Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Custom-made transcriptional biosensors for metabolic engineering

Mathilde Koch[1,4], Amir Pandi[1,4], Olivier Borkowski[2], Angelo Cardoso Batista[1] and Jean-Loup Faulon[1,2,3]

Transcriptional biosensors allow screening, selection, or dynamic regulation of metabolic pathways, and are, therefore, an enabling technology for faster prototyping of metabolic engineering and sustainable chemistry. Recent advances have been made, allowing for routine use of heterologous transcription factors, and new strategies such as chimeric protein design allow engineers to tap into the reservoir of metabolite-binding proteins. However, extending the sensing scope of biosensors is only the first step, and computational models can help in fine-tuning properties of biosensors for custom-made behavior. Moreover, metabolic engineering is bound to benefit from advances in cell-free expression systems, either for faster prototyping of biosensors or for whole-pathway optimization, making it both a means and an end in biosensor design.

Addresses
[1] Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France
[2] Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France
[3] SYNBIOCHEM Center, School of Chemistry, University of Manchester, Manchester, UK

Corresponding author: Faulon, Jean-Loup (jean-loup.faulon@inra.fr)
[4] Equal contributions.

Current Opinion in Biotechnology 2019, **59**:78–84

This review comes from a themed issue on **Tissue, cell and pathway engineering**

Edited by **Marjan De Mey** and **Eveline Peeters**

https://doi.org/10.1016/j.copbio.2019.02.016

Introduction

Metabolic engineering allows the production of value-added compounds from renewable sources, therefore, making it a key discipline for a greener and more sustainable chemistry. As the domain of synthetic biology has matured, numerous techniques have been developed and applied in metabolic engineering, allowing for cheaper and faster DNA synthesis, sequencing, and assembly. It is nowadays faster to design and build constructs than to characterize them as testing often involves expensive mass spectrometry analyses. This has led to an increased interest in biosensors, which can allow fast and real-time screening, selection, or dynamic regulation engineering of metabolic pathways. Cells harboring fluorescent proteins as the reporter of the biosensor allow screening of a huge number of variants, both for experimental growth conditions or genetic constructs (enzymes, RBS, promoters). Moreover, dynamic regulation can be used to monitor intermediates, final products, or quorum molecules, allowing for optimal pathway balancing and resource consumption.

The advantages of using biosensors in metabolic engineering have been extensively reviewed before [1–3] and will not be detailed further. Moreover, a wide array of techniques now exists to develop biosensors, from FRET [4] to riboswitches [5,6]: the interested reader is referred to those two excellent reviews that cover the strengths and limitations of the above-mentioned technologies [7•,8••]. In this review, we will focus on transcriptional biosensors in three different aspects. First, we will review techniques for discovery and engineering of transcriptional biosensors for new compounds, second, we will present how computer-assisted modeling can facilitate the tuning of biosensors for custom-made behavior, and third we will review the advances and advantages of using cell-free systems for biosensor characterization and metabolic engineering.

Designing a transcriptional biosensor to detect a compound of interest

Engineering allosteric transcription factors

The first step to engineer a biosensor, whether homologous or heterologous, is to identify the transcription factor (TF) and promoters that respond to it. Strategies involving transcriptional micro-arrays and identification of the upregulated or downregulated genes in response to the ligand of interest provide first leads. These approaches can suffer from important limitations for metabolic engineering use: the identified genes can be either indirectly regulated by the ligand of interest, or very unspecific. This strategy has been successfully applied for 1-butanol detection [9]. Another strategy for the identification of potential TF-promoter pair comes from Zhang *et al.* [10••] who identified pairs that could detect lactam derivatives: they used a chemo-informatics approach to reveal operons listed in BRENDA (Braunschweig Enzyme Database) [11] that detected similar chemicals, and identified the

gene likely coding the transcription factor. We recently published [12•] a dataset of detectable metabolites (Figure 1a). This dataset includes a manually curated list of experimentally validated detectable metabolites and information from databases of regulation, which contain known or putative detectable metabolites. Other strategies for mining parts have been discussed in a previous review [13].

Once a potential TF/promoter pair is identified, the bioengineering workflow involves modifying the promoter, RBS and binding sites to improve selectivity, dynamic, operational range, fold change and leakiness. A number of successful biosensors have been developed in recent years, including heterologous TF despite the challenges faced to adapt the transcriptional machinery. This technology is becoming increasingly mature, as shown by the numerous examples in Table 1. In addition, engineering of specific biosensors for Malonyl-CoA is reviewed by Johnson *et al.* [30], while Ambri [31] describes in details an implementation of bacterial TF in yeast. Voigt's group recently published an *Escherichia coli* strain containing twelve genomically integrated small molecule sensors, using a directed evolution strategy. It has been developed as a synthetic biology tool but the presented methods are applicable to metabolic engineering [32].

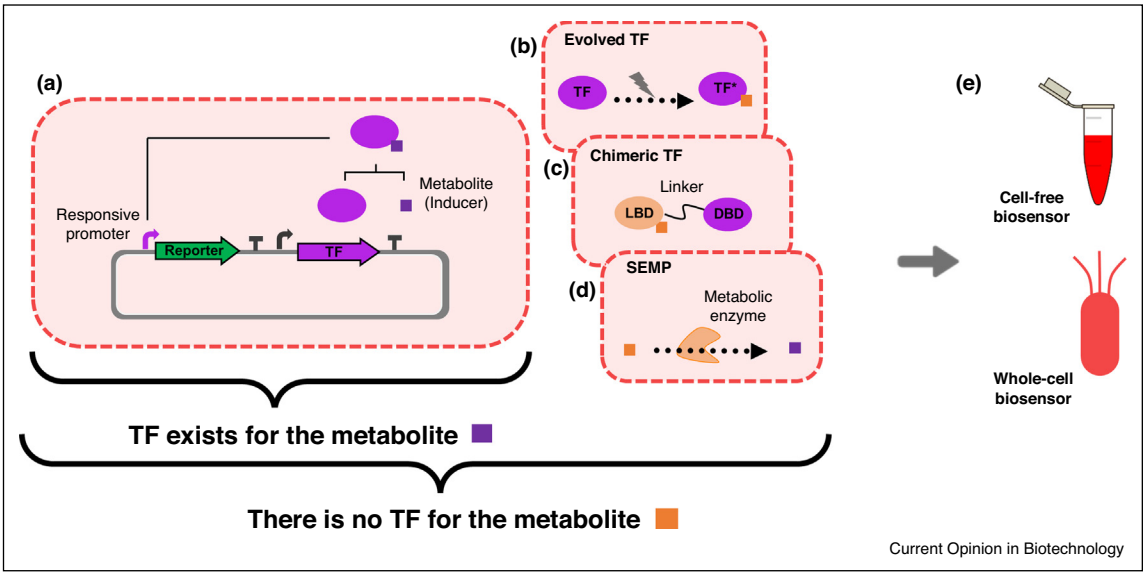
However, the above-mentioned strategies are only applicable if a natural transcription factor-biosensor pair exists for a given compound. We will now review strategies to extend the chemical scope of transcriptional biosensors.

Extending the chemical space for biosensors

A strategy to extend the chemical scope is to start from a known transcription factor and apply rounds of protein engineering to change its specificity (Figure 1b). For example, to design a biosensor for lactulose, LacI was altered using saturation mutagenesis, with rounds of selection to ensure specificity to lactulose [33]. Taylor *et al.* [34••] used computer-assisted protein design, followed by saturation or random mutagenesis to modify LacI to sense either fucose, gentiobiose, lactitol or sucralose. The promiscuous MphR transcription factor has been modified with a similar strategy to change its selectivity towards various macrolides [29]. Despite their successes, these examples still rely on well-known transcription factors and labor-intensive mutagenesis or computationally assisted protein design to change the specificity of a transcription factor to, still, a chemically similar molecule.

Several groups have tried radical approaches, fusing DNA binding domains (DBD) to determine ligand binding domains in different ways (Figure 1c). This strategy has been successfully applied to maltose [35] and benzoate [36] by testing various linkers and DBD systematically. Another strategy, also applied to maltose, was to randomly insert the DBD into the metabolite binding protein, using transposon insertion reaction, to select constructs presenting biosensor-like behavior [37•]. In a recent study [38•], the authors use a ligand-dependent stabilization strategy, fusing LacI (respectively MphR) to the Zif268 DBD and RNA polymerase ω-subunit

Figure 1



Different strategies to develop a TF based biosensor for a given metabolite. There is either an existing TF for a metabolite **(a)** or it could be engineered using evolved TF **(b)**, chimeric protein **(c)**, or a metabolic pathway (SEMP) **(d)**. A designed biosensor could be implemented in whole-cell or cell-free system **(e)**. Abbreviations: TF, transcription factor; LDB, ligand binding domain; DBD, DNA binding domain; SEMP, sensing-enabling metabolic pathways.

Table 1

Successful homologous and heterologous biosensor design based identified on transcription factor/promoter pairs

Compound	Original organism	Implementation organism	Design strategy	Biosensor application	Reference
Itaconic acid	*Yersinia pseudotuberculosis*	*Escherichia coli*	Identified TF and promoter from catabolism pathways	Used for enzyme improvement in pathway prototyping	[14]
Vanillin	*Escherichia coli*	*Escherichia coli*	Natural *E. coli* regulator tuned with mathematical modeling	Used for library screening	[15]
Syringaldehyde	*Escherichia coli*	*Escherichia coli*	Natural *E. coli* regulator tuned with mathematical modeling	Used for library screening	[15]
Muconic acid	*Acinetobacter* sp. ADP1	*Saccharomyces cerevisiae*	Identified from a previous publication	Used for selection of high producing strains	[16]
Pinocembrin	*Herbaspirillum seropedicae*	*Escherichia coli*	Tuned with the help of a mathematical model	Can be used for metabolic engineering	[17]
Pamamycin	*Streptomyces alboniger*	*Streptomyces alboniger*	Improved from native genetic elements	Can be used for metabolic engineering	[18]
p-coumaric acid	*Bacillus subtilis*	*Escherichia coli*	Identified from literature and library design of RBS to tune the repressor properties	Used for screening a producer strain in microfluidic droplets	[19]
Formaldehyde	*Escherichia coli*	*Escherichia coli*	Optimized from native regulatory elements.	Used to identify promising enzymes for methanol assimilation.	[20]
N-acetylneuraminic acid	*Escherichia coli*	*Escherichia coli*	Modularization of the native biosensing system	Used for screening high-producing strains	[21]
Putrescine	*Escherichia coli*	*Escherichia coli*	Modularization of the native biosensing system	Used for screening high-producing strains	[22]
L-phenylalanine	*Escherichia coli*	*Escherichia coli*	Modularization of the native biosensing system	Used for screening high-producing strains	[23]
Shikimic acid	*Corynebacterium glutamicum*	*Corynebacterium glutamicum*	Using the promoter from native genetic elements, considering the transcription factor to be naturally expressed	Used for screening high-producing strains	[24]
Cellobiose	*Thermobifida fusca*	*Escherichia coli*	Identified from literature and expressed in *E. coli*	Used to identify promising cellulases	[25]
Naringenin	*Herbaspirillum seropedicae*	*Escherichia coli*	Identified from literature, modularized and expressed in *E. coli*	Can be used for metabolic engineering	[26]
Naringenin	*Acinetobacter* sp. ADP1	*Saccharomyces cerevisiae*	Identified from literature, modularized and expressed in *E. coli*	Used for pathway prototyping - screening	[27]
Various aromatic blocks	*Sphingobium* sp. SYK-6	*Escherichia coli*	Identified from literature, modularized and expressed in *E. coli*	Used to screen for lignin degrading enzymes	[28]
Various macrolides	MphR, isolated from wastewater treatment plant	*Escherichia coli*	Directed evolution and random mutagenesis to improve selectivity	Can be used for metabolic engineering	[29]

transcription-activating domain. Those constructs are quickly degraded unless the ligand is present. The authors managed to engineer biosensors responding to IPTG and D-glucose with satisfying dose-response (respectively erythromycin with a modest response). However, to underline the difficulty of this approach, they report that in two structurally similar periplasmic binding proteins, a similar mutation did not confer ligand-dependent stabilization. Another similar approach was developed recently, it uses both ligand-dependent stabilization and protein dimerization: two ligand binding domains (that can homodimerize, but bind different ligands) are fused respectively to the activation domain and the DBD. Upon ligand binding, the two proteins are stable and can homodimerize, resulting in biosensing. This system allows for better range tuning and possible orthogonal biosensing of different ligands [39].

Other known metabolite responsive proteins are two-component systems, which have also been used as biosensors. By fusing the transmembrane sensing domain of another species detecting methanol with the cytoplasmic phosphorylation domain of *E. coli*, binding of methanol activates a phosphorylation cascade enabling biosensing [40]. In an elegant study, transmembrane and cytosolic receptors for caffeine were built by fusing single-domain antibodies to monomeric DBDs [41]. Different DBDs were used, proving the scalability of the method. These

two platforms should allow bioengineers to tap into the vast reservoir of two-component systems and antibodies to design new sensors.

A radically different approach to engineer the sensing scope of bacteria was coined Sensing-Enabling Metabolic Pathways (SEMP) (Figure 1d). The principle of this method is to metabolically convert an undetectable ligand into an already detectable one. This method makes the most of existing biosensors as well as of the impressive accumulated knowledge on metabolic reactions. It has been successfully applied in a metabolic engineering project to produce 3-hydroxypropionate [42], and its modularity was shown by Libis *et al.* [43]. A web-server is now available to design SEMP for compounds of interest [44].

Computer-assisted fine-tuning of biosensor properties

While the scientific community agrees that biosensors need to be fine-tuned for selectivity, sensitivity and dynamic range, tuning strategies are usually based on labor-intensive and costly rounds of selection and mutagenesis. Controlling those properties is especially interesting for metabolic engineering as the specifications of a biosensor needed during various stages of the process will change, from detecting micromolar amounts before pathway optimization to g/L titers in later development stages. Therefore, after engineering a biosensor with new specificity, its properties also need to be fine-tuned to match the metabolic engineer's needs.

A detailed mechanistic model of the ArsR arsenic biosensor was developed by Berset *et al.* [45], which recapitulates the sensor behavior under various circuit configurations, different ArsR alleles, promoter strengths, and presence or absence of arsenic efflux in the bioreporters. This model was then used to predict a circuit variant with steeper response at low arsenite concentrations. A thermodynamical model was developed in a recent study [46••], which was used to tune the dynamic range of ligand-inducible promoters (mainly AraC and LasR), using binding energies calculated for different promoter sequences. Both studies proved that with sufficiently detailed models, tailoring biosensor properties for custom-made behavior can be achieved. Another interesting study based on the Lac system and involving extensive phenomenological modeling sought to find theoretical constraints for biosensor design, notably a maximum achievable dynamic range and exposing tunable parameters for orthogonal control of dynamic range and response threshold [47]. As impressive as these studies are, they are based on well-characterized and known systems and such modeling cannot be applied easily to a new biosensor.

However, a simpler formalism (Michaelis–Menten) for mathematical modeling was used to tune a biosensor used for selection of lignin transforming enzymes, giving insights on parameters influencing sensitivity, such as TF concentrations or copy number [15]. The role of plasmid copy number on sensitivity and fold-change of a pinocembrin and naringenin biosensor was investigated through a mathematical modeling [17], using the common Hill framework, allowing for better understanding of the biosensor behavior and suggestions for further tuning of properties according to desired outputs. Landry *et al.* [48] used mathematical modeling with Hill formalism to tune the detection range of a two-component system. They successfully applied it to improve their detection threshold up to two orders of magnitude. These later studies showed that simple mathematical models can help to understand and tune specific properties of a biosensor, even in less known systems.

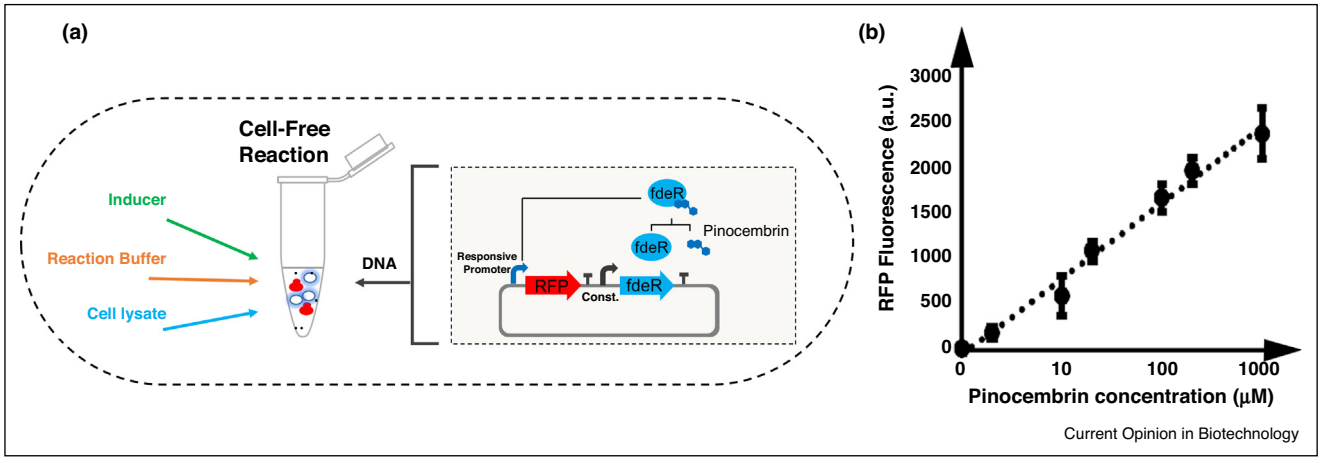
Computer-assisted design does not always yield the expected results, as current models are often more explicative a posteriori than predictive a priori. Therefore, we believe investing the time needed to develop reliable models for a library of constructs can only be worthwhile in the long run for designing biosensors, as formalized knowledge is more easily translatable to other situations.

Custom-made biosensors' new application domain: cell-free metabolic engineering

Despite the advances presented in this review, biosensor design still necessitates rounds of trial and error. This limitation can be significantly sped up by using cell-free systems (Figure 1e). Moreover, cell-free systems are poised to become a key characterization tool in the metabolic engineering workflow before *in vivo* implementation. Cell-free systems lead to quicker responses, simpler cloning and larger combinatorial libraries screening, without requiring transformation steps. These systems can also be an appropriate platform for production because of lower noise and toxicity and absence of resource competition between pathway and cell growth. To date, cell-free systems have been applied to implement pathways for violacein [49], 4-BDO [50], polyhydroxyalkanoates bioplastics [51], mevalonate [52], *n*-butanol [53] and raspberry ketone [54], using either transcription-translation (TX-TL) systems, overexpressed enzymes in the crude extract or purified enzymes. Advantages and possibilities of cell-free systems for metabolic engineering have been reviewed elsewhere [55], and a methods chapter for pathway prototyping in cell-free systems has recently been published [56].

Cell-free biosensors for various applications have been reviewed elsewhere [57] and we will focus on strategies applicable to metabolic engineering. In a recent study, a vanillin biosensor was developed in cell-free systems [58••]. The authors first used computational protein design and then rapid cell-free prototyping to develop

Figure 2



Pinocembrin cell-free biosensor. Cell-free reaction consists of TX-TL cell lysate, reaction buffer and DNA plus inducer for the biosensor **(a)**. **(b)** The graph shows a dose response RFP fluorescence after 9 hours incubation in a plate reader at 30°C. 40 nM of biosensor plasmid is added with 0, 1, 2, 10, 20, 100, 200, or 1000 μM of pinocembrin in 10.5 μl of cell-free reaction. RFP fluorescence points and error bars are the mean and standard deviation of three measurements.

a biosensor for this toxic effector, which was subsequently used in dynamic control loops *in vivo* to alleviate toxicity.

Another use of cell-free systems for biosensor engineering is discussed by Halleran *et al.* [59]: to develop complex consortia for pathway distribution among cell populations, metabolic engineers need reliable quorum sensing systems. This study characterized cross-talk between cell-free system and *in vivo* and discovered significant correlation between cell-free and *in vivo* measurements, validating the use of cell-free systems as a successful and fast characterization testbed. Recently, we have proposed a method to optimize the response of TF-based cell free biosensors. We also proved that SEMP are modularly implementable in cell-free systems, and exhibit high sensitivity, fast response times and broad dynamic range [60].

For this review, we implemented our *in vivo*-characterized pinocembrin biosensor [17] in a cell-free system (Figure 2a). The cell-free biosensor exhibited a linear correlation between input concentration and fluorescence intensity as well as a wider dynamic and operational range (Figure 2b) compared to its *in vivo* counterpart [17]. These tools could be used for real-time screening and speed up the design-build-test-learn workflow for metabolic engineering.

Cell-free systems provide fascinating new opportunities for metabolic engineering, not only for faster biosensor development, notably for toxic products, but also for prototyping whole pathways. Cell-free based metabolic engineering can benefit from all advantages of biosensor-based screening or dynamic regulation engineering, as does traditional metabolic engineering.

Conclusion

Thanks to extensive efforts by the research community, it has never been easier to develop transcriptional biosensors for new compounds, either from existing TF or engineering strategies. We believe the next frontier in custom-made biosensor design resides in efficient fine-tuning of properties, which is greatly advanced by modeling efforts. Moreover, metabolic engineering might be entering a new phase, with cell-free systems enabling faster prototyping of biosensors and even whole pathways. The current advances in biosensors for high-throughput screening will truly allow the field to move from the Design-Build-Test cycle to the Design-Build-Test-Learn cycle.

Conflict of interest statement

Nothing declared.

Acknowledgements

JLF acknowledges support from BBSRC/EPSRC (grant number BB/M017702/1). ACB and JLF also acknowledge funding provided by the ANR SINAPUV grant number ANR-17-CE07-0046. MK is supported by DGA (French Ministry of Defense) and Ecole Polytechnique and AP is supported by INRA (National Institute for Agricultural Research) and idEx interdisciplinary scholarship from Paris-Saclay. OB is supported by Genopole 'Allocation Recherche 2017' and CRI Paris 'Short-term Fellows'.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Liu D, Evans T, Zhang F: **Applications and advances of metabolite biosensors for metabolic engineering**. *Metab Eng* 2015, **31**:35-43.

2. de Frias UA, Pereira GKB, Guazzaroni M-E, Silva-Rocha R: **Boosting secondary metabolite production and discovery through the engineering of novel microbial biosensors**. *Biomed Res Int* 2018, **2018**:7021826.

3. Liu Y, Liu Y, Wang M: **Design, optimization and application of small molecule biosensor in metabolic engineering**. *Front Microbiol* 2017, **8**:2012.

4. Ameen S, Ahmad M, Mohsin M, Qureshi MI, Ibrahim MM, Abdin MZ, Ahmad A: **Designing, construction and characterization of genetically encoded FRET-based nanosensor for real time monitoring of lysine flux in living cells**. *J Nanobiotechnol* 2016, **14**:49.

5. Xiu Y, Jang S, Jones JA, Zill NA, Linhardt RJ, Yuan Q, Jung GY, Koffas MAG: **Naringenin-responsive riboswitch-based fluorescent biosensor module for *Escherichia coli* co-cultures**. *Biotechnol Bioeng* 2017, **114**:2235-2244.

6. Ruscito A, McConnell EM, Koudrina A, Velu R, Mattice C, Hunt V, McKeague M, DeRosa MC: ***In vitro* selection and characterization of DNA aptamers to a small molecule target**. *Curr Protoc Chem Biol* 2017, **9**:233-268.

7. • Carpenter AC, Paulsen IT, Williams TC: **Blueprints for biosensors: design, limitations, and applications**. *Genes* 2018, **9**.

A recent full review on all types of biosensors with detailed classifications and examples.

8. •• Rogers JK, Taylor ND, Church GM: **Biosensor-based engineering of biosynthetic pathways**. *Curr Opin Biotechnol* 2016, **42**:84-91.

A review on application of biosensors for metabolic engineering with a focus on screening and selection.

9. Shi S, Choi YW, Zhao H, Tan MH, Ang EL: **Discovery and engineering of a 1-butanol biosensor in *Saccharomyces cerevisiae***. *Bioresour Technol* 2017, **245**:1343-1351.

10. •• Zhang J, Barajas JF, Burdu M, Ruegg TL, Dias B, Keasling JD: **Development of a transcription factor-based lactam biosensor**. *ACS Synth Biol* 2017, **6**:439-445.

The authors present a method for identifying transcription factor/promoter pairs based on chemical similarity search in enzymatic databases.

11. Jeske L, Placzek S, Schomburg I, Chang A, Schomburg D: **BRENDA in 2019: a European ELIXIR core data resource**. *Nucleic Acids Res* 2019, **47**:D542-D549.

12. • Koch M, Pandi A, Delépine B, Faulon J-L: **A dataset of small molecules triggering transcriptional and translational cellular responses**. *Data Brief* 2018, **17**:1374-1378.

A dataset of detectable metabolites, consisting of manually extracted examples of biosensors in synthetic biology as well as gathered data from related databases.

13. Libis V, Delépine B, Faulon J-L: **Sensing new chemicals with bacterial transcription factors**. *Curr Opin Microbiol* 2016, **33**:105-112.

14. Hanko EKR, Minton NP, Malys N: **A transcription factor-based biosensor for detection of itaconic acid**. *ACS Synth Biol* 2018, **7**:1436-1446.

15. Ho JCH, Pawar SV, Hallam SJ, Yadav VG: **An improved whole-cell biosensor for the discovery of lignin-transforming enzymes in functional metagenomic screens**. *ACS Synth Biol* 2018, **7**:392-398.

16. Snoek T, Romero-Suarez D, Zhang J, Skjoedt ML, Sudarsan S, Jensen MK, Keasling JD: **An orthogonal and pH-tunable sensor-selector for muconic acid biosynthesis in yeast**. *ACS Synth Biol* 2017 http://dx.doi.org/10.1101/229922.

17. Trabelsi H, Koch M, Faulon J-L: **Building a minimal and generalizable model of transcription factor-based biosensors: showcasing flavonoids**. *Biotechnol Bioeng* 2018, **115**:2292-2304.

18. Rebets Y, Schmelz S, Gromyko O, Tistechok S, Petzke L, Scrima A, Luzhetskyy A: **Design, development and application of whole-cell based antibiotic-specific biosensor**. *Metab Eng* 2018, **47**:263-270.

19. Siedler S, Khatri NK, Zsohár A, Kjærbølling I, Vogt M, Hammar P, Nielsen CF, Marienhagen J, Sommer MOA, Joensson HN: **Development of a bacterial biosensor for rapid screening of yeast p-coumaric acid production**. *ACS Synth Biol* 2017, **6**:1860-1869.

20. Woolston BM, Roth T, Kohale I, Liu DR, Stephanopoulos G: **Development of a formaldehyde biosensor with application to synthetic methylotrophy**. *Biotechnol Bioeng* 2018, **115**:206-215.

21. Peters G, De Paepe B, De Wannemaeker L, Duchi D, Maertens J, Lammertyn J, De Mey M: **Development of N-acetylneuraminic acid responsive biosensors based on the transcriptional regulator NanR**. *Biotechnol Bioeng* 2018, **115**:1855-1865.

22. Chen X-F, Xia X-X, Lee SY, Qian Z-G: **Engineering tunable biosensors for monitoring putrescine in *Escherichia coli***. *Biotechnol Bioeng* 2018, **115**:1014-1027.

23. Liu Y, Zhuang Y, Ding D, Xu Y, Sun J, Zhang D: **Biosensor-based evolution and elucidation of a biosynthetic pathway in *Escherichia coli***. *ACS Synth Biol* 2017, **6**:837-848.

24. Liu C, Zhang B, Liu Y-M, Yang K-Q, Liu S-J: **New intracellular shikimic acid biosensor for monitoring shikimate synthesis in *Corynebacterium glutamicum***. *ACS Synth Biol* 2018, **7**:591-601.

25. Kwon KK, Yeom S-J, Lee D-H, Jeong KJ, Lee S-G: **Development of a novel cellulase biosensor that detects crystalline cellulose hydrolysis using a transcriptional regulator**. *Biochem Biophys Res Commun* 2018, **495**:1328-1334.

26. De Paepe B, Maertens J, Vanholme B, De Mey M: **Modularization and response curve engineering of a naringenin-responsive transcriptional biosensor**. *ACS Synth Biol* 2018, **7**:1303-1314.

27. Skjoedt ML, Snoek T, Kildegaard KR, Arsovska D, Eichenberger M, Goedecke TJ, Rajkumar AS, Zhang J, Kristensen M, Lehka BJ *et al.*: **Engineering prokaryotic transcriptional activators as metabolite biosensors in yeast**. *Nat Chem Biol* 2016, **12**:951-958.

28. Machado LFM, Dixon N: **Development and substrate specificity screening of an *in vivo* biosensor for the detection of biomass derived aromatic chemical building blocks**. *Chem Commun* 2016, **52**:11402-11405.

29. Kasey CM, Zerrad M, Li Y, Cropp TA, Williams GJ: **Development of transcription factor-based designer macrolide biosensors for metabolic engineering and synthetic biology**. *ACS Synth Biol* 2018, **7**:227-239.

30. Johnson AO, Gonzalez-Villanueva M, Wong L, Steinbüchel A, Tee KL, Xu P, Wong TS: **Design and application of genetically-encoded malonyl-CoA biosensors for metabolic engineering of microbial cell factories**. *Metab Eng* 2017, **44**:253-264.

31. Ambri F, Snoek T, Skjoedt ML, Jensen MK, Keasling JD: **Design, engineering, and characterization of prokaryotic ligand-binding transcriptional activators as biosensors in yeast**. *Methods Mol Biol* 2018, **1671**:269-290.

32. Meyer AJ, Segall-Shapiro TH, Glassey E, Zhang J, Voigt CA: ***Escherichia coli* Marionette strains with 12 highly optimized small-molecule sensors**. *Nat Chem Biol* 2018, **15**:196-204 http://dx.doi.org/10.1038/s41589-018-0168-3.

33. Wu J, Jiang P, Chen W, Xiong D, Huang L, Jia J, Chen Y, Jin J-M, Tang S-Y: **Design and application of a lactulose biosensor**. *Sci Rep* 2017, **7**:45994.

34. •• Taylor ND, Garruss AS, Moretti R, Chan S, Arbing MA, Cascio D, Rogers JK, Isaacs FJ, Kosuri S, Baker D *et al.*: **Engineering an allosteric transcription factor to respond to new ligands**. *Nat Methods* 2016, **13**:177-183.

The authors present a method for computer-assisted mutagenesis to change a transcription factor's specificity.

35. Younger AKD, Dalvie NC, Rottinghaus AG, Leonard JN: **Engineering modular biosensors to confer metabolite-responsive regulation of transcription**. *ACS Synth Biol* 2017, **6**:311-325.

36. Juárez JF, Lecube-Azpeitia B, Brown SL, Johnston CD, Church GM: **Biosensor libraries harness large classes of**

binding domains for construction of allosteric transcriptional regulators. *Nat Commun* 2018, **9**:3101.

37. • Younger AKD, Su PY, Shepard AJ, Udani SV, Cybulski TR, Tyo KEJ, Leonard JN: **Development of novel metabolite-responsive transcription factors via transposon-mediated protein fusion**. *Protein Eng Des Sel* 2018, **31**:55-63.
The authors use a chimeric protein strategy by inserting a ligand binding domain into a DNA binding protein to create repressors.

38. • Brandsen BM, Mattheisen JM, Noel T, Fields S: **A biosensor strategy for *E. coli* based on ligand-dependent stabilization**. *ACS Synth Biol* 2018, **7**:1990-1999 http://dx.doi.org/10.1021/acssynbio.8b00052.
The authors fuse DNA binding, ligand binding and transcription recruiting domains to generate activator, by achieving ligand-dependent stabilization.

39. Jester B, Tinberg CE, Rich MS, Baker D, Fields S: **Engineered biosensors from dimeric ligand-binding domains**. *ACS Synth Biol* 2018, **7**:2457-2467 http://dx.doi.org/10.1021/acssynbio.8b00242.

40. Selvamani V, Ganesh I, Maruthamuthu MK, Eom GT, Hong SH: **Engineering chimeric two-component system into *Escherichia coli* from *Paracoccus denitrificans* to sense methanol**. *Biotechnol Bioprocess Eng* 2017, **22**:225-230.

41. Chang H-J, Mayonove P, Zavala A, De Visch A, Minard P, Cohen-Gonsaud M, Bonnet J: **A modular receptor platform to expand the sensing repertoire of bacteria**. *ACS Synth Biol* 2018, **7**:166-175.

42. Rogers JK, Church GM: **Genetically encoded sensors enable real-time observation of metabolite production**. *Proc Natl Acad Sci U S A* 2016, **113**:2388-2393.

43. Libis V, Delépine B, Faulon J-L: **Expanding biosensing abilities through computer-aided design of metabolic pathways**. *ACS Synth Biol* 2016, **5**:1076-1085.

44. Delépine B, Libis V, Carbonell P, Faulon J-L: **SensiPath: computer-aided design of sensing-enabling metabolic pathways**. *Nucleic Acids Res* 2016, **44**:W226-W231.

45. Berset Y, Merulla D, Joublin A, Hatzimanikatis V, van der Meer JR: **Mechanistic modeling of genetic circuits for ArsR arsenic regulation**. *ACS Synth Biol* 2017, **6**:862-874.

46. •• Chen Y, Ho JML, Shis DL, Gupta C, Long J, Wagner DS, Ott W, Josić K, Bennett MR: **Tuning the dynamic range of bacterial promoters regulated by ligand-inducible transcription factors**. *Nat Commun* 2018, **9**:64.
The authors use a thermodynamical model to guide the optimization of their biosensor properties.

47. Mannan AA, Liu D, Zhang F, Oyarzún DA: **Fundamental design principles for transcription-factor-based metabolite biosensors**. *ACS Synth Biol* 2017, **6**:1851-1859.

48. Landry BP, Palanki R, Dyulgyarov N, Hartsough LA, Tabor JJ: **Phosphatase activity tunes two-component system sensor detection threshold**. *Nat Commun* 2018, **9**:1433.

49. Nguyen PHB, Wu Y, Guo S, Murray RM: **Design space exploration of the violacein pathway in *Escherichia coli* based transcription translation cell-free system (TX-TL)**. *bioRxiv* 2015 http://dx.doi.org/10.1101/027656.

50. Wu YY, Culler S, Khandurina J, Van Dien S, Murray RM: **Prototyping 1,4-butanediol (BDO) biosynthesis pathway in a cell-free transcription-translation (TX-TL) system**. *bioRxiv* 2015 http://dx.doi.org/10.1101/017814.

51. Kelwick R, Ricci L, Chee SM, Bell D, Webb AJ, Freemont PS: **Cell-free prototyping strategies for enhancing the sustainable production of polyhydroxyalkanoates bioplastics**. *bioRxiv* 2017 http://dx.doi.org/10.1101/225144.

52. Dudley QM, Anderson KC, Jewett MC: **Cell-free mixing of *Escherichia coli* crude extracts to prototype and rationally engineer high-titer mevalonate synthesis**. *ACS Synth Biol* 2016, **5**:1578-1588.

53. Karim AS, Heggestad JT, Crowe SA, Jewett MC: **Controlling cell-free metabolism through physiochemical perturbations**. *Metab Eng* 2018, **45**:86-94.

54. Moore SJ, Tosi T, Hleba YB, Bell D, Polizzi K, Freemont P: **A cell-free synthetic biochemistry platform for raspberry ketone production**. *bioRxiv* 2017 http://dx.doi.org/10.1101/202341.

55. Jiang L, Zhao J, Lian J, Xu Z: **Cell-free protein synthesis enabled rapid prototyping for metabolic engineering and synthetic biology**. *Synth Syst Biotechnol* 2018, **3**:90-96.

56. Karim AS, Jewett MC: **Cell-free synthetic biology for pathway prototyping**. *Methods Enzymol* 2018, **608**:31-57.

57. Soltani M, Davis BR, Ford H, Nelson JAD, Bundy BC: **Reengineering cell-free protein synthesis as a biosensor: biosensing with transcription, translation, and protein-folding**. *Biochem Eng J* 2018, **138**:165-171.

58. •• de los Santos ELC, Meyerowitz JT, Mayo SL, Murray RM: **Engineering transcriptional regulator effector specificity using computational design and *in vitro* rapid prototyping: developing a vanillin sensor**. *ACS Synth Biol* 2016, **5**:287-295.
The authors use computational design and cell-free prototyping to develop a biosensor for a toxic compound.

59. Halleran A, Murray RM: **Cell-free and in vivo characterization of Lux, Las, and Rpa quorum activation systems in *E. coli***. *ACS Synth Biol* 2017, **7**:752-755 http://dx.doi.org/10.1021/acssynbio.7b00376.

60. Voyvodic PL, Pandi A, Koch M, Faulon J-L, Bonnet J: **Plug-and-play metabolic transducers expand the chemical detection space of cell-free biosensors**. *bioRxiv* 2018 http://dx.doi.org/10.1101/397315.