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Mining and design of biosensors for engineering microbial cell factory

Lei Qin[1,2], Xia Liu[3], Ke Xu[4] and Chun Li[1,2,3]

Improving the yield of target product is the purpose in the construction and optimization of microbial cell factory. Conferring biosensor-based intelligence on the strains is an important way to solve the engineering problems such as imbalance of metabolic flux and accumulation of toxic intermediates. In recent years, genetically encoded biosensors for both metabolites and environmental signals are rapidly created and developed via rational design, directed evolution or machine learning. In addition to being a rapid detection method for high-throughput screening, biosensors are more and more applied for directed evolution of strains and dynamic regulation of metabolic pathway. Biosensor-based intelligence will play more important role in the construction of microbial cell factory.

Addresses
[1] Key Lab for Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing, 100084, PR China

[2] Center for Synthetic & Systems Biology, Department of Chemical Engineering, Tsinghua University, Beijing, 100084, PR China
[3] Key Laboratory of Medical Molecule Science and Pharmaceutics Engineering, Ministry of Industry and Information Technology, Institute of Biochemical Engineering, School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing, 100081, PR China
[4] Tangshan Key Laboratory of Agricultural Pathogenic Fungi and Toxins, Department of Life Science, Tangshan Normal University, Tangshan, 063000, PR China

Corresponding author: Li, Chun (lichun@tsinghua.edu.cn)

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Introduction

Genetically encoded biosensor in synthetic biology refers to a biological element that can convert some chemical or physical signals (inputs) to directly measurable outputs, such as fluorescence or gene expression. One of the most common scenarios for biosensor is the fast detection of target products, which is usually accompanied with the high-throughput screening process, such as droplet microfluidics, to select high production strains [1–3] and to greatly improve the engineering efficiency of microbial cell factory compared to the long and costly chromatograph methods. Although a LC–MS method for rapid product detection has been developed, it is still limited by high cost for widely application [4].

Another application of biosensor is the dynamic regulation of microbial cell factory. Maximizing the yield of target product is the primary goal in the construction and optimization of microbial cell factory. The enhancement of target synthesis pathway often encounters problems such as low pathway flux, imbalance of metabolic flux or accumulation of toxic intermediates, which inhibit production or cell growth. Improving the intelligence of the microbial cell factory is an important way to solve the above problems, and biosensor is the basis of strains intelligence. Through autonomous regulation of transcription, translation or protein level of microorganisms under the control of sensing specific chemicals or signals, metabolic flow can be radically altered and production can be greatly improved [5,6].

Herein, we reviewed the recent advances in the engineering of biosensors for improving the performance and the application in dynamic regulation and directed evolution of microbial cell factory (Figure 1).

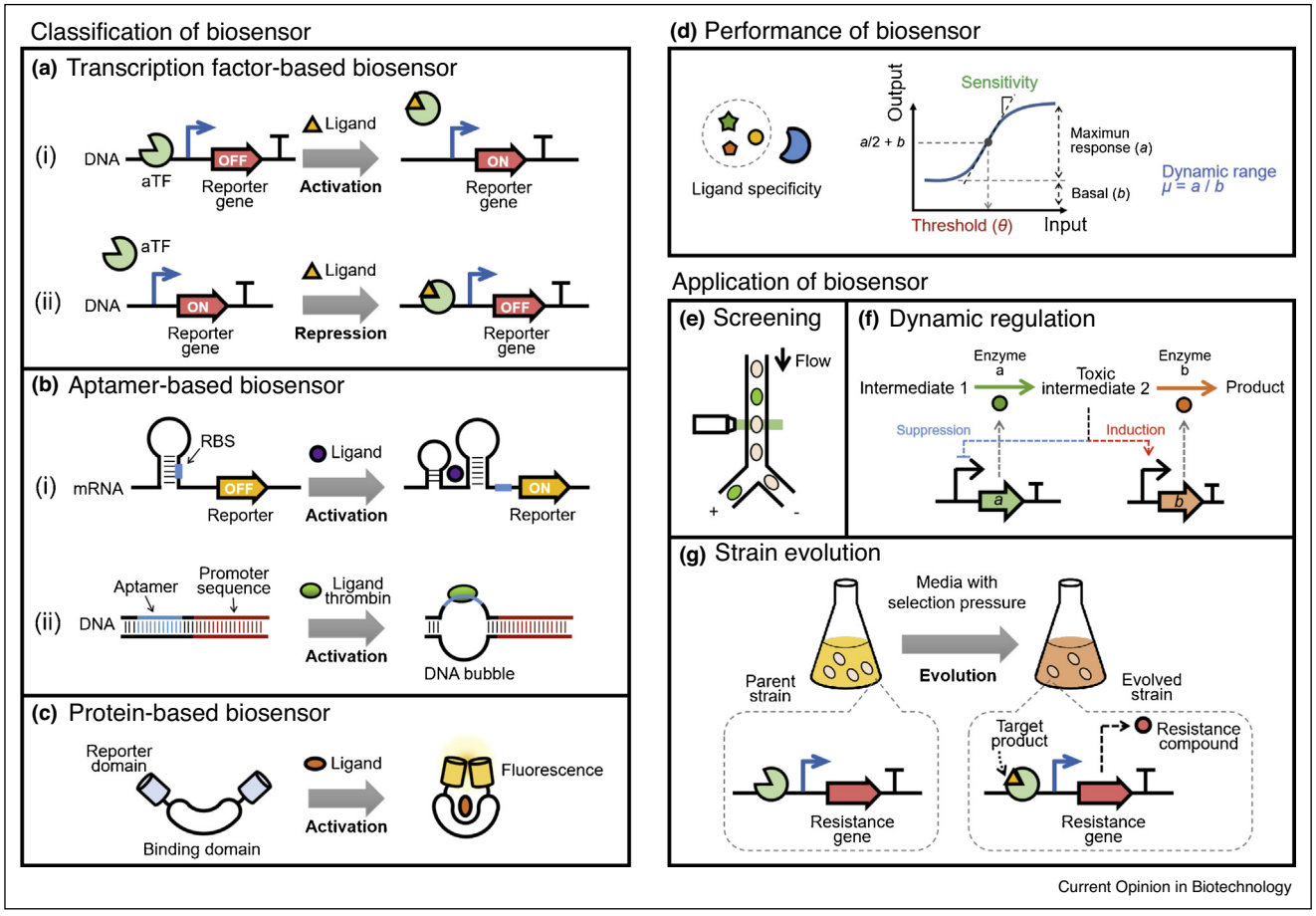
Mining and engineering of biosensors

There are mainly three types of biosensors according to mechanism: allosteric transcription factor (aTF)-based biosensor, aptamer-based biosensor and protein-based biosensor (Figure 1a–c and Table 1) [5,6]. The engineering of biosensor is always essential to improve the detection performance, because biosensors are generally excavated from the original species and transplanted into the target species or *de novo* designed artificially. Some important factors determine the effectiveness and applicability of a biosensor, including the ligand specificity, dynamic range, response threshold, and sensitivity, and so on (Figure 1d) [7].

aTF-based biosensors

aTF-based biosensor, as the type of the largest number and widest application, functions depending on the sensing module for the interaction between the metabolite and the TF, and the regulation module where the TF controls the expression of a target gene. Biosensing for small molecule metabolites has been found in nature for decades [8]. The list of compounds for which biosensors have been developed is growing rapidly, including

Figure 1



Classification, performance and application of genetically encoded biosensors.

precursors of biosynthetic pathways and secondary metabolites. Researchers have systematically sorted out the aTFs that can specifically interact with target metabolites. Through mining a series of aTFs requiring the participation of ligands (small molecule metabolites) and their promoters in the database, 15 metabolite-induced biosensors were obtained, and the orthogonality between each other was verified [9••]. Whereas, appropriate aTF cannot be found for all compounds in nature. Directed evolution and rational design of the aTF binding pocket are effective approaches to expand scope of responsive ligands, change the ligand specificity and enhance dynamic range [10,11]. For example, P_{hucR} is a uric acid inducible promoter found in *Deinococcus radiodurans*. Uric acid can bind to HucR protein and promote P_{hucR} transcription. Through saturation mutagenesis of the key sites of HucR, a mutant HucR-V7 was obtain which realized the induction by vanillin and ferulic acid [12•]. In addition, some TFs have promiscuous ligands that causing signal interference [13]. LysG is an aTF binding to both L-lysine, L-histidine and L-arginine discovered from *Corynebacterium glutamicum*. To narrow the ligand specificity, LysG was engineered semi-rationally toward no binding of L-lysine, while maintaining its L-histidine-binding and L-arginine-binding capabilities by molecular dynamics simulations [14].

Besides protein engineering to modify the binding kinetics between the metabolite and aTF, promoter engineering to modify the transcriptional activity can also improve the biosensor performance [15,16]. The rational design of TF binding site (TFBS) position and number [17•,18], and optimization of the interval spacing [19] were fruitful on tuning the dynamic range, signal strength and sensitivity of biosensor signals. In many cases, TFBS was arbitrarily inserted in the unannotated position of promoter due to the lack of explicit mechanism of aTF:DNA binding affinity, which may result in the ignoration of some efficient sites. Therefore, the scanning of TFBS positions at single-nucleotide resolution was shown

Table 1

Examples of recently developed biosensors

Biosensor	Input	Chassis	Biological effects	Refs
aTF-based biosensor				
EryD/P_{DBS}	Erythritol	*E. coli*	High-throughput screening for erythritol producing *Yarrowia lipolytica*	[3]
OapR/$P_{H16\text{-}RS01330}$	β-alanine	*E. coli* and *P. putida*	The addition of β-alanine resulted in up to 40-fold increase in fluorescence output	[9••]
MphR/P_{mhpR}	Clarithromycin	*E. coli*	Directed evolution of MphR led to specificity to clarithromycin	[10]
HucR-V7/P_{hucR}	Vanillin and ferulic acid	*E. coli*	Response to ferulic acid and vanillin in different dynamic ranges	[12•]
Saro_0803/P_{nov1}	Stilbenes and cannabinoids	*E. coli*	Detection of stilbenes with resorcinol groups, and cannabidiolic acid with a β-resorcylic acid functional group	[13]
LysG/P_{lysE}	L-lysine, L-histidine, and L-arginine	*E. coli*	LysG mutant used to isolate L-histidine-producing strains by fluorescence activated cell sorting (FACS)	[14]
PadR/P_{padC}	*p*-coumaric acid	*E. coli*	PadR mutant exhibited increased dynamic range and sensitivity	[16]
PdhR/P_{43}	Pyruvate	*B. subtilis*	Mutation of PdhR-binding site improved dynamic range	[17•]
MalR/P_{simh}	Malate	*Bacillus licheniformis*	Output was amplified by introducing a strong constitutive promoter and iteratively tuning the action sites	[18]
MarR/P_{marO}	Salicylic acid	*E. coli*	Hybrid promoter exhibited improved responsive strengths and shifted dynamic ranges	[21]
BenM/$P_{CYC1\text{-}BenO}$	*cis,cis*-muconic acid	*S. cerevisiae*	Evolution of BenM changed ligand specificity and increased dynamic range	[23]
$P_{ZEV\text{-}AR}$	*β*-estradiol	*S. cerevisiae*	Artificial promoters were predicted via training an ensemble of convolutional neural networks	[25••]
FdeR/$P_{FdeO\text{-}TEF}$	Naringenin	*Yarrowia lipolytica*	The hybrid promoter was composed of FdeR binding site FdeO and the core promoter of TEF	[49••]
Aptamer-based biosensor				
p15-ribo727	Tryptophan	*E. coli*	165.9% improvement of tryptophan titer via fluorescence activated cell sorting	[2]
Thrombin-binding aptamer	Thrombin	*B. subtilis*	The expression level of egfp increased by 8.23-fold	[27]
Histamine riboswitch	Histamine	Artificial cell	A riboswitch isolated via SELEX	[28]
Adenine riboswitch	Adenine	*E. coli*	A riboswitch isolated via SELEX	[30]
Protein-based biosensor				
LHGFR	L-2-Hydroxyglutarate	*E. coli*	A FRET sensor constructed using L-2-hydroxyglutarate binding protein LhgR	[32]
HGPRT-GFP	5-phospho-α-D-ribose 1-diphosphate (PRPP)	*E. coli*	The ligand pocket can be redesigned to recognize D-erythrose 4-phosphate (E4P) and glycerate-3-phosphate	[33]
LOCKER	Artificial α-helix	*In vitro* and in yeast	*De novo* design of protein switches	[35••,36–38]

effective and necessary to improve the response of the biosensor [20•]. aTF-based biosensor can usually function in 'plug-and-play' manner and in cross-species manner due to its mechanism. Constructing hybrid promoters by placing TFBS into constitutive strong promoter can significantly increase signal strength [16,21]. Besides, aTF-based biosensors in prokaryotes can be transferred into eukaryotes by inserting TFBSs in eukaryotic promoters [22•] followed by the evolution of aTF [23].

At present, it is still difficult to one-time successfully rationally design the promoter depending on experts' experience, as the specific molecular mechanism of biosensor is intricate. Iterative optimization is usually needed to improve biosensor performance, which enlarged research period. With the development of artificial intelligence and big data, machine learning (ML), as a separate data-driven approach, can fast predict and design promoter sequences without the requirement of detailed mechanistic information. Through the ML of promoter sequence, both prokaryotic and eukaryotic promoters have been well-predicted, and unnatural sequence with higher activity can be design [24,25••]. Researchers profiled the expression dynamics of over 8000 rationally designed, IPTG-inducible promoters in *Escherichia coli* and fit a statistical mechanics model to predict the gene expression and optimized the promoter response [19]. Meanwhile, ML of eukaryotic promoters is quite more difficult than prokaryotic ones due to the very long sequence and unclear motif function. To solve this problem, artificial promoters were designed by fixing conserve motifs (ZEV, a TFBS responding to β-estradiol, TATA box and TSS) and other sequence was randomly synthetized to create a library consisting of 327 thousand

promoter sequences in yeast. By training the dataset using convolutional neural networks, high predictive accuracy on sequence-activity was achieved, and higher-responsive unnatural promoters can be generated [25••].

Aptamer-based biosensors

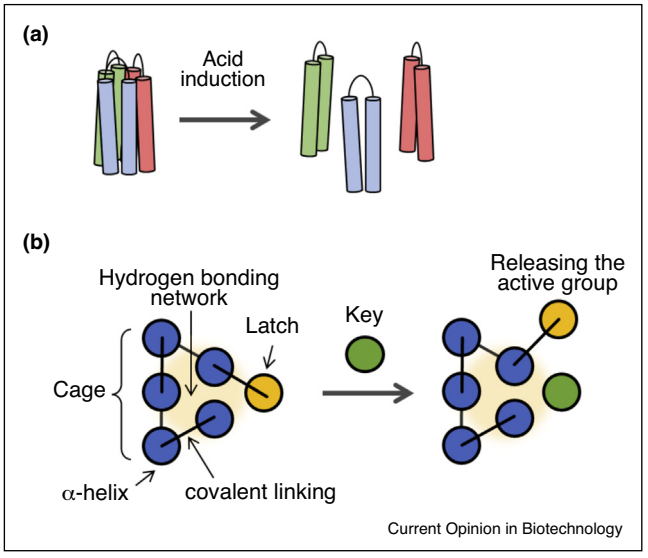
Aptamer-based biosensor functions by changing nucleic acid (DNA or RNA) secondary structure through the interaction between ligand and nucleic acid sequence. RNA aptamer biosensor, namely riboswitch, kinetically controls translation initiation through conformation change with the present of ligand (Figure 1b) [26]. Researchers developed an aptamer-based biosensor that can dynamically upregulate and downregulate the expression of target genes in response to the ligand at transcriptional and translational levels, respectively. Thrombin can bind to the corresponding DNA aptamer sequence and induce unwinding of the complementary DNA strand positioned upstream of promoter to increase the expression of downstream gene (Figure 1b). On the contrary, when thrombin-bound aptamer was inserted upstream of ribosome binding site (RBS), the translation process was inhibited by steric hindrance [27]. The method of mining and engineering riboswitches for chemicals called SELEX or the modified Capture-SELEX, an iterative process where specific binding RNA molecules can be selected from a very extensive library [28–30].

Protein-based biosensors

The allosteric regulatory function of proteins widely exists in nature. These proteins bind to the ligand causing changes in protein fluorescence in various manners [31]. The ligand binding domain can be design to sense different small molecules [32,33].

In addition to natural protein-based biosensors, David Baker' group designed a series of protein regulatory elements *de novo* based on α-helix structure. The specific heterodimer or homotrimer of α-helix can be protonated and depolymerize under acidic conditions, and then destroyed phospholipid membrane, which formed an acid-induced system (Figure 2a) [34]. Protein switches were also designed based on the homotrimer of α-helix: the addition of a 'key' protein released the 'latch' in the 'cage' (Figure 2b). By replacing the 'latch' with a degron (which can be recognized by ubiquitin protein and degraded), the target protein fused with the switch can be induced to degrade. Cas protein and signal transduction pathway were regulated by this protein switch [35••,36,37]. Because of the orthogonality between the heterodimers, various protein-based logic gates were designed [38]. The functions of these logic gates are rarely affected by the host species, which greatly enriches the available elements of synthetic biology.

Figure 2

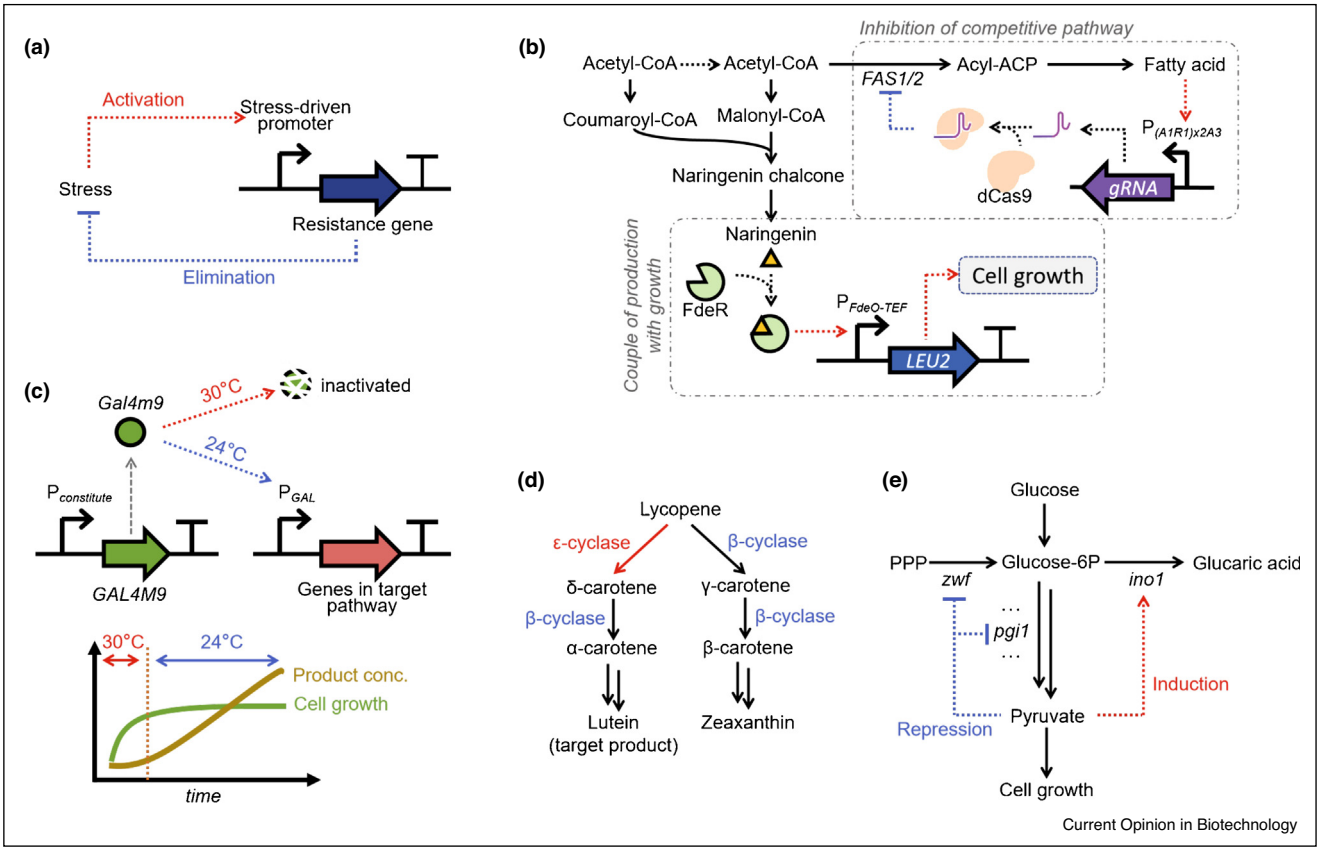


De novo design of regulatory proteins. **(a)** Acid-induced depolymerization of α-helix heterodimer; **(b)** Protein switch system.

Engineering of biosensors for environmental stress and signals

There are various stress factors in the industrial fermentation environment, such as high temperature, oxidation, and pH fluctuation, and so on, which will affect the growth and production of microorganisms [39]. Strictly controlling the industry fermentation conditions can maintain production stability, whereas it will also increase the costs including the energy consumption and the use of cooling water and acid/base. Therefore, improving the intelligence and robustness of industrial strains to environment stress is the fundamental solution to improve production and reduce cost. To improve yeast tolerance to high temperature, heat-inducible promoters were mined from transcriptome and were used to strengthen glutathione synthesis pathway. As glutathione can eliminate the inducer stress (reactive oxygen species), a dynamic feedback regulation system was built (Figure 3a) [40•]. Similarly, a genetic pH shooting (GPS) system was constructed to strengthen the self-pH-adjustment ability of *E. coli*. The system consisted of a glutaminase gene *glsA* (catalyzing glutamine to produce ammonia) under the control of the acid-induced promoter P_{asr} and a lactate dehydrogenase gene *ldhA* (catalyzing the production of lactic acid) under the control of the base-induced promoter P_{atpB} from *C. glutamicum*. This system has been shown to significantly improve cell growth and lycopene production while reducing the acid/base usage in the fermenter by stabilizing the environmental pH [41,42]. Some intracellular stresses often cause protein unstable. A biosensor detecting protein stability was developed. The target protein is

Figure 3



Dynamic regulation of microbial cell factory based on biosensor. **(a)** Stress-driven feedback regulation of resistance genes to eliminate stress; **(b)** Coupling metabolic addiction with negative autoregulation to improve pathway yield; **(c)** Temperature-induced two-stage fermentation process; **(d)** Sequential expression of genes to obtain the target product based on the temperature-induced biosensor; **(e)** Regulation of metabolic flow using the pyruvate-responsive biosensor.

inserted into the permissive site within the $CysG^A$ biosensor. If the target protein is stable, $CysG^A$ reassembles into its functional conformation and catalyzes formation of red fluorescent trimethylpyrrocorphin and sirohydrochlorin. In contrast, an unstable protein is prone to aggregation or proteolysis, resulting in elimination of functional $CysG^A$ and therefore weak or no fluorescence [43].

Environmental signals also act as more favorable inducers to regulate metabolism, instead of adding chemicals during fermentation [44]. A cold-inducible biosensor was developed in *E. coli* through directed evolution of transcription inhibitor cI434 being low temperature-inactivated and protease from tobacco etch virus being high temperature-inactivated [45•]. Similarly, protein Gal4 from *Saccharomyces cerevisiae* was evolved to obtain a temperature-sensitive mutant Gal4m9, which lost the function on inducing GAL promoter at 30°C, while restored the function at 24°C [46]. Using this temperature-responsive regulation system, the decouple of production from cell growth was achieved that enhanced the production of carotenoids [47].

Application of biosensors on directed evolution and dynamic regulation of microbial cell factory

Biosensor not only facilitated high-throughput screening but also enabled the directed evolution of microbial cell factory. One strategy of metabolic regulation is to couple growth and production, also called metabolic addiction [48,49••]. This mechanism aims to reward high-yield strains in a mixed cell population, in which only high-yield strains can survive, so as to eliminate low-yield strains and to screen out high-yield strains. For example, using a 3-hydroxypropionic acid (3-HP) biosensor, the production of 3-HP was converted to antibiotic resistance. Through adaptive laboratory evolution in antibiotic with increasing concentrations, high-yield strains were enriched and screened (Figure 1g) [50•]. Similarly, a

naringenin biosensor induced the expression of essential gene *LEU2*, which coupled naringenin production with cell growth (Figure 3b) [49••]. Yeast pleiotropic drug resistance (PDR) promoter can respond to abundant of natural products and xenobiotics. Researchers designed a PDR promoter-based biosensor and related the PDR response to the cell growth. Through screening of the yeast deletion library, key genes that affect the transcriptional induction of PDR transporters were identified [51]. Researchers constructed an acid induced cell death system. Acid induced-promoter P_{asr} was used to regulate the expression of toxic protein doc. By optimizing the promoter strength, the cells grew stably at pH = 7 and stopped growing at pH = 5 [52]. Therefore, strains producing some chemicals with specific pH values can be screened.

For many intracellular products, the number of intracellular products is directly proportional to the cell density, so the accumulation of biomass is important. The dynamic two-stage fermentation allowed cell growing to a high level first and then initiated product accumulation (Figure 3c) [47]. This two-stage strategy was also suitable for the toxic products [12•]. The temporal expression of genes is also a solution to avoid competitive pathway and to accumulate the target product. During construction of lutein-producing yeast, α-carotene formation through asymmetric ε- and β-cyclization of lycopene was found as the main limiting step, attributed to intrapathway competition of the cyclases for lycopene, forming β-carotene instead. To avoid β-carotene producing, temperature-responsive expression of β-cyclase was coupled to constitutive expression of ε-cyclase for flux redirection to α-carotene by allowing ε-cyclization to occur first (Figure 3d) [53•].

In regulation of metabolic flux, biosensor plays a key role in regulating cellular feedback control circuits [54,55]. Xylonic acid is a toxic intermediate metabolite in the synthesis of 1,2,4-butanol from xylose. The acid-induced promoter P_{cadBA} and transcription factor LacI form a 'NOR' gate to regulate xylonic acid synthesis genes *xdh* and *xylC*. The increase of xylonic acid concentration will induce P_{cadBA}, thereby inhibiting the expression of xylonic acid synthesis genes and forming feedback inhibition [56]. The researchers also discovered the xylonic acid-induced promoter P_{yjhI}, which was used to induce the expression of the genes of the downstream pathway of xylonic acid [57]. These two regulations reduced the accumulation of xylonic acid and increased the yield of 1,2,4-butanol. Another study created a set of programmable and bifunctional pyruvate-responsive genetic circuits for dynamic dual control of central metabolism in *Bacillus subtilis*. The competitive relationship between the central carbon metabolism pathway and glucaric acid synthesis pathway is one of the bottlenecks for the synthesis of glucaric acid. Using the pyruvate-activated promoters to enhance expression of the *ino1* gene involving in glucaric acid synthesis, and the pyruvate-inhibited promoter to repress the expression of the genes *pgi* and *zwf*, which are involved in glycolysis and the pentose phosphate pathway, respectively, the production of glucaric acid was greatly improved (Figure 3e) [17•].

Conclusions and perspectives

Biosensor-based intelligent biological manufacturing has gradually attracted attention, which will play a revolutionary role in the industrial fermentation process. A variety of biosensors have been developed to respond to different signals. The ligand specificity, dynamic range and sensitivity are important criteria to evaluate whether it can be applied to practical production. Some important breakthroughs have been made in the design of biosensors, including machine learning on DNA sequence design and the *de novo* design of regulatory proteins. The computer-aided biological design automation of biosensor will be a direction of further development. For the dynamic regulation of metabolism, the biosensor-based autonomous dynamic regulation played important roles in trade-off between growth and production and increasing the yield of fermentation. For the biosensor-based high-throughput screening and directed evolution, biosensor played an important role in signal transformation. The specificity of biosensors is critical to avoid interference of structural analogues in screening and evolution process. Conclusively, developing biosensors for a wider range of chemicals and environmental signals and improving biosensor performance are significant to the construction of microbial cell factory.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Lei Qin: Writing – original draft, Visualization. **Xia Liu:** Investigation, Resources. **Ke Xu:** Writing – review & editing. **Chun Li:** Conceptualization, Supervision, Writing – review & editing.

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