

REVIEW

Current Technologies of Synthetic Biosensors for Disease Detection: Design, Classification and Future Perspectives

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Abstract Synthetic biology aims to endow living cells with new functions by incorporating functional gene networks into them. By overexpressing, blocking and rewiring native gene pathways, synthetic biologists have harnessed this promising technology to reprogram cells to perform diverse tasks such as drug discovery, biopharmaceutical manufacturing, gene therapy and tissue engineering, etc. In this review, we focus on current technologies of synthetic biosensors for disease detection. We start with the design principle of synthetic biosensors. Then we move towards the characteristics of simple synthetic biosensors, which can respond to a single input signal, and complex synthetic biosensors including Boolean gate biosensors, cascade biosensors, time-delay biosensors, oscillator biosensors and hysteretic biosensors, which can respond to more than two input signals and perform complex tasks. Synthetic biosensor has showed great potential in disease detection, but it is still in its infancy stage. More efforts should be made in identifying and constructing clinically relevant regulation systems. Computational tools are also needed in the design process in order to guarantee the precision of the synthetic biosensor. The ultimate goal of a synthetic biosensor is to act as a therapeutic sensor-effector device that connects diagnostic input with therapeutic output and therefore provides all-in-one diagnostic and therapeutic solutions for future gene- and cell-based therapies.

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CONVENTIONAL disease diagnostics rely on iconography or antibody-based platform which are slow and costly.¹ Synthetic biology which enables cells to perform designed novel tasks by modifying the native gene pathways has provided an alternative approach for disease detection. A gene pathway can be regarded as a network consisting of a series of functional gene modules. Synthetic biologists reprogram cells to perform designed tasks by integrating different functional gene modules into cells. This promising technology has been successfully utilized to reprogram a wide range of different cell species from prokaryotes like *Escherichia coli* and lower eukaryotes like yeast to higher eukaryotes like mammalian cells, to perform novel tasks with great diversity, such as producing new chemicals and proteins, drug discovery. In this review, we give an overview of recent advances in the application of synthetic biosensors for disease detection. The design principles and characteristics of synthetic biosensors with different architectures are summarized.

DESIGN PRINCIPLES OF SYNTHETIC BIOSENSORS

An ideal synthetic biosensor for disease detection is reminiscent of a precise regulation system. It consists of two functional modules: the detecting module and the responding module. The detecting module precisely detects biomarker signals and triggers the responding module to express desired reporters. Biology enriches with plenty of perfect gene regulation systems. The first regulation system, lac operon in *Escherichia coli* was discovered by Jacob and Monod in 1961.² The lac operon exhibits a subtle regulatory control of lactose metabolism. There are three structural genes of lac operon, lacZ, lacY, and lacA. They encode enzymes necessary for lactose catabolism. In the absence of lactose, lac repressor which is constitutively expressed in *E. coli* binds to the DNA sequence, called lac operator, downstream of the promoter and inhibits the transcription of lacZ, lacY, and lacA and consequently halts the production of the enzymes encoded. In the presence of lactose, on the contrary, a lactose metabolite called allolactose, binds to the lac repressor, causing an allosteric shift. Then the repressor is released from the DNA sequence, allowing the transcription of lac genes and thereby leading to higher levels of the enzymes. The lac operon shows how biol-

ogy control cellular metabolism in an efficient, precise and energy saving manner.

Since the discovery of the lac operon, more and more regulation systems have been discovered and identified, most of which are in prokaryotes.³ Inspired by electronic circuits, synthetic biologists have integrated different functional parts of these regulation systems into gene circuits which act in a biosensor manner. These heterologous synthetic biosensors enabled cells precisely and efficiently respond to signals from their microenvironment. A synthetic biosensor for disease detection is composed of three key elements: a signal, a transcription factor, and an operator.

In the disease detection manner, a signal is the biomarker representing the pathological conditions of the cell and/or system. It triggers the binding or releasing of a transcription factor from the operator, which then controls the expression of the reporter. To date, a great number of prokaryotic transcription factors have been identified that respond to a variety of signals including antibiotics,⁴⁻⁶ amino acids,⁷ food additives,⁸ metabolites, hormones, vitamins, cell-cell signaling molecules⁹⁻¹¹ and metals.¹²

Transcription factors can be classified into two distinct classes: the activator and the repressor. In the activation manner, prokaryotic transcription factors are fused to a transactivation domain *Herpes simplex* virus virion protein 16 (VP16),¹³ forming a functional mammalian chimeric transcription factor. It acts as a transcription activator of target genes by binding to the operator of the promoter. The DNA binding activity of the chimeric transcription factor can be activated or repressed by signals according to different allosteric shifts, therefore forming an ON type or an OFF type system (**Fig. 1A**). In the repression manner, prokaryotic regulators are fused to transcription-silencing domain Krueppel associated box (KRAB) to repress the transcription of target genes when bound to operators located in the vicinity of the promoter. The DNA binding of the chimeric transcription factor can be active or repressed by the signal according to different allosteric shifts, therefore constructing an OFF type or an ON type system (**Fig. 1B**).

An operator is a DNA sequence that the transcription factor can bind to, which will modulate the activity of the target gene. An operator can be composed of a single copy or multiple tandem copies of the minimal DNA-binding sequence and can be located either upstream and/or downstream of the promoter.

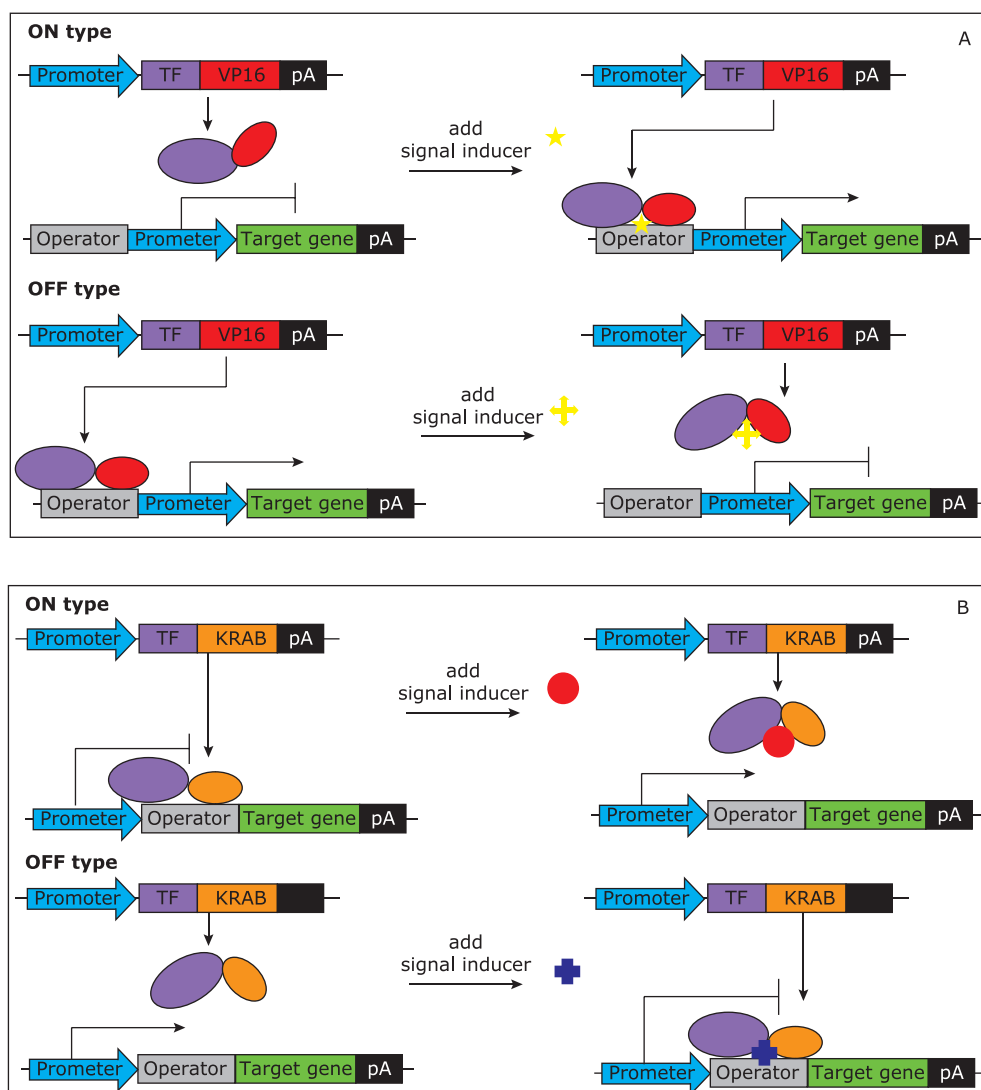


Figure 1. Design principles of synthetic biosensors.

A. Activation-based ON and OFF synthetic biosensor. A prokaryotic transcription factor is fused to a transactivation domain, Herpes simplex virus protein 16 (VP16), forming a functional mammalian chimeric transcription factor. It acts as a transcription activator of target genes by binding to the operator of the promoter. In an ON-type synthetic biosensor, the signal inducer triggers the binding of the chimeric transcription factor to the operator thus turn on the transcription of the target gene. In an OFF-type synthetic biosensor, the signal inducer represses the binding of the chimeric transcription factor to the operator thus turn off the transcription of the target gene.

B. Repression-based ON and OFF synthetic biosensor. A prokaryotic regulator is fused to transcription-silencing domain Krueppel associated box (KRAB) to repress the transcription of target genes when bound to operators located in the vicinity of the promoter. In an ON-type synthetic biosensor, the signal inducer represses the binding of chimeric transcription factor to the operator thus turn on the transcription of the target gene. In an OFF-type synthetic biosensor, the signal inducer triggers the binding of the chimeric transcription factor to the operator thus turn off the transcription of the target gene.

SIMPLE SYNTHETIC BIOSENSORS

Simple synthetic biosensors are the basic biosensor device composed of a signal sensing module and a responding module. The tetracycline (Tet) on/off synthetic biosensor is the most commonly used

one. It is derived from the prokaryotic tetracycline repressor (TetR) transcription system. TetR transcriptional repressors constitute one of the most abundant transcription family of prokaryotic regulators.^{3,12} It responds to antibiotic tetracycline and the homologous. In the absence of tetracycline, TetR forms a di-

mer that strongly binds to the TetR operator sequence (tetO). In the presence of tetracycline, the dimer is disrupted, TetR dissociates from the DNA, and gene expression is activated.

In a synthetic biosensor, TetR can be constructed as either a repressor, or converted into an activator by fusing it to the transactivation domain VP16. Single to multiple copies of tetO are placed upstream of the promoter, referred to as a Tetracycline Response Element or TRE. In a "Tet-Off" system, the reporter expression is activated when TetR is bound to the TRE, and becomes inactivated upon the addition of doxycycline which realizes the binding of TetR to TRE. In a "Tet-On" system, the TetR transcription factor is reversely fused to VP16, referred to as rtTA. In this fashion, the reporter expression is activated upon addition of doxycycline.¹⁴ These synthetic biosensors typically exhibit a large dynamic range (from 10 to several thousand-fold induction) and have been shown to function in a wide range of cell lines, including embryonic stem cells,^{15,16} CHO,¹⁷ HEK,¹⁸ HeLa¹⁹ and MCF-7²⁰ cells.

During the past decade, synthetic biosensors have been rapidly developed from simple control devices to multi-gene/protein-based transcription and signaling networks, from linear manner regulation to looped manner regulation. These developments not only broadened the application of biosensors, but also improved the performance of biosensors. Leakiness was the typical problem of the early generation biosensor. A series of studies focused on this issue by investigating different promoters, optimizing the number of operator modules, the relative spacing of the operator modules to the promoter, and the resulting torsion angle of the operator-bound transactivator in order to eliminate the leakiness. Side effects was another issue of the early generation biosensor because most trigger molecules, e.g. antibiotics, hormones, immunosuppressive drugs exhibit therapeutic side effects. To overcome this problem, adjustable transgene expression circuits controlled by endogenous physiologic metabolites, food derived compounds, vitamins, or pathologic signals have been gradually designed.²¹ With more regulation system identified, a series of synthetic biosensors responding to various signals have been built based on similar design principles of the Tet on/off system. **Table 1** summarizes detail characteristics of various simple synthetic biosensors.

COMPLEX SYNTHETIC BIOSENSORS

All of the simple synthetic biosensors mentioned above are minimal control devices that can only perform simple tasks involving one signal input. These simple control devices can be assembled into higher order gene circuits which can respond to multiple signal inputs and perform more sophisticated tasks. Multi-signal inputs with complex trigger controlled topology have been designed, including cascade biosensors, Boolean gate biosensors,²² time-delay biosensors,²³ hysteretic biosensors and oscillator biosensors.²⁴⁻²⁶

Cascade biosensors

A cascade synthetic biosensor consists of an artificial stepwise signal cascade whereby the transcription factor at the upper level controls expression of the transcription factor at the lower level that modulates transcription of the target gene at the lowest level.²⁷ Each transcription factor at different levels can be induced in an active or a repressive manner by specific signals, thus providing discrete multilevel control in response to different input signals. **Fig. 2A** illustrates a three-level cascade synthetic biosensor. At the first level, the TET-responsive promoter P_{TET} drives expression of the TET-triggered transactivator tTA and erythromycin (EM)-triggered transactivator, ET1. At the second level, ET1 activates the EM-responsive promoter P_{ERT} which controls transcription of the streptogramin-triggered transactivator, a pristinamycin I (PI)-VP16 fusion protein (PIT). At the third level, PIT activates the expression of the reporter, human placental secreted alkaline phosphatase (SEAP). This signaling cascade synthetic biosensor can be interrupted by TET, EM, or PI. They specially inactivate the transcription factor at each level, which are tTA, ET1, and PIT, respectively. The cascade bioreactor is the most basic complex biosensor. It is utilized to process entirely external signals to create the desired function in host by integrating a series of artificial synthetic networks to endogenous physiologic signals.

Boolean gate biosensors

Boolean gate biosensors consist of different compatible ON-type or OFF-type control devices interacting in a parallel or serial combination manner. It can respond to two different input signals (input A, input B) and the relationship of its inputs and outputs can be

Table 1. Characteristics of various simple synthetic biosensors

Classification of signal	On/Off	Signals	Regulation factor	Regulation factor fused domain	Chimeric regulation factor	Applications	Reference
Antibiotic							
	On and Off	Macrolide (erythromycin, clarithromycin, and roxithromycin)	MphR(A)	VP16 and KRAB	ET	Gene therapy, tissue engineering, in vivo gene function analyses, drug discovery, biopharmaceutical manufacturing	5
	On and Off	Pristinamycin I	Pip	VP16 and KRAB	PIT	Compatible with the Tet-OFF system, thus two different gene activities can be controlled in the same cell	6
Amino acid							
	On	L-arginine	ArgR	ART	VP16	Gene therapy and manufacturing of protein pharmaceuticals	7
	On	Tryptophan	TrpR	VP16	TRT	Targeted gene expression control	57
Small molecular							
	On and Off	Cumate	CymR	VP16	cTA, rcTA	Regulation of gene expression level and duration	58
Gas							
	On	Acetaldehyde	AlcR	-	AlcR	Therapeutic transgene dosing and biopharmaceutical manufacturing	59
	On	6-hydroxy-nicotine	HdnoR	VP16	-	Drug-responsive homologs in basic research, therapeutic cell engineering and biopharmaceutical manufacturing.	60
	On	pH/CO ₂	TDAG8	-	-	Remote control of cellular behavior inside microfluidic devices; CO ₂ -triggered production of biopharmaceuticals in standard bioreactors	61
Drug compound							
	On	2-phenylethyl-butyrate	EthR	VP16	-	Generic screening platform to discover drug candidates	62
Quorum sensing signal							
	On	Butyrolactones	ScbR SpbR	VP16	SCA, SPA	Clinical application	9
	On	3-oxo-C ₈ -HSL	TraR	NF-κB p65	p65NTraR	Versatile gene expression control	10
Metabolites							
	On	Uric acid	HucR	KRAB	mUTS	Self-sufficient control of pathologic metabolites, gene- and cell-based therapies	54
	On	Bile acids	TGR5	-	p65NTraR	Liver injuries protection	44
	On	Fatty acid	TtgR	-	LSR	Metabolic disorders treatment	63
Food additive							
	Off	Phloretin	TtgR	VP16	TtgA	Biopharmaceutical manufacturing, gene- and cell- based therapies	8
	On	Vanillic acid	MOR9-1	-	-	Cell differentiation	64
	On and Off	Vanillic acid	VanR	VP16 and KRAB	VanA ₁ and VanA ₄	Biopharmaceutical manufacturing, gene- and cell- based therapies	65

Continue to Table 1. Characteristics of various simple synthetic biosensors

Classification of signal	On/Off	Signals	Regulation factor	Regulation factor fused domain	Chimeric regulation factor	Applications	Reference
Allergic biomarkers							
	On	Histamine	HRH2	-	-	Allergy diagnostics	40
	On	Acrylate	AcuR	-	-	Investigate characteristics of inducible transcriptional regulators	66
	On	Glucarate	CdaR	-	-		
	On	Erythromycin	MphR	-	-		
	On	Naringenin	TtgR	-	-		
Vitamin							
	Off	Biotin	BirA	-	-	Cell therapy and biopharmaceutical manufacturing	67
	On	Biotin	BirA	VP16	BIT	Gene therapy, tissue engineering, biopharmaceutical manufacturing	68
Hormone	On and Off	Thyroid hormone	TSHR	-	TSR	Thyroid hormone regulation	52
Inflammation factor	On	NF-kB	NF-kB-responsive elements	-	-	Inflammation monitor	56

Note: MphR(A), erythromycin repressor; VP16, Herpes simplex virus virion protein 16; KRAB, Krueppel associated box; ET, erythromycin-dependent transactivator; Pip, pristinamycin-induced protein; PIT, Pristinamycin I-dependent transactivator; ArgR, arginine regulator; ART, arginine-regulated transgene; TrpR, L-tryptophan repressor; TRT, L-tryptophan-dependent transactivator CymR, cumate repressor; cTA, transactivator formed by the fusion of CymR with VP16; rcTA, reverse cumate activator; AlcR, acetaldehyde repressor; HdnR, 6HNic-responsive repressor; TDAG8, T cell death associated gene 8; EthR, Baeyer–Villiger monooxygenase repressor; ScbR, *Streptomyces coelicolor* quorum-sensing receptor; SCA, transactivator formed by the fusion of ScbR with VP16; SPA, *S. pristinaespiralis*-derived transactivator; SpbR, *S. pristinaespiralis* butyrolactone transcriptional repressor; HucR, bacterial transcriptional repressor that binds a DNA sequence motif (hucO); mUTS, mammalian urate-dependent transsilencer; TGR5 (GPBAR1), G protein-coupled bile acid receptor 1; LSR, intracellular lipid-sensing receptor; TtgR, naringenin repressor; TtgA, activator binds and activates transcription from chimeric promoter P_{TtgR1} harboring O_{TtgR} ; MOR9-1, vanillic acid-sensitive olfactory G protein-coupled receptor; VanR, vanillic acid-responsive transcriptional repressor; VanA, activator consisting of VanR fused to VP16; HRH2, the histamine receptor H2; AcuR, acrylate repressor; CdaR, glucarate repressor; MphR, erythromycin repressor; BirA, biotin ligase; BIT, biotin-dependent transactivator; TSHR, thyroid-stimulating hormone receptor; TSR, thyroid-sensing receptor; NF-kB, nuclear factor-k-gene binding.

described using the Boolean operator truth table.^{28,29} To date, almost all kinds of electronic Boolean gates topology can be realized in synthetic gene networks, including (i) AND gates which are activated when both input A and input B are present,³⁰ (ii) OR gates which are induced when either input A or input B are present, (iii) NOR gates which are repressed when either input A or input B are present, (iv) NOTIF gates which are exclusively induced if input A is present but NOT input B and (v) NAND gates which are always on unless input A and input B are present (**Fig. 2B**). These basic Boolean

gate biosensors can be further assembled to perform more complex tasks by varying the interconnectivity.³¹

Time-delay biosensors

In time-delayed biosensors, expression of the reporter can be controlled in a time delayed manner. When applying a small amount of signals to the system, persistence of the reporter can be observed even after removal of the signal. Time-delay biosensors are utilized to modulate essential natural biologic patterns, such as control of rhythmic gene expression,³² operation

of the circadian clock,³³ spacio-temporal coordination of cell differentiation and development, etc. **Fig. 2C** illustrates a time-delay biosensor.²³ The output signal is controlled by biotin in a time-delay manner. The TetR and VP16 domains are respectively fused to streptavidin (SA) and AviTag peptide (AT), forming two new transcriptional regulators: TetR-SA and AT-VP16. In the presence of the *Escherichia coli* biotin ligase BirA, biotin is able to bind to SA and AT, thereby reconstituting a functional heterodimerize transcription factor, TetR-SA-biotin-AT-VP16, activating the expression of the output signal SEAP. Since the heterodimerization is reversible, the transcription factor TetR-SA-biotin-AT-VP16 can retain its activation function after removal of the signal, biotin, thereby the expression of the downstream reporter SEAP exhibits a time-delay manner.

Oscillator biosensors

Oscillation is of prime importance in biology. In mammals, several processes including endocrine production and release, body temperature modulation, and immune responses that show circadian oscillatory behavior.^{34,35} The aim of oscillator biosensor is to achieve self-sustaining oscillation of reporter(s) between two separate states. Oscillator biosensors can dynamically control the reporter expression in a periodic pulsed manner without triggering the signal via utilization of different transcriptional activators and repressors. **Fig. 2D** illustrates a mammalian oscillator biosensor regulated by the tetracycline-dependent transactivator (tTA). It consists of three independent transcription modules: (i) a tTA controlled by a dual promotor system. In this module, the expression of tTA is triggered by the tetracycline-responsive promoter ($P_{hCMV^{*}-1}$) and inhibited by the streptogramin-responsive promoter (PPIR), (ii) a $P_{hCMV^{*}-1}$ -driven PIT (streptogramin-dependent transactivator) and (iii) a $P_{hCMV^{*}-1}$ -driven destabilized green fluorescent protein (dGFP). The tTA regulation module and the generate a autoregulated oscillating expression loop, therefore the dGFP level whose expression is triggered by tTA shows an oscillatory fashion.²⁴

Hysteretic biosensors

Classic synthetic biosensors provide a dose-dependent graded expression profile in response to signals. In hysteretic biosensors, the threshold required to switch from one state to another depends on the historical environment. Hysteretic biosensor can be utilized for

many important natural processes such as the cell cycle³⁶ and cell fate control.³⁷ It exhibits the following two characteristics: (i) Threshold levels for 'ON' and 'OFF' states depend on the starting state rather than the absolute concentration of the inducer. (ii) Different inducer concentrations are required for the system switching from OFF state to the ON state and from the ON state to the OFF state. **Fig. 2E** illustrates a typical hysteretic biosensor. It consists of a transactivator TetR-VP16 inducing the expression of reporter SEAP and a trans-silencer E-KRAB inhibiting the expression of reporter SEAP. TetR-VP16 and E-KRAB compete for binding to the same hybrid promoter P_{HYBRID} which drives the expression of the reporter SEAP and the transactivator TetR-VP16. E-KRAB is constitutively expressed, represses promoter P_{HYBRID} in an erythromycin (EM)-triggered manner. The expression pattern of reporter SEAP depends on the starting state of EM concentrations. High historical EM levels result in high TetR-VP16 levels that require greater E-KRAB activity and lower EM concentrations for an ON-to-OFF switch. Conversely, low historical EM concentrations result in minimal TetR-VP16 levels that require maximum inactivation E-KRAB, hence relatively much higher EM concentrations, for an OFF-to-ON switch.

APPLICATION OF BIOSENSORS IN DISEASE DETECTION

Application of synthetic biosensor as an alternative therapy to conventional pharmacotherapy is promising.³⁸ The basic principle is to utilize the biosensor as a sensor-actuator device which could monitor disease signals in vivo and make therapeutic responses automatically.³⁹ Here, we highlighted some synthetic biology strategies that have been developed to target allergy, liver injuries, insulin resistance, Graves' disease, urate homeostasis and inflammation.

Histamine-specific allergy

Histamine is the biochemical that is released as part of an allergic reaction, causing swelling, rashes and itching, sneezing, sickness, diarrhea, stomach pains and respiratory problems. Histamine mediates allergic symptoms by binding and activating a family of G protein-coupled receptors, histamine receptors.⁴⁰ Ausländer *et al.* constructed a synthetic allergy biosensor by rewiring histamine input to the production of reporter protein, thereby integrating histamine levels with re-

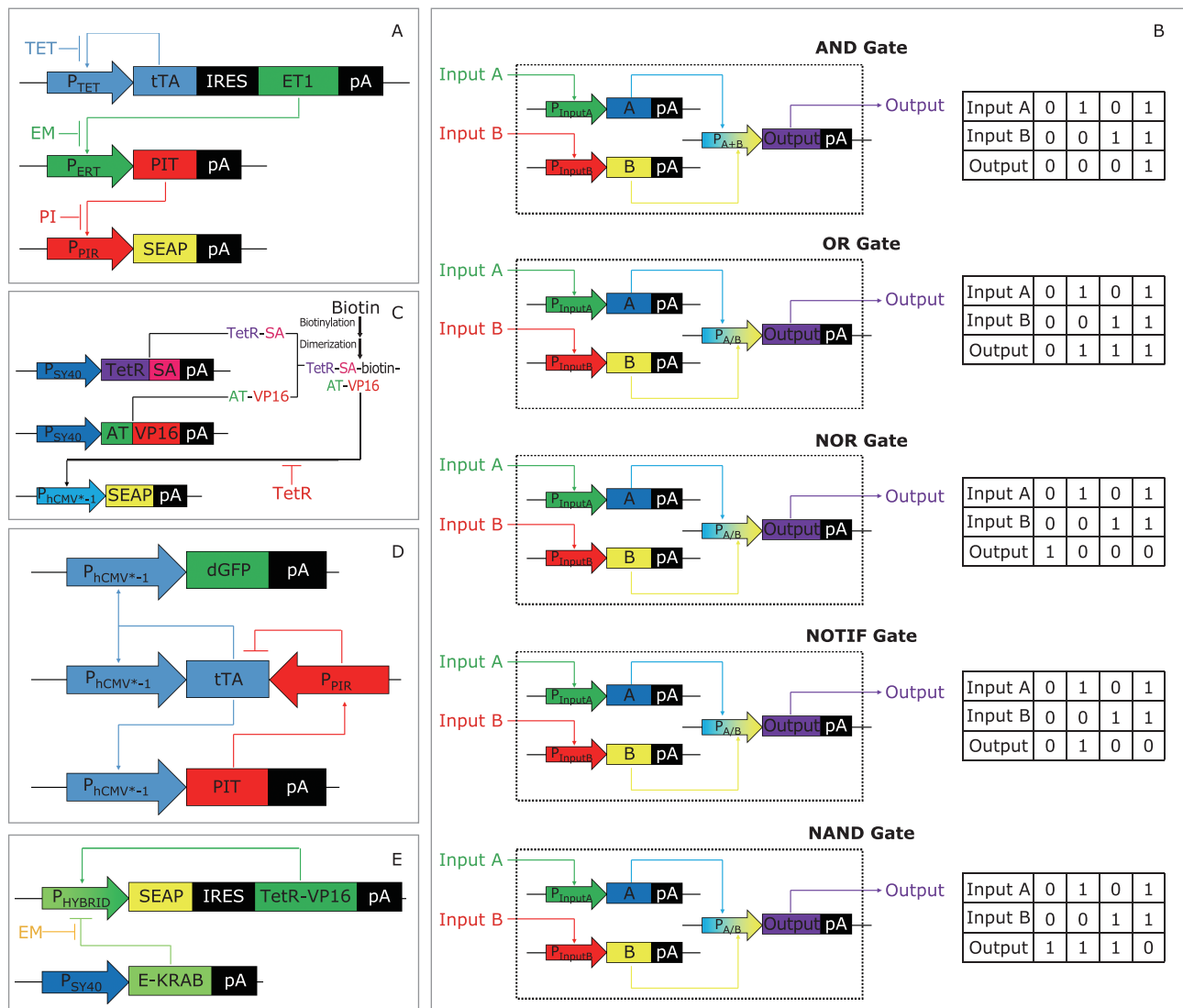


Figure 2. Schematic diagrams of five complex synthetic biosensors.

A. Cascade biosensor. The expression of the output reporter SEAP can be fine-tuned by a three level transcriptional gene circuit and can be interrupted by TET, EM, or PIP. pA, poly(A) tail; IRES, internal ribosome entry site. **B.** Boolean gate biosensors. P_{A+B} represents a promoter that can be triggered when both A and B are present. $P_{A/B}$ represents a promoter that can be triggered by A or by B. **C.** Time-delay biosensor. The expression of downstream reporter SEAP shows a time-delay manner after removal of biotin from the culture environment. **D.** Oscillator biosensor. The reporter dGFP level shows an oscillatory manner. **E.** Hysteretic biosensor. The expression of reporter SEAP exhibits a bistable hysteretic manner. Inducer EM required to switch the reporter from OFF state to ON state is higher than that required for ON to OFF switch.

markable sensitivity and a wide dynamic range.⁴⁰ This biosensor consists of a synthetic histamine-responsive signaling cascade in which the G protein-coupled receptor senses extracellular histamine levels and triggers G protein-mediated activation of adenylate cyclase, which in turn converts ATP to cyclic AMP (cAMP). This second messenger molecule binds regulatory subunits of protein kinase A, whose catalytic subunits translocate into the nucleus where they phosphorylate the cAMP-re-

sponsive binding protein, which binds and activates synthetic promoter P_{CRE} driving reporter gene expression.

Liver injury

Liver injuries impair the clearance of bile acids from the hepatic portal vein which leads to activation of the G protein-coupled bile acid receptor TGR5 and therefore initiation of a variety of hepatoprotective processes.⁴¹⁻⁴³ Peng *et al.* created a closed-loop bio-

sensor which could sense the excessive bile acid levels associated with liver injuries and automatically produce a therapeutic protein in response⁴⁴. They linked activation of ectopically expressed TGR5 to an artificial promoter, P_{CRE} , which controls transcription of the hepatocyte growth factor in a self-sufficient, reversible and dose-dependent manner.

Insulin resistance

Obesity-induced insulin resistance is the key etiologic defect of the metabolic syndrome, a cluster of clinical findings including hypertension and dyslipidaemia, increasing the imminence of cardiovascular disorders and type 2 diabetes.^{45,46} Ye *et al.* designed an insulin biosensor aiming to combat the insulin resistance.⁴⁷ This biosensor consists of an insulin receptor which senses the insulin level and a reporter gene driven by transcription factor TetR-ELK1.^{48,49} It was designed by rewiring the insulin level to the native IRS-1-Ras-MAPK pathway, activation of which phosphorylates TetR-ELK1, thus triggers transcription from synthetic promoters containing multiple TetR-ELK1-binding sites.^{49,50}

Graves' disease

Graves' disease is caused by autoantibodies that activate the thyroid-stimulating hormone (TSH) receptor and trigger a chronic increase in thyroid hormone levels.⁵¹ Saxena *et al.* designed a biosensor to sense thyroid hormone levels and produce an engineered variant of the human TSH, thereby restore the thyrotrophic feedback control of the hypothalamus–pituitary–thyroid axis.⁵² This biosensor consists of a hormone-sensing receptor (TSR) which precisely monitors thyroid hormone levels and a responding module which regulates the activation of the thyroid hormone receptor. In the absence of the thyroid hormones, TSR is supposed to bind to its cognate promoter and repress gene expression by recruiting endogenous corepressors so that to trigger histone deacetylation and inhibit gene repression. In the presence of thyroid, TSR interacted with coactivators that trigger histone acetylation and mediate gene expression.

Urate homeostasis

Urate is the end-product of purine metabolism. Elevated uric acid levels are associated with gouty arthritis.⁵³ Kemmer *et al.* engineered a synthetic biosensor to restore uric acid homeostasis in the blood by sensing and

responding to uric acid level.⁵⁴ The detection module of the biosensor is a bacterial transcriptional repressor HucR that binds a DNA sequence motif *hucO* in the absence of uric acid. The signaling module is a secretion-engineered *Aspergillus flavus* urate oxidase regulated by *hucO*. When uric acid is present, HucR dissociates from DNA, thereby allowing expression of a downstream gene, urate oxidase that converts uric acid to allantoin, thereby restoring uric acid homeostasis in the blood.

Inflammation

The design principle of the inflammation biosensor is converting the presence of proinflammatory cytokines to anti-inflammatory cytokines. Schukur *et al.* designed a biosensor which could sense the presence of tumor necrosis factor (TNF) and interleukin 22 (IL22) levels and produce the anti-inflammatory cytokines IL4 and IL10 with AND-gate-expression logic. This biosensor is applied as an antipsoriatic cytokine converter.⁵⁵ Based on the same principle, Smole *et al.* also created an inflammation biosensor comprising a sensor, an amplifier and an effector. This biosensor could be autonomously activated by inflammatory signals and reset externally by a chemical signal.⁵⁶

FUTURE PERSPECTIVE AND CHALLENGES

Synthetic biology has showed great potential in disease detection due to its compatibility, orthogonality and fine-tuning ability. Biomedical and clinical applications will necessitate more complex synthetic circuits and construct to perform sophisticated tasks. Although a variety of regulation systems have been identified, most of them are from microbes. Signals associated with clinical diseases are still limited. Therefore, more efforts should be made in identifying and constructing clinically relevant regulation systems. Moreover, as the complexity of a synthetic biosensor increases, computational tools need to be developed to aid the design process in order to guarantee the precision of the synthetic biosensor. The ultimate goal of a sophisticated synthetic biosensor is to play as a therapeutic sensor–effector device that connects diagnostic input with therapeutic output and therefore provides all-in-one diagnostic and therapeutic solutions for future gene- and cell-based therapies.

Conflict of interests statement

All authors declared no conflict of interests.

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