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Introduction

1.1 Motivation and Applications

Exponential development of computing systems based on silicon materials and binary algorithms formulated as the "Moore's law" [1] (Figure 1.1) is coming to the end being limited by further component miniaturization and by the speed of operation. Conceptually novel ideas are needed to break through these limitations. The quest for novel ideas in the information processing has resulted in several exciting directions in the general area of unconventional computing [2–4], including research in quantum computing [5] and biologically inspired molecular computing [6–9]. Molecular computing systems, generally motivated by mimicking natural biological information processing [10, 11], are not necessarily based on biomolecules and could be represented by synthetic molecules with signal-controlled switchable properties. Synthetic molecular systems and nano-species have been designed to mimic the operation of Boolean logic gates and demonstrate basic arithmetic functions and memory units. However, despite progress achieved in assembling synthetic molecular systems performing basic Boolean operations and simple computations, these systems have limited complexity, and further increase of their complexity is very challenging. A new advance in the development of molecular information systems has been achieved with the use of biomolecular species [12] (Figure 1.2) such as DNA/RNA [13–16], oligopeptides [17], proteins [18], enzymes [2, 19, 20], antigens/antibodies [21], and even whole biological cells/organisms [22–24] capable of operating in a biological environment [25], borrowing some ideas from systems biology [26]. The advantage of the biomolecular computing systems is their ability to be integrated in artificially designed complex reacting processes mimicking multistep information processing networks. These systems are still far away from the natural information processing in cells but are already much more complex than pure synthetic molecular systems. In fact, biochemical reactions are at the core of the mechanism of life itself, and therefore one could set rather ambitious expectations for how far can (bio)chemical reaction systems be scaled up in complexity, if not speed, for information processing. While in a long perspective a "biocomputer" might become a reality [27], particularly for some special applications, e.g., for solving complex combinatorial problems [28], potentially promising to have an advantage over silicon-based electronic computers

Enzyme-Based Computing Systems, First Edition. Evgeny Katz.

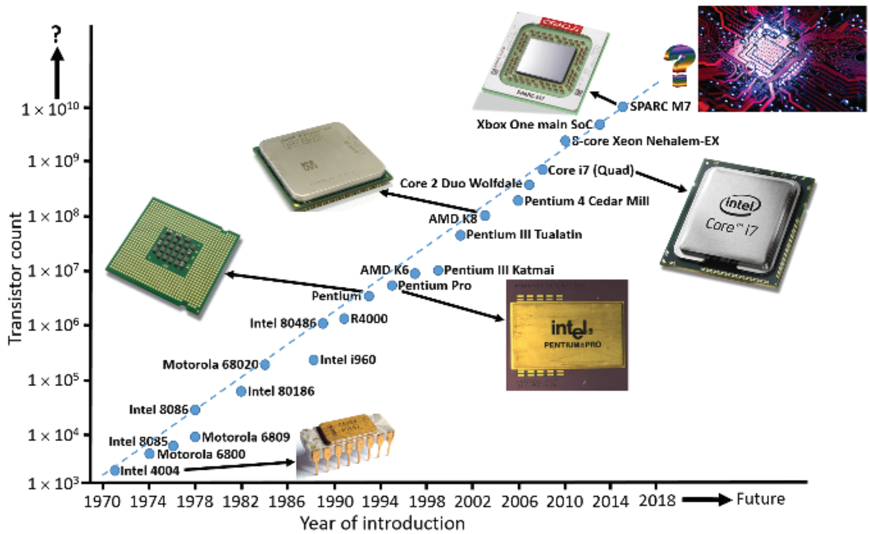

Figure 1.1 Moore's law – exponential increase of transistors on integrated circuit chips. (The plot shown in the figure is based on the data provided by Wikipedia: https://en.wikipedia.org/wiki/Moore%27s_law.) Source: Katz 2018 [2]. Adapted with permission of John Wiley and Sons.

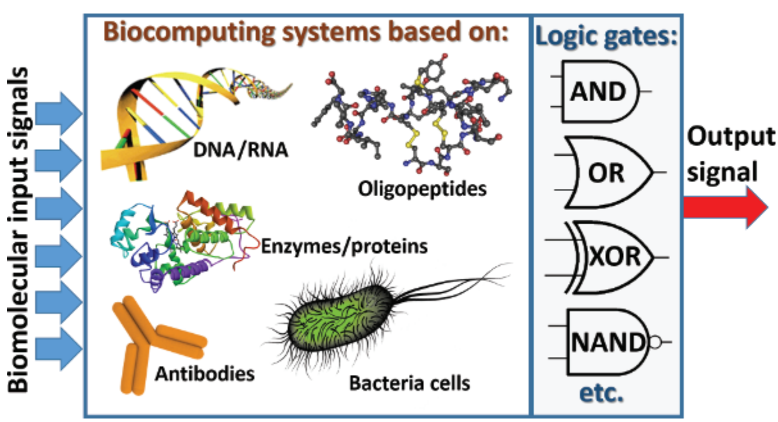

Figure 1.2 Biomolecular computing systems mimicking operation of different Boolean logic gates and circuitries can be based on various species including oligopeptides, enzymes/proteins, DNA/RNA, antibodies, and even whole biological (e.g., microbial) cells. Source: Katz 2018 [2]. Adapted with permission of John Wiley and Sons.

due to parallel computing performed by numerous biomolecular units, the present level of technology does not allow any practical use of biomolecular systems for real computational applications. For achieving any practical result soon, some other applications, different from making a biocomputer, should be considered using the (bio)molecular systems with a limited complexity. One of the immediate possible applications for molecular logic systems is a special kind of biosensing [29–31] where the multiple input signals are logically processed through chemical reactions resulting in YES/NO decisions in the binary (**0**,**1**) format. In this subarea of biomolecular logic systems, practical results are already possible at the present level of the system complexity, particularly for biomedical applications [32, 33]. Overall, the research in molecular/biomolecular information processing, which has been motivated originally to progress unconventional computing applications, is broadly developing to areas not directly related to computing in its narrow definition. This research is bringing us to novel areas in sensing/biosensing [29–31], switchable "smart" materials controlled by logically processed signals [34–36], bioelectronic devices (e.g., biofuel cells) controlled by external signals [37, 38], signal-controlled release processes [39–43], etc.

1.2 Enzyme-Based Logic Gates and Short Logic Circuits

While the major research efforts have been directed to the DNA-based computing systems [10, 13–15], mostly aiming at computing applications in their direct narrow definition [27, 44–50] and expecting acceleration of the computing process due to massively parallel data processing [28, 51], enzyme logic systems [19, 20] received smaller attention since they are less promising for real large-scale computational applications. Growing interest to the enzyme logic systems is based on their activation with physiologically relevant biomolecular signals (metabolites) appearing at physiological concentrations [52–54] allowing low-scale information processing for biomedical applications, such as binary (YES/NO format) biosensing [32, 33], signal-controlled materials, and implantable bioelectronic devices [55].

Enzyme-based logic gates are usually realized through relatively simple enzyme-catalyzed reactions [19, 20] (Figure 1.3). Rapid progress in enzyme-based information processing systems has resulted in the design of biocatalytic cascades mimicking various Boolean logic gates, including AND [52, 56–59], OR [59, 60], NAND [61], NOR [57, 61], CNOT [62], XOR [57, 59, 63–65], INHIB [57, 59], Identity [57], and Inverter [57] gates. In order to digitalize chemical processes, the reacting species considered as logic input signals were applied at two levels of their concentrations: their physical absence (zero concentration) was defined as logic **0** input, while logic **1** input was defined as experimentally optimized and conveniently high concentration, thus allowing significant separation in the produced output signals when inputs **0** and **1** were applied in different combinations. Depending on specific needs set by applications, the input signals were defined as variable concentrations of substrates and/or cofactors reacting with enzymes [57] or different concentrations of the biocatalytic enzymes added to a "soup" of substrates/cofactors being ready to react with the enzymes [59]. The non-variable part of the system was considered as a "machinery" operating with the variable input signals applied in various combinations. Multistep biocatalytic cascades activated by several

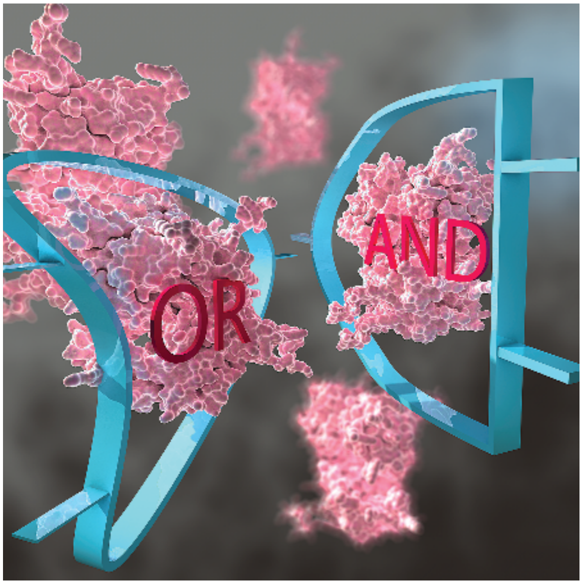

Figure 1.3 Enzyme-based Boolean logic gates – artistic vision. Source: Katz 2018 [2]. Adapted with permission of John Wiley and Sons.

(more than two) input signals have been assembled to mimic logic circuits composed of concatenated logic gates [66–69]. Various reaction cascades have been designed to mimic different combinations of concatenated logic gates; however, they usually do not include more than three or four logic steps. Due to noise formation and amplification through the reaction steps, the number of logic steps is limited, and theoretical estimation limits the system complexity by approximately 10 logic steps (which have never been realized experimentally in those enzyme-biocatalyzed reactions) [70]. Complex branched biocatalytic reactions realized in flow cell systems have been used to mimic operation of reversible logic gates, such as Feynman gate, Double Feynman gate, Toffoli gate, Peres gate, and Fredkin gate [71–74].

The following chapters present different logic systems based on the enzyme reactions, their optimization, and applications. While the designed systems demonstrated many different logic/computing processes, their operation provided only low-scale information processing, which is not sufficient for building a biomolecular computer. However, the designed systems have found important applications in various signal-controlled bioelectronic devices, biosensors, and stimuli-responsive materials. The research in the biomolecular computing, particularly using enzyme reactions for information processing, motivated initially by computational goals and expected to compete with silicon-based microelectronics, finally moved to signal-switchable devices processing a few signals in limited complexity processes. Therefore, the results obtained in the research area represent limited interest for pure computational applications but offer highly promising applications in bioelectronics, particularly operating in biological environment and being adaptive to biological processes.

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