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The Blueprint of Logical Decisions in a NF-κB Signaling System

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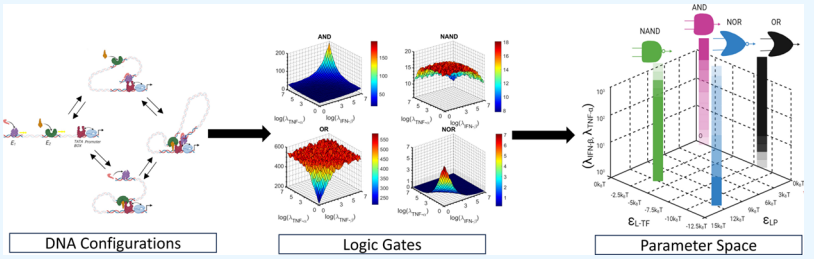

ABSTRACT: Nearly identical cells can exhibit substantially different responses to the same stimulus that causes phenotype diversity. Such interplay between phenotype diversity and the architecture of regulatory circuits is crucial since it determines the state of a biological cell. Here, we theoretically analyze how the circuit blueprints of NF-κB in cellular environments are formed and their role in determining the cells' metabolic state. The NF-κB is a collective name for a developmental conserved family of five different transcription factors that can form homodimers or heterodimers and often promote DNA looping to reprogram the inflammatory gene response. The NF-κB controls many biological functions, including cellular differentiation, proliferation, migration, and survival. Our model shows that nuclear localization of NF-κB differentially promotes logic operations such as AND, NAND, NOR, and OR in its regulatory network. Through the quantitative thermodynamic model of transcriptional regulation and systematic variation of promoter−enhancer interaction modes, we can account for the origin of various logic gates as formed in the NF-κB system. We further show that the interconversion or switching of logic gates yielded under systematic variations of the stimuli activity and DNA looping parameters. Such computation occurs in regulatory and signaling pathways in individual cells at a molecular scale, which one can exploit to design a biomolecular computer.

INTRODUCTION

Signal transduction and information processing are fundamental steps for any cellular decision-making, which happens via binding transcription factors (TFs) to the regulatory unit of DNA.[1,2] When TFs bind to the regulatory unit of DNA, the information from environmental and developmental cues is relayed into gene expression outcomes. A frequently used conceptual and quantitative model to explore gene expression is that TFs combinatorially recruit or replace RNA polymerase (RNAP) that binds to the promoter by direct physical interactions. TFs and other biomolecules bind with DNA and often form a complex programmable assembly, which is critical in converting the TF inputs into a switching-like transcriptional output.[3,4] Exploring such inherent networks is crucial since they act as information processing units at the cellular level.[5−7] We refer to such inherent network architecture of biomolecules at thermodynamic equilibrium as the **blueprint** of a genetic response.[8,9]

Combinatorial control is the hallmark of cellular signaling and gene regulation.[10] In many instances, cellular signaling and transcriptional regulation involve switch-like molecular responses to the presence of signaling molecules.[4] Therefore, creating layered regulatory cascades that define sequential transcription programs can coordinate complex phenotypic changes. Over the past decade, the construction of transcriptional logic circuits has continued in earnest, revealing both the potential and limitations of this approach. Through a particular combination, transcriptional regulatory networks could give rise to the Boolean logic operations from a specific set of biomolecular assemblies. Complex self-assembly that enables tuning of two input circuits performs molecular computations, forming logic gates. Many studies have been performed in this direction in the past.[3,10−13] Out of them, an elegant study explores the combined effect of two distinct TFs on the transcriptional activity of a given promoter depending upon their respective binding strengths and the cooperative interactions between them.[3] Their study reveals that tuning of binding strength and cooperative parameters can create AND, OR logic gates.[3] Nevertheless, the logic can also be non-Boolean in biological systems, as demonstrated in a study for the bacteriophages.[14]

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A

Input		AND		OR		NAND		NOR	
E_1	E_2	A	LP	A	LP	A	LP	A	LP
L	L	0	0	0	0	1	0	1	0
H	L	0	0/1	1	1	1	0	0	0
L	H	0	0/1	1	1	1	0	0	0
H	H	1	2	1	2	0	0	0	0

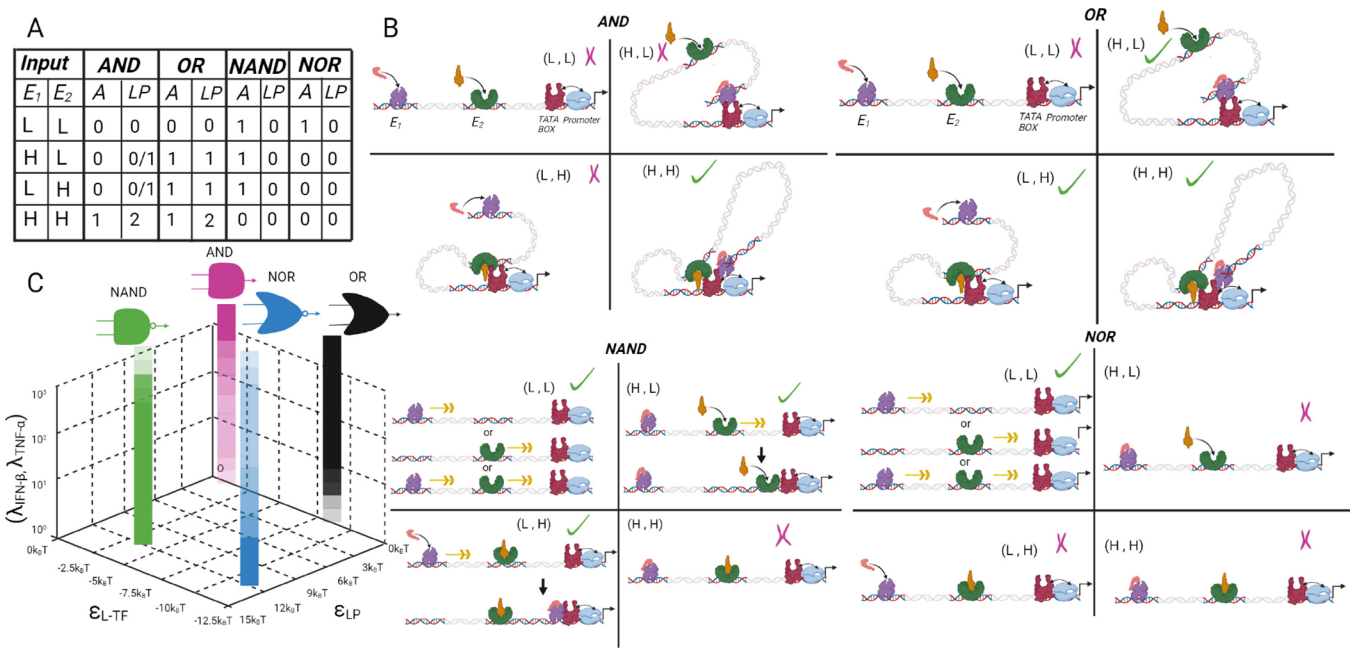

Figure 1. Schematic for forming various possible logic gates configurations for the NF-κB system as formed by long-distance TF promoter interaction through DNA looping and by the diffusion of TF to the promoter. (A) H and L indicate the high and low stimuli activities in the table. The symbols E1 and E2 are the two enhancer elements of NF-κB system, namely, IRE, κB sites. The symbol A is the Boolean expression of the corresponding gate operations, and LP is the number of enhancer−promoter loops. (B) Various configurations for AND, OR, NAND, and NOR logical operations. The active configurations are marked with the tick symbols in the figure. Here, we use the purple and green colors cartoon for the protein, IRF, and NF-κB. These two proteins are stimulated by the IFN-β and TNF-α, as shown by the orange and brown color cartoons. The red and light blue cartoons are used to indicate the heterodimeric complex of p300-AP-1 and RNAP molecules. The yellow symbol ⇢ indicates the facilitated tracking diffusion and translation modes of TFs. (C) Schematic view of various logic gates in parameter space. We show the logic gates as a function of a few controllable parameters such as free energies for the stimuli-induced protein activation, $\epsilon_{IFN-\beta/TNF-\alpha}$, the strength of DNA loops ϵ_{LP} and the activities of stimuli, $\lambda_{IFN-\beta}$ and $\lambda_{TNF-\alpha}$. The gradients in the color bars are used to show the gene expression level.

The engineering of synthetic genetic logic to achieve simple yet robust and independent control over biological processes is an active area of research. The study of genetically encoded logic is employed to fine-tune the adaptability of living systems by exploring biomolecular computations to improve cellular therapeutics.[15−17] To date, a significant number of synthetic designs[17−20] for gene architecture have been built to achieve various logic gate operations and have shown the astounding ability to utilize computational circuits in a biological cell to manipulate the cell signaling.[15,21] Despite the advancement of such gene circuits, they lack modularity or are dependent on their host anatomy.[20,22] Their designs could also be more challenging to scale up, a problem originating from the reuse of biomolecules in the self-mixed environment of individual cells. These drawbacks stave off the incorporation of such designs for significantly bigger biological systems to acquire other complex logic operations. In principle, having a genetic logic device that facilitates its reuse and makes it reliable for a wide range of host gene frameworks is desirable. Further, tethering elements are employed to induce enhancer−promoter interactions in eukaryotes that promote the formation of a programmable DNA loop, a crucial characteristic for controlling gene regulation.[23,24] Despite the lack of devices to customize DNA loops, the essential role of DNA loops in regulating the expression is critical for biological computation. A recent report suggests that the CRISPR-based DNA looping method offers a promising platform to customize or manipulate DNA loops.[25] In this method, dcas9 complexes activate genes by reconnecting DNA to bring distant regulatory elements in the proximity of the gene promoters. Such methods offer massive flexibility for DNA manipulation for various cell types.

Multimodule enhancers, which are several hundred thousand base pairs away from the promoter of interest, regulate the expression of genes.[26,27] One such system that exhibits differential regulation is nuclear factor κB (NF-κB), which activates enhancers and plays a crucial role in antigen-dependent B cell differentiation.[28−31] The NF-κB system exploits various transcriptional machinery for producing threshold and graded responses controlled by typical promoter-TF interactions.[32−35] In particular, the long-distance DNA−protein associations involving multiple factors, such as BRD4 and MED1 in the adjunct enhancer regions, underlie phase separation that enables high-density transcription reactions.[36,37] Few experimental studies confirm that the involvement of such distance-dependent gene regulation happens via the interplay of enhancer−promoter interactions.[38−42] Furthermore, NF-κB often form a heterodimer with interferon regulatory factors (IRF), where these two TFs bind to their respective cognate sites (κB and IRE), and they are activated in macrophages after exposure to pathogens. Such binding can achieve specific gene regulation, an organizing principle for understanding the logic of gene regulatory circuits.[43] Further, Wang et al. have reported that the IRF combinatorially controlled NF-κB target genes through their computational modeling and identified the existence of AND and OR logic gates for this system.[13] Tumor necrosis factor-alpha (TNF-α) activates NF-κB, but not IRF, and interferon-beta (IFN-β) activates IRF but not NF-κB, thereby showing stimulus specificity.[44] Upon activation using respective stimuli,

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both of the TFs fluctuate between active and inactive states[45,46] and that promote binding to their respective enhancer elements of target genes.[43] Typically, the source of these stimuli are pathogens, which are considered a powerful pro-inflammatory agent, and a potent activator in monocytes/macrophages.[47,48] However, the biological function of NF-κB is complex, producing diverse cellular variability in response to stimuli, but the mechanisms behind the selective participation of NF-κB to enhancers remain unclear.[49,50]

Here, we explore the diversity of combinatorial logic responses that can be affected by long-distance interactions between NF-κB and p300 via DNA looping. The p300, a part of the TATA-binding protein, interacts with the AP-1 TF and initiates to form a preinitiation complex that often facilitates DNA loop formation between enhancer and promoter in a dose-dependent manner.[51] The DNA loops offer an intriguing opportunity for the existence of enhancer−promoter interactions,[52,53] and the literature supports the contribution of such programmable DNA loops to the regulation of logic gates.[54−56] In particular, the DNA looping, the facilitated tracking or translational modes of TFs[57,58] on the DNA produces transient biomolecular self-assembly that performs molecular logic operations in a cell. We develop a statistical thermodynamical model to characterize the Boolean logic to understand how these responses change and how they can be controlled. We demonstrate from our calculation that DNA looping and the cooperative interactions among proteins generate various analog and Boolean computations without considering specific regulatory architecture or energy expenditure. We further demonstrate how altering DNA flexibility, which can occur through changes in its surroundings or chemical modification, can cause a switch in the logic behavior of a protein−DNA complex assembly, such as transitioning from AND into OR behavior. Finally, we discussed the growing experimental work on natural and de novo-designed molecular logic gates. Our obtained results hint at the simple mechanisms in biological systems, which can be used to refine their combinatorial control.

THEORY AND MODEL

We model the system using grand canonical ensemble formalism. We first demonstrated the logic gate operations model and then discussed our prototypical system, NF-κB. We assume that (a) the average behavior of the network is invariant over time and (b) the binding kinetics is much faster than other cellular processes, such as cell growth, in our calculation. We perform these calculations using partition function and Monte Carlo (MC) simulation under a grand canonical ensemble. The details of the technicalities of our theoretical approach and simulation[59,60] are presented in the Supporting Information (SI). In addition, the parameters employed for the calculations for this system are given in Table S1 in the SI. Furthermore, we have given Table S2 in the SI, which contains a total of 36 microstates presented against their statistical weights for the binding of various biomolecules or TFs, i.e., IRF and NF-κB on respective IRE and κB sites in the presence of IFN-β and TNF-α stimuli, respectively.

Model. Figure 1 shows various configurations of AND, OR, NAND, and NOR logic gates for the NF-κB system. The origin of various configurations of complex assemblies in the parameter space relies on the free energy of interactions and the activities of biomolecules. In this model, we aim to control the population of multiple configurations by varying stimuli activities, the activation free energies of stimuli to the TF, and the DNA looping energies. We define the active and inactive states of TFs depending on whether the stimuli randomly activate or not. We further consider that the binding of active TFs is more potent to bind with DNA than its inactive form. The TFs can access the promoter region by various modes and play a critical role in forming active configurations, thereby modifying gene regulation. An active configuration produces a unit of mRNA, demonstrating that the AND and NOR gates produce 25% of mRNA and OR and NAND produce 75% of mRNA on average. In the case of AND and OR gates, the TFs access the promoter by forming loops, whereas the TFs access the promoter region by diffusion for NAND and NOR gates.

The complex assembly forms an active configuration in the presence of external stimuli. The activity of stimuli is controllable; one can achieve various gates by tuning them. The TFs can bind to the enhancer elements and participate in mRNA production when they access the promoter region of the genes. The DNA forms two loops between enhancer and promoter regions for an AND operation through interacting TFs with the TATA-binding protein. We refer to it as an active configuration since it can produce a unit of mRNA (Figure 1). This particular configuration is possible when the DNA is flexible ($\epsilon_{LP} = 0\ k_BT$) and a saturated level of stimuli (IFN-β and TNF-α) weakly induces TFs ($\epsilon_{L-TF} = -1\ k_BT$). One can control the population of active TFs by increasing stimuli activities. The presence of stimuli promotes enhancer-TF interaction, further facilitating DNA loop formation between enhancer and promoter regions through protein−protein interactions. Therefore, one can realize a unique configuration for AND assembly containing two DNA loops at high values of stimuli activities. Note that the protein−protein cooperative interactions among TFs and TATA-binding proteins become very strong in this case, which rules out the formation of a single DNA loop configuration. Therefore, it promotes only one active configuration with two DNA loops to produce a single mRNA molecule. However, a strong induction of stimuli to TFs ($\epsilon_{L-TF} = -8\ k_BT$), which have bound to the enhancer elements of flexible DNA, allows to form three unique configurations of assemblies containing a single loop or double loops in the parameter space. Production of such configurations corresponds with the OR-like gates. In the case of the OR gate, we notice three active configurations produce three mRNA molecules.

As we decrease the flexibility of DNA ($\epsilon_{LP} = 12\ k_BT$), the long-distance TF-p300 interactions through DNA looping are stopped. Under this situation, only the facilitated tracking or diffusion-like mode allows inactive TFs to reach proximity to the promoter region and control the gene expression. Depending on the strong ($\epsilon_{L-TF} = -11\ k_BT$) and weak ($\epsilon_{L-TF} = -3.5\ k_BT$) stimuli-induced activation of TF, we obtain another set of unique configurations in the parameter space upon varying stimuli activities. With a small to moderate stimuli activity, it weakly induces TF that promotes the accessibility of inactive TFs toward the core promoter region and forms three unique configurations of complex assemblies that produce three mRNA molecules. We found this signature for the NAND gate. As stimuli strongly induce TF, the active TFs rarely visit the promoter region. Therefore, the inactive TFs visit the promoter region only at low stimuli activities and form a unique configuration of complex assembly for the NOR gate. However, at the saturated level of stimuli activities, the movement of TFs is entirely restricted for both NAND and

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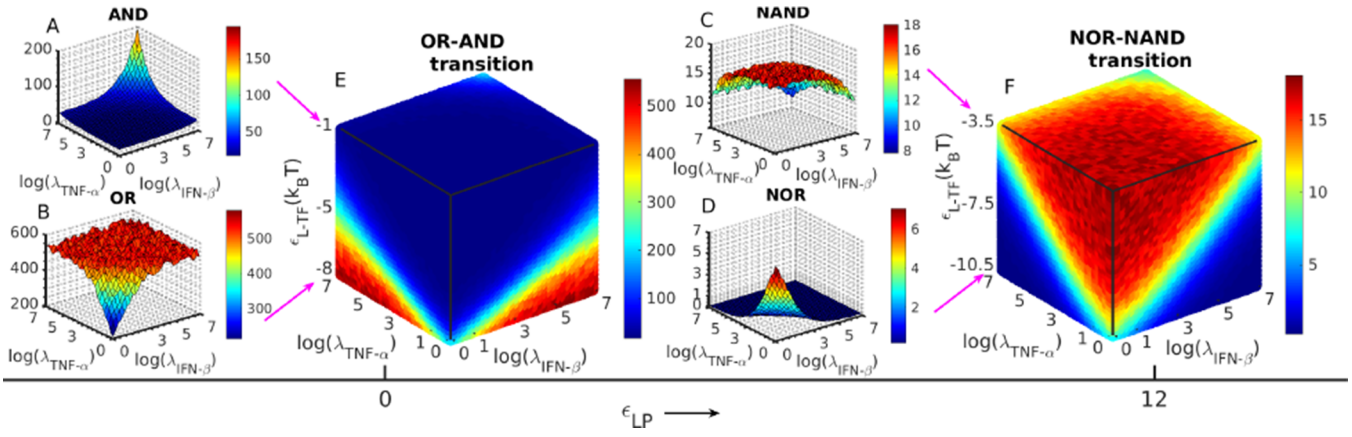

Figure 2. Transition of the various logic gates in the parameter space. Panels A, B, C, and D refer to the population of the active assemblies for the AND, OR, NAND, and NOR logic gates as a function of stimuli activities ($\lambda_{TNF-\alpha}$ and $\lambda_{IFN-\beta}$). Panels E and F are the logic gates switching between AND to OR and NAND to NOR. Note that the switching only happens as the strength of interaction between stimuli and TF (ϵ_{L-TF}) varies. The switching from OR-AND to NOR-NAND happens through the variation of DNA stiffness parameter (ϵ_{LP}) as a function of stimuli activities. The color bars show the population of all active configurations formed in the parameter space. The contour maps corresponding to the top view of logic gates transition maps (shown in panels E and F) for OR-AND and NOR-NAND transitions for varying ϵ_{L-TF} values are shown in Figures 3 and 4, respectively.

NOR gates, and the gene stays almost at the off state that corresponds with a basal mRNA level.

NF-κB. One can realize the above prototypical model for logic operations in many natural and synthetic systems.[43,61] We consider the NF-κB gene regulatory system that exhibits two enhancer elements, TATA-box and promoter regions. We show the network of interactions in Figure 1. Typically, the network has two enhancer regulatory elements, κB and IRE, and a promoter region. Two activators, NF-κB and IRF, bind to the κB and the IRE enhancer elements, respectively. The biological function of NF-κB is complex, producing diverse cellular variability in response to stimuli such as TNF-α, LPS, etc.[49] Typically, the source of these stimuli are pathogens, which primarily activate NF-κB and IRF and bind with the respective enhancer elements of target genes.[4] It can perform logic gate operations and thus support our model. Here, IFN-β and TNF-α act as stimuli[44,49] that stimulate the IRF and NF-κB proteins to form their active state. Experiments show that the active form of IRF and NF-κB proteins prefer to bind to their respective enhancer binding sites.[43] Another crucial component of this system is the TATA-binding protein, p300, which binds with the TATA-box region of the regulatory unit. The binding of the p300 promotes DNA looping through an interaction with the bound enhancer proteins. The enhancer−promoter looping is controllable dose-dependent as found in stimuli-dependent osteopontin expression for various immune cells.[43,62]

RESULTS

Characterization of AND, OR, NAND, and NOR Gates.

Figure 2 (panels A, B, C, D) presents various gate results obtained from partition function calculations and MC simulations in the parameter space. Here, we show the formation of various active protein−DNA assemblies that change depending on free energy parameters and stimuli activities in the parameter space. The weak activation of TF limits the population of active TFs, but a significant population of active TFs on enhancers is observed at high stimuli activities. The binding of the active TFs to the enhancer elements does not necessarily mean that the configuration is active unless they reach the promoter region and alter the mRNA production. As discussed above, various mechanisms can reach the promoter region: (a) by long-distance protein−protein interaction through DNA looping and (b) by diffusion-like mode.

In the case of AND configuration, binding of active NF-κB and IRF to the enhancer elements promotes the form of DNA loops between enhancer and promoter through a cooperative interaction among the bound NF-κB, IRF, and p300 molecules. Note that an active TF binding to the promoter rarely happens; therefore, the long-distance interaction through DNA looping and cooperative interaction among protein molecules are essential for an active AND assembly, as revealed from our study. Since TFs access the promoter region by this mechanism, they become an active assembly because they can produce mRNA or participate in gene regulation. Therefore, we find a narrow region in the parameter space where the active complex assemblies of AND-like configurations are observed when a large quantity of stimuli weakly induces TF. Overall, two DNA loops formed at high stimuli activity values and weak stimuli-induced TF activation characterize AND assembly. Similarly, the origin of the OR-like gate's configurations relies upon tuning the free energy of activation between the TF and stimuli. As we increase the free energy of activation between TF and stimuli, the strong interaction between them allows TFs to reach proximity to the promoter region by single or double DNA loops. Therefore, we obtain a broad region of responses as both stimuli activities increase.

In contrast to the above two gates, the NAND and NOR gates rely on the stiffness of DNA. We increased the stiffness of DNA by increasing the elastic parameter for the DNA chain and observed complementary AND and OR logical responses. We show that binding TFs to the enhancer elements allows only their translation along with DNA. Such translation motion of TFs along DNA is crucial since it allows them to access the promoter region for activation or repression of the gene regulation. As mentioned in the previous sections, the nature of binding between TFs and DNA creates NAND and NOR complexes in the parameter space. We find that the weak

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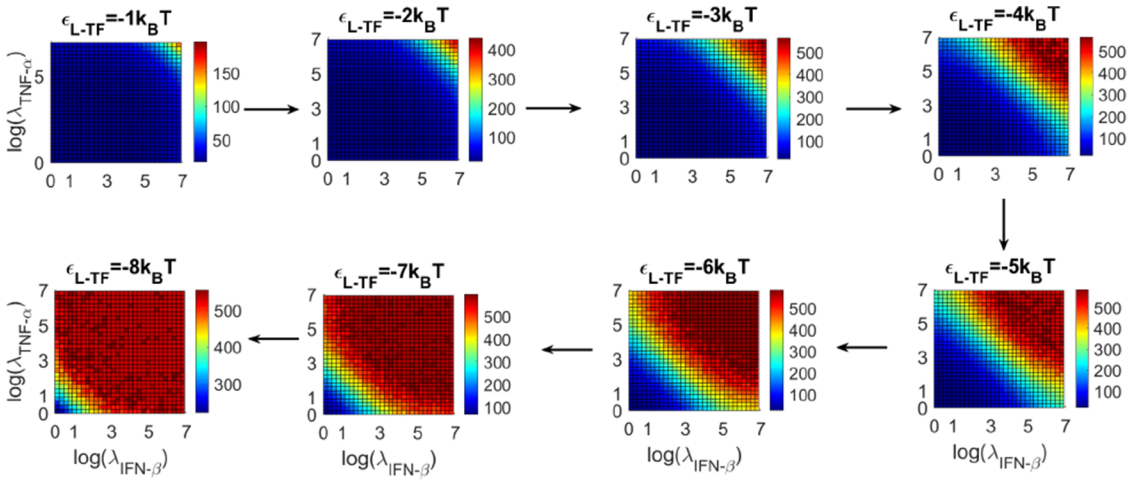

Figure 3. Transition of AND-OR logic gates switching as a function stimuli activities. The two-dimensional contours are taken from Figure 2E at a particular value of ϵ_{L-TF}, shown on each panel's top. The switching between the two gates is visible from the analysis. The color bars show the population of all active configurations formed in the parameter space.

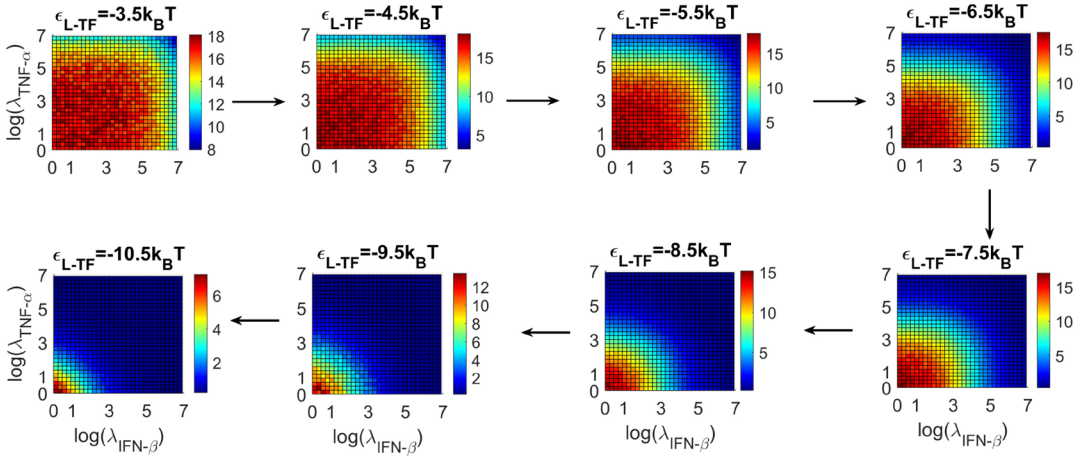

Figure 4. Switching of NAND to NOR logic gates as a function TNF-α and IFN-β activities. The two-dimensional contours are taken from Figure 2F at a particular value of ϵ_{L-TF}, shown on each panel's top. The switching between the two gates is visible from the analysis. The color bars show the population of all active configurations formed in the parameter space.

activation of TFs and moderate levels of their activities produce NAND-like gates. However, one can control the accessibility of TFs to the promoter region of DNA by tuning the activation free energy between TF and stimuli. As we increase the free energy of activation, the TFs rarely visit the promoter region. As a result, we find the NOR responses only at high values of stimuli activities. Further, it is evident from our analysis that the movement of TFs is restricted at very high values of stimuli activities that appear as inactive NAND and NOR-like responses. We have presented the theoretical results related to the logic gates for a NF-κB signaling system in Figure S1 in the SI and have observed a fair correlation with the simulation results. The analytical (marked by solid lines) and simulation results (marked by circles) in Figure S3 in the SI match well and indicate the symmetry in the expressions between both TFs.

It is clear from the above analysis that the diverse range of logical computations by the interactions between protein and DNA through the formation of specific complexes is quite possible in the parameter space. We show a specific complex responsible for a specific logical response in a narrow range of parameters. Such parameter variations are common in cellular biology as the activities of biomolecules change through many biological processes such as cooperative binding, post-translational modifications, oligomerization, etc. Therefore, the observed output patterns for various logic expressions switch among themselves because of the existence of such parameter variations in cellular systems. We discuss them below.

Switching between Logic Gates. Figure 2 (panels E and F) shows the switching among various gates. Here, we define switching as changing protein−DNA assembly configurations from one logic gate to another. The observed pattern of various logical responses switches over upon variation of DNA looping and free energies for the stimuli-induced TF activation. The OR to AND or the NOR to NAND transitions are observed as the stimuli weakly induce TFs (ϵ_{L-TF}). The population of OR (or NOR) assembly switches to AND (or NAND) assembly as we increase the stimuli activities ($\lambda_{IFN-\beta}$ and $\lambda_{TNF-\alpha}$) and weakly induces TFs for a fixed value of looping energy. We further show that the switching between OR-AND to NOR-NAND as the DNA becomes rigid. The increase in stiffness inhibits the formation of DNA loops; thereby, the long-distance enhancer−promoter interactions are stopped. We demonstrate this in Figure 2 (panels E and F) for

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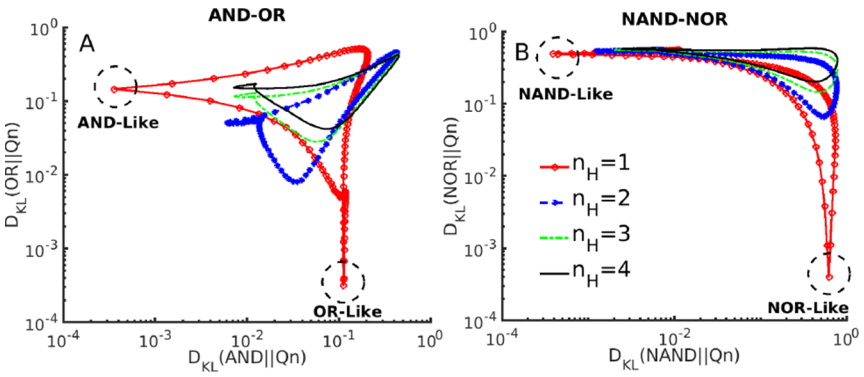

Figure 5. Behavior space for the complete set of available assembly configurations is plotted as K-L divergence (D_{KL}): similarity between theoretical model computed output surfaces and the Boolean surfaces obtained from Monte Carlo simulations. The regions are marked for the exclusive AND to OR, and NAND to NOR logic gates in the panels A and B,respectively. The calculations are done by varying degrees of oligomerization (n_H) to explore the robustness of the switching among gates.

two different values of DNA looping free energy ϵ_{LP}, i.e., 0 k_BT and 12 k_BT. We show the pattern of various logic gates at the extreme parameter values in panels A, B, C, and D. Therefore, the tunable protein−DNA interactions switch from one logical gate to another, providing an ideal platform for preparing a biocomputing machine.

To explore the transition of the switching from one gate to another one, we plot multiple contours for different values of ϵ_{L-TF} in Figures 3 and 4. The figure shows a clear signature of switching between the OR- and AND-; NOR- and NAND-like logical responses. The arrows indicate the ϵ_{L-TF} variation corresponding to different logic gates. The transformation from OR to AND and from NOR to NAND, like logical responses, are noticeable as a function of ϵ_{TF-L} parameter from Figures 3 and 4.

The reason behind switching between OR to AND-like logical responses is the formation of the number of DNA loops that vary for AND and OR assemblies. The formation of two DNA loops promotes cooperative interactions among proteins in the locally formed complexes on the DNA. On the other hand, the OR-like gate requires either or both TFs to interact with the promoter to form the DNA loops. It happens because the stimuli strongly induce TF that enhances the occupancy of active TFs to enhancers even at low values of stimuli activities. As the population of active TFs increases at their low values, the probability of the formation of DNA loops increases in the absence of TF-TF cooperative interaction. Further, our analysis revealed the importance of cooperative interaction, which is crucial for forming an AND-like response. However, forming an OR-like gate requires no cooperative effect through the TF-TF interaction. As a result, either single or double DNA loops at low stimuli values activate the promoter region and provide an OR-like response.

Like OR-AND switching, NOR-NAND is also observed upon variation of ϵ_{L-TF}. In this case, we first set the high DNA looping energy (ϵ_{LP}). Then, we performed a continuous variation of ϵ_{L-TF}, $\lambda_{IFN-\beta}$, and $\lambda_{TNF-\alpha}$ on the active complex. The high values of ϵ_{L-TF} disfavor the long-distance interaction through DNA looping; TFs only access the promoter region by translation mode along the DNA. However, the movement of TFs is controlled by the binding between TF and DNA: the enhancement of interactions produces NOR, and its suppression produces NAND logical responses. Therefore, the origin of the NOR-NAND switching again lies in the variation of the ϵ_{L-TF} parameter. Modulation of interaction between stimuli and TF controls the movement of the TFs on the DNA, which is the origin of the NOR and NAND logical responses. As we increase the $\lambda_{IFN-\beta}$ and $\lambda_{TNF-\alpha}$, the population of activated TFs is enhanced. Such enhancement of the population of TFs increases the TF-DNA interactions that stop their accessibility to the promoter region of the regulatory motif. As demonstrated earlier, we find the NAND response with a low to moderate level of stimuli activities. In contrast, the NOR response is observed only at low values of $\lambda_{IFN-\beta}$ and $\lambda_{TNF-\alpha}$. We find a narrow red region for the NOR response spreads over and transforms to NAND response upon variation of ϵ_{L-TF} and the stimuli activities. To validate the transitions among logic gates, we present the analytical results corresponding to Figures 3 and 4 in S4 and S5, demonstrating a close correspondence with the simulation results and a clear view of the transitions of the logic gate in the SI file.

D_{KL} Analysis. We characterize the switching between two logical responses by calculating the D_{KL} function to explore the behavior space for the complete set of available configurations of assemblies. It measures the similarity between the results obtained from MC simulations and the theoretical logical functions obtained from our partition function calculation. Plotting D_{KL} as a scatter in Figure 5 revealed that AND and OR Boolean-like computations are contained in flexible DNA, whereas rigid DNA can compute NAND and NOR responses. We find a divergence region for both the AND-OR and NAND-NOR switching. It is a clear sign of the interconversion between the OR and AND, like logical responses, which are detectable in the parameter space. The signature of wide divergence for the AND-OR switching suggests that they are distinguishable, and the exclusive AND and OR-like logical responses are detectable in the behavior space from our analysis. The conversion between NAND and NOR switching is less detectable, as found from the D_{KL} analysis since the divergence for the NOR-NAND switching is narrow. We have presented the D_{KL} calculations performed for the variation ϵ_{L-TF} parameter for the various logic gate conditions, i.e., AND, OR, NAND, and NOR in Figure S2 in the SI. We further vary the degree of oligomerization of NF-κB to achieve robustness of gate switching. We find that the oligomerization of NF-κB does not enhance switching robustness; instead, their monomers provide robust switchings from AND to OR or NAND to NOR. We find from the analysis that the formation of the higher-order oligomers perturbs the logic gate

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operations, a signature that moves away from the precise computation.

DISCUSSIONS AND CONCLUSIONS

Biomolecular computers or biocomputers offer an outstanding potential over silicon-based computers because of their small size and high efficiency, the chip permanence and the reliability of biological computers, storage and parallel processing of biological computers, heating and signal interference, etc.[63−66] Inspired by living cells, the elementary computational unit of these computers are proteins, which appear to have as their primary function the transfer and processing of information, rather than the chemical transformation of metabolic intermediates or the building of cellular structures.[5] It uses a simple but profound logic where the protein and DNA can form functional self-assembly to participate in gene expression and produce the mRNA.[65,67,68] One can control their structures by stimulating TFs externally. Here, we exploit the formation of controllable self-assembly and the programmable DNA loops for Boolean logic gate operations (AND, OR, NAND, and NOR) that provide a roadmap of digital paradigms for biomolecular computing.[68] Switching over logic gates from one another is mediated via DNA loop and stimuli-induced assembly and disassembly of the structures corresponding to various gates.[25] We show that complex multi-bit processing devices, which communicate through chemical wires to perform computations in multicellular assemblies, can be engineered. We have successfully merged transcription and translation controllers in a combinatorial plug-and-play manner to achieve synthetic networks that form logic gates for executing fundamental arithmetic operations.[22] One can design such biomolecular computers to control and monitor a wide range of biological systems.[65]

In biomolecular computing, we engineer cues/stimuli as input and the mRNA molecules as output for a given gene regulatory circuit.[67] Using a Boolean logic gate requires grouping signals in low and high expression. The engineering of gene network design for the logic gate that is transcriptor based, as we have modeled above for the NF-κB gene regulatory system, symbolizes the advancements toward the digitalization of signals.[22,68] We have employed and developed the theoretical model that accounts for all complex digital behavior of this architecture. In particular, we found how the programmable DNA loops and the stimuli-induced TF activation play a crucial role in controlling the output of the biological system. We show that a specific combination of DNA loop and stimuli-induced TF activation marks forming a Boolean logic gate operation.

Various organisms, ranging from prokaryotes to eukaryotes, employ gene regulatory networks (GRNs) as a blueprint or map of molecular interactions despite the underlying complexity associated with the regulatory mechanisms of TFs in GRNs. A key question about how these intertwined connections among biomolecules cooperatively contribute to deciding the expression level or cellular state is unanswered.[69] To comprehend how individual cells can execute molecular arithmetic functions using modulated self-assemblies for the NF-κB signaling system, we build a biophysical thermodynamic model that describes the role of biomolecular self-assembly and DNA stiffness in generating logical gene expression responses and the feasibility of switching among these gates in a stimulus-dependent manner. Biomolecular assemblies influence the regulatory mechanisms because of the formation of the vast range of complexes,[70] and such enhancer−promoter logic contributes to the gene expression output and thus controls the regulatory design features such as network architecture and hierarchical organizations[71] and, therefore making biomolecular assemblies an important biophysical event regulating cellular growth, development, and reproduction.

We demonstrate the possibility of creating a biomolecular computer using our theoretical calculations for the NF-κB system. The unique regulatory feature of the NF-κB system shares the possibility of forming active assemblies under various stimuli conditions.[43,62] We explored the building blocks of such biomolecular computers by exploiting the programmed DNA loop and variability of stimuli-dependent TF activation. We show that various active self-assemblies formed under two input conditions, a feature mimicking modern computer chips. As defined before, an active assembly is where a TF interacts with a promoter by a few mechanisms so that they participate in mRNA production. We manipulate the stiffness of DNA that allows us to create programmable DNA loops, a crucial factor for AND, OR, NAND, and NOR Boolean operations. Since we can control DNA flexibility externally, gates are interconvertible in the parameter space. Therefore, a single assembly unit can perform sequential operation, a feature absent in silicon-based computers. Integration of such logic gates may offer high-level biomolecular computation in a cellular system. These computers have the potential to identify and analyze disease-related genes associated with cancer.[67]

We show that these combinatorial circuits integrated a two-molecule input and performed digital computations with AND, OR, NAND, and NOR expression logic in single cells. The work demonstrates that biomolecular self-assemblies have the potential to capture digital information in the form of mRNA molecules. The modularity of the design facilitates improving each computer component independently. Our findings demonstrate that individual cells can execute molecular arithmetic functions using modulated self-assembly. This feature has been demonstrated by Bashor et al., showing how a complex signal is processed in synthetic gene circuits using cooperative regulatory assemblies.[3] These machines do precise and robust computation, which may offer new treatment strategies and bioelectronic interfaces in future gene-based and cell-based therapies.[64]

Such logic gates in NF-κB are known and demonstrated previously.[13,43] Cheng et al. revealed unexpected cross-regulation between the NF-κB and IRF that coordinate innate immune responses.[43] Wang et al. developed a mechanistic modeling framework and computational workflow to determine the identifiability of all possible combinations of synergistic (AND) or nonsynergistic (OR) gene regulatory strategies involving TFs.[13] They found that a much greater fraction of genes is combinatorially controlled than previously reported by considering compensation among TFs. Specifically, they revealed that a group of known NF-κB target genes may also be regulated by IRF, which is supported by their chromatin immuno-precipitation analysis. However, they have yet to explore the switchable logic gates, a crucial feature we explored in our study.

As literature suggests the involvement of synthetically programmed DNA loops in altering or computing Boolean logic at the gene level.[25,54−56] Our modeling approach emphasizes the role of biomolecular self-assembly and DNA

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stiffness in controlling the formation of various types of gates and interconversion among them. Although we have performed a computational analysis in this work on the NF-κB signaling system, this model can be easily transformed to apply to any other gene regulatory systems for enhancer−promoter interactions, e.g., RXR and RAR system where 9-cis-retinoic acid and retinoic acid act stimuli to regulate the expression, respectively.[72] Many studies have shown that mRNA-based biocomputers can detect disease indicators, including mRNA of genes linked with lung cancer and prostate cancer.[67,73−75] Here, we delineated such a biological system for NF-κB as a building block and how self-assembly and programmable DNA loop lead to forming a typical computer system, which we can call a biological microprocessor. This device considers stimuli as input information and then rewires the GRNs through a modulated self-assembly that produces the Boolean output as the population of mRNA.

However, there are many areas for improvement in the experimental design of such computers. A few of them we list here, but several others can be found elsewhere.[64] For example, maintaining biological components' distinct and robust modular structure is difficult, and unexpected phenomena arise in large networks. There is no sophisticated procedure for automation that generates network blueprints with arbitrarily defined input. It is challenging to generalize for any arbitrary system. Finding a set of correct parameters where the computation occurs is complex. We cannot avoid noise in biological systems since they are inherent in cells and work unpredictably.[68] The designing of such machines must address safety, reliability, and reproducibility if they are being used for medical applications. Nevertheless, these issues are surmountable since naturally occurring gene regulatory circuits and biomolecular assemblies exist under different conditions in a cell. We have shown that such modularity of the self-assemblies exists in cells at different conditions. Our work will improve understanding of such computation in great detail.

■ ASSOCIATED CONTENT

Data Availability Statement

Data and relevant code for these analyses are available at: https://zenodo.org/records/10300210.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00049.

Detailed theoretical calculations and simulation methodology to obtain the fractions of various active assemblies for the different networks of logic gates; results obtained from the theoretical model-based logic gates in the parameter space; D_{KL} calculations performed for the variation of ϵ_{L-TF} interaction energy; expressions for the variation of different stimuli activities; theoretical results for switching AND to OR logic gates; theoretical results for switching NAND to NOR logic gates; different values of parameters used in our calculations; and a total of 36 microstates presented along with their statistical weights (PDF)

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Author Contributions

S.K.S. and P.G. designed the research, performed the research, and wrote the paper. P.G. analyzed the data.

Notes

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