Structure of Feed-Forward Realizations with Enzymatic Processes

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Abstract — **Basic bio-inspired information-processing motifs, such as feed forward can be useful in complex biochemical networks for signal processing and biosensing. We propose an experimental design and a numerical approach to a synthetic enzyme cascade-based biochemical system for feed-forward loop implementation that demonstrates an ability to delay and stabilize the changes in the output signal in response to chaotic fluctuations in the input signal.**

Keywords *— Feed-forward loop; enzymatic processes.*

I. INTRODUCTION

Information processing with networked biomolecular reactions, termed "biocomputing" [1][2], is a type of unconventional computing [3] that offers interesting new applications for multi-input biosensing [4]-[6] and generally complex signal processing [7][8] without involving electronics at each step. Most experimental efforts aimed to implementing biocomputing utilize the analog/digital approach because it is fully understood and has fault-tolerant scalability. This approach has been successfully applied to many enzyme-based systems offering a selection of binary logic gates [9]-[12]. Small biochemical networks have also been explored, utilizing concatenated enzymatic reactions to carry out Boolean functions [1][2][13]-[15].

However, biomolecular processes also offer tools for considering new unconventional network elements and architectures that are bio-inspired but are vastly simpler than those in nature. Indeed, recent research has involved consideration of non-binary network elements for improving noise handling of binary steps, and also utilization of several bio-inspired memory elements (memristors, etc.) for designs of electronic circuitry for specific applications [16][17]. The former development [2][18]-[27] allowed to incorporate biochemical "filtering" in biocatalytic reactions and reaction cascades, leading to a considerable reduction of the noise transmission factor. The resulting output response as a function of the input was transformed to sigmoid shape [2][18][19][21].

Realization of such synthetic biochemical systems in the framework of biocomputing [1][2][28]-[30] has enabled their implementation in practical applications, primarily in biosensing. The availability of biomolecular "building blocks" offers a toolbox of processes to experiment with optimization of networking involved in signal processing with biomolecular reaction cascades [1][21].

Several research groups have studied biocomputing, using synthetic deoxyribonucleic acid (DNA) chains (oligonucleotides) [31][32], various proteins (including enzymes) [12][14][18]-[21][33]-[36], and other bio-objects [10][37][38] (even whole cells). Compatibility of enzymes with physiological processes and electronics has favored their use in biocomputing designs aimed at near-term applications. Enzyme reactions are also particularly suitable for interfacing with electronics [39][40] in electrochemical settings. Therefore, even small networks with several enzymatic steps offer applications [41], e.g., for biosensing [4]-[6][31][32], for the point-of-care [33][34] and security purposes [35][36].

Information processing in nature is another successful approach, quite different from the analog/digital paradigm. Systems biology studies aspects of nature's information processing [8][37]-[39]. Bio-inspired approaches have resulted in interesting research involving memory, learning, etc. Some of the considered "network elements" enable novel electronic circuit designs [16][17] and suggest novel biocomputing approaches [40][41].

Here, we review recent ideas [42] and report new results on enzymatic-process realizations of feed-forward loops which are the most common network motifs in nature's information processing [8][42]-[46]. Specifically, feed-forward loops are abundant as signal processing steps in cellular processes ranging from regulatory mechanisms [47] to cell differentiation [48]. The functionality of feed-forward loops and other complicated network motifs is largely determined by the connectivity of the various network components including biochemical processes [43][49][50]. The study of the feed-forward dynamics usually focuses on

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specific network architectures of interest [45][50][51]. Such research is typically devoted to modeling of the temporal or spatial features of constituent processes [43]-[45][50][51], and to their networking [45][52][53].

There are several types of feed-forward loops. The most common examples of the feed-forward loop network motif are the coherent and incoherent feed-forward loops [8], that have been extensively studied theoretically [43][44][54]. More complicated "topologies" have also been considered [49][50], with different types of activation [44][51]. However, only limited experimental realizations are known to date for feed-forward loops as synthetic biochemical processes, specifically, with DNA [55].

Modeling allows exploration of the potential future incorporation of bio-inspired network elements into biochemical circuits [8][42][56][57], for instance, for biocomputing [52][58][59]. Numerical approaches include biochemical systems theory [60], kinetic modeling [42][43], Gaussian models [49], cellular automata simulation [50], etc.

In Section II we discuss possible realizations of feed-forward biochemical system. Section III is devoted to proposed experimental design and numerical approach to the synthetic biochemical system with enzyme-catalyzed biochemical reactions that demonstrate feed-forward response. In Section IV offers a concluding discussion.

II. DISCUSSION OF THE FEED-FORWARD REALIZATION

In this work, we propose a realization of feed-forward systems with few coupled enzymatic reactions [42], which, if realized, will be a much simpler implementation than those in nature. Let us discuss an example of a possible design and offer a general introduction. The details of the actual biochemistry of this and other systems are explained in [42]. Unlike the various earlier-realized biocomputing gates, feed forward in most cases is not functioning as a binary gate [60]-[63]. We expect that the considered designs [42] will initiate research into synthetic information processing setups not aimed at "artificial life" [64]-[66], but rather at a more limited and hopefully more tractable task of mimicking the nature's information processing.

The challenge of attempting enzyme-process realizations of the feed-forward loop is that it has two "signal transduction" steps (see the scheme in Figure 1), each involving the input signal, X, usually not being directly converted into the output Z. Instead, in the primary (direct) step, X acts as the activator (promoter), denoted by →, or repressor (inhibitor), denoted by ⊣, of ongoing processes that generate the output signal, Z. The secondary (indirect) pathway in the loop consists of the input X affecting (activating or repressing) the processes that produce another, intermediate signal, Y. This intermediate signal, Y, in turn activates or represses the ongoing processes of the production of the output, Z. In Figure 1, two enzyme-catalyzed biochemical processes (shown in boxes) constantly produce chemicals that are signals Y and Z.

For biochemical realizations, we will consider the simplest situation when X *or* Y, rather than X *and* Y together activate the output signal, Z, production. The latter is another feed-forward option. In the simplest classification [44][62], there are 8 different loop types:

$$X \to Z \text{ or } X \dashv Z, \quad X \to Y \text{ or } X \dashv Y, \quad Y \to Z \text{ or } Y \dashv Z. \qquad (1)$$

The feed-forward loop is "coherent" or "incoherent" depending on whether the net effect of X on the production of Z in the secondary pathway is the same as in the primary. The most common feed-forward loops in nature involve three activations [44][47][48].

We present a potential realization of such a process [42] with enzymatic reactions in Figure 1. Such systems require at least two biochemical processes that generate signals Y and Z. The chemical reaction rates of these processes are then controlled by the input at time t, $X(t)$. Here chemical input signal X activates (promotes) the production of both Z and Y, whereas Y promotes the production of Z.

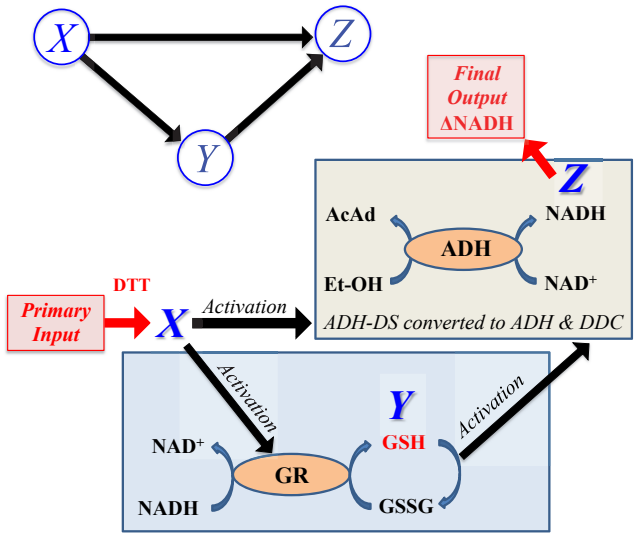

Fig. 1. Feed-forward loop with *activation* in all the signal transduction steps. The top scheme shows the activations involved. The biochemical processes are explained in Section III, with additional details founds in [42].

In some situations, activation can be made rather sharp as a function of parameters, and inhibition can also be made sharp. Then, the feed-forward loop can approximate binary logic gates [67]. However, generally feed forward is not binary. Its primary network function, specifically with three promotions (Figure 1), is to provide a stabilizing role.

Indeed, feed-forward loop as a network element has the capability to delay the changes in the output signal, $Z(t)$, in response to erratic fluctuations and fast variations in the environmental input signal, $X(t)$ [42][45][47][51][52]. This frequently enables threshold behavior [8][44], whereby the response begins and/or stops only when one or several of the chemical concentrations cross (up or down) activation thresholds. Otherwise, the system does not respond to input signal variations. This property avoids waste of resources in

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natural-pathway responses. The secondary pathway provides the postponing of the effect of changes in the input signal, X, in its net impact on Z. The system's response can then also have its own response time scale(s) [44][68]-[70].

In an experimental realization, we have to control the availability of the input chemical, $X(t)$, inputting or deactivating this compound by physical or (bio)chemical methods, at the rate, R_{ext}, that can be negative or positive,

$$\frac{dx}{dt} = R_{ext}(t) + \text{reaction terms}, \qquad (2)$$

where the "reaction terms" describe the kinetics of a possible consumption of X by the biochemical processes of the feed-forward loop itself. The quantification of the feed-forward effect will consist of detecting how the response time dependence of $Z(t)$ is affected by the presence of the secondary transduction step, $X \to Y \to Z$. This pathway can be enabled at various degrees of activity.

The stabilizing effects expected of feed-forward functions have never been realized in simple "synthetic" enzymatic systems. Here, we highlight the challenges involved in such realizations, see [42]. Ref. [42] also offers an example of an enzymatic system with all the transductions being repression, ⊣, steps.

III. DESIGN AND MODELLING

Let us outline the principles of feed-forward design [42] based on enzymatic cascades. Specifically, consider the system shown in Figure 1. This cascade includes the functioning of two enzymes with activations in all steps: Glutathione reductase (GR), which biocatalytically converts glutathione from its oxidized form, Glutathione disulfide (GSSG), to the reduced form, Glutathione (GSH). Then, GSH, acts as the intermediate signal, Y, in the feed-forward functioning. In parallel, β-nicotinamide adenine dinucleotide is converted from its reduced form, NADH, to the oxidized form, NAD^+. Alcohol dehydrogenase (ADH), biocatalytically oxidizes ethanol (Et-OH) to yield acetaldehyde (AcAd), while β-nicotinamide adenine dinucleotide is converted from its oxidized form, NAD^+, to the reduced state NADH. These processes can yield the increase in the amount of NADH that can be measured optically by changes in absorption. Thus, as expected for feed forward, signals Z and Y are generated continuously once the reactions are started. In fact, the rate of production of NADH must be kept in check, to avoid rapid build-up of signal Z: The excess concentration of NADH,

$$\Delta\text{NADH}(t) = \text{NADH}(t) - \text{NADH}(0), \qquad (3)$$

is the measured output signal, $Z(t)$. The names of the additional chemical compounds in Figure 1, are abbreviated as follows: dithiothreitol (DTT), diethyldithiocarbamate (DDC), disulfiram (DS), and their role is detailed in [42].

The full realization and characterization of the proposed enzymatic cascade will require addressing several challenges, even though some of the processes have already been studied in the literature. Possible experimental realizations and preliminary tests are described in [42].

Generally, the structure of enzymatic feed-forward realizations is shown in Figure 2. We will next discuss a kinetic modelling approach to such systems. In modelling of feed-forward loops, one can set up coupled rate equations [42] describing signal and other compound variations. This approach can yield the expected features, including the delayed response of the output to the input's variations/fluctuations, and other properties. For enzymes, the resulting systems of equations will be more complicated and contain different terms than those considered in purely phenomenological formulations [7][62].

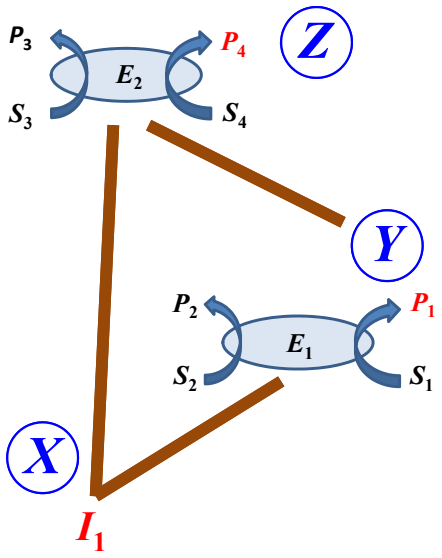

Fig. 2. Principle of an enzymatic-cascade feed-forward design in terms of the constituent enzymatic processes. Activations or repressions (promotions or inhibitions) are shown by brown lines.

We use the standard Michaelis-Menten type model, which focuses on the dominant enzyme bio-catalysis mechanism. The first enzyme, E_1, binds a substrate, S_1, to produce a complex, C. This complex can either on its own or by binding another substrate, S_2, produces the product(s) $P_{1,2}$ (Figure 2), restoring the enzyme to its original form. In the chemical reaction notation, we have,

$$S_1 + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C, \quad S_2 + C \xrightarrow{k_2} E_1 + P_1 + P_2. \qquad (4)$$

The second step can usually be assumed irreversible, but the first one requires two rate constants. These process parameters, here $k_{\pm 1}$, k_2, are generally not known individually and have to be fitted from experiments.

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Activation/repression can involve several mechanisms, one of which can be a complex formation, for example,

$$I_1 + E_1 \underset{r_{-1}}{\overset{r_1}{\rightleftarrows}} \overline{E_1} + W. \quad (5)$$

Here, the "complex" is the modified enzyme $\overline{E_1}$ with a different activity, with larger or smaller rate constants, $\overline{k_{\pm 1}}$, $\overline{k_2}$ in processes similar to those in Equation (4), and it can be restored to the original form, E_1, for example by reacting with some other chemical, here denoted W. If, for instance, I_1 is our input, X, then the added "reaction terms" in Equation (2) enter via such chemical processes,

$$\frac{dI_1}{dt} = R_{ext}(t) - r_1 I_1(t) E_1(t) + r_{-1} W(t)\, \overline{E_1}\,(t), \quad (6)$$

whereas the time-dependence of the quantities entering here is in turn set by their own rate equations, for example,

$$\frac{dE_1}{dt} = -\, r_1 I_1 E_1 + r_{-1} W \overline{E_1} - k_1 S_1 E_1 + k_{-1} C, \quad (7)$$

etc. Note that in the next stage, when writing the rate equation for S_1, for instance, terms resulting from its reaction with both the original and modified enzymes will enter, with their respective rates,

$$\frac{dS_1}{dt} = -\, k_1 S_1 E_1 + k_{-1} C - \overline{k_1} S_1 \overline{E_1} + \overline{k_{-1}}\, \bar{C}. \quad (8)$$

Even within this relatively simple chemical kinetics description, enzymatic cascades lead to systems of numerous chemical rate equations, with parameters that depend on the physical and chemical conditions of the experiment, and that are documented only to a very limited extent; typically, at most a single parameter, calculated in our notation from the quantities $k_{\pm 1}$ and $S_2(0)k_2$, call the Michaelis-Menten constant, is tabulated.

Attempts can be made to consider much more simple enzymatic feed-forward realizations. Let us consider the cascade sketched in Figure 3, where X is one of the substrates for enzyme E_1, but also for enzyme E_2. The former enzyme outputs Z as one of its products, whereas the latter outputs Y. However, Y is a substrate for enzyme E_3, which also outputs Z. Such systems are easier to design for experimental realizations because they involve process cascades of the type already realized in the binary-biocomputing-gate research [1][2]. The "activation" by X and by Y consists of them simply being the actual inputs enabling the reactions. The availability of the primary input substrate, $X(t)$, was controlled by adjusting $R_{ext}(t)$, to yield the shown time dependence.

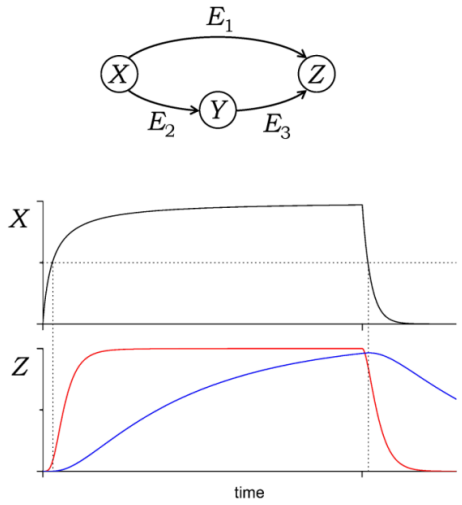

Fig. 3. The top scheme shows a three-enzyme system, which was numerically modelled as a candidate for a simplified all-activations feed forward. Our modelling results indicate that a limited feed-forward-type delayed response is possible, as illustrated by the blue curve.

In this model, adjustable quantities (concentrations of those chemicals that are not designated as input/output signals) can be selected as needed to achieve variants of delayed response, Figure 3, to various protocols, $R_{ext}(t)$, of controlling the input signal availability. However, this simplified approach cannot provide the full-featured feed-forward realization even for the all-activations case. More complicated cascades [42], such as that shown in Figure 1, will have to be explored in future work.

IV. CONCLUSION

We surveyed the conceptual design for cascades of enzyme-catalyzed biochemical reactions that promise realizations of the feed-forward response. This design of synthetic biochemical system with few coupled enzymatic reactions demonstrated the activation in all the signal transduction steps with a capability to delay the changes in the output signal. Such enzymatic-process realization of feed-forward loops is expected to have a stabilizing effect in the response to chaotic fluctuations in the input signals.

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REFERENCES

[1] V. Privman, O. Zavalov, L. Halámková, F. Moseley, J. Halámek, and E. Katz, "Networked Enzymatic Logic Gates with Filtering: New Theoretical Modeling Expressions and Their Experimental Application," J. Phys. Chem. B 2013, vol. 117, pp. 14928–14939.

[2] V. Privman, B. E. Fratto, O. Zavalov, J. Halámek, and E. Katz, "Enzymatic AND Logic Gate with Sigmoid Response Induced by Photochemically Controlled Oxidation of the Output," J. Phys. Chem. B 2013, vol. 117, pp. 7559–7568.

[3] A. Adamatzky, L. Bull, B. De Lacy Costello, S. Stepney, and C. Teuscher (eds.), Unconventional Computing 2007, Luniver Press, Bristol, UK, 2007.

[4] J. Wang and E. Katz, "Digital Biosensors with Built-in Logic for Biomedical Applications – Biosensors Based on Biocomputing Concept," Anal. Bioanal. Chem. 2010, vol. 398, pp. 1591–1603.

[5] J. Wang and E. Katz, "Digital Biosensors with Built-in Logic for Biomedical Applications," Israel J. Chem. 2011, vol. 51, pp. 141–150.

[6] V. Privman, O. Zavalov, and A. Simonian, "Extended Linear Response for Bioanalytical Applications Using Multiple Enzymes," Anal. Chem. 2013, vol. 85, pp. 2027–2031.

[7] D. D. Seaton and J. Krishnan, "Effects of Multiple Enzyme-Substrate Interactions in Basic Units of Cellular Signal Processing," Phys. Biol. 2012, vol. 9, Article #045009.

[8] U. Alon, An Introduction to Systems Biology. Design Principles of Biological Circuits, Chapman & Hall/CRC Press, 2007.

[9] J. Halámek, et al., "Realization and Properties of Biochemical-Computing Biocatalytic XOR Gate Based on Enzyme Inhibition by a Substrate," J. Phys. Chem. B 2011, vol. 115, pp. 9838–9845.

[10] M. N. Stojanovic, T. E. Mitchell, and D. Stefanovic, "Deoxyribozyme-Based Logic Gates," J. Am. Chem. Soc. 2002, vol. 124, pp. 3555–3561.

[11] E. Gdor, E. Katz, and D. Mandler, "Biomolecular AND Logic Gate Based on Immobilized Enzymes with Precise Spatial Separation Controlled by Electrochemical Scanning Microscopy," J. Phys. Chem. B 2013, vol. 117, pp. 16058–16065.

[12] T. Miyamoto, S. Razavi, R. DeRose, and T. Inoue, "Synthesizing Biomolecule-Based Boolean Logic Gates," ACS Synthetic Biology 2013, vol. 2, pp. 72–82.

[13] M. Kahan, B. Gil, R. Adar, and E. Shapiro, "Towards Molecular Computers that Operate in a Biological Environment," Physica D 2008, vol. 237, pp. 1165–1172.

[14] G. Ashkenasy and M. R. Ghadiri, "Boolean Logic Functions of a Synthetic Peptide Network," J. Am. Chem. Soc. 2004, vol. 126, pp. 11140–11141.

[15] E. Katz, "Biomolecular Information Processing: From Boolean Logic Gates to Complex Networked Systems," Int. J. Unconventional Computing 2012, vol. 8, pp. 339–346.

[16] M. Di Ventra and Y. V. Pershin, "The Parallel Approach," Nature Phys. 2013, vol. 9, pp. 200–202.

[17] Y. V. Pershin and M. Di Ventra, "Solving Mazes with Memristors: A Massively Parallel Approach," Phys. Rev. E 2011, vol. 84, Article #046703.

[18] J. Halámek, O. Zavalov, L. Halámková, S. Korkmaz, V. Privman, and E. Katz, "Enzyme-Based Logic Analysis of Biomarkers at Physiological Concentrations: AND Gate with Double-Sigmoid "Filter" Response," J. Phys. Chem. B 2012, vol. 116, pp. 4457–4464.

[19] O. Zavalov, V. Bocharova, V. Privman, and E. Katz, "Enzyme-Based Logic: OR Gate with Double-Sigmoid Filter Response," J. Phys. Chem. B 2012, vol. 116, pp. 9683–9689.

[20] O. Zavalov, et al., "Two-Input Enzymatic Logic Gates Made Sigmoid by Modifications of the Biocatalytic Reaction Cascades," Int. J. Unconv. Comput. 2012, vol. 8, pp. 347–365.

[21] S. Bakshi, O. Zavalov, J. Halámek, V. Privman, and E. Katz, "Modularity of Biochemical Filtering for Inducing Sigmoid Response in Both Inputs in an Enzymatic AND Gate," J. Phys. Chem. B 2013, vol. 117, pp. 9857−9865.

[22] J. Halámek, et al., "Biomolecular Filters for Improved Separation of Output Signals in Enzyme Logic Systems Applied to Biomedical Analysis," Anal. Chem. 2011, vol. 83, pp. 8383–8386.

[23] M. Pita, et al., "Towards Biochemical Filter with Sigmoidal Response to pH Changes: Buffered Biocatalytic Signal Transduction," Phys. Chem. Chem. Phys. 2011, vol. 13, pp. 4507–4513.

[24] V. Privman, et al., "Biochemical Filter with Sigmoidal Response: Increasing the Complexity of Biomolecular Logic," J. Phys. Chem. B 2010, vol. 114, pp. 14103–14109.

[25] S. P. Rafael, et al., "Employing the Metabolic "Branch Point Effect" to Generate an All-or-None, Digital-Like Response in Enzymatic Outputs and Enzyme-Based Sensors," Anal. Chem. 2012, vol. 84, pp. 1076–1082.

[26] A. Vallée-Bélisle, F. Ricci, and K. W. Plaxco, "Engineering Biosensors with Extended, Narrowed, or Arbitrarily Edited Dynamic Range," J. Am. Chem. Soc. 2012, vol. 134, pp. 2876−2879.

[27] D. Kang, A. Vallée-Bélisle, K. W. Plaxco, and F. Ricci, "Re-engineering Electrochemical Biosensors to Narrow or Extend Their Useful Dynamic Range," Angew. Chem. Int. Ed. 2012, vol. 51, pp. 6717–6721.

[28] E. Katz and V. Privman, "Enzyme-Based Logic Systems for Information Processing," Chem. Soc. Rev. 2010, vol. 39, pp. 1835–1857.

[29] V. Privman, M. A. Arugula, J. Halámek, M. Pita, and E. Katz, "Network Analysis of Biochemical Logic for Noise Reduction and Stability: A system of Three Coupled Enzymatic AND Gates," J. Phys. Chem. B 2009, vol. 113, pp. 5301–5310.

[30] S. Mailloux, O. Zavalov, N. Guz, E. Katz, and V. Bocharova, "Enzymatic Filter for Improved Separation of Output Signals in Enzyme Logic Systems towards 'Sense and Treat' Medicine," Biomaterials Science 2014, vol. 2, 184–191.

[31] R. Pei, E. Matamoros, M. Liu, D. Stefanovic, and M. N. Stojanovic, "Training a Molecular Automaton to Play a Game," Nature Nanotechnol. 2010, vol. 5, pp. 773–777.

[32] M. N. Stojanovic and D. Stefanovic, "Chemistry at a Higher Level of Abstraction," J. Comput. Theor. Nanosci. 2011, vol. 8, pp. 434–440.

[33] V. Privman, "Biomolecular Computing: Learning Through Play," Nature Nanotechnology 2010, vol. 5, pp. 767–768.

[34] Y. Benenson, "Biomolecular Computing Systems: Principles, Progress and Potential," Nature Rev. Genetics 2012, vol. 13, pp. 455–468.

[35] E. Katz (ed.), Biomolecular Information Processing – From Logic Systems to Smart Sensors and Actuators, Willey-VCH, Weinheim, 2012.

[36] R. Unger and J. Moult, "Towards Computing with Proteins," Proteins 2006, vol. 63, pp. 53–64.

[37] Y. Benenson, "RNA-Based Computation in Live Cells," Curr. Opin. Biotechnol. 2009, vol. 20, pp. 471–478.

[38] M. L. Simpson, G. S. Sayler, J. T. Fleming, and B. Applegate, "Whole-Cell Biocomputing," Trends Biotechnol. 2001, vol. 19, pp. 317–323.

[39] E. Katz, V. Bocharova, and M. Privman, "Electronic Interfaces Switchable by Logically Processed Multiple Biochemical and Physiological Signals," (Highlight Article). J. Mater. Chem. 2012, vol. 22, pp. 8171–8178.

[40] A. Poghossian, et al., "Integration of Biomolecular Logic Gates with Field-Effect Transducers," Electrochim. Acta 2011, vol. 56, pp. 9661–9665.

 ISBN: 978-1-61208-380-3

[41] L. Halámková, et al., "Analysis of Biomarkers Characteristic of Porcine Liver Injury – From Biomolecular Logic Gates to Animal Model," Analyst 2012, vol. 137, pp. 1768–1770.

[31] E. Katz, J. Wang, M. Privman, and J. Halámek, "Multi-Analyte Digital Enzyme Biosensors with Built-in Boolean Logic," (Feature Article). Anal. Chem. 2012, vol. 84, pp. 5463–5469.

[32] E. Katz and J. Wang, in: Biomolecular Information Processing – From Logic Systems to Smart Sensors and Actuators, Willey-VCH, Weinheim, 2012, E. Katz (ed.), chap. 5, pp 81–101.

[33] M. Zhou, et al., "A self-powered "Sense-Act-Treat" system that is based on a biofuel cell and controlled by Boolean logic," Angew. Chem. Int. Ed. 2012, vol. 51, pp. 2686–2689.

[34] N. Zhou, et al., "Enzyme-Based NAND Gate for Rapid Electrochemical Screening of Traumatic Brain Injury in Serum," Anal. Chim. Acta 2011, vol. 703, pp. 94–100.

[35] V. Bocharova, et al. "Alert-Type Biological Dosimeter Based on Enzyme Logic System," Talanta 2011, vol. 85, pp. 800–803.

[36] M.-C. Chuang, et al., "High-Fidelity Simultaneous Determination of Explosives and Nerve Agent Threats via a Boolean Biocatalytic Cascade," Chem. Commun. 2011, vol. 47, pp. 3087–3089.

[37] T. Melham, "Modeling, Abstraction, and Computation in Systems Biology: A View from Computer Science," Prog. Biophys. Molec. Biol. 2013, vol. 111, pp. 129–136.

[38] S. Navlakha and Z. Bar-Joseph, "Algorithms in Nature: The Convergence of Systems Biology and Computational Thinking," Molec. Systems Biol. 2011, vol. 7, Article #546.

[39] D. D. Seaton and J. Krishnan, "Multispecific interactions in enzymatic signalling cascades," Information Processing in Cells and Tissues, Publisher: Springer, 2012, pp. 67–73.

[40] K. MacVittie, J. Halámek, V. Privman, and E. Katz, "A Bioinspired Associative Memory System Based on Enzymatic Cascades," Chem. Commun. 2013, vol. 49, pp. 6962–6964.

[41] V. Bocharova, et al., "Realization of Associative Memory in an Enzymatic Process: Toward Biomolecular Networks with Learning and Unlearning Functionalities," J. Phys. Chem. Lett. 2012, vol. 3, pp. 1234–1237.

[42] V. Privman and E. Katz, "Can bio-inspired information processing steps be realized as synthetic biochemical processes?" Physica Status Solidi A, 2014, http://dx.doi.org/10.1002/pssa.201400131.

[43] N. Wagner and G. Ashkenasy, "Systems Chemistry: Logic Gates, Arithmetic Units and Network Motifs in Small Networks," Chemistry Eur. J. 2009, vol. 15, pp. 1765–1775.

[44] S. Mangan and U. Alon, "Structure and Function of the Feed-Forward Loop Network Motif," Proc. Natl. Acad. Sci. USA 2003, vol. 100, pp. 11980–11985.

[45] S. S. Shen-Orr, R. Milo, S. Mangan, and U. Alon, "Network Motifs in the Transcriptional Regulation Network of Escherichia Coli," Nat. Genet. 2002, vol. 31, pp. 64–68.

[46] J. Macía, S. Widder, and R. Solé, "Specialized or flexible feed-forward loop motifs: a question of topology," BMC Systems Biology 2009, vol. 3 pp. 84–102.

[47] N. Kashtan and U. Alon, "Spontaneous evolution of modularity and network motifs," Proc. Natl. Acad. USA 2005, vol. 102, pp. 13773–13778.

[48] J. S. Mattick, "A New Paradigm for Developmental Biology," J. Exp. Biol. 2007, vol. 210, pp. 1526–1547.

[49] W. H. de Ronde, F. Tostevin, and P. R. ten Wolde, "Feed-forward loops and diamond motifs lead to tunable transmission of information in the frequency domain," Phys. Rev. E 2012, vol. 86, Article #021913.

[50] A. A. Apte, J. W. Cain, D. G. Bonchev, and S. S. Fong, "Cellular automata simulation of topological effects on the dynamics of feed-forward motifs." J. Biol. Eng. 2008, vol. 2, pp. 2–14.

[51] E. Dekel, S. Mangan, and U. Alon, "Environmental Selection of the Feed-Forward Loop Circuit in Gene-Regulation Networks," Phys. Biol. 2005, vol. 2, pp. 81–88.

[52] F. Tostevin and P. R. ten Wolde, "Mutual Information between Input and Output Trajectories of Biochemical Networks," Phys. Rev. Lett. 2009, vol. 102, Article #218101.

[53] A. U. Igamberdiev and M. R. Roussel, "Feedforward non-Michaelis-Menten mechanism for CO2 uptake by Rubisco: Contribution of carbonic anhydrases and photorespiration to optimization of photosynthetic carbon assimilation," BioSystems, 2012, vol. 107, pp. 158–166.

[54] S. Huang, "Back to the biology in systems biology: what can we learn from biomolecular networks," Brief Funct Genomics Proteomics 2004, vol. 2, pp. 279–297.

[55] D. Y. Zhang, A. J. Turberfield, B. Yurke, and E. Winfree, "Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA," Science 2007, vol. 318, pp. 1121–1125.

[56] E. Katz, V. Privman, and J. Wang, "Towards Biosensing Strategies Based on Biochemical Logic Systems," Proc. Quantum, Nano and Micro Technol. 2010. ICQNM '10. Fourth Int. Conf. 2010, pp.1-9.

[57] V. Privman, G. Strack, D. Solenov, M. Pita, and E. Katz, "Optimization of Enzymatic Biochemical Logic for Noise Reduction and Scalability: How Many Biocomputing Gates Can Be Interconnected in a Circuit?" J. Phys. Chem. B 2008, vol. 112, pp. 11777–11784.

[58] U. Pischel and B. Heller, "Molecular logic devices (half-subtractor, comparator, complemementary output circuit) by controlling photoinduced charge transfer processes," New J. Chem. 2008, vol. 32, pp. 395–400.

[59] M. Amelia, M. Baroncini, and A. Credi, "A Simple Unimolecular Multiplexer/Demultiplexer," Angew. Chem. Int. Ed. 2008, vol. 47, pp. 6240–6243.

[60] M. Georgiopoulos, C. Li, and T. Kocak, "Learning in the Feed-Forward Random Neural Network: A Critical Review," Performance Eval. 2011, vol. 68, pp. 361–384.

[61] A.-J. Annema, Feed-Forward Neural Networks: Vector Decomposition Analysis, Modelling and Analog Implementation, Springer Science, 1996, New York.

[62] U. Alon, "Network Motifs: Theory and Experimental Approaches," Nature Rev. 2007, vol. 8, pp. 450–461.

[63] A. Arkin and J. Ross, "Computational functions in biochemical reaction networks," Biophysical Journal 1994, vol. 67, pp. 560–578.

[64] D. Wolf and A. Arkin, "Motifs, modules and games in bacteria," Curr. Opin. Microbiol. 2003, vol. 6, pp. 125–134.

[65] J. J. Collins, et al., "Synthetic biology: How best to build a cell" Nature 2014, vol. 509, pp. 155–157.

[66] M. Shein-Idelson, E. Ben-Jacob, and Y. Hanein, "Engineered Neuronal Circuits: A New Platform for Studying the Role of Modular Topology," Frontiers in Neuroengineering 2011, vol. 4, pp. 10–21.

[67] L. Qian and E. Winfree, "A Simple DNA Gate Motif for Synthesizing Large-Scale Circuits," J. R. Soc. Interface 2011, vol. 8, pp. 1281–1297.

[68] M. A. Savageau, Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology, Addison–Wesley, Reading, MA, 1976.

[69] N. Rosenfeld and U. Alon, "Response delays and the structure of transcription networks," J. Mol. Biol. 2003, vol. 329, pp. 645–654.

[70] N. Rosenfeld, M. B. Elowitz, and U. Alon, "Negative Autoregulation Speeds the Response Times of Transcription Networks," J. Mol. Biol. 2002, vol. 323, pp. 785–793.

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