamics. We shall focus on examining the basic dynamical properties and functional roles in network dynamics of those enzymes with profound slow conformational changes. Therefore, we adopt minimal models representing the enzymes and networks, and leave enzyme models that are more sophisticated and complex (but also may be distracting) for future studies (27).

METHODS

All calculations were performed through solving the corresponding rate equations in the form of ordinary and stochastic differential equations with the software MATLAB (The MathWorks, Natick, MA). Numerical details, model equations, and model parameters can be found in the Supporting Material. The corresponding MATLAB codes are available upon request.

RESULTS

Enzyme with slow conformational changes can serve as noise filter and adaptation motifs

We first analyzed the representative enzymatic reaction shown in the upper-right panel of Fig. 1. We set the rate constants for those reactions represented by dashed lines to be zero for simplicity, and chose other rate constants subject to detailed balance requirements. The substrates S are injected into the system, and the products P are removed from the system at a certain rate, therefore the system is kept at a nonequilibrium steady state. Each enzyme molecule has two possible conformations; each can bind the substrate and catalyze the reaction but with different activity. The enzyme can stochastically convert between these two conformations. Fig. 2 *a* shows that this simple motif with slow enzyme conformational change (see the Supporting Material for detailed mathematical equations) filters high-frequency stochastic fluctuations of the substrate concentration [S]. The time course for the product concentration [P] (*lower panel* of Fig. 2 *a*) shows slower fluctuations than the ones for the [S] (*upper panel* of Fig. 2 *a*). The power spectra in Fig. 2 *b* further shows that the same motif can also filter both high and low frequencies for certain parameters. Assuming the input substrate concentration subject to white-noise-like fluctuations (*upper panel*), the product P only fluctuates at the midrange of the frequency space (*lower panel* of Fig. 2 *b*).

For further analysis, we examined the system response to monochromatic sinuous [S] fluctuations. Although this analysis is normally used for linear systems, our usage is justified, noting that nonlinear contribution to the responses is negligible for the system we examined. As shown in Fig. 2 *c*, an enzyme with slow conformational changes has a finite response time, thus variation of the product concentration ΔP can only follow the low- (*black*) but not high- (*pink*) frequency substrate fluctuations. The comparison of monochromatic sinuous fluctuations demonstrates that the high

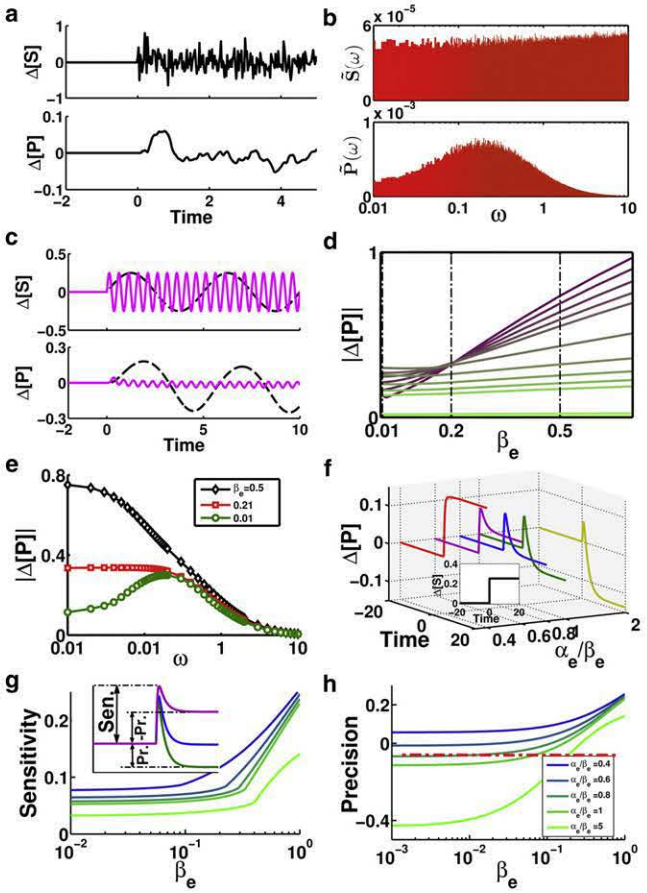

FIGURE 2 Enzyme with slow conformational change (motif *a* in Fig. 1 without the dashed connections for simplicity) may serve as noise filter and adaptive motif. (*a*) A typical trajectory of substrate and product derivation from their steady-state values with fluctuations. (*b*) Power spectrum of the substrate and product fluctuations. (*c*) Mono frequency analysis of fluctuation buffer with slow enzyme conformational changes. (*Upper panel*) Regulatory signal. (*Lower panel*) Downstream product response. (*Dashed line*) Low frequency. (*Solid line*) High frequency. The enzyme conformational transition rate $\alpha = 0.01$. (*d*) The product oscillation amplitude ΔP_m under sinuous substrate concentration fluctuations versus the transition rate constants between ES and $E'S$, β_e. The substrate oscillation frequency takes the value $\omega = 0.02$, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 from dark to light lines. (*e*) The product oscillation amplitude versus ω for the three β_e values shown in panel *c*. (*f*) Trajectories of product response upon sudden change of substrate concentration (*inset plot*) under different values of α_e/β_e (the ratio between transition rate constants $ES \rightarrow E'S$ and $E'S \rightarrow ES$). Enzyme fluctuation can generate positive (*red line*), adaptive (*purple*, *blue*, and *green lines*), and negative responses (*yellow line*). (*g* and *h*) Sensitivity and precision (defined by the *inset plot*) of the response curves in panel *h* versus β_e at a given value of α_e/β_e.

variation frequency could be filtered due to the slow response of the enzymes. To further analyze the effects of the response rate of the enzyme activity fluctuations, we examined the conformational change rate-constant systematically. Fig. 2 *d* shows that ΔP_m, the maximum change of product concentration under sinuous variation of [S], increases with the conformational change rate constant between ES and $E'S$, β_e. In these calculates, the rate for the reverse reaction between ES and $E'S$ α_e changes proportionally to keep α_e/β_e constant. The change of product concentration has higher dependence on β_e at a lower frequency of substrate variation but lower dependence on β_e at a higher frequency. Consequently, as shown in Fig. 2 *e*, at different fixed β_e values, the ΔP_m can be either a monotonic decreasing function of the frequency ω at the [S] fluctuation (similar to Fig. 2 *a*), or reach a maximum at resonant frequency, similar to what observed in Fig. 2 *b*.

Fig. 2, *f–h*, shows another property of the motif. Upon a sudden and sustained increase of [S], the product concentration [P] relaxes to a new steady state, which may be higher than, close to, or counterintuitively lower than the original steady state. These results are in agreement with the recent study on inhibitor-activator switching with nonequilibrium binding (28). We examined the system dynamics upon varying the three rate constants shown in Fig. 1 (the *motif* in the *upper-right panel*), α_e, β_e, and k_1, while keeping other parameters fixed. The detailed balance requirement constrains the three parameters further that $\alpha_e/(\beta_e\ k_1)$ must be kept constant. Within certain parameter ranges (see the Supporting Material for details), [P] increases temporally then decreases from a maximum value. The ability of the system to respond to a change, then relax to a preset value, is essentially adaptation. Adaptation behavior is an important dynamic property observed in many biological systems. Numerous studies have been focused on identifying those network structures that give rise to adaptation (2,5,29).

Note here that it can be achieved by a single enzymatic reaction. Following standard measures on adaptation, Fig. 2, *g* and *h*, shows how sensitivity (defined as the difference between the peak response and initial value) and precision (defined as the difference between the final and initial values) vary with enzyme conformational change rate. Adaptation requires slower conformational changes. When conformational change is no longer rate-limiting, ΔP simply increases with ΔS, as one expects intuitively. Physically, one can understand the dynamic property of the system as follows. Note that in the network of Fig. 1 *a*, a complete enzymatic cycle can either go through $E' \rightarrow E \rightarrow ES \rightarrow$, or $E' \rightarrow E'S \rightarrow ES$. The two pathways compete for the limited source of enzyme molecules. At low [S], there is sufficient time for a newly released enzyme in the form E' from a complex $E'P$ to convert to E before binding to a substrate molecule, and thus it takes the first pathway. Upon increasing [S], however, more reactions may take the second pathway. In the case where $E'S \rightarrow ES$ is the rate-limiting step, the effective turnover rate drops because many enzyme molecules are trapped in the $E'S$ state. Fig. 2 *h* confirms this picture. With a fixed value of β_e, increasing α_e (and therefore k_1) leads to more enzymes trapped in the state $E'S$ and thus lower [P].

We also examined the network in Fig. 2 *b*, which can similarly filter and modulate substrate fluctuations. Below

we use several examples to examine the functional roles of slowly changing enzymes as molecular noise filters, and possible building blocks of larger networks with certain dynamic properties.

Filtering and buffering upstream or environmental fluctuations

Numerous enzymes show hysteretic response to pH fluctuations. One example is nitrate reductase, which catalyzes the reduction of nitrate to nitrite, a rate-limiting (thus a regulatory) step in the nitrate assimilation process in higher plants and algae (30–33). Another example, alkaline phosphatase (AP), is physiologically more important. Phosphatases are a large class of enzymes responsible for removing phosphate groups from other molecules. They counteract with kinases that add phosphate groups to proteins, and play important roles in cell regulation. AP's pH optimum is >7. For human beings, AP is present in all tissue throughout the body. Experiments show that upon changing environmental pH values, AP activity adapts hysteretically to the change, at rates as slow as 2 h (17).

We generalize the classical diprotonic model for enzyme pH dependence based on the alkaline phosphatase properties (35), as shown in Fig. 3 *a*. Each enzyme molecule has two protonable sites where only the monoprotonated form is catalytically active. Each molecule stochastically interconverts between two conformations with different proton affinity. The model correctly gives the pH dependence of enzyme catalytic activity (see Fig. 3 *b*). The active conformation EH has an optimal pH response curve, which is consistent with the experimental observations (17). Subject to transient pH changes, Fig. 3 *c* shows that that slower conformational interconversion rate leads to slower enzyme response, which provides response time for the intracellular pH regulation toolkits before resulting in detrimental effects. Fig. 3 *d* shows that the longer the pH change is retained, the smaller is the value needed for the pH change to reach the same enzyme activity response.

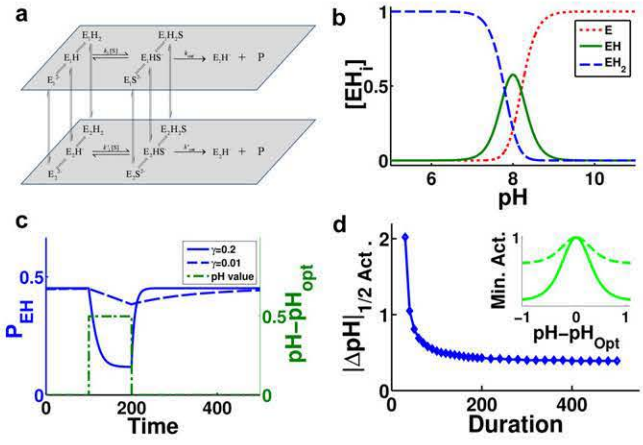

FIGURE 3 Slow conformational change allows enzymes to buffer pH fluctuations. Model parameters are chosen to resemble alkaline phosphatase. (*a*) A simple diprotonic model with conformational change, which is abstracted from the real alkaline phosphatase system. (*b*) The steady-state percentage of the active form EH has an optimum pH value. (*c*) Change of P_{EH} for an enzyme with fast (*solid line*) and slow (*dashed line*) conformational change upon sudden and transient pH change (*dot-dashed line*). The steady-state P_{EH} is normalized to 1 here. (*d*) pH change needed for $\Delta P_{EH} = 1/2$ in panel *c* versus duration of the pH change. (*Inset plot*) ΔP_{EH} versus $\Delta pH = pH - pH_{opt}$ with sustained (*solid line*) and transient (*dashed line*) pH fluctuation.

Reconciling ultrasensitivity and robustness

Cells often need to change their phenotypes and subphenotypes upon environmental change. Fig. 4 *a* shows one example from brine shrimp *Artemia*. Changing the intracellular pH value of brine shrimp *Artemia* embryos from >7.9 to 6.3 (e.g., due to deprivation of oxygen) leads the cell to switch from a metabolically active state to a dormant state with very low metabolic activities. The embryo can survive in this dormant state for several years and switch back to normal state upon changing the intracellular pH value back to alkaline levels (36). Fig. 4 *b* shows two basic dynamic behaviors with dramatic system property changes frequently found in regulatory networks (37). The upper panel shows that a sigmoidally shaped response curve allows the system to respond to pH-value change sensitively.

However, the system may switch back and forth undesirably between the two high and low enzyme activity states upon fluctuations of pH near point *c*. The lower panel shows a bistable response. That is, the system may have two steady states with a controlling parameter, e.g., pH, at a given value

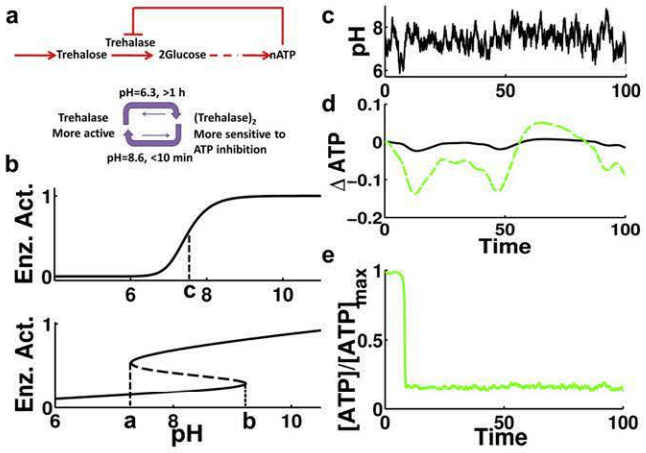

FIGURE 4 Model study of the Brine Shrimp Embryo metabolic regulatory network. (*a*) Basic network from *Artemia* embryo. (*b*) Two typical response curves for biological systems switching between two distinct states. (*Upper panel*) Sigmoidal response curve corresponding to the *Artemia* embryo network in panel *a*. (*Lower panel*) A typical bistable response curve. (*c*) pH fluctuations lead to different ATP production fluctuation patterns with sigmoidal response curve for *Artemia* embryo (*d*), and hypothetical bistable response curve (*e*). (In panel *d*, *solid line*, slow Trehalase conformational change; *dashed line*, fast conformational change.)

between a and b in the lower panel of Fig. 4 *b*. Bistability allows a system to resist small amplitude fluctuations with the capacity increases that occur upon increasing the value (b-a), and avoids frequent dramatic changes of the cell state. For example, the system initially staying at the lower branch remains until a pH value $>b$. However, larger capacity to resist fluctuations also means a larger value of (b-a) and a less-sensitive response to pH changes. Therefore, robustness to transient fluctuations, and sensitivity to (sustained) changes of the controlling parameters, are two seemingly opposite requirements to the regulatory network.

For *Artemia* embryos, Hand and Carpenter (36) found that this transition is mediated by a hysteretic enzyme trehalase. As illustrated in Fig. 4 *a*, the main energy source used by the embryo, the disaccharide trehalose, is converted to glucose by the enzyme trehalase. The end-product ATP molecules can inhibit this conversion reaction. Trehalase can form a polymerized complex with doubled mass and reduced enzyme activity. Remarkably, at basic conditions the monomer form is more stable, and the dimer-to-monomer transition takes place in <10 min. In contrast, at pH $= 6.3$, it takes >1 h for the monomer-to-dimer transition to finish. Our model studies show that with the available experimentally measured parameter values, the system can give rise to a sigmoidal response curve to the pH value (*upper panel* of Fig. 4 *b*), but not bistability with the network shown in Fig. 4 *a*.

For transient fluctuations around the transition point c for a sigmoidal response (Fig. 4 *c*), the hysteretic enzymes cannot respond quickly before the large fluctuations die off (see the *solid line* of Fig. 4 *d*). Consequently, an *Artemia* embryo in the dormant state can respond to pH changes sensitively and converts to the active state. Once in the active state, the embryo is robust against transient fluctuations without switching back to the dormant state. On the other hand, Fig. 4 *e* shows a hypothetical system with bistable response. With certain rare large-amplitude fluctuation, the system response may jump from one branch to another one, and be trapped for a prolonged period before another, rare large-amplitude fluctuation brings it back. The coupling between the sigmoidal response in the protein-protein network and the slow response enzyme dynamics in *Artemia* embryo gives rise to the required properties that are so hard to be fulfilled by only a simple regulatory network at the intermolecular level: the cell should be robustly shielded from transient fluctuations, but at the same time be sensitive to (sustained) changes of pH value.

Insulating local network fluctuations

Biological networks are highly interconnected, with a structure analogous to a spider's web. When one stripe of the spider's web is pulled, will it disturb the whole network?

Clearly it would be generally detrimental to the proper function of a biological network. For metabolic networks, some metabolic intermediates are highly toxic to cells. Large concentration fluctuations of these species are undesirable to cells. There are many ways to restrain their damage to cells. One possibility is to carefully insulate their dynamics from fluctuations of other parts of the network. Most hysteretic enzymes identified in the early days are regulatory enzymes in metabolic networks. Frieden (21) summarized some typical network motifs with hysteretic enzymes abstracted from realistic networks such as *Escherichia coli* Threonine synthesis pathway. To test whether these hysteretic enzymes may function as fluctuation insulators, we analyzed two of them, as shown in Fig. 5. The basic metabolic network showed in Fig. 5 is two parallel pathways from substrate A to product F and F' and two pathways are branched at intermediate species B. The difference between upper and lower networks of the center column in Fig. 5 is the location of the end-product inhibition from F.

For both networks, as well known from previous network motif studies (38–41), the end-product inhibition mechanism can remove fluctuations of F (different patterns are shown in the *left column* of Fig. 5), but at the expense of inducing fluctuations of F' at another parallel pathway sharing common ancestor metabolites (*right column* of Fig. 5). On the other hand, hysteretic enzymes reduce the coupling between these two pathways and lead to smaller F' responses, as can be seen from Fig. 5 and Fig. S1 in the Supporting Material. The two networks shown in Fig. 5 respond to F fluctuations differently.

In the first network (*upper*), longer F fluctuations (*upper panel* of *left-column*) lead to larger and more sustained F' fluctuations (*upper-right panels*), thus F' responses to the duration of F fluctuations. In the second network (*lower*),

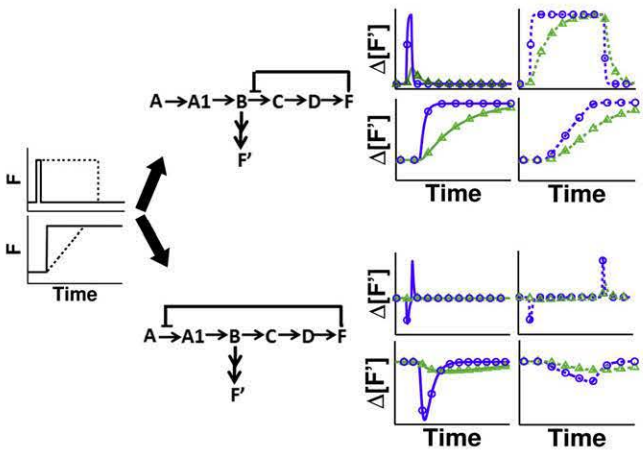

FIGURE 5 Dynamics of metabolic networks with end-product inhibition. (*Left*) Different patterns of introduced end-product F fluctuations. (*Middle*) Two typical metabolic networks. The regulatory enzymes are modulated by end-product F and are hysteretic. (*Right*) Induced F' fluctuations. (*Curves* with *circles* and *triangles*) Results with fast and slow enzyme conformational changes, respectively. (*Solid* and *dashed curves*) F' fluctuations induced by the corresponding F fluctuations (shown in the *left panel*).

F' responses to the rate of F change, i.e., faster F change results in larger F' response (*lower-right panels*). Fig. S1 provides additional results supporting the above observations. The dependence of F' variation on F fluctuation frequency shows high-frequency filtering with network 1 (*middle column*, *upper panel* in Fig. 5), and resonance with network 2 (*middle column*, *lower panel* in Fig. 5) (see Fig. S1 *a*). With network 1, the change of F' concentration, $\Delta[F']$, with different conformational changing rates γ, converge to the same value upon increasing the F fluctuation duration. With network 2, $\Delta[F']$ is larger with larger γ-values (Fig. S1 *b*). The change of $[F']$ is less sensitive to γ with more sustained F fluctuations in the network 1. On the other hand, the $\Delta[F']$ value is more sensitive to γ with both shorter and more sustained F fluctuations in the network 2 (see Fig. S1 *c*). As Fig. S1 *d* shows, although the main difference between the two networks is the location of end-product inhibition, they respond to the increasing rate of the F concentration changes very differently. The observed differential and integral response behaviors are essentially the same as those of the more complicated kinetic insulation networks of Behar et al. (3).

Building blocks for larger networks

As already illustrated by some of the above examples, incorporating hysteretic enzymes into larger network structures can lead to desirable dynamic properties. To further demonstrate the motiflike behavior of the hysteric enzymes in the networks, we show two additional examples in Fig. 6, *a* and *b*. Fig. 6 *a* shows a cascade of two or more enzymatic reactions with hysteric enzyme behaviors. Fig. 6 *b* shows a combination of hysteric enzymes and regulatory networks. As demonstrated in Fig. 6 *c*, cascades of hysteretic enzyme-catalyzed reactions show sharper filtering. With more units of cascades of hysteric enzyme-catalyzed reactions, it could filter the noisy high-frequency noises even better. It provides a possible mechanism for cells to filter the higher-frequency fluctuations with high efficiency without constructing complicated interaction networks. Fig. 6 *d* shows the resonance spectra on increasing the cascade length (more cascade units). Coupling a hysteretic-catalyzed reaction with another reaction with sigmoidally shaped response (Fig. 6 *b*) also leads to sharper resonance spectrum, as shown in Fig. 6 *e*. This combined system could filter both high and low frequencies of fluctuations at a very high efficiency. Overall, by simply adding-on one or two types of hysteretic enzyme kinetics, it can be easy to form functional motifs that could need a much larger molecular network to fulfill similar functions.

DISCUSSION

In this work, we have demonstrated that intramolecular conformational fluctuations can be coupled to cellular network dynamics, and give rise to important physiological functions. Specifically we show that enzymes can use slow intramolecular conformational changes to buffer transient system fluctuations, functionally substituting some more complex network motifs involving multiple molecule species. For example, compared to the example in Fig. 4, a corresponding widely discussed network motif selectively responding to sustained signals is the coherent feed-forward loop motif (42). The network dynamics discussed in Fig. 5 closely resemble that of Behar et al. (3). We used several examples to illustrate possible functions of hysteretic enzymes, and suggest that one can also use hysteretic enzyme-catalyzed reactions to engineer networks with certain dynamic properties.

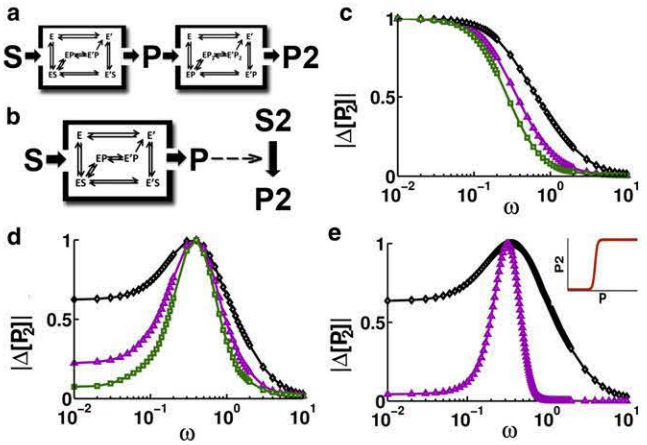

FIGURE 6 Construct networks with hysteretic enzymes. (*a*) Cascade of enzymatic reactions. (*b*)The product of reaction 1 with hysteretic enzyme regulates reaction 2 sigmoidally. (*c*) Normalized ΔP_2 versus frequency ω of [S] oscillations for the cascade of one (*diamond*), two (*triangle*), and three (*square*) enzymatic reactions motifs. (*d*) Same as panel *c* except with different parameters to have resonance. (*e*) ΔP_2 versus ω for network in panel *b*.

Recently, intramolecular dynamics of the *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpB (14) and the Von Willebrand factor-binding protein for blood coagulation (43) have been experimentally characterized. Slow conformational changes may likely play similar buffering roles in these systems. Existing experimental information on slow conformational fluctuations focuses on enzymes. One expects that it may be ubiquitous for proteins and other macromolecules to have conformational fluctuations spanning a broad distribution of timescales. It requires further experimental studies to test this hypothesis. The molecular fluctuation dynamics, especially some regulatory proteins, is likely under selection pressure. We suggest that the biological consequences of the phenomenon can only be fully understood in the context of network dynamics of biochemical systems. In Fig. 3, we demonstrated that slow conformational changes buffer transient pH fluctuations. This may have significant physiological functions. The catalytic activity of each enzyme is pH-sensitive, and drops

dramatically when the environmental pH value deviates away from its optimal pH value.

For example, a change as small as 0.05 pH unit can substantially inhibit the enzyme activity of phosphoructokinase, an important enzyme in the glycolysis cycle (44,45). However, the number of intracellular free protons is small. As a very crude estimation without considering many factors such as compartmentation, with a volume of 1 μm^3 and an average intracellular pH value of 7.6 corresponding to an *E. coli* cell, the number of corresponding protons is ~15. The variation of 0.1 in pH value leads to ~20% changes of the proton number. Despite tight regulations, intracellular pH inevitably fluctuates transiently, especially locally around a given complex (46,47), which may be detrimental to the cell. The negative feedback mechanism frequently appearing in cellular regulation networks is not practical here, given the large number of enzymes involved. Instead Nature may have designed a much simpler solution, using slow conformational changes. The slower enzyme response could protect the cell from a large transient fluctuation of pH values (it could be significantly large based on the estimation above), and, at the same time, allow a response to the sustained long-term pH changes so that the cell can be adaptive to the environmental fluctuation.

We leave other possible functional roles of slow intramolecular fluctuation for future studies. For example, at low copy numbers, the observed enzyme catalytic activity fluctuations may contribute to noises in a network. Xing (27) also proposed possible roles of conformational dynamics in allosteric regulation. Frauenfelder et al. (48) provided another perspective on the function of protein conformational fluctuations. Their work also suggests possible mechanisms of protein-protein interactions through couplings among the dynamics of proteins, solvation shell, and the solvent. The concept of dynamic disorder can also be further generalized in cell network studies. Suppose that the dynamics of a network is described by a set of ordinary differential equations $d\mathbf{x}/dt = f(\mathbf{x},c)$, with $\mathbf{x}$ the concentrations of species involved. The set of constants, such as rate constants and total molecule concentrations, are often slowly varying quantities, due to conformational fluctuations as discussed in this work, stochastic synthesis and degradation, etc. (49). In cell biology this phenomenon of dynamic disorder is usually called "nongenetic heterogeneity" (50). When the corresponding deterministic dynamics of a network is near a transition point, e.g., a bifurcation point, consequences of the transient parameter fluctuations can be further amplified and temporarily frozen (51,52), as illustrated in Fig. 4 *e*, which may explain the observed larger and slower timescale (in days or longer) stochasticity (53).

In conclusion, we suggest that it is an important yet often overlooked subject to examine the coupling between macromolecular and intercellular dynamics. Advance of this subject requires cooperative efforts between experimentalists and theoreticians; some quantitative measurements of fluctuation correlations within a network context, e.g., in metabolic networks, will be greatly needed.

SUPPORTING MATERIAL

System equations and one figure are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(12)00868-5.

We thank Drs. Hans Frauenfelder, Haw Yang, and Michelle M. Elekonich for helpful discussions.

This work is supported by National Science Foundation grant No. EF-1038636.

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