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REVIEW

Recent advances and opportunities in synthetic logic gates engineering in living cells

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Abstract Recently, a number of synthetic biologic gates including AND, OR, NOR, NOT, XOR and NAND have been engineered and characterized in a wide range of hosts. The hope in the emerging synthetic biology community is to construct an inventory of well-characterized parts and install distinct gene and circuit behaviours that are externally controllable. Though the field is still growing and major successes are yet to emerge, the payoffs are predicted to be significant. In this review, we highlight specific examples of logic gates engineering with applications towards fundamental understanding of network complexity and generating a novel socially useful applications.

Keywords Synthetic biology · Logic gate · Gene networks · Gene regulation · Therapy

Abbreviations

AHL	Acyl-homoserine lactone
ATc	Anhydrotetracycline
AU	Arbitrary units
C4-HSL	*N*-Butyryl-homoserine lactone
IPTG	Isopropyl β-D-1-thiogalactopyranoside
MAPK	Mitogen-activated protein kinase
RBS	Ribosomal binding site
RNAP	RNA polymerase
TF	Transcription factor
eGFP	Enhanced green fluorescent protein

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Introduction

Synthetic Biology arose out of a desire to implement engineering-level precision in constructing a biological circuit. This gave rise to the concept of parts standardization, composition standards, logic gates, reprogramming pathways and Computer Aided Design of biological networks (Endy 2005; Moe-Behrens 2013). A number of promoters (Lutz and Bujard 1997; Alper et al. 2005), proteins and RNAs (Basu et al. 2005; Pfleger et al. 2006; Win and Smolke 2008) and scaffolds (Park et al. 2003; Dueber et al. 2009) have been designed and characterized. Additionally, synthetic devices such as oscillators (Elowitz and Leibler 2000; Stricker et al. 2008; Tigges et al. 2009; Danino et al. 2010), toggle switch (Gardner et al. 2000; Atkinson et al. 2003), riboregulators (Isaacs et al. 2004; Rodrigo et al. 2012, 2013; Na et al. 2013) and riboswitches (Winkler et al. 2002; Tucker and Breaker 2005; Blount and Breaker 2006) have been constructed and characterized in a wide range of hosts. All of these existing synthetic parts, devices and circuits have been started to further implement for rewiring (Miyamoto et al. 2013; Singh 2014; Soma et al. 2014; Xu et al. 2014; Sowa et al. 2014) or coupling (Prindle et al. 2014) of intracellular networks or manipulating the cellular functions at certain scale.

Another aspect of synthetic biology has been probing into the mechanism of biological complexity of logic gate circuitry, improved computational speed and potential biotechnological applications. A logic gate in electronics, is a

physical device which is implemented with a Boolean function based on input and output signals (0 and 1). It executes a logical function on one or more inputs that produce a single output (Jaeger 1997). It was initially implemented using diodes or transistors acting as electronic switches and further designed using electromagnetic relays (relay logic), fluidic logic, pneumatic logic, optics and mechanical elements. Therefore, a number of logic gates have been used to create a circuit or device such as multiplexers, registers, arithmetic logic units and computer memory (Tinder 2000). Logic gates are used for storing the data that can be constructed by connecting several gates in a Flip-flops circuit which is a central building block of digital electronics systems in computers and communications.

However, taking the advantage of electronics, synthetic biologists are engineered a number of biologic gates which comprise of coding DNA, promoter, transcription factor, RNA polymerase, non-coding RNA, DNA binding elements and small signalling molecules. These genetic elements are interacted with regulatory protein to switch a gene ON or OFF while RNA or protein concentration can serve as input or output depending upon the level of abstraction. Therefore, a long-term goal of synthetic biology that is the ability to reprogram the decision-making gene networks in order to implement them as logic gates in living systems. Whereas Boolean logic gates are at the core of the operational machinery which makes biocomputer a reality, biologic gates become a necessity (Miyamoto et al. 2013). Biocomputation in living systems is a revolutionary step in biotechnology for improving the existing cellular process and enabling a new application (Brophy and Voigt 2014). In recent years, a number of biological gates have been constructed and characterized in a number of hosts including mammalian cells (Seelig et al. 2006; Sayut et al. 2011; Tamsir et al. 2011; Moon et al. 2012; Goñi-Moreno and Amos 2012; Bonnet et al. 2013; Shis and Bennett 2013; Miyamoto et al. 2013; Bonnet et al. 2013). An advanced level programming language has been implemented in *Escherichia coli* which is a digital circuit that functions on the principles of modern computing. It counts user-defined inputs with a range of frequencies that can be expanded to count higher numbers (Friedland et al. 2009).

A set of synthetic transcription-translation controllable combinatorial circuits have been rewired in mammalian cells. It was integrated into two molecule inputs leading to the digital logic gates function, including NOT, AND, NAND and N-IMPLY in a single cell (Ausländer et al. 2012). It was further implemented in a more complex 16 two-input based logic functions from combinations of the same type of NOR gates in mammalian cells (Gaber et al. 2014). Additionally, DNA-based logic gates including AND, OR and AND-NOT have been designed and characterized using wiring into a three-levels circuit that exhibits XOR function (Frezza et al. 2007). The 16 two-input based complex logic gates have been designed in *E. coli* cells with long-term maintenance of memory (Siuti et al. 2013; Purcell and Lu 2014). A synthetic multicellular biological computer has been engineered with specific task using cell–cell conjugation systems (Goñi-Moreno et al. 2013; Macia and Sole 2014). Additionally, application of synthetic AND gate in mammalian cell for targeting the cancer cells has been demonstrated earlier (Nissim and Bar-Ziv 2010). In the present review, we highlight the synthetic logic gates with predictive digital output for tight controlling the cellular function and also useful for diagnostic, therapy, tissue engineering and more biotechnological applications.

Table 1 Truth table of AND gate

Input A	Input B	Output AB
0	0	0
1	0	0
0	1	0
1	1	1

Biologic gates engineering in bacteria

The AND gate

Recent efforts in synthetic biology have also focused on the designing and implementation of logical functions within a living cell. The AND gate gives a high output (1) only if both the inputs are high and represented as A·B or AB (Table 1). Recently, AND gate in *E. coli* has been constructed using a novel regulatory component derived from *Pseudomonas syringae*. It consists of *hrpR* and *hrpS* genes which are expressed under the control of two different promoters which is considered as inputs. The σ54 dependent hrpL promoter drove the output during activation. When both the genes (*hrpR* and *hrpS*) were expressed that showed a digital AND gate function (Wang et al. 2011). As shown in Fig. 1, AND gate was demonstrated to be modular by applying a new regulatory input promoters. That connects output to a NOT gate producing a combinatorial NAND gate. The effect of temperature variation was studied by characterizing a promoter at two different temperatures (30 and 37 °C). The effect of change in temperature on the three promoters was different while a small change in strength of P_{BAD} was observed by changing the temperature from 30 to 37 °C. Plux became as leakier at 30 °C than at 37 °C while Plac shows a higher response at 37 °C than 30 °C. These variations were recorded because of different effect of temperature shift on

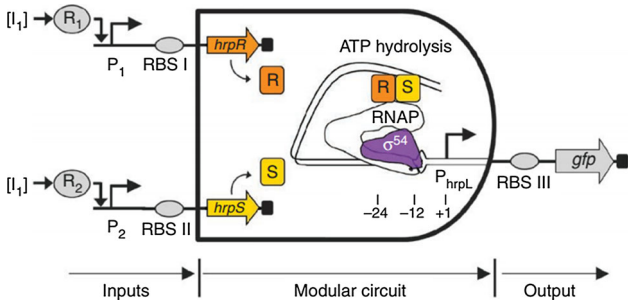

Fig. 1 AND gate design based on σ54-dependent hrpR/hrpS. Two environment-responsive promoters, P1 and P2 that acts as inputs to drive the transcription of hrpR and hrpS, and respond to the small molecules I 1 and I 2. The transcription hrpL promoter is turned on when HrpR and Hrps are present. It binds to the upstream activator sequence to remodel the closed σ54-RNAP-hrpL transcription complex through ATP hydrolysis. GFP was used as output while RBS for fine tuning. Figure reproduced with permission from Nature (Wang et al. 2011) © 2011 Macmillan Publishers Ltd

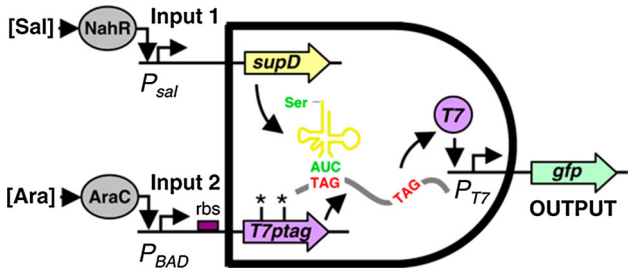

Fig. 2 Design of AND gate based on two promoters. The first promoter was linked with transcription of amber suppressor tRNA supD and second T7 RNA polymerase. Polymerase was modified to contain two amber stop codons and translated as serine when supD was transcribed and T7 pol was expressed when both SupD and T7ptag mRNA are present. Figure reproduced with permission from Anderson et al. (2007)

the binding affinities between TFs on their DNA-binding sites. This study demonstrated that Plac is weaker promoter as compared to P_{BAD} and Plux (Wang et al. 2011).

Similarly, a synthetic AND gate has been constructed in *E. coli* and combined from two promoters as an input that leads to the activation of output promoter. Both the input promoters have been transcriptionally integrated by interaction between mRNA and tRNA (Anderson et al. 2007). As shown in Fig. 2, the first promoter controls T7 RNA polymerase (T7 RNA pol) expression with two internal amber stop codons while second promoter controls the amber suppressor tRNA *supD* when both components are transcribed. T7 RNA polymerase was produced and activated the T7 promoter. Therefore, transitions between ON and OFF states were steep, thus producing AND gate function. No detectable expression was observed in the absence of either inducer or a 1,000-fold induction when both inducers are present (Anderson et al. 2007). This approach can be further expanded in a wide range of hosts which enables the future designing of a nutrient status-dependent logic gate that can regulate a number of cellular functions.

Recently, an AND logic gate has been engineered using a set of synthetic microbe based biosensors. It comprises of sensory, signal processing and actuation systems that was engineered by distinct signalling sensory modules. It was used to identify by different chemical signals and combinations with a fluorescent reporter. *E. coli* based biosensor was constructed for detecting the environmental hazardous metals such as arsenic, mercury and copper (Wang et al. 2013). In a relevant study, the synthetic genetic circuits have been constructed by integrating intercellular signalling molecule. It is based on Plux quorum-sensing promoter and the lac repressor (Sayut et al. 2009). Bagh et al. (2010) developed a minimal genetic device with AND gate function in *E. coli*. It is based on external sensing molecules IPTG and aTc while eGFP used as reporter for measuring logic function. The signal can be amplified by using arabinose and tuned by different concentrations of sensing molecules. It is a versatile AND gate which can be useful for tunable biological production in an adjustable manner.

A 2-input AND gate has been engineered for generating a more complex cellular programming (Moon et al. 2012). In the experiment, 3-input and 4-input AND gates have been built by different combinations of 2-input gates (Fig. 3a). The 3-input gate has the potential for an error when shifting from a +Ara/+IPTG/−aTc [110] to [011] state. An alternative logic combination have been produced the same functions which is used for gate layering. In both the cases, output was ON only when all of inducers were present in the medium. In the 3-input gate, the output was 4.5-fold more highest OFF state (Fig. 3b) when all inducers were available (Moon et al. 2012). The cytometry data for all sets of input states were shown in Fig. 3c (−), thick line indicates a set of inducers. Figure 3d represents 4-input AND gate and in Fig. 3e, output fluorescence for different permutations of inputs. A total four inducers (Ara (5 mM), IPTG (0.1 mM), 3OC6 (5 μM) and aTc (10 ng/ml) were used for ON input and in Fig. 3f, shown cytometry data for all inputs (Moon et al. 2012).

Rodrigo et al. (2012) have developed automated designing of RNA-based AND gate by placing the sRNA and the 5′ UTR-mRNA-GFP under the control of tunable promoters (PLtetO1 and PLlacO1) which are repressed by TetR and LacI (Lutz and Bujard 1997). However, these promoters are induced by aTc and IPTG molecules and showed AND gate function. Rodrigo et al. (2013) reviewed that automated synthetic RNA devices can be used for

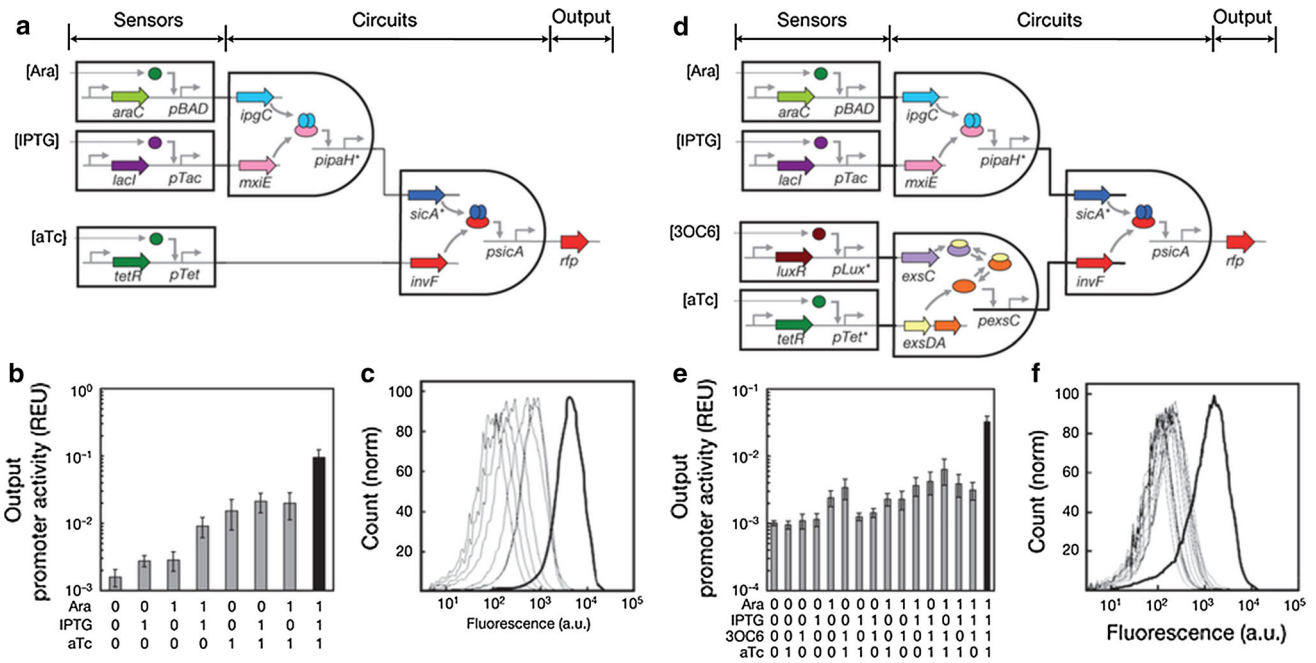

Fig. 3 Design of layering AND gate. **a** The 3-input AND gate. It consists of 3 sensors which is integrated circuit and a reporter gene. **b** The fluorescence was measured from cells containing the 3-input AND gate. The three inducers were used for on (1) input were Ara (5 mM), IPTG (0.1 mM) and aTc (10 ng/ml). **c** Cytometry data used for all sets of input states. **d** 4-input AND gate and **e** output fluorescence for different combinations of inputs. Ara (5 mM), IPTG (0.1 mM), 3OC6 (5 μM) and aTc (10 ng/ml) were used as inducers. **f**, Raw cytometry data used for all inputs. Figure reproduced with permission from Nature (Moon et al. 2012) © 2012 Macmillan Publishers Ltd

manipulating the post-transcriptional regulation and also conditional detection of cellular biomolecules. An automated designing approach can be further applied for generating a number of logic gates including NAND, XOR, OR and more.

Recently, Temme et al. (2012) have implemented AND gate function in multigene metabolic pathways for tight regulation and control of complex multigene expression over time and condition. In this study, authors initially engineered AND gate using a variant of T7 RNA polymerases which is induced by IPTG and aTc that was monitored by downstream expression of red fluorescent protein (RFP). It was further implemented for controlling of two pigments producing biosynthetic pathways (lycopene and deoxychromoviridans) in *E. coli*. The small amount of lycopene was produced via 1-deoxy-D-xylulose-5-phosphate (DXP) pathway following the insertion of heterologous carotenoid genes *crtEBI* that was improved by overexpressing *dxs* and *idi* genes. When the IPTG is present then lycopene is synthesized while no effect of aTc molecule. Whereas, deoxychromoviridans was produced by extension of L-tryptophan by inserting *vioABE* genes when both inducers molecules (IPTG and aTc) are present (Temme et al. 2012). This study suggests that deoxychromoviridans pathway is tightly controllable under the AND logic gate but it can be further improved by implementing not only AND gate but also OR, NAND, NOR, XOR logic function for tight controlling of other multigene pathways. Moser et al. (2012) also tested AND logic gate at microreactor to bioreactor levels by changing selection of media, strain, and growth rate. In *E. coli* DH10B, the AND gate is non functional in minimal media. However, AND gate activity could be rescued when supplementing media with yeast extract or tryptone. When AND gate was used in industrial strain *E. coli* DS68637 where normal function was observed in minimal media. This study suggests that DH10B is not considered as good strain for industrial application of top value molecules production the under control of AND gate (Moser et al. 2012). This study can be applied in metabolic engineering for tunable and high production renewable biofuels (Singh et al. 2014) at industrial level scale.

Recent application of AND logic gate has been demonstrated by cell–cell communication through conjugation systems. AND gate was constructed using a number of inducible promoters and transcription factors for environmental monitoring and degradation of hazardous pollutants. In this study, linking between two *Pseudomonas putida* strains was used for signal communication through benzoic acid as the wiring molecule. A sender strain carried TOL pathway for biodegradation of aromatics processed toluene as first input and generates benzoate as

Table 2 Truth table of NAND gate

Input A	Input B	Output AB
0	0	1
1	0	1
0	1	1
1	1	0

output signal. Whereas, diffusion of metabolic intermediate to the medium was then sensed by a second strain (receiver) that used benzoate as second input for producing final AND gate function (Silva-Rocha and de Lorenzo 2014). Therefore, an urgent need arises to expand cell–cell conjugation approach not only in environmental science but also in medical science for controlling of pathogenic bacteria via logic gate engineering.

The NAND gate

The NAND logic gate is equal to an AND gate followed by a NOT gate (Table 2). The output of NAND gate is high if any of inputs are low. It was constructed based on protein–protein interactions through coupling of DNA looping (Zhan et al. 2010). Multiplex of logic gate have been constructed by same designing but different combinations of sequence variants. A combinatorial library (125 synthetic networks) were designed and constructed using a small set of transcriptional regulators (LacI, TetR and lambda CI) and their corresponding promoters. The binding of LacI and TetR have been changed by a small molecules inducers IPTG and aTc (Guet et al. 2002). Five promoters were regulated by their corresponding proteins covering a broad range of regulatory activities such as repression, activation, leakiness and strength. Two of promoters were repressed by LacI, one was repressed by TetR, and the remaining two were regulated by λ CI, one positively and one negatively (Guet et al. 2002). It can be easily introduced into cells and served as a powerful tool for creating a versatile biocomputer. Therefore, a handful of interacting genetic elements can create a large diversity of complex behaviours. Logic gate and its memory play a key role to memorize cell functionality. The recombinases based approach has been used for construction of logic function in *E. coli* (Siuti et al. 2013). Using this approach, AND, NAND, XOR, OR, NOR and N-IMPLY have been constructed and also characterized. The 16 two-input Boolean logic functions in *E. coli* have been implemented and showed long-term memory (90 cell generations). Integrated logic and memory system may enable the implementation of complex cellular machines, behaviours and pathways (Siuti et al. 2013; Purcell and Lu 2014) for therapeutic, diagnostic and other biotechnological applications in near future.

Table 3 Truth table of NOR gate

Input A	Input B	Output A + B
0	0	1
1	0	0
0	1	0
1	1	0

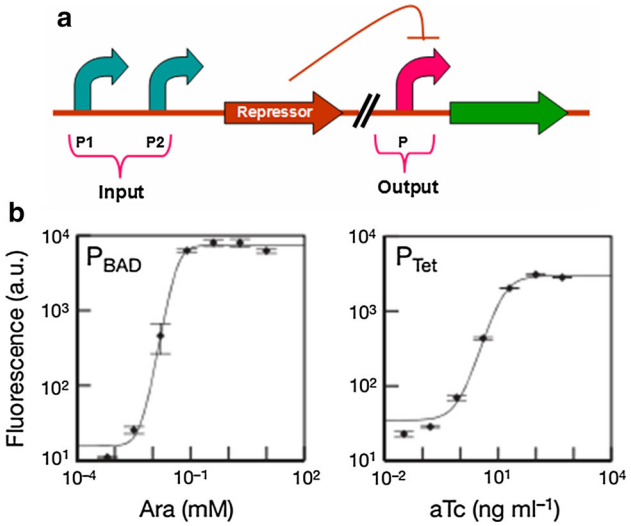

Fig. 4 NOR gate was engineered based on two tandem promoters (P1 and P2) express repressor and turn off downstream promoter (Pout) (**a**). **b** Different inducer concentrations were used for tandem promoter and NOR gate characterizations. Figure reproduced with permission from Nature (Tamsir et al. 2011) © 2011 Macmillan Publishers Ltd

The NOR gate

NOR gate is equivalent to OR gate followed by a NOT gate. The outputs of NOR gates are low, if any of the inputs are high. Table 3 provides the truth table for NOR gate. It requires at least two inputs (A and B) that produces output in form of fluorescent protein or enzyme as signal detection. Recently, Tamsir et al. (2011) engineered NOR gate which is 'ON' only when both the inputs are 'OFF'. Tandem promoter is common in prokaryote genome and is expected to generate OR gate which present in same orientation that drives the expression of repressor (Fig. 4a). Multiple gates have been combined for generating a complex biological operation. Inputs and outputs have been used to wire the different orthogonal quorum-sensing sender and receiver cells (Tamsir et al. 2011). In their work (Fig. 4b), Tamsir et al. (2011) activated P_{BAD} and Ptet promoters in presence of arabinose (Ara) and aTc. Five different concentrations of arabinose and aTc were used for characterizing the NOR gate and individual transfer functions of P_{BAD} and Ptet were measured. An OR gate was constructed by placing P_{BAD} and

Ptet promoters tandemly. P_{BAD}-Ptet demonstrated the OR logic with 7,000-fold induction between the 'OFF' state (2 Ara, 2 aTc) and the 'ON' state (1 Ara, 1aTc). For conversion of the OR into NOR gate; the CI was placed under the control of P_{BAD}-Ptet and YFP expressed from second plasmid under the control PR promoter.

Recently, an alternative approach has been applied for constructing NOR logic function using cell–cell conjugation systems. It required two strains (sender or donor and receiver) when the sender cell comes into contact with receiver. The plasmid from sender cell was transferred to receiver by conjugation mechanism. However, inducers F2 are produced inside the receiver by plasmid pmd^{i}, which induces the expression of reporter gene (G2) and show GFP expression (Goñi-Moreno et al. 2013). With reference to metabolic engineering point of view, the performance of NOR logic function was tested at the industrial level by changing different strains and media. NOR gate was constructed using two inputs arabinose and aTc while GFP used as a reporter for monitoring logic function. It was transformed in *E. coli* DH10B and also industrial strain DS68637 and performance was tested by changing the media conditions. NOR is functional in all used media and also showed 76-fold induction in LB media. NOR gate induction differs 16 % between DH10B and DS68637 strains. This study suggests that NOR gate is more reliable output irrespective to media and strains at industrial scale (Moser et al. 2012).

The XOR gate

Exclusive-OR gate is also known as XOR gate which requires two input molecules. It gives a high output if anyone signal is present (Table 4). As shown in Fig. 5a, XOR gate was engineered using a combination of three NOR gates. The output of XOR gate was found to be ON only when either input are present. There are four strains used, each carrying a different logic gate to build XOR gate. These strains were spotted on agar plate in a spatial arrangement to perform XOR function (Fig. 5b) (Tamsir et al. 2011). The cell 1 contains a NOR gate and used Ara and aTc as inputs to express LasI output that allows cell 1 to wire with NOR gates in cells 2 and 3 by means of 3OC12-HSL. Cells 2 and 3 were used Ara and aTc as their second inputs and the output of NOR gate in cells 2 and 3 was RhlI which produced C4-HSL (*N*-butyryl-homoserine lactone). Cell 4 continues as buffer gate by integrating the outputs from cells 2 and 3 by C4-HSL molecule. The complete logic gate was designed in four strains that behave as XOR gate with respect to inputs inducers Ara and aTc (Fig. 5c, d). Each intermediate colony executes a digital logical behaviour by replacing each output gene with YFP (Fig. 5c) (Tamsir et al. 2011). In this study, the distributed cellular computation has used global communication by quorum sensing to implement the "wiring" between cell types. Individual cell performs a specific subtask in which they are communicated to other cell types for further processing. The manner in which outputs are communicated that is slow processing of logic gates function overall success of such a system.

Table 4 Truth table of XOR gate

Input A	Input B	Output A + B
0	0	0
1	0	1
0	1	1
1	1	0

Recently, Goñi-Moreno et al. (2013) used an alternative approach for logic gate engineering based on conjugation system that allows for the direct transfer of conjugative plasmid from cell to cell. As depicted in Fig. 6a, cell NOR_1 (donor or sender) is inputs from concentration of molecules A and B while F1 is output. The inputs for cell NOR_2 (recipient) are molecule A and F1. Similarly, inputs for cell NOR_3 (recipient) are B and F1. The output for the XOR function, F2 is permutation of outputs from NORs 2 and 3. As shown in Fig. 6b, the truth table for how NOR gates based circuits changes in XOR function within the cells. Figure 6c, shows inside logic of NOR_1, in the case 0–0 (no inputs), gene G3 expresses FimB and turns, alters the plasmid present in strain by inverting the promoter. G1 expresses FimE that inverts the promoter region of plasmid pointing towards F2; whereas G1 expresses repressor X which stops the production of FimB (Goñi-Moreno et al. 2013). As shown in Fig. 6d, inside logic of cell NOR_2, once plasmid is inside the cell (connected), if no inputs present, GFP are expressed. If not, Cre is produced (A bound to promoter pA and/or inducer F1 bound to pF1) and deleted F2 from plasmid. Cell NOR_3 has the same circuitry but sensitive to input B instead of A (Goñi-Moreno et al. 2013). This study suggests that multicellular population is able to compute, in a distributed fashion of XOR function. Although a number of challenge remain for the biological system based computation, pursuing this concept still has its attraction. Conjugation-based logic multicellular system can be expanded for detecting and destroying not only environmental pathogen but also in animal pathogen for controlling of diseases.

The OR, NOT and more logic gates

The OR gate is an electronic circuit that gives a high output (1) if one or more of its inputs are high. A plus (+) is used to show the OR operation (Table 5). The NOT gate is an

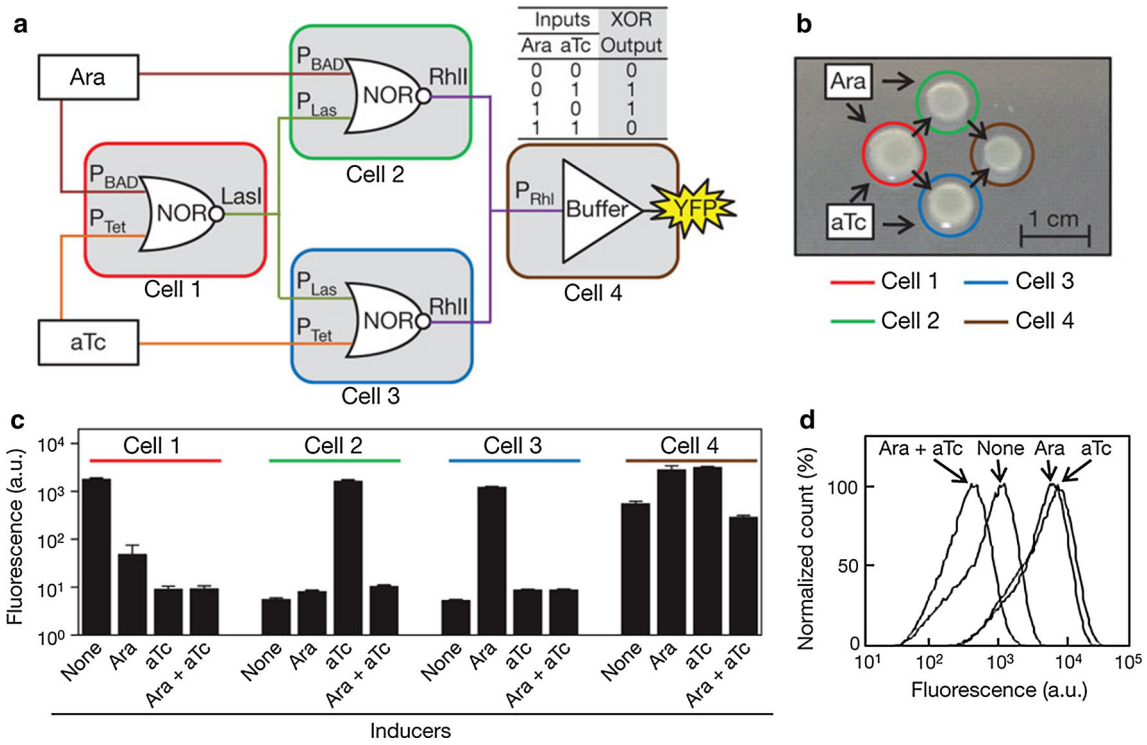

Fig. 5 Design of XOR gate. **a** Four colonies, each contains a single gate and spatially arranged on agar plate. Ara and aTc inputs were added in plate. **b** Spatial arrangement of the colonies. **c** Each colony responds to the combinations of input signals. Fluorescence values and their error bars were calculated (mean ± SD). **d** Cytometry data showed for XOR (cell 4). Figure reproduced with permission from Nature (Tamsir et al. 2011) © 2011 Macmillan Publishers Ltd

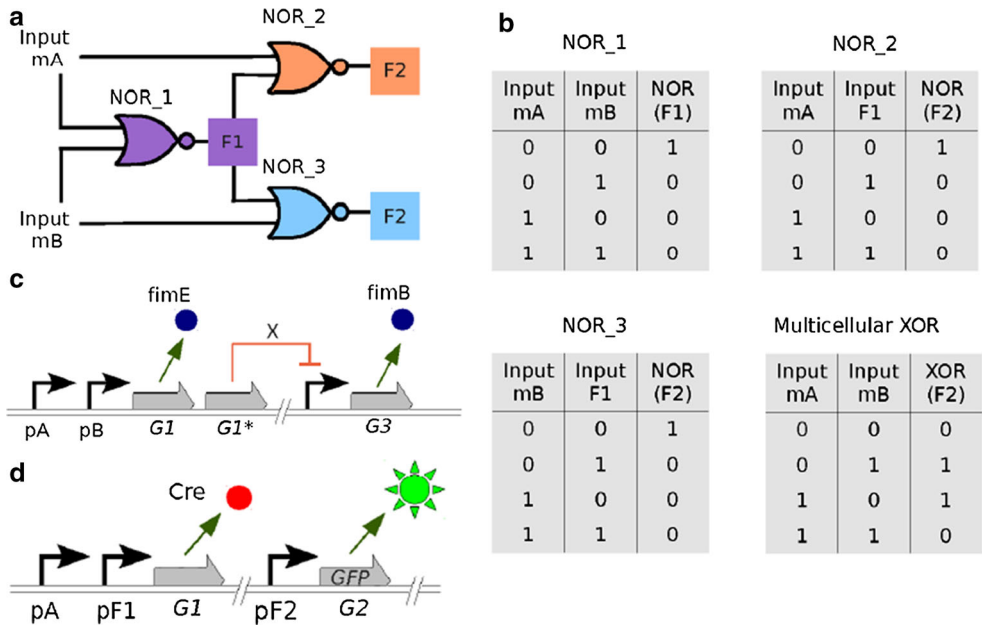

Fig. 6 Multicellular design of a distributed XOR circuit by conjugation of three-strain population. **a** Cell–cell communications by sender and receiver. **b** Truth tables for each NOR also XOR gate. **c** NOR_1 (in the case 0–0), gene G3 expresses FimB and turns to alter plasmid present in strain by inverting the promoter in random direction. G1 expresses FimE and inverts the promoter region of plasmid towards gene F2 and G1 expresses X that stops expression of FimB. **d** NOR_2, once plasmid is in the cell that is connected, if no inputs present thus, GFP were expressed. If not, Cre is produced that removed F2 from the plasmid. Whereas Cell NOR_3 has the same network but sensitive to input *B* instead of *A*. Figure reproduced with permission from Goñi-Moreno et al. (2013)

Table 5 Truth table of OR gate

Input A	Input B	Output A + B
0	0	0
1	0	1
0	1	1
1	1	1

electronic circuit that produces an inverted version of input at its output. It is also known as inverter gate such as if the input variable is A, then inverted output is known as NOT A. It is the simplest biochemical circuit which has a single input signal. The biological application of NOT gate, the enzyme RNA polymerase binds to promoter that produces a desired protein. It can be achieved by combining separately logic gates. Recently, a part mining exploration has been performed in bacterial genome and constructed a library of 73 TetR family repressors. On the basis of these libraries, a number of synthetic promoters have been engineered, out of these, 16 were identified that strongly repressed their cognate promoters (5–207-fold) and reveal less cross interactions. Each repressor-promoter pair has been converted into NOT gate function. Out of 16, more than 10 gates have been built by changing the pattern of input and output promoters (Stanton et al. 2014).

Moving forward towards logic function, two-input logic gates have been engineered at molecular level including OR, AND, XOR, NOR, NAND and XNOR. These logic gates have been designed to perform the set-reset function by applying an additional input (Park et al. 2012). A number of logic gates (NOT, AND, and OR) have been recently engineered and also characterized. Two NOT gates, two AND gates, and an OR gate were connected in a network to generate XOR function (Gerasimova and Kolpashchikov 2012). A three-terminal device was developed (Transcriptor) that uses bacteriophage serine integrases for controlling flow of RNA polymerase and DNA. In this study, a low-copy plasmids was used for construction of logic gates such as AND, OR, XOR, NAND, NOR, and XNOR by a standard strong prokaryotic promoter (input signal) and GFP as a reporter (Bonnet et al. 2013). Amplifying AND, NAND, OR, XOR, NOR, and XNOR gates actuated across widespread control signal ranges in order to logic supporting independent cell–cell communications. The single-layer logic gate has been constructed which facilitates the design of amplifying logic gates for controlling of transcription rate (Bonnet et al. 2013). Output signal was vary within and among the different logic function. These variations may be possible because of differences in RNA secondary structures that influence the mRNA stability and translation initiation rates. There is a need to expand and more apply logic gates circuit in therapeutic, diagnostic and cell rewiring in near future.

Biologic gates in yeast

Yeast (*Saccharomyces cerevisiae*) is an important model organism which is widely used in biotechnology applications from alcoholic beverage industry, biofuels and fine chemicals (Nevoigt 2008; Huang et al. 2010; Krivoruchko et al. 2011). However, programming of yeast was performed by assembling of biologic gates. A synthetic RNA device has been developed to perform a higher-order cellular processing. It is composed of three functional units such as RNA aptamer based sensor and a hammerhead ribozyme-utilizing actuator united by a transmitter component. The engineered synthetic RNA systems can function as logic gates (AND, NAND, OR, and NOR) by using theophylline molecule (Win and Smolke 2008). A logically different form of complex Boolean logic gates (NOR and NAND) were constructed and well characterized. It was implemented using a library of engineered yeast cells which combine in multiple ways. Each logic function and combining cells are connected to allow a building more complex circuits (Regot et al. 2011). As shown in Fig. 7a, gene circuit for AND logic was involved in two cell types responding to two stimuli (NaCl and oestradiol) by pheromone (alpha factor). The availability of NaCl was stimulated the Cell 1 to produce pheromone (IDENTITY) that was received by Cell 2. Thus, Cell 2 has ability to sense another external input (oestradiol) by producing and activating Fus3 mitogen-activated protein kinase (MAPK) production when both the inputs are available (Regot et al. 2011).

Giot et al. (1999) investigated the mutations that can enhance the weak mating defect of MATa ste2-T326 cells. These cells were (10-fold) more sensitive to alpha-factor pheromone because of ste2-T326 encoded a truncated alpha-factor. NOR gate (Fig. 7b) was used by a different pair of cell types, each cell responded to a doxycycline and inhibitor of Fus3 as kinase. Only in the absence of both stimuli, a positive output was obtained (Regot et al. 2011). The first three-cell circuit was an OR logic gate and two inputs are NaCl and galactose. In the cell 1 and 5 functions which correspond to the presence of NaCl (input 1) or galactose (input 2) that leads to the production of molecule that induced output in Cell 6 (GFP). But in the presence of any input (galactose or NaCl), the output was generated corresponding to an OR gate (Fig. 7c). While as shown in Fig. 7d, NAND gate shows function when doxycycline and glucose are present (Regot et al. 2011). A theoretical quantitative of a cellular biologic gates have been implemented in *S. cerevisiae* using endogenous MAP kinase

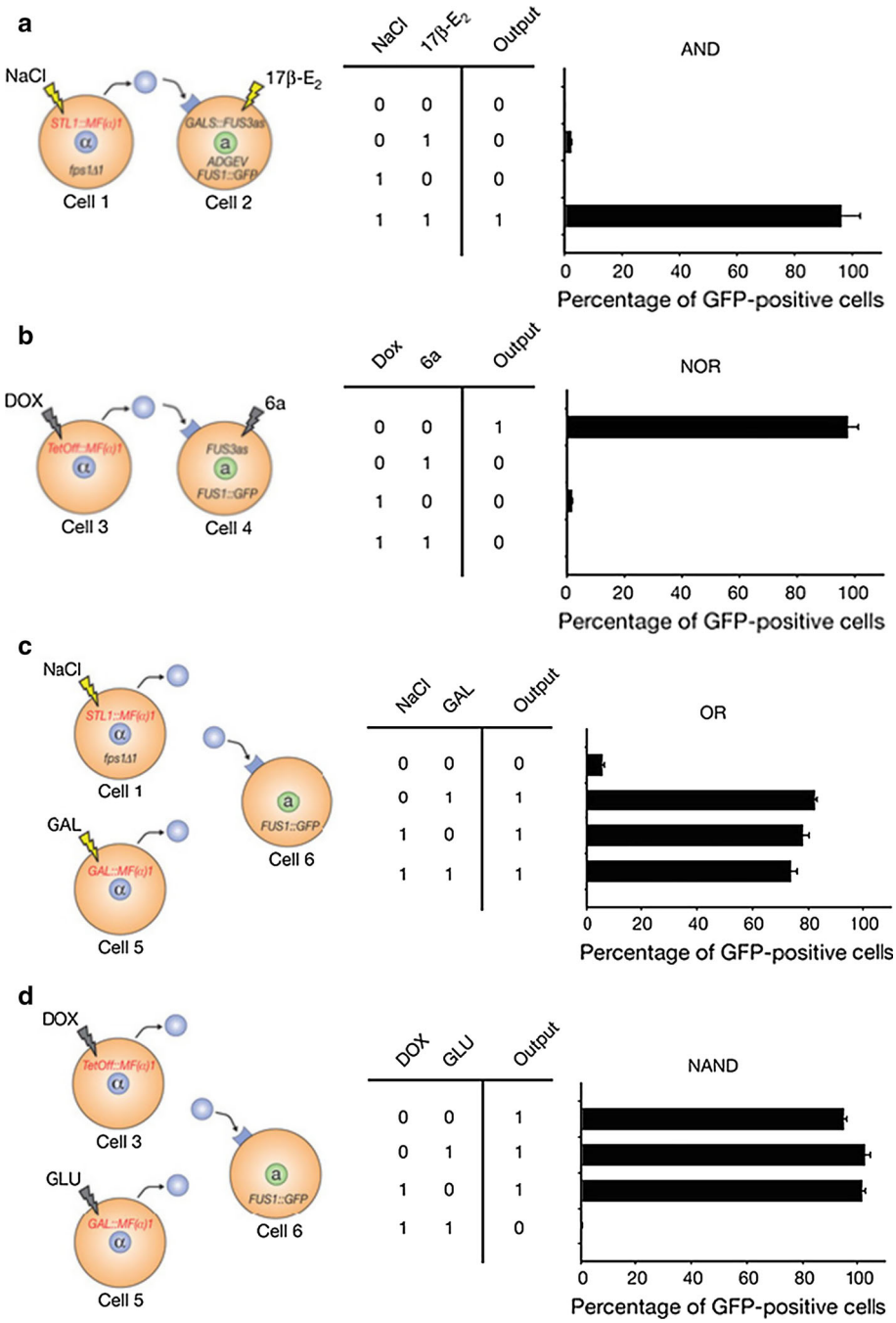

Fig. 7 Logic gate design in yeast. **a** Cells were mixed together with inputs (NaCl and oestradiol). **b** Panel ordered as in (**a**) following NOR logic. Cells have been treated with inputs doxycycline. **c** OR gate based strains have been treated with inputs 0.4 M NaCl and 2 % galactose (GAL). **d** NAND gate based strains treated with inputs doxycycline and 2 % glucose. Figure reproduced with permission from Nature (Regot et al. 2011) © 2011 Macmillan Publishers Ltd

signaling pathways. The kinetic models of multicellular IDENTITY, NOT, OR, and IMPLIES logic gates were modelled using both deterministic and stochastic (Hoffman-Sommer et al. 2012). Therefore, yeast has become a versatile and attractive choice of model organism not only in biotechnological application but also in synthetic biology and metabolic engineering with a huge potential for many applications including therapeutic, vaccine, biofuels, pigment, chemicals.

Biologic gates in mammalian cells

One of the landmark papers in mammalian synthetic biology was reported by Fussenegger and colleagues (Weber and Fussenegger 2011) for designing of biologic gate. It was compatible with expression control systems that laid the foundations for assembly of transcription control. It has been used to design combinatorial transcription control such as promoters (up to three operator sites for regulatory

proteins) and serial connecting of two gene regulatory systems. A combination of tetracycline, streptogramin, macrolide and butyrolactone have been used for generating NOT, AND, NAND, OR, NOR and INVERTER logic gates (Kramer et al. 2004). The combinatorial gene circuits have been integrated into a two-molecule input performing a digital function, including NOT, AND, NAND and N-IMPLY. The IMPLY gate is a basic logic gate and a universal gate that has ability for implementing any logic function. It requires two input signals molecules that is a repressor while an inducer for activation. If both the input signals are absent, the gene is transcribed. However, function was interconnected by two N-IMPLY variants, resulting in bit-wise intracellular XOR operations and a combinatorial arrangement of three logic gates that allow independent cells to implement the programmable half-subtractor and half-adder computations (Ausländer et al. 2012).

In this study, a number of transcription control system have been used to share a common two-component design. However, the synthetic TF contains a trigger-controlled DNA-binding fused with a *trans*-activation domain that binds to a specific operator by activating adjacent minimal promoters (Weber and Fussenegger 2011). It could allow the design of complex human machine interfaces and provides diagnostic information. It also provides a therapeutic intervention in future gene-based and cell-based treatment of human serious diseases (Weber and Fussenegger 2011). Therefore, transcription activator-like repressors was used for optimizing the designing of orthogonal NOR gate. All 16 two-input logic functions was implemented by combinations of same type of NOR gates in mammalian cells. Moreover, a logic circuit where one input is used for selecting AND and OR function to process the data input using the same circuit (Gaber et al. 2014). In this novel engineering effort, synthetic biologists are more focused to build a logic gene circuit with a more precise, predictive and controllable manner. However, one of the major drawbacks of the existing synthetic logic gates based circuits is it requires a long period of time (minutes to hours) to perform the logic function. It is because of long processing time inherent in transcription and translation machinery.

Conclusion and opportunities

A number of logic gates circuits and devices have been already developed and implemented in electronics including multiplexers, registers, arithmetic logic units, and computer memory. It is also used as storage data (computer memory) as they vary in their performance based on speed, complexity and reliability of storage. Discovery of these physical systems can give a strong motivation to the synthetic biologists for constructing a combination of biologic gate that can be further implemented into a controllable biological systems including memory. Previously, synthetic biologists are much focused on constructing a part, device and circuits. However, there is a immense opportunities for designing of logic gate based sensor for detecting and targeting not only cancer cells (Nissim and Bar-Ziv 2010) but also monitoring of environmental pollutant (Wang et al. 2011; Silva-Rocha and de Lorenzo 2014), toxic chemicals, pathogenic organism to detect and destroy. AND logic has been implemented in *E. coli* for tuning and controllable production of pigment molecules such as lycopene and deoxychromoviridans using metabolic engineering approach (Temme et al. 2012). AND and NOR gates were tested in *E. coli* for industrial level applications that could be functionally reliably during fermentation process (Moser et al. 2012).

Siuti et al. (2013) recently constructed the 16 two-input complex logic gates in *E. coli* cells that show long-term maintenance of memory for at least 90 cell generations. Goñi-Moreno et al. (2013) engineered a conjugation based logic multicellular system that can be useful for detecting and destroying bacterial pathogen not only environmental but also animal gut for controlling of serious diseases. Wang et al. (2011) suggested that biologic gates can be useful as a sensor that swim inside arteries, detecting the build up of harmful plaque and rapidly delivering medications to the affected area. Therefore, an urgent need arises for designing of a library of logic gates circuits that may contain the high speed, processing and potential biotechnological applications. A long road ahead to go before synthetic biology-based clinical treatments becomes a reality but it holds a promise and a great future possibilities. There is currently an unmet need to engineer biologic gate with different combination for targeting the desired cell with specific functionality. With more data and innovative approaches coming in, it hopes that synthetic biology methods and reference inventory data will provide a template for precision engineering to construct a more complex biological system in future.

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