Biological Switches: past and future milestones of transcription factor-based biosensors

De Paepe Brecht[1], De Mey Marjan[1,*]

[1] Centre for Synthetic Biology, Ghent University, Ghent, 9000, Belgium

Corresponding author:

M. De Mey

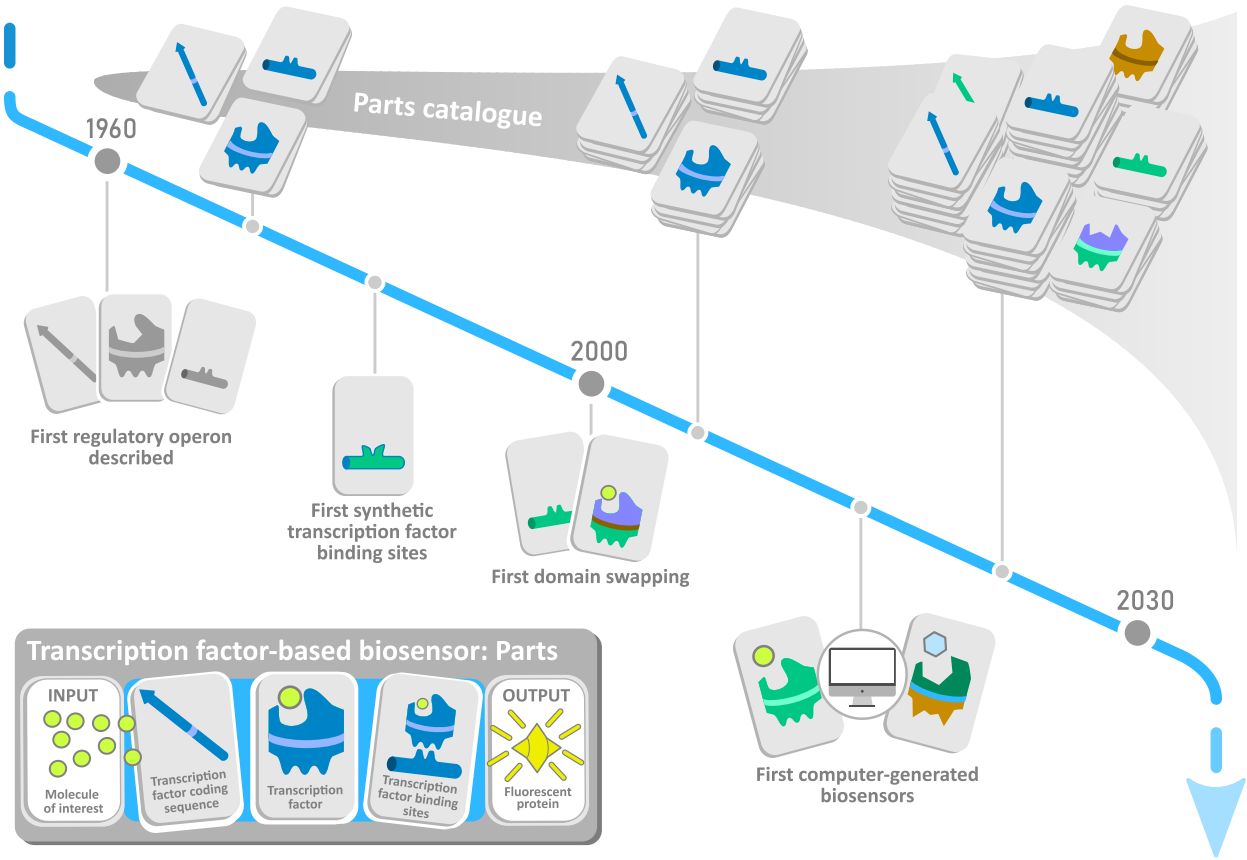
Coupure Links 653, 9000 Ghent, Belgium

marjan.demey@ugent.be

Tel: +32 9 2646028

Abstract

Since the description of the *lac* operon in 1961 by Jacob and Monod, transcriptional regulation in prokaryotes has been studied extensively and has led to the development of transcription factor-based biosensors. Due to the broad variety of detectable small molecules and their various applications across biotechnology, biosensor research and development increased exponentially over the past decades. Throughout this period, key milestones in fundamental knowledge, synthetic biology, analytical tools, and computational learning have led to an immense expansion of the biosensor repertoire and its application portfolio. Over the years, biosensor engineering became a more multi-disciplinary discipline, combining high-throughput analytical tools, DNA randomization strategies, forward engineering and advanced protein engineering workflows. Despite these advances, many obstacles remain to fully unlock the potential of biosensor technology. This review analyzes the timeline of key milestones on fundamental research (1960s to 2000s) and engineering strategies (2000s onward), on both the DNA and protein level of biosensors. Moreover, insights into the future perspectives, remaining hurdles and unexplored opportunities of this promising field are discussed.



Keywords: Biosensors, transcription factor, historical overview, inducible systems, prokaryotes, engineering strategies, computational techniques

Introduction

Extending from the earliest civilizations to the 21st century, any progress in scientific exploration, research, and engineering has been rooted in the principle of scientific observation. In other words, a clear and intricate link is present between the advancement of engineering disciplines and the progress in the field of measurement devices – or sensors -, enabling the imperative steps in the design-build-test-learn cycle. On the one hand, the development of increasingly precise and accurate sensors opens the door to a more detailed, profound understanding of nature which in turn facilitates higher control over various engineering parameters. On the other hand, envisioning new science and engineering objectives

necessitates the development of new sensors, tailored to specific performance criteria to make the desired innovation feasible. This tandem continuously boosts progress in all fields of research and development. This principle holds true in the field of bio-engineering and synthetic biology at least as strongly as it does in other engineering disciplines [1].

Classic analytic techniques such as (detector-coupled) chromatography, mass spectrometry, X-ray crystallography, capillary electrophoresis, nucleic acid sequencing, and immunological techniques have greatly expanded our understanding of biological systems. Besides boosting fundamental knowledge and biotechnological applications, this increasingly in-depth understanding also paved the way for the development of biological sensors, *i.e.*, using biological components such as metabolites, DNA sequences, proteins, and microbial cells to build sensor devices instead of pure physical and/or chemical components. Biological components bring specific advantages to the sensor-building table as they inherently function on the same scale, sense more direct, allow for less or no destructive analytical workflows and enhance portability and miniaturization [2–7].

One of the most interesting and diverse biological parts in nature's repertoire is the prokaryotic transcription factor (TF) from the widespread one-component transcriptional regulatory systems [8,9]. These proteins contain two domains: (*i*) a ligand-binding domain (LBD) which binds a specific small ligand molecule of interest and (*ii*) a DNA-binding domain which binds to a specific DNA sequence (*i.e.*, TF binding sites, TFBS) to exert control over transcription of a specific output gene of choice in a concentration-dependent manner (see Figure 1) [3–5,10–12]. Therefore, to build a TF-based biological sensor, generally a (genome-) or plasmid-based genetic circuit is constructed (or altered) comprising two operons: (*i*) a detector module expresses the TF which detects the ligand of interest and (*ii*) an effector module holds the TFBS and control elements to regulate the transcription of the output gene of choice (see Figure 1) [3–5,10–12]. This output gene can code for a fluorescent protein to allow for easy detection and quantification of the sensor output, an antibiotic resistance gene to allow for live-dead screening, an enzyme to allow

for metabolic influences or many other options [6]. As these transcription factors are able to sense all types of triggers such as sugars, amino acids, metal ions, antibiotics, fatty acids, specialized plant metabolites, and even pH, temperature, oxygen, osmotic pressure, and light, it is evident that bolstering these parts to build biological sensors opens up a world of possibilities in biotechnology, environmental monitoring, medical diagnostics and beyond [3–6,10–12].

These transcription regulatory systems are so-called one-component systems, *i.e.*, a single transcription factor detects the ligand molecule and transduces the resulting signal. In two-component systems, an additional protein transduces the signal through phosphorylation [13,14]. Many of these systems are involved in sensing and transducing extracellular signals, having the intrinsic advantage of circumventing any transport requirements of specific molecules of interest. Moreover, many global regulators, *e.g.* involved in stress response, are two-component systems, creating additional biosensor opportunities. Although two-components systems have been exploited for these biosensor opportunities, one-component systems demonstrate a higher abundance with a much broader diversity in sequences, TF structures, and ligands to be sensed [15–19]. This vast richness of TFs is the perfect foundation for biosensor development with many successful applications and corresponding engineering strategies throughout history, and therefore the focus of this review article [15,20–23].

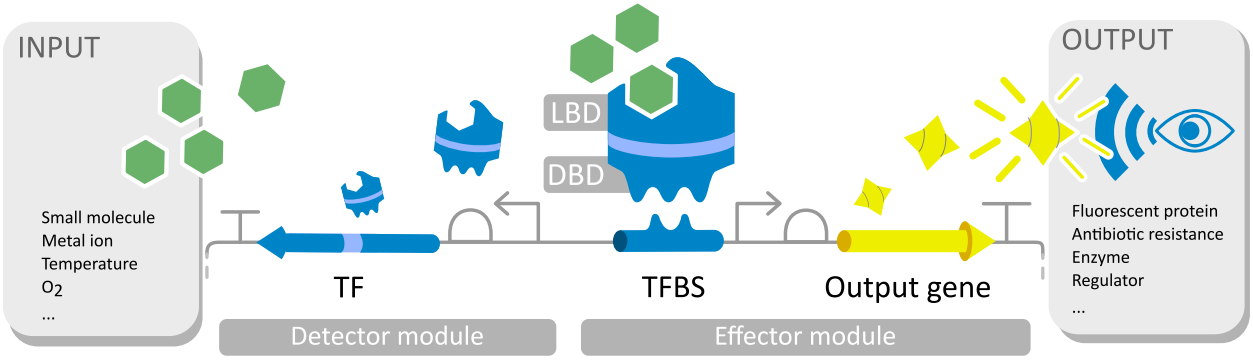


Figure 1: Schematic representation of a transcription factor-based biological sensor. Inputs of such biosensors can range from small molecules over metal ions to physical conditions such as temperature. In general, a biosensor circuit consists of a detector

module and effector module. Through genetic control from a promoter and ribosome binding site (RBS) sequence (see SBOL symbols), the detector module expresses the transcription factor gene (TF). The TF detects the input of interest with its ligand-binding domain (LBD) which causes a conformational change. This change alters the interaction of its DNA-binding domain with the transcription factor binding sites (TFBS) in the effector module and, therefore, regulates transcription of the output gene. This module also holds the necessary promoter and RBS sequences to control the expression of the output gene of choice such as a fluorescent protein, antibiotic resistance gene, enzyme of interest or another regulatory element. Adapted with permission from Demeester *et al.* [12].

The biosensor repertoire, corresponding development strategies and resulting applications have come a long way since the first description of an inducible promoter by Jacob and Monod in 1961 [24,25]. Over the past 60 years, many diverse and transformative milestones, in- and outside the field of biology, have contributed to boosting the promising field of biosensor technology. This review will provide an overview of key milestones, future opportunities, and remaining obstacles to illustrate the impact of biosensor technology. For a comprehensive overview of the various successful applications of biosensors, please see the recent reviews of Moraskie *et al.* and Chaisupa and Wright [6,266].

1960s to 2000s- Expansion of fundamental knowledge

Following the discovery of the DNA double helix structure in 1953 [26] and, in the subsequent years, the unraveling of the link between genes and protein synthesis, the introduction of the operon concept in 1961 by Jacob and Monod truly marked the start of focused research on inducible systems [24,25,27]. This work on the lactose-inducible *lac* operon in *Escherichia coli* paved the way for understanding transcriptional regulatory systems and added the extra layer of gene regulation to the central dogma of molecular biology. They described the response curve, or input/output relationship, and ligand specificity of this inducible system, the two fundamental characteristics of a biosensor. Together with the description of the triptych: (*i*) transcription factor (TF), (*ii*) transcription factor binding sites (TFBSs) and (*iii*) ligand molecule, they established the framework wherein biosensors and their parts will be studied, developed

and characterized in the decades after. For a historical overview of the milestones that were foundational for biosensor research (1950s - 2000s), please see Figure 2.

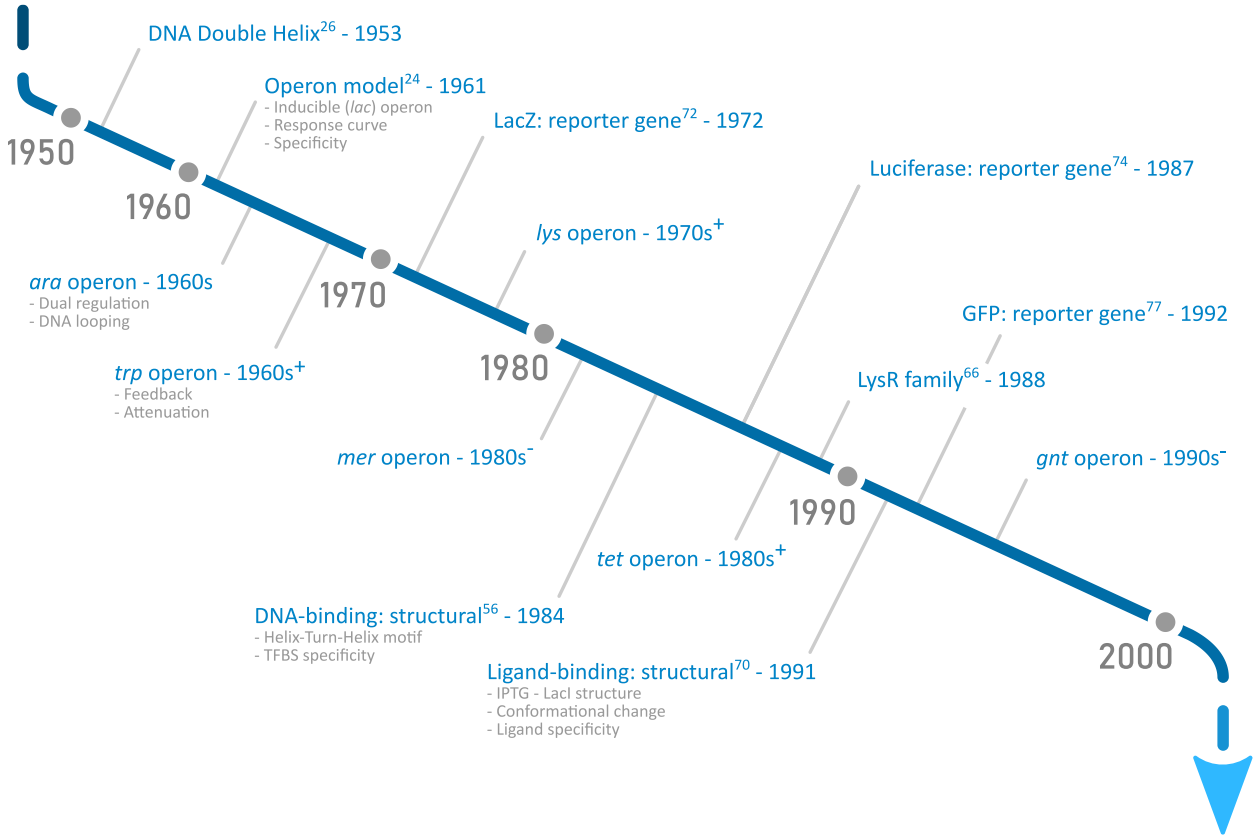


Figure 2: Schematic timeline from the 1950s to the 2000s, highlighting key milestones on the expansion of fundamental knowledge on inducible circuits, foundational to biosensor technology. Specific milestones include keywords representing significant advancements or concepts introduced. DNA = deoxyribonucleic acid, TFBS = transcription factor binding site, IPTG = isopropyl, GFP = Green Fluorescent Protein, 19XXs^{-} = early 19XXs, 19XXs^{+} = late 19XXs.

Genetic architecture (DNA level) Shortly after the work of Jacob and Monod on the *lac* operon, the repertoire of, and knowledge on, inducible circuits already started to expand through the studies on the arabinose-inducible *araBAD* operon [28–32] and the tryptophan-inducible *trp* operon [33–40], from the 1960s onward. Until the study of the *araBAD* operon, negative regulation (repression/de-repression) was the dominant model, as exemplified by the *lac* operon and the Lambda phage repressor [32]. However, it was discovered that the *araBAD* operon is regulated in a positive, even dual regulatory manner [32,41,42]. More

specifically, the AraC TF functionally represses transcription in the absence of arabinose and functionally activates transcription in the presence of arabinose. This regulation occurs through a DNA looping mechanism, which was unraveled later in 1984 through TFBS deletions and *in vitro* TF-DNA binding experiments [32,43]. With accumulating studies, this DNA looping and dual regulation appeared to be widespread throughout nature [44,45]. The tryptophan-regulated *trp* operon also played a pivotal role in biosensor development, serving as the first example of both the concept of feedback inhibition and that of transcriptional attenuation [34–36]. By studying these two concepts in the 1970s and 1980s, Yanofsky and his team shed more light on the complexity of transcriptional regulatory networks as well as the intricate interplay of DNA and RNA, respectively [34–37,39,40]. During the same period, molecular biology tools such as plasmids and restriction enzymes [46], and Sanger sequencing in 1977 [47] boosted biological research even further. In a second wave of operon studies, the *lys* operon in the late 1970s [48,49], the *mer* operon in the early 1980s [50–52], the *tet* operon in the late 1980s [53], and the *gnt* operon in the early 1990s [54,55] were studied extensively. This research deepened our understanding of the broad repertoire of transcriptional regulatory mechanisms, the diversity of triggers, ranging from environmental signals to central metabolites and the mechanisms of antimicrobial and metal resistance.

Transcription factor structure (protein level) For all these inducible operons, the control elements, TFBS sequences, and corresponding TF amino acid sequences were systematically annotated. It was only in the period between 1980 and 1983 that the first three-dimensional structures of site-specific DNA-binding proteins were published, namely for the three prokaryotic TFs, Cro and cI Lambda phage repressor proteins and the CAP protein of *E. coli* [56–62]. In 1984, based on the combined information of these available TFBSs and 3D TF structures, Pabo and Sauer proposed the helix-turn-helix (HTH) motif as the key structure responsible for DNA-binding in these proteins [56]. Subsequent sequence and secondary structure analysis of regulators throughout nature revealed that this HTH motif is common in all domains of life and is the

most prevalent DNA-binding motif in prokaryotes, especially in transcription factors [63,64]. This structural data aided immensely in the following studies for DNA-binding specificity and regulatory mechanisms.

Because these DNA-binding domains are conserved, their sequence similarities form the basis of TF family designation, with the first characterized member giving its name to the family [9]. The LysR, MerR, TetR, GntR, AraC/GalR, LacI, LuxR, and many other families were defined in the late 1980s and 1990s, with their numbers expanding rapidly in the decades thereafter [15,65–69]. Established in 1988 as one of the first TF families, the LysR family is now the largest with over 852,000 predicted structures, demonstrating a vast diversity of ligand molecules to be sensed [15]. LysR family members can even account for up to 20% of all regulators in a bacterial genome [15,66]. Categorizing transcription factors into families enhances our understanding of their evolutionary relationships, aids in predicting their functions, and strengthens design strategies for biosensors. Genomic studies on TFs revealed that prokaryotes have the most divergent and numerous transcriptional regulatory systems in nature, especially for small molecule-binding TFs, emphasizing their importance for expanding the biosensor repertoire [8,9].

The next advancement in the field of TF structural research was the first crystal structure of a TF-ligand complex, namely that of the LacI repressor in complex with its inducer, isopropyl β-D-1-thiogalactopyranoside (IPTG), elucidated in 1991 [70]. Besides confirming the presence of the HTH motif in LacI, this study revealed the conformational changes that occur when tetrameric LacI binds IPTG and how this process regulates transcriptional activation [70]. Structural knowledge on ligand-binding domains, and more specifically ligand-binding pockets, of TFs is invaluable for rational protein engineering strategy to alter or enhance the molecular specificity of TFs, and therefore biosensors [3,71].

Reporter genes Development of sensitive, fast, cheap, non-invasive and easy-to-measure reporter genes is critical for biosensor development, and biological research in general. Before the discovery and omnipresent use of fluorescent proteins, other various systems were developed and used to study

transcriptional regulation. Besides the gained knowledge on the architecture and mechanism of action, the initial studies on the *lac operon* also introduced the *lacZ* gene, expressing the β-galactosidase enzyme [24]. In 1972, Miller published the first use of this enzyme as a measurable readout for gene expression which quickly became, and still is, a widely used reporter gene in all biological disciplines [72]. More specifically, this colorimetric assay allows for the quantification of gene expression with *O*-nitrophenyl-β-D-galactoside (ONPG) as substrate to be converted into a yellow color [72]. In 1979, Casadaban and Cohen used the *lacZ* gene for the first time as a reporter for gene expression and hinted at its use for functional and mutational studies of transcription [73].

A decade later, a new important player in the field of reporter genes was introduced in 1987 with the discovery and use of luciferase genes from the firefly *Photinus pyralis* which generates light after conversion of a specific substrate, *e.g.*, luciferin [74]. This reporter gene allows for higher sensitivity and real-time monitoring of gene expression [74]. Although other reporters like chloramphenicol acyltransferase from 1982 [75] and alkaline phosphatase from 1985 [76] have their value, a true reporter revolution was ushered in 1992 by the work of Chalfie and coworkers [77]. In their Nobel prize-winning research, the green fluorescent protein (GFP) from *Aequorea Victoria* was used in *E. coli* and *Caenorhabditis elegans* as a fully self-sufficient genetic reporter [77–79]. GFP was rapidly adopted throughout all biological research disciplines for precisely studying gene expression dynamics and patterns, protein localization, and molecular interactions through protein fusions, and much more, in live cells [77,79]. Within only a few years, researchers such as Tsien and coworkers created mutated versions of GFP with different fluorescence spectra, efficiencies, and stabilities [79]. This led to the development of an ever-expanding family of fluorescent proteins (FPs) with diverse characteristics, facilitating applications such as experimental setups with multiple readouts [80–82]. In 1996, GFP was used for the first time as genetic output of an inducible system [83]. More specifically, the IPTG-inducible *lac* system was used to screen for GFP reporter mutants demonstrating optimized fluorescence using fluorescence-activated cell sorting (FACS) [83]. A year later, the same set-up was used for

high-throughput selection of transcriptionally active promoter regions induced by specific macrophage triggers, demonstrating its use for mining biosensor parts [84]. Since its invention in 1976 and subsequent optimization in the decades after [85], FACS has proven to be a highly valuable tool in biosensor-based strategies, for both developing biosensors and applying them in high-throughput screening set-ups [4,5,86].

2000s onward- Engineering biosensor circuits for custom characteristics

Based on all this foundational work, researchers started to explore and build new, synthetic inducible circuits, unlocking the potential of biosensor technology. With the development of the polymerase chain reaction (PCR) in 1983, the rise of automated sequencing, improved computational tools in the mid-1990s, and the continuous increase in throughput in biological research in general, the expansion of our catalog of biological parts and our understanding of them surged from the 2000s onward [27,87,88]. For a historical overview of the milestones that advanced biosensor engineering from the 1990s onward, please see Figure 3.

As stated earlier, a biosensor consists of multiple parts, both on the DNA level and the protein level, *i.e.*, promoters, ribosome binding sites (RBSs), transcription factor binding sites (TFBSs) as well as the transcription factors (TFs) and their domains [2,3]. Since the discovery and study of the *lac* operon, researchers have been altering these parts, primarily to gain fundamental insights of the underlying biological mechanisms [24,89]. Since the 1990s, these biosensor parts were being modified to modulate the two key biosensor characteristics, *i.e.*, response curve and ligand specificity, in a variety of ways to meet the researcher's needs [3–5,11,90].

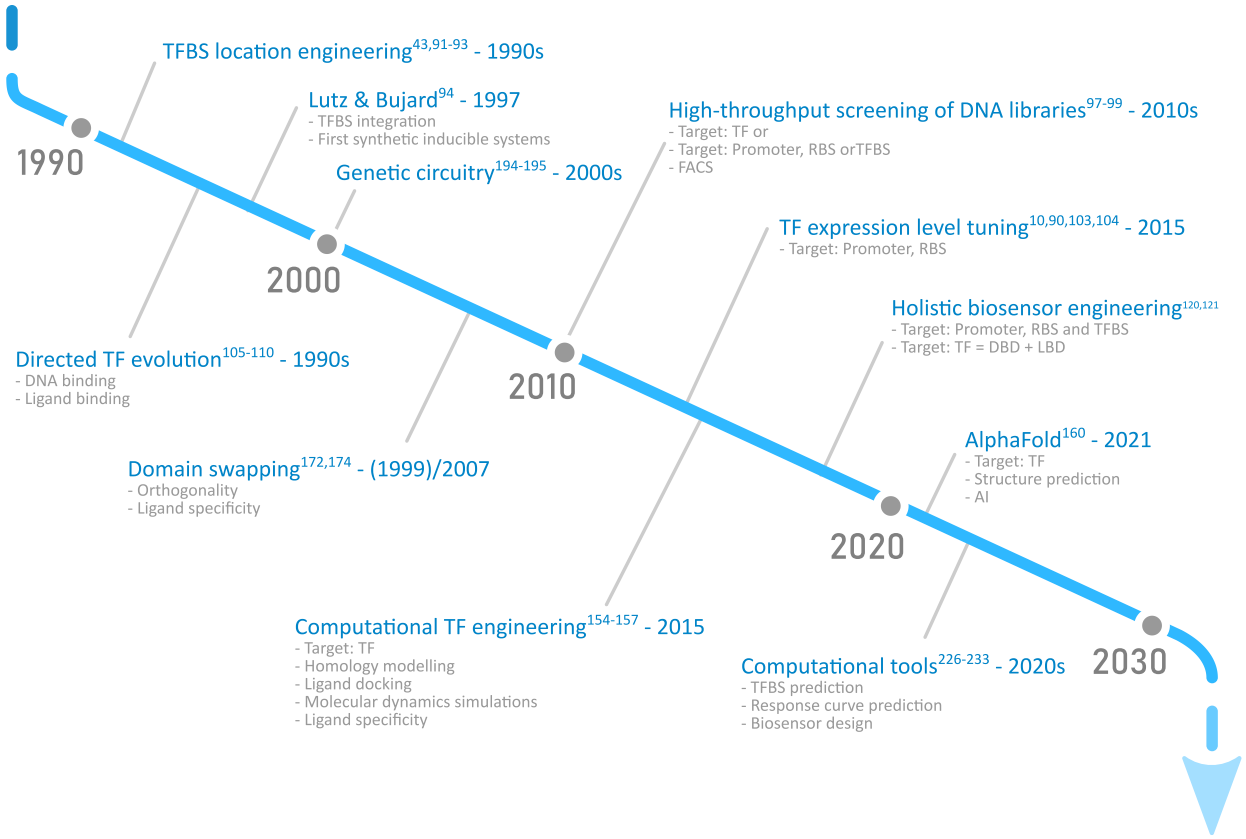


Figure 3: Schematic timeline from the 1990s onward, highlighting key milestones on the engineering of biosensors for custom characteristics such as response curve and ligand specificity. Specific milestones include keywords representing significant advancements or concepts introduced. TF = transcription factor, TFBS = transcription factor binding site, LBD = Ligand-binding domain, DBD = DNA-binding domain, RBS = ribosome binding site, AI = artificial intelligence, FACS = fluorescence-activated cell sorting.

TFBS-TF interaction at the DNA level Through the work of Dunn *et al.* in 1984 [43], Lanzer *et al.* in 1988 [91] and Lee *et al.* in 1989 [92], and Muller *et al.* in 1996 [93], the TFBS position relative to the other control elements emerged as an engineering target for inducible systems. Extrapolating from this concept, Lutz and Bujard published a landmark research paper in 1997 that significantly advanced the development of inducible systems with stronger expression, tighter control, and a broader dynamic range [94]. To this end, they created three hybrid inducible systems in *E. coli* by integrating specific operator sequences from the *tet*, *ara*, and *lac* system into the well-established pL promoter from Lambda bacteriophage [94]. More

specifically, they developed new TetR/anhydrotetracycline (aTc)-, LacI/IPTG-, and even a cascaded LacI/IPTG+AraC/arabinose-inducible systems with unprecedent dynamic ranges, up to 5000-fold with tight control. In addition, Lutz and Bujard hint at plasmid copy number, in direct correlation with TFBS copy number, as yet another additional biosensor engineering target [94–96].

Around the 2010s, DNA randomization strategies to create promoter, RBS, and TFBS libraries became more established concurrently with high-throughput screening methods to select the desired phenotype [97,98]. In 2012, Garcia and coworkers performed an in-depth analysis of the effect of operator sequence and location and hypothesized that even the underlying regulatory mechanism can change with altering operator location [99]. Altering the operator sequence in concert with key promoter sequences in a high-throughput combinatorial screening strategy, enabled Jha and coworkers in 2014 to optimize a biosensor's response and make it more compatible with the transcription machinery of the non-native host [100]. In a more extensive study that year, Stanton and coworkers genomically mined TFBS sequences for TetR family TFs and designed synthetic ones based on that information [22]. Next, these synthetic TFBS-TF pairs were screened for minimal cross-interference when co-expressed which enabled the creation of a collection of orthogonal biosensors to build a variety of genetic circuits from [22]. In 2015, Liu *et al.* tuned the response curve of a FapR-based biosensor by introducing random mutations in the core promoter and TFBS controlling the output gene [101]. Further developments in computational learning are a great addition to the available high-throughput engineering strategies and allowed for the development of predictive models through machine learning in the late 2010s. In 2019, Liu and coworkers developed a complete workflow to generate new TFBS sequences, characterize their functionality in the context of different biosensors within the TetR family and, finally, apply machine learning to build a predictive model coupling sequence determinants with biosensor induction[102]. Interestingly, the generated insights suggests that the TFBS sequences also have an influence on ligand sensitivity and cooperativity, properties generally attributed to solely the TF [102].

Biosensor characteristics can not only be altered by directly changing the TF-TFBS interaction. Modulating the expression levels of the TF or the output gene also have a direct influence on the biosensor response curve. This was demonstrated clearly through the work of Wang *et al.* in 2015 in which a diverse set of response curves was achieved for both the TetR-based and LuxR-based biosensor, solely by modulating the expression levels of the TFs [103]. In 2018, the same principle was demonstrated by creating a collection of response curves for a flavonoid-responsive LysR-type biosensor by altering the ribosome binding sites of either the TF or the output gene, independently from any other control element or TFBS [10]. Varying the expression levels of the TF or output gene has now become an established, sometimes only small part of many biosensor engineering strategies [3,104]. With the advent of deep learning tools, Ding *et al.* in 2020 focused on varying the RBS sequences, controlling the TF and output gene, to alter the dynamic range of a glucarate-responsive biosensor [90]. After screening, sorting and sequencing thousands of RBSs, a convolutional neural network was trained and allowed to predict dynamic range of other biosensor based on the RBS sequences, to a certain extent [90].

TFBS-TF interaction at the protein level Going into the protein level of biosensor engineering, the TF-TFBS interaction can also be altered through changing the amino acid sequence of the TF, *e.g.*, at the DBD. Some of the earliest examples of biosensor engineering can be attributed to Sartorius, Lehming, and coworkers in 1987, 1989 and 1990 [105–107], to Bass and coworkers in 1988 [108], and to Helbl and coworkers in 1998 [109,110] for their work on creating mutant LacI, Trp, and TetR regulators, respectively, with affinities for new TFBS sequences [105–110]. In 2003, Swint-Kruse *et al.* created several LacI mutants with enhanced inducer response and, moreover, observed the importance of long-range effects of LBD mutations on DNA-binding affinity [111]. Scholz and coworkers (2004) performed DNA shuffling across the full TetR protein sequence to successfully generate TetR variants that even have a reversed regulatory mechanism [112]. The discovered mutations for activity reversal are clustered around the linker region between DBD and LBD, emphasizing the importance of this region as well [112]. Toward a similar goal, LacI variants with reversed functionality

were created between 2011 and 2019 through mutational strategies, together with the generation of new, alternate TFBSs [113–116]. To customize the biosensor response curve itself, Lakshmi and Rao (2008) evolved LacI into a variant that reaches ten times higher output expression levels than wild-type LacI for the same IPTG concentration [117]. In a different study in 2016, mutagenesis across the full DmpR TF sequence was successfully performed, purely to reduce the detection limit of the biosensor response [118]. A similar mutational strategy was performed in 2020, to boost the operational range of a XylR-based biosensor [119].

Combining all of the available high-throughput engineering and screening techniques, researchers started tackle biosensor development on a larger, holistic scale. In 2018, Meyer and coworkers developed a directed evolution strategy to create a set of 12 high-performance, orthogonal biosensors that can be used simultaneously in a single *E. coli* cell [120]. This strategy targeted the promoter and RBS sequences of both the effector and detector module, the TFBS sequence, and even coding sequence part of the DBD of the respective TF [120]. In the same year, a similar holistic strategy was applied to evolve 14 Trp-based biosensors by evolving ligand specificity, DNA specificity and TFBS sequences [121].

Ligand specificity engineering With more tools and structural knowledge available on ligand-binding domains, researchers started generating mutant TFs with altered ligand specificity [122]. Since the mid-1990s, the repertoire of mutant TFs has steadily expanded, with research mainly focusing on a small set of well-characterized TFs. Throughout this whole period, the applied engineering strategy for altering ligand specificity was predominantly one of only a few variations on combining directed evolution and iterative FACS with dual selection [122–124]. Various TF variants from different TF families were created, namely for DmpR [125–127], AlkS [128], XylR [126,129,130], XylS [131], AraC [132–137], TetR [138–140], NahR [141–143], DntR [144], LuxR [145–147], PcaV [148], PbrR [149], PobR [150], CadR [151], and TraR [152], among many others, highlighting the effectiveness of protein engineering strategies for biosensor customization. Moreover, these experiments demonstrate the potential held within a single TF to form the starting point of new, altered TFs and even the potential to transfer specificities within the same TF family [3,153]. This expansion of mutant TFs directly correlates

with the expansion of the catalog of ligand molecules that can be detected and quantified by biosensors, *i.e.*, metal ions, aromatic acids, antibiotics, phenols, alkanes, lactones, aromatic aldehydes, mevalonate, and many more.

Protein engineering has always evolved in tandem with computational techniques. Due to the development of protein modelling techniques, ligand docking simulations, and molecular dynamics simulations, rational design of ligand-binding pockets became easier. Especially from the 2010s, the impact of computational tools on protein engineering for biosensor development became unmistakable. In 2015, Taylor *et al.*, de los Santos *et al.,* and Jha *et al.* computationally designed new LacI, QacR, and PobR variants, respectively, to bind non-native ligands, namely fucose, lactitol, and sucralose (LacI), vanillin (QacR) and 3,4-dihydroxybenzoate (PobR) [154–157]. They applied the Rosetta model [158,159] for structure prediction through homology modelling, ligand docking simulations, and subsequent prediction of key amino acid residues interacting with the ligand of interest for targeted mutagenesis [154–157]. These computational tools rapidly evolved further, became more easily accessible and were augmented with artificial intelligence tools, such as AlphaFold from 2021 onward [160,161]. In the past 5 years, they became a key element in most TF engineering strategies, which is demonstrated by the work of Della Corte *et al.* in 2020 [162], Flachbart et al. in 2021 [163], d'Oelsnitz *et al.* [104,164], and Cole *et al.* in 2022 [165], either to identify amino acid targets or as post-analysis after random mutagenesis for fundamental knowledge. With the goal of expanding the biosensor repertoire further, these studies successfully engineered new ligand specificities for the LysG TF toward histidine [162], the HcaR TF toward various aromatic acids [163], the CamR TF toward bicyclic monoterpenes [104], the RamR TF toward therapeutic alkaloids [164], and the RolR TF toward phloroglucinol and other aromatics [165]. Recently in 2024, a full computational workflow was applied for the developed of adipic acid-responsive biosensor by modelling, ligand docking, molecular dynamics simulations and rational engineering of the BenM TF [166].

Domain swapping Instead of mutating the LBD for custom ligand specificity or the DBD for altered TFBS specificity, swapping domains of homo- or even heterologous TFs has also proven to be a valuable strategy to create biosensors with new or altered characteristics [3,154]. More specifically, domain swapping allows for pairing up the ligand specificity of one TF with the DNA specificity of another. This strategy also enables the creation of orthogonal biosensors, *i.e.*, independent functionality from the host regulatory network and/or from any other biosensors in the same cell, enabling complex genetic circuitry [167]. Furthermore, novel insights are garnered on interdomain contacts, multimerization, and conformational changes [3,168–171].

The first TF domain swapping for altering specificity can be attributed to the work of Brockelhurst *et al.* in 1999, in which zinc-responsiveness was transferred from one MerR family member to another, non-zinc responsive one [172]. Albeit not domain swapping in the strict sense, Garmendia *et al*. in 2001 (and subsequently in 2006) shuffled LBD parts between XylR homologues to transfer specificities [171,173]. In 2007, Tungtur *et al.* successfully combined the LBD of the PurR with the DBD (and linker) of LacI, both members of the LacI family [174]. Expanding on this research in the following decade, multiple chimeric TFs within the LacI family were created and characterized through domain swapping [168–170]. From this chimeric collection, boasting more than 40 chimeric TFs, various multi-input, single-output systems were built by co-expressing TFs that share an identical DBD sequence (binding on the same TFBS) but have unique LBD sequences (detecting different ligands of interest) [167,170,175]. Based on the available sequence data on LacI homologues (>70,000 sequences in 2019), Dimas and coworkers even developed a computational model for computing compatibility between any DBD and LBD and predicting resulting repressor efficiency [167]. Around 2020, chimeric transcription factors were developed for additional TF families, namely for the LysR [71] and TetR family [176] in 2019, the LuxR family in 2020 [177], and the MerR family in 2023 [178]. In addition, domain swapping can also be used to enhance other biosensor engineering strategies. In 2020, Snoek and coworkers combined domain swapping with directed evolution of the LBD to create a library of BenM

variants with shifted ligand specificities but that still retained their original DNA-binding affinity, functionality, and even portability [179].

Tools and concepts in support of biosensor technology

Besides different engineering strategies to tune biosensor characteristics, many auxiliary tools were developed that supported biosensor technology directly or indirectly, such as databases, experimental and computational tools, and standardization efforts. In the realm of analytical tools, some experimental methods were transformative for biosensor technology, mainly for analyzing protein-DNA interactions. Since its first description in 1988 [180], chromatin immunoprecipitation (ChIP) has evolved immensely and has been coupled with next-generation sequencing workflows in 2007 to create ChIP-Seq [181,182], which allows for genome-wide scanning of transcription factor-bound DNA sequences. Electrophoretic mobility shift assays (EMSA), established in 1981, are used to confirm DNA-binding of a purified TF on putative TFBS sequences [183,184]. This tool and variants hereof to boost experimental throughput are still integrated in fundamental research studies today [185,186]. In 2010, a high-throughput systematic evolution of ligands by exponential enrichment (HT-SELEX) assay was developed to map the DNA-binding specificities of TFs [187,188]. This method was successfully applied to map the TFBS sequence specificities of 182 putative TFs in *Pseudomonas aeruginosa* [189]. For an overview on DNA-binding motif detection methods, please see the review article of Inukai and coworkers [190].

Genetic circuitry When expanding the focus of engineering biosensors, synthetic biologists quickly started combining these inducible circuits into more complex genetic circuitry, mimicking the natural complexity of regulation [191]. Since its conceptualization in 1995 [191], genetic circuitry has been developed for a wide array of goals such as enabling biological computation, metabolic engineering for optimizing microbial cell factories, and many more [192,193]. The first examples of true genetic circuitry were developed in 2000, *i.e.*, a genetic toggle switch [194] and an oscillator circuit [195]. Since then, this field has evolved immensely

throughout the past three decades with the review article of Gao *et al.* (2023) giving a comprehensive overview of the state-of-the-art [192]. In biosensor technology specifically, genetic circuitry can be used as a way to customize the response curve. This is exemplified by the enhancement of sensor stability through autoregulation in 2000 [196], the boost in sensitivity and temporal delay through cascading in 2005 [197] and signal amplification through positive feedback in 2010 and 2014 [198,199], among others [192]. In addition, genetic circuitry can enhance specific biosensor screening strategies, which was exemplified by d'Oelsnitz in 2022 [164]. Their SELIS (seamless enrichment of ligand-inducible sensors) method augments the efficiency of FACS-coupled directed evolution of TFs by implementing an additional inverter circuit that ensures the repressive power of any mutant TF throughout the library screen [164].

In silico databases With the rise of computational power, *in silico* databases were being created to compile the ever-increasing amount of data collected and to aid subsequent research. Following Margaret Dayhoff's pioneering work on developing the first-ever protein sequence database in 1965 [200], the RCSB Protein Data Bank (PDB) published in 1971 marked a significant advancement, and it continues to be one of the foremost repositories for protein structure information [201,202]. The GenBank database, currently containing over 250 million genetic sequences in general, became publicly available in 1982 with just over 600 DNA sequences at that time [203–205]. Throughout the past decades, also more specialized databases were built, containing manually curated data, computationally predicted data or a combination of both on topics like transcriptional regulation, binding motifs and protein structures. Some of them halted support after a certain time, such as PRODORIC [206] and RegTransBase [207]. Noteworthy databases regarding prokaryotic transcriptional regulation are EcoCyc (est. 1996) [208,209], RegulonDB (est. 1997) [210], DBTBS (est. 1999) [211], CoryneRegNet (est. 2006) [212,213], RegPrecise (est. 2009) [214,215], P2TF (est. 2012) [216], CollecTF (est. 2014) [217,218] and more recently iModulonDB (est. 2021) [219] and GroovDB (est. 2022) [220].

Computational tools Besides databases, different types of computational tools emerged and evolved for either analytical or predictive purposes. As a key computational tool, the first sequence alignment method

published by Needleman and Wunsch in 1970 had a great impact on DNA and protein research and is widely used to this day [221]. Decades apart, sequencing algorithms evolved further, with the Smith-Waterman method in 1981 [222] and the Basic Local Alignment Search Tool (BLAST) in 1990, the most-cited *in silico* tool [223,224]. From the 2000s on, the diversity and number of computational tools increased exponentially [224]. In 2023, Cai and coworkers created SynBioTools, a comprehensive repository of computational tools, databases and experimental methods in synthetic biology, currently holding more than a thousand tools [224]. For a brief history of bioinformatics, please see the work of Gauthier and coworkers [225].

Besides all general computational tools related to protein modelling and protein-DNA interactions, biosensor-specific tools have emerged throughout recent years as well. For example, SensiPath (est. 2016) and DetSpace (est. 2024) allow to compute "sensing-enabling" metabolic pathways to convert a non-detectable ligand to a detectable one, based on known TFs [226–228]. In 2023, the TFBMiner [229] and Sensbio [230] tools were published and allow for the identification and design of biosensors for a ligand of choice. The year after, the Snowprint [231] and Ligify [232] tools were published by d'Oelsnitz and coworkers for predicting and designing TFBS-TF pairs and for automating biosensor design for a specific ligand input. Databases and especially predictive tools, such as DeepTFactor [233], greatly benefitted from the recent advances in artificial intelligence (AI) and machine learning. For a comprehensive overview of the various ways AI is aiding the field of biosensor technology, please see the recent review by Ding *et al.*[161] .

Standardization efforts The characterization of biological systems like a biosensor can be tackled in a variety of ways, for which something seemingly simple as a device setting or DNA part definition can influence the interpretation of the resulting data and limit cross-lab transferability [2,234,235]. As in any engineering discipline, standardization efforts are imperative for pushing synthetic biology into the next phase of maturity [2,234,235]. In 2003, the BioBrick standardized DNA assembly method was published with corresponding standardized part design [236,237]. The Standard European Vector Architecture (SEVA),

published in 2013, consists of a framework and database for standardizing plasmid design [238–240]. Later in 2015, the Synthetic Biology Open Language (SBOL) was published to enable a universal standard to symbolize DNA parts based on functionality [241]. To standardize fluorescence-based characterization experiments, De Wannemaeker *et al.* published an easy-to-use, standardized strategy in 2023 [234]. That same year, Demeester, De Baets, and coworkers published the Modular Biological Sensor (MoBioS) platform to enable a standardized plasmid architecture and assembly to easily build, characterize and even fine-tune biosensors [12]. In addition, for each of the eight biosensors development as proof-of-concept, "ID sheets" were created which hold all necessary biosensor information in a standardized way, ranging from experimental information, biosensor characterization data and DNA sequence information [12]. These type of ID sheets, datasheets [120] or performance cards [242] are of great importance to standardize communication about inducible circuits and enhance their repeatability and transferability [12,120,242].

Future outlook and obstacles

Since the 1960s, research on inducible systems has expanded greatly, both toward in-depth fundamental knowledge on transcriptional regulation and toward engineering strategies to transform these natural systems into biosensors, customized for specific applications. A broad array of techniques and studies have contributed to biosensor development, characterization, optimization and repertoire expansion, ranging from directed evolution to predictive computation. Biosensors have clearly demonstrated their potential as quantification tools for a wide variety of molecules, as key controller circuits to optimize microbial cell factories and as high-throughput screening method in various bio-engineering strategies [3,5,6,11,243,244]. The scope of this review article is on prokaryotic one-component transcription factors as these proteins hold a vast richness of structural diversity and potential repertoire of detectable molecules. Consequently, these natural regulatory systems dominate the field of transcription-based biosensor development.

Most biosensor development and application up till now has been limited to model organisms such as *E. coli*. However, many opportunities are still to be explored in non-model organisms, *e.g.*, for optimizing production of industrially relevant biomolecules [245,246]. Moreover, the archaeal and eukaryotic domains of life hold many promising opportunities as well, both as resources for (extremophilic) biosensor parts and as hosts for potential biosensor applications [179,247–249]. Instead of expanding biosensor research across life, cell-free systems have also clearly proven their potential for biosensing in fundamental research, *in vitro* applications, and even in biosensor development strategies [155,245,250–260].

With recent research on bioelectricity and bio-electrical sensors, bridging the gap between the digital and biological world is becoming more realistic, with biosensors playing a key role [261]. A biosensor-enabled cell culture generating an electrical output instead of a fluorescent protein can be linked into other electronic systems more directly with many advantages as a result and yet to be fully explored [262–264].

Despite great advances in biological knowledge, there are still many obstacles to overcome before the full potential of biosensor technology can be unlocked. Knowledge on protein structure, especially multimerization, remains a significant hurdle. Further, fundamental research cannot be overlooked in order to clearly understand, model and tune all DNA-protein, protein-protein and ligand-protein interactions within a biosensor system. On a larger scale, it is imperative to shed more light on the differences of the single cell versus population reference frame regarding dynamics and biological variability of inducible systems as well. Biological variability originates at different scales, including TF-TFBS interactions, plasmid copy number fluctuations, per-cell differences in detectable inducer molecules, and metabolic, kinetic, and regulatory disparities across individual cells within a population. Therefore, interpreting biosensor output variability as a measure of general biological variability reflects the cumulative impact of these fluctuations. Gaining deeper knowledge of variability at each scale and integrating it into a holistic view of genetic circuits at the population level is a formidable challenge but is essential to advance synthetic biology.

In general, as our fundamental knowledge deepens, so does our appreciation of the immense complexity and variability of biological systems. Despite numerous efforts to address it, this intrinsic complexity leads to low predictability [265]. With the continuous rise of computational power, the impact of machine learning, artificial intelligence and even quantum computing will certainly expand further to boost predictability. This runs synchronous with the increase in experimental automation which, together, brings us to the verge of a new expanse in the biosensor repertoire [251]. To ensure this, a focus toward both fundamental research and real-life applications should remain. Besides the potential to have cheap, fast, on-line, portable, sustainable sensor technology, the power of biosensors lies even more in the wide diversity of ligand molecules they can specifically detect. Synthetic biologists collaborating with researchers in green chemistry could be of benefit as well to expand the biosensor repertoire further, with a focus on the ligand molecules. Exploration of strategies and applications of biosensors capable of truly detecting any molecule of interest must be the primary goal if we want to push biosensor technology into its next era.

Author contributions

B.D.P. and M.D.M. were involved in the conceptualization and design of the manuscript. B.D.P. drafted the manuscript, which was followed by critical revision by all authors.

Acknowledgements

B.D.P. holds an FWO postdoctoral fellowship (1246323N). The authors thank the UGent Core Facility “HTS for SynBio” for training, support, and access to the instrument park.

Notes

The authors declare no competing financial interest.

Bibliography

(1) Feynman, R. P. There's Plenty of Room at the Bottom. *Journal of Microelectromechanical Systems* **1992**, *1* (1), 60–66. https://doi.org/10.1109/84.128057.

(2) Decoene, T.; De Paepe, B.; Maertens, J.; Coussement, P.; Peters, G.; De Maeseneire, S. L.; De Mey, M. Standardization in Synthetic Biology: An Engineering Discipline Coming of Age. *Crit Rev Biotechnol* **2018**, *38* (5), 647–656. https://doi.org/10.1080/07388551.2017.1380600.

(3) De Paepe, B.; Peters, G.; Coussement, P.; Maertens, J.; De Mey, M. Tailor-Made Transcriptional Biosensors for Optimizing Microbial Cell Factories. *J Ind Microbiol Biotechnol* **2017**, *44* (4–5), 623–645. https://doi.org/10.1007/S10295-016-1862-3.

(4) Mahr, R.; Frunzke, J. Transcription Factor-Based Biosensors in Biotechnology: Current State and Future Prospects. *Appl Microbiol Biotechnol* **2016**, *100* (1), 79–90. https://doi.org/10.1007/s00253-015-7090-3.

(5) Tellechea-Luzardo, J.; Stiebritz, M. T.; Carbonell, P. Transcription Factor-Based Biosensors for Screening and Dynamic Regulation. *Front Bioeng Biotechnol* **2023**, *11*, 1118702. https://doi.org/10.3389/fbioe.2023.1118702.

(6) Chaisupa, P.; Wright, R. C. State-of-the-Art in Engineering Small Molecule Biosensors and Their Applications in Metabolic Engineering. *SLAS Technol* **2024**, *29* (2), 100113. https://doi.org/10.1016/J.SLAST.2023.10.005.

(7) Dietrich, J. A.; Shis, D. L.; Alikhani, A.; Keasling, J. D. Transcription Factor-Based Screens and Synthetic Selections for Microbial Small-Molecule Biosynthesis. *ACS Synth Biol* **2013**, *2* (1), 47–58. https://doi.org/10.1021/sb300091d.

(8) Seshasayee, A. S. N.; Sivaraman, K.; Luscombe, N. M. An Overview of Prokaryotic Transcription Factors: A Summary of Function and Occurrence in Bacterial Genomes. *Subcell Biochem* **2011**, *52*, 7–23. https://doi.org/10.1007/978-90-481-9069-0_2.

(9) Minezaki, Y.; Homma, K.; Nishikawa, K. Genome-Wide Survey of Transcription Factors in Prokaryotes Reveals Many Bacteria-Specific Families Not Found in Archaea. *DNA Research* **2006**, *12* (5), 269–280. https://doi.org/10.1093/dnares/dsi016.

(10) 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* (5), 1303–1314. https://doi.org/10.1021/acssynbio.7b00419.

(11) Zhou, G. J.; Zhang, F. Applications and Tuning Strategies for Transcription Factor-Based Metabolite Biosensors. *Biosensors (Basel)* **2023**, *13* (4), 428. https://doi.org/10.3390/bios13040428.

(12) Demeester, W.; De Baets, J.; Duchi, D.; De Mey, M.; De Paepe, B. MoBioS: Modular Platform Technology for High-Throughput Construction and Characterization of Tunable Transcriptional Biological Sensors. *Biosensors (Basel)* **2023**, *13* (6), 590. https://doi.org/10.3390/bios13060590.

(13) Nixon, B. T.; Ronson, C. W.; Ausubel, F. M. Two-Component Regulatory Systems Responsive to Environmental Stimuli Share Strongly Conserved Domains with the Nitrogen Assimilation Regulatory Genes NtrB and NtrC. *Proc Natl Acad Sci U S A* **1986**, *83* (20), 7850. https://doi.org/10.1073/PNAS.83.20.7850.

(14) Cozzone, A. J. Protein Phosphorylation in Prokaryotes. *Annu Rev Microbiol* **1988**, *42* (1), 97–125. https://doi.org/10.1146/annurev.mi.42.100188.000525.

(15) Demeester, W.; De Paepe, B.; De Mey, M. Fundamentals and Exceptions of the LysR-Type Transcriptional Regulators. *ACS Synth Biol* **2024**. https://doi.org/10.1021/acssynbio.4c00219.

(16) Ong, N. T.; Tabor, J. J. A Miniaturized *Escherichia coli* Green Light Sensor with High Dynamic Range. *ChemBioChem* **2018**, *19* (12), 1255–1258. https://doi.org/10.1002/cbic.201800007.

(17) Zhao, J.; Sun, H.; Wang, G.; Wang, Q.; Wang, Y.; Li, Q.; Bi, S.; Qi, Q.; Wang, Q. Engineering Chimeric Chemoreceptors and Two-Component Systems for Orthogonal and Leakless Biosensing of Extracellular γ-Aminobutyric Acid. *J Agric Food Chem* **2024**, *72* (25), 14216–14228. https://doi.org/10.1021/acs.jafc.4c00041.

(18) Daeffler, K. N.; Galley, J. D.; Sheth, R. U.; Ortiz-Velez, L. C.; Bibb, C. O.; Shroyer, N. F.; Britton, R. A.; Tabor, J. J. Engineering Bacterial Thiosulfate and Tetrathionate Sensors for Detecting Gut Inflammation. *Mol Syst Biol* **2017**, *13* (4), 923. https://doi.org/10.15252/msb.20167416.

(19) Schmidl, S. R.; Ekness, F.; Sofjan, K.; Daeffler, K. N. M.; Brink, K. R.; Landry, B. P.; Gerhardt, K. P.; Dyulgyarov, N.; Sheth, R. U.; Tabor, J. J. Rewiring Bacterial Two-Component Systems by Modular DNA-Binding Domain Swapping. *Nature Chemical Biology 2019 15:7* **2019**, *15* (7), 690–698. https://doi.org/10.1038/s41589-019-0286-6.

(20) He, W.; Yuan, S.; Zhong, W.-H.; Siddikee, Md. A.; Dai, C.-C. Application of Genetically Engineered Microbial Whole-Cell Biosensors for Combined Chemosensing. *Appl Microbiol Biotechnol* **2016**, *100* (3), 1109–1119. https://doi.org/10.1007/s00253-015-7160-6.

(21) Zhang, F.; Keasling, J. Biosensors and Their Applications in Microbial Metabolic Engineering. *Trends Microbiol* **2011**, *19* (7), 323–329. https://doi.org/10.1016/j.tim.2011.05.003.

(22) Stanton, B. C.; Nielsen, A. A. K.; Tamsir, A.; Clancy, K.; Peterson, T.; Voigt, C. A. Genomic Mining of Prokaryotic Repressors for Orthogonal Logic Gates. *Nature Chemical Biology 2013 10:2* **2013**, *10* (2), 99–105. https://doi.org/10.1038/nchembio.1411.

(23) Juárez, J. F.; Lecube-Azpeitia, B.; Brown, S. L.; Johnston, C. D.; Church, G. M. Biosensor Libraries Harness Large Classes of Binding Domains for Construction of Allosteric Transcriptional Regulators. *Nat Commun* **2018**, *9* (1). https://doi.org/10.1038/S41467-018-05525-6.

(24) Jacob, F.; Monod, J. Genetic Regulatory Mechanisms in the Synthesis of Proteins. *J Mol Biol* **1961**, *3* (3), 318–356. https://doi.org/10.1016/S0022-2836(61)80072-7.

(25) De Baets, J.; De Paepe, B.; De Mey, M. Delaying Production with Prokaryotic Inducible Expression Systems. *Microb Cell Fact* **2024**, *23* (1), 249. https://doi.org/10.1186/s12934-024-02523-w.

(26) WATSON, J. D.; CRICK, F. H. C. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. *Nature* **1953**, *171* (4356), 737–738. https://doi.org/10.1038/171737a0.

(27) Cameron, D. E.; Bashor, C. J.; Collins, J. J. A Brief History of Synthetic Biology. *Nature Reviews Microbiology 2014 12:5* **2014**, *12* (5), 381–390. https://doi.org/10.1038/nrmicro3239.

(28) Helling, R. B.; Weinberg, R. COMPLEMENTATION STUDIES OF ARABINOSE GENES IN *ESCHERICHIA COLI*. *Genetics* **1963**, *48* (10), 1397–1410. https://doi.org/10.1093/genetics/48.10.1397.

(29) Sheppard, D. E.; Englesberg, E. Further Evidence for Positive Control of the L-Arabinose System by Gene AraC. *J Mol Biol* **1967**, *25* (3), 443–454. https://doi.org/10.1016/0022-2836(67)90197-0.

(30) Englesberg, E.; Sheppard, D.; Squires, C.; Meronk, F. An Analysis of "Revertants" of a Deletion Mutant in the C Gene of the l-Arabinose Gene Complex in *Escherichia coli* B/r: Isolation of Initiator Constitutive Mutants (Ic). *J Mol Biol* **1969**, *43* (2), 281–298. https://doi.org/10.1016/0022-2836(69)90268-X.

(31) Gielow, L.; Largen, M.; Englesberg, E. INITIATOR CONSTITUTIVE MUTANTS OF THE L-ARABINOSE OPERON *(OIBAD)* OF *ESCHERICHIA COLI* B/r. *Genetics* **1971**, *69* (3), 289–302. https://doi.org/10.1093/genetics/69.3.289.

(32) Schleif, R. Regulation of the L-Arabinose Operon of *Escherichia coli*. *Trends in Genetics* **2000**, *16* (12), 559–565. https://doi.org/10.1016/S0168-9525(00)02153-3.

(33) Yanofsky, C.; Horn, V. Role of Regulatory Features of the Trp Operon of *Escherichia coli* in Mediating a Response to a Nutritional Shift. *J Bacteriol* **1994**, *176* (20), 6245–6254. https://doi.org/10.1128/jb.176.20.6245-6254.1994.

(34) Yanofsky, C. Attenuation in the Control of Expression of Bacterial Operons. *Nature* **1981**, *289* (5800), 751–758. https://doi.org/10.1038/289751a0.

(35) Yanofsky, C. Transcription Attenuation: Once Viewed as a Novel Regulatory Strategy. *J Bacteriol* **2000**, *182* (1), 1–8. https://doi.org/10.1128/JB.182.1.1-8.2000.

(36) Imamoto, F.; Yanofsky, C. Transcription of the Tryptophan Operon in Polarity Mutants of *Escherichia coli*. *J Mol Biol* **1967**, *28* (1), 25–35. https://doi.org/10.1016/S0022-2836(67)80074-3.

(37) Miozzari, G. F.; Yanofsky, C. Translation of the Leader Region of the *Escherichia coli* Tryptophan Operon. *J Bacteriol* **1978**, *133* (3), 1457–1466. https://doi.org/10.1128/jb.133.3.1457-1466.1978.

(38) Kelley, R. L.; Yanofsky, C. Mutational Studies with the Trp Repressor of *Escherichia coli* Support the Helix-Turn-Helix Model of Repressor Recognition of Operator DNA. *Proceedings of the National Academy of Sciences* **1985**, *82* (2), 483–487. https://doi.org/10.1073/pnas.82.2.483.

(39) Oxender, D. L.; Zurawski, G.; Yanofsky, C. Attenuation in the *Escherichia coli* Tryptophan Operon: Role of RNA Secondary Structure Involving the Tryptophan Codon Region. *Proceedings of the National Academy of Sciences* **1979**, *76* (11), 5524–5528. https://doi.org/10.1073/pnas.76.11.5524.

(40) Bennett, G. N.; Schweingruber, M. E.; Brown, K. D.; Squires, C.; Yanofsky, C. Nucleotide Sequence of the Promoter-Operator Region of the Tryptophan Operon of *Escherichia coli*. *J Mol Biol* **1978**, *121* (2), 113–137. https://doi.org/10.1016/S0022-2836(78)80001-1.

(41) Englesberg, E.; Irr, J.; Power, J.; Lee, N. Positive Control of Enzyme Synthesis by Gene C in the <scp>l</Scp> -Arabinose System. *J Bacteriol* **1965**, *90* (4), 946–957. https://doi.org/10.1128/jb.90.4.946-957.1965.

(42) GREENBLATT, J.; SCHLEIF, R. Arabinose C Protein: Regulation of the Arabinose Operon in Vitro. *Nat New Biol* **1971**, *233* (40), 166–170. https://doi.org/10.1038/newbio233166a0.

(43) Dunn, T. M.; Hahn, S.; Ogden, S.; Schleif, R. F. An Operator at -280 Base Pairs That Is Required for Repression of AraBAD Operon Promoter: Addition of DNA Helical Turns between the Operator and Promoter Cyclically Hinders Repression. *Proceedings of the National Academy of Sciences* **1984**, *81* (16), 5017–5020. https://doi.org/10.1073/pnas.81.16.5017.

(44) Saiz, L.; Vilar, J. M. DNA Looping: The Consequences and Its Control. *Curr Opin Struct Biol* **2006**, *16* (3), 344–350. https://doi.org/10.1016/j.sbi.2006.05.008.

(45) Cournac, A.; Plumbridge, J. DNA Looping in Prokaryotes: Experimental and Theoretical Approaches. *J Bacteriol* **2013**, *195* (6), 1109–1119. https://doi.org/10.1128/JB.02038-12.

(46) Cohen, S. N.; Chang, A. C. Y.; Boyer, H. W.; Helling, R. B. Construction of Biologically Functional Bacterial Plasmids *In Vitro*. *Proceedings of the National Academy of Sciences* **1973**, *70* (11), 3240–3244. https://doi.org/10.1073/pnas.70.11.3240.

(47) Sanger, F.; Nicklen, S.; Coulson, A. R. DNA Sequencing with Chain-Terminating Inhibitors. *Proc Natl Acad Sci U S A* **1977**, *74* (12), 5463–5467.

(48) Stragier, P.; Richaud, F.; Borne, F.; Patte, J. C. Regulation of Diaminopimelate Decarboxylase Synthesis in *Escherichia coli*. I. Identification of a LysR Gene Encoding an Activator of the LysA Gene. *J Mol Biol* **1983**, *168* (2), 307–320. https://doi.org/10.1016/S0022-2836(83)80020-5.

(49) Boy, E.; Richaud, C.; Patte, J. C. Multiple Regulation of DAP-Decarboxylase Synthesis in *Escherichia coli* K-12. *FEMS Microbiol Lett* **1979**, *5* (4), 287–290. https://doi.org/10.1111/j.1574-6968.1979.tb03322.x.

(50) Foster, T. J.; Brown, N. L. Identification of the MerR Gene of R100 by Using Mer-Lac Gene and Operon Fusions. *J Bacteriol* **1985**, *163* (3), 1153–1157. https://doi.org/10.1128/jb.163.3.1153-1157.1985.

(51) Summers, A. O.; Kight-Olliff, L.; Slater, C. Effect of Catabolite Repression on the *mer* Operon. *J Bacteriol* **1982**, *149* (1), 191–197. https://doi.org/10.1128/JB.149.1.191-197.1982.

(52) Barrineau, P.; Summers, A. O. A Second Positive Regulatory Function in the *mer* (Mercury Resistance) Operon. *Gene* **1983**, *25* (2–3), 209–221. https://doi.org/10.1016/0378-1119(83)90225-1.

(53) Hillen, W.; Berens, C. MECHANISMS UNDERLYING EXPRESSION OF TN *10* ENCODED TETRACYCLINE RESISTANCE. *Annu Rev Microbiol* **1994**, *48* (1), 345–369. https://doi.org/10.1146/annurev.mi.48.100194.002021.

(54) Miwa, Y.; Fujita, Y. Promoter-Independent Catabolite Repression of the *Bacillus subtilis gnt* Operon. *The Journal of Biochemistry* **1993**, *113* (6), 665–671. https://doi.org/10.1093/oxfordjournals.jbchem.a124100.

(55) Reizer, A.; Deutscher, J.; Saier, M. H.; Reizer, J. Analysis of the Gluconate (*gnt*) Operon of *Bacillus subtilis*. *Mol Microbiol* **1991**, *5* (5), 1081–1089. https://doi.org/10.1111/J.1365-2958.1991.TB01880.X.

(56) Pabo, C. O.; Sauer, R. T. PROTEIN-DNA RECOGNITION. *Annu Rev Biochem* **1984**, *53* (1), 293–321. https://doi.org/10.1146/annurev.bi.53.070184.001453.

(57) Pabo, C. O.; Lewis, M. The Operator-Binding Domain of λ Repressor: Structure and DNA Recognition. *Nature* **1982**, *298* (5873), 443–447. https://doi.org/10.1038/298443a0.

(58) McKay, D. B.; Steitz, T. A. Structure of Catabolite Gene Activator Protein at 2.9 Å Resolution Suggests Binding to Left-Handed B-DNA. *Nature 1981 290:5809* **1981**, *290* (5809), 744–749. https://doi.org/10.1038/290744a0.

(59) Ohlendorf, D. H.; Anderson, W. F.; Matthews, B. W. Many Gene-Regulatory Proteins Appear to Have a Similar Alpha-Helical Fold That Binds DNA and Evolved from a Common Precursor. *J Mol Evol* **1983**, *19* (2), 109–114. https://doi.org/10.1007/BF02300748.

(60) Ohlendorf, D. H.; Anderson, W. F.; Fisher, R. G.; Takeda, Y.; Matthews, B. W. The Molecular Basis of DNA-Protein Recognition Inferred from the Structure of Cro Repressor. *Nature* **1982**, *298* (5876), 718–723. https://doi.org/10.1038/298718A0.

(61) Sauer, R. T.; Yocum, R. R.; Doolittle, R. F.; Lewis, M.; Pabo, C. O. Homology among DNA-Binding Proteins Suggests Use of a Conserved Super-Secondary Structure. *Nature* **1982**, *298* (5873), 447–451. https://doi.org/10.1038/298447a0.

(62) Steitz, T. A.; Ohlendorf, D. H.; McKay, D. B.; Anderson, W. F.; Matthews, B. W. Structural Similarity in the DNA-Binding Domains of Catabolite Gene Activator and Cro Repressor Proteins. *Proc Natl Acad Sci U S A* **1982**, *79* (10), 3097–3100. https://doi.org/10.1073/PNAS.79.10.3097.

(63) Adhya, S. Regulatory Genes. In *Encyclopedia of Genetics*; Elsevier, 2001; pp 1652–1657. https://doi.org/10.1006/rwgn.2001.1092.

(64) Aravind, L.; Anantharaman, V.; Balaji, S.; Babu, M. M.; Iyer, L. M. The Many Faces of the Helix-Turn-Helix Domain: Transcription Regulation and Beyond. *FEMS Microbiol Rev* **2005**, *29* (2), 231–262. https://doi.org/10.1016/J.FMRRE.2004.12.008.

(65) Weickert, M. J.; Adhya, S. A Family of Bacterial Regulators Homologous to Gal and Lac Repressors. *Journal of Biological Chemistry* **1992**, *267* (22), 15869–15874. https://doi.org/10.1016/S0021-9258(19)49615-4.

(66) Henikoff, S.; Haughn, G. W.; Calvo, J. M.; Wallace, J. C. A Large Family of Bacterial Activator Proteins. *Proceedings of the National Academy of Sciences* **1988**, *85* (18), 6602–6606. https://doi.org/10.1073/PNAS.85.18.6602.

(67) Brown, N. L.; Stoyanov, J. V.; Kidd, S. P.; Hobman, J. L. The MerR Family of Transcriptional Regulators. *FEMS Microbiol Rev* **2003**, *27* (2–3), 145–163. https://doi.org/10.1016/S0168-6445(03)00051-2.

(68) Colclough, A. L.; Scadden, J.; Blair, J. M. A. TetR-Family Transcription Factors in Gram-Negative Bacteria: Conservation, Variation and Implications for Efflux-Mediated Antimicrobial Resistance. *BMC Genomics* **2019**, *20* (1), 1–12. https://doi.org/10.1186/s12864-019-6075-5.

(69) Maddocks, S. E.; Oyston, P. C. F. Structure and Function of the LysR-Type Transcriptional Regulator (LTTR) Family Proteins. *Microbiology (N Y)* **2008**, *154* (12), 3609–3623. https://doi.org/10.1099/MIC.0.2008/022772-0.

(70) Pace, H. C.; Lu, P.; Lewis, M. Lac Repressor: Crystallization of Intact Tetramer and Its Complexes with Inducer and Operator DNA. *Proc Natl Acad Sci U S A* **1990**, *87* (5), 1870–1873. https://doi.org/10.1073/PNAS.87.5.1870.

(71) De Paepe, B.; Maertens, J.; Vanholme, B.; De Mey, M. Chimeric LysR-Type Transcriptional Biosensors for Customizing Ligand Specificity Profiles toward Flavonoids. *ACS Synth Biol* **2019**, *8* (2), 318–331. https://doi.org/10.1021/ACSSYNBIO.8B00326.

(72) Miller, J. H. Experiments in Molecular Genetics. *Published in **1972** in Cold Spring Harbor (NY) by Cold Spring Harbor laboratory* **1972**, 352–355.

(73) Casadaban, M. J.; Cohen, S. N. Lactose Genes Fused to Exogenous Promoters in One Step Using a Mu-Lac Bacteriophage: In Vivo Probe for Transcriptional Control Sequences. *Proceedings of the National Academy of Sciences* **1979**, *76* (9), 4530–4533. https://doi.org/10.1073/pnas.76.9.4530.

(74) de Wet, J. R.; Wood, K. V.; DeLuca, M.; Helinski, D. R.; Subramani, S. Firefly Luciferase Gene: Structure and Expression in Mammalian Cells. *Mol Cell Biol* **1987**, *7* (2), 725–737. https://doi.org/10.1128/MCB.7.2.725-737.1987.

(75) Gorman, C. M.; Moffat, L. F.; Howard, B. H. Recombinant Genomes Which Express Chloramphenicol Acetyltransferase in Mammalian Cells. *Mol Cell Biol* **1982**, *2* (9), 1044–1051. https://doi.org/10.1128/MCB.2.9.1044-1051.1982.

(76) Manoil, C.; Beckwith, J. TnphoA: A Transposon Probe for Protein Export Signals. *Proceedings of the National Academy of Sciences* **1985**, *82* (23), 8129–8133. https://doi.org/10.1073/PNAS.82.23.8129.

(77) Chalfie, M.; Tu, Y.; Euskirchen, G.; Ward, W. W.; Prasher, D. C. Green Fluorescent Protein as a Marker for Gene Expression. *Science (1979)* **1994**, *52* (13 Suppl), 1766–1767. https://doi.org/10.1126/SCIENCE.8303295.

(78) SHIMOMURA, O.; JOHNSON, F. H.; SAIGA, Y. Extraction, Purification and Properties of Aequorin, a Bioluminescent Protein from the Luminous Hydromedusan, *Aequorea*. *J Cell Comp Physiol* **1962**, *59* (3), 223–239. https://doi.org/10.1002/JCP.1030590302.

(79) Tsien, R. Y. The Green Fluorescent Protein. *Annu Rev Biochem* **1998**, *67*, 509–544. https://doi.org/10.1146/ANNUREV.BIOCHEM.67.1.509.

(80) Heim, R.; Tsien, R. Y. Engineering Green Fluorescent Protein for Improved Brightness, Longer Wavelengths and Fluorescence Resonance Energy Transfer. *Current Biology* **1996**, *6* (2), 178–182. https://doi.org/10.1016/S0960-9822(02)00450-5.

(81) Ormö, M.; Cubitt, A. B.; Kallio, K.; Gross, L. A.; Tsien, R. Y.; Remington, S. J. Crystal Structure of the *Aequorea victoria* Green Fluorescent Protein. *Science (1979)* **1996**, *273* (5280), 1392–1395. https://doi.org/10.1126/SCIENCE.273.5280.1392.

(82) Kilgard, R.; Heim, A. B.; Tsien, R. Y. Improved Green Fluorescence. *Nature 1995 373:6516* **1995**, *373* (6516), 663–664. https://doi.org/10.1038/373663b0.

(83) Cormack, B. P.; Valdivia, R. H.; Falkow, S. FACS-Optimized Mutants of the Green Fluorescent Protein (GFP). *Gene* **1996**, *173* (1), 33–38. https://doi.org/10.1016/0378-1119(95)00685-0.

(84) Valdivia, R. H.; Falkow, S. Fluorescence-Based Isolation of Bacterial Genes Expressed Within Host Cells. *Science (1979)* **1997**, *277* (5334), 2007–2011. https://doi.org/10.1126/SCIENCE.277.5334.2007.

(85) Herzenberg, L. A.; Sweet, R. G.; Herzenberg, L. A. Fluorescence-Activated Cell Sorting. *Sci Am* **1976**, *234* (3), 108–117. https://doi.org/10.1038/SCIENTIFICAMERICAN0376-108.

(86) Schallmey, M.; Frunzke, J.; Eggeling, L.; Marienhagen, J. Looking for the Pick of the Bunch: High-Throughput Screening of Producing Microorganisms with Biosensors. *Curr Opin Biotechnol* **2014**, *26*, 148–154. https://doi.org/10.1016/j.copbio.2014.01.005.

(87) Saiki, R. K.; Gelfand, D. H.; Stoffel, S.; Scharf, S. J.; Higuchi, R.; Horn, G. T.; Mullis, K. B.; Erlich, H. A. Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. *Science* **1988**, *239* (4839), 487–491. https://doi.org/10.1126/SCIENCE.2448875.

(88) Saiki, R. K.; Scharf, S.; Faloona, F.; Mullis, K. B.; Horn, G. T.; Erlich, H. A.; Arnheim, N. Enzymatic Amplification of β-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia. *Science (1979)* **1985**, *230* (4732), 1350–1354. https://doi.org/10.1126/SCIENCE.2999980.

(89) Müller-Hill, B.; Crapo, L.; Gilbert, W. Mutants That Make More Lac Repressor. *Proc Natl Acad Sci U S A* **1968**, *59* (4), 1259. https://doi.org/10.1073/PNAS.59.4.1259.

(90) Ding, N.; Zhou, S.; Deng, Y. Transcription-Factor-Based Biosensor Engineering for Applications in Synthetic Biology. *ACS Synth Biol* **2021**, *10* (5), 911–922. https://doi.org/10.1021/acssynbio.0c00252.

(91) Lanzer, M.; Bujard, H. Promoters Largely Determine the Efficiency of Repressor Action. *Proc Natl Acad Sci U S A* **1988**, *85* (23), 8973–8977. https://doi.org/10.1073/PNAS.85.23.8973.

(92) Lee, D. H.; Schleif, R. F. In Vivo DNA Loops in *araCBAD*: Size Limits and Helical Repeat. *Proc Natl Acad Sci U S A* **1989**, *86* (2), 476. https://doi.org/10.1073/PNAS.86.2.476.

(93) Müller, J.; Oehler, S.; Müller-Hill, B. Repression of Lac Promoter as a Function of Distance, Phase and Quality of an Auxiliary Lac Operator. *J Mol Biol* **1996**, *257* (1), 21–29. https://doi.org/10.1006/jmbi.1996.0143.

(94) Lutz, R.; Bujard, H. Independent and Tight Regulation of Transcriptional Units in *Escherichia coli* Via the LacR/O, the TetR/O and AraC/I1-I2 Regulatory Elements. *Nucleic Acids Res* **1997**, *25* (6), 1203–1210. https://doi.org/10.1093/NAR/25.6.1203.

(95) Williams, S. Repressor Titration: A Novel System for Selection and Stable Maintenance of Recombinant Plasmids. *Nucleic Acids Res* **1998**, *26* (9), 2120–2124. https://doi.org/10.1093/nar/26.9.2120.

(96) Brewster, R. C.; Weinert, F. M.; Garcia, H. G.; Song, D.; Rydenfelt, M.; Phillips, R. The Transcription Factor Titration Effect Dictates Level of Gene Expression. *Cell* **2014**, *156* (6), 1312–1323. https://doi.org/10.1016/j.cell.2014.02.022.

(97) Alper, H.; Fischer, C.; Nevoigt, E.; Stephanopoulos, G. Tuning Genetic Control through Promoter Engineering. *Proc Natl Acad Sci U S A* **2005**, *102* (36), 12678–12683. https://doi.org/10.1073/PNAS.0504604102/SUPPL_FILE/04604TABLE_1.PDF.

(98) De Mey, M.; Maertens, J.; Lequeux, G. J.; Soetaert, W. K.; Vandamme, E. J. Construction and Model-Based Analysis of a Promoter Library for *E. coli*: An Indispensable Tool for Metabolic Engineering. *BMC Biotechnol* **2007**, *7* (1), 34. https://doi.org/10.1186/1472-6750-7-34.

(99) Garcia, H. G.; Sanchez, A.; Boedicker, J. Q.; Osborne, M.; Gelles, J.; Kondev, J.; Phillips, R. Operator Sequence Alters Gene Expression Independently of Transcription Factor Occupancy in Bacteria. *Cell Rep* **2012**, *2* (1), 150–161. https://doi.org/10.1016/j.celrep.2012.06.004.

(100) Jha, R. K.; Kern, T. L.; Fox, D. T.; M. Strauss, C. E. Engineering an *Acinetobacter* Regulon for Biosensing and High-Throughput Enzyme Screening in *E. coli* via Flow Cytometry. *Nucleic Acids Res* **2014**, *42* (12), 8150–8160. https://doi.org/10.1093/nar/gku444.

(101) Liu, D.; Xiao, Y.; Evans, B. S.; Zhang, F. Negative Feedback Regulation of Fatty Acid Production Based on a Malonyl-CoA Sensor–Actuator. *ACS Synth Biol* **2015**, *4* (2), 132–140. https://doi.org/10.1021/sb400158w.

(102) Liu, X.; Gupta, S. T. P.; Bhimsaria, D.; Reed, J. L.; Rodríguez-Martínez, J. A.; Ansari, A. Z.; Raman, S. De Novo Design of Programmable Inducible Promoters. *Nucleic Acids Res* **2019**, *47* (19), 10452. https://doi.org/10.1093/NAR/GKZ772.

(103) Wang, B.; Barahona, M.; Buck, M. Amplification of Small Molecule-Inducible Gene Expression via Tuning of Intracellular Receptor Densities. *Nucleic Acids Res* **2015**, *43* (3), 1955–1964. https://doi.org/10.1093/nar/gku1388.

(104) d'Oelsnitz, S.; Nguyen, V.; Alper, H. S.; Ellington, A. D. Evolving a Generalist Biosensor for Bicyclic Monoterpenes. *ACS Synth Biol* **2022**, *11* (1), 265–272. https://doi.org/10.1021/acssynbio.1c00402.

(105) Lehming, N.; Sartorius, J.; Kisters-Woike, B.; Von Wilcken-Bergmann, B.; Muller-Hill, B. Mutant Lac Repressors with New Specificities Hint at Rules for Protein--DNA Recognition. *EMBO J* **1990**, *9* (3), 615. https://doi.org/10.1002/J.1460-2075.1990.TB08153.X.

(106) Lehming, N.; Sartorius, J.; Niemöller, M.; Genenger, G.; v Wilcken-Bergmann, B.; Müller-Hill, B. The Interaction of the Recognition Helix of Lac Repressor with Lac Operator. *EMBO J* **1987**, *6* (10), 3145–3153. https://doi.org/10.1002/J.1460-2075.1987.TB02625.X.

(107) Sartorius, J.; Lehming, N.; Kisters, B.; von Wilcken-Bergmann, B.; Müller-Hill, B. Lac Repressor Mutants with Double or Triple Exchanges in the Recognition Helix Bind Specifically to Lac Operator Variants with Multiple Exchanges. *EMBO J* **1989**, *8* (4), 1265–1270. https://doi.org/10.1002/j.1460-2075.1989.tb03500.x.

(108) Bass, S.; Sorrells, V.; Youderian, P. Mutant Trp Repressors with New DNA-Binding Specificities. *Science* **1988**, *242* (4876), 240–245. https://doi.org/10.1126/SCIENCE.3140377.

(109) Helbl, V.; Hillen, W. Stepwise Selection of TetR Variants Recognizing Tet Operator 4C with High Affinity and Specificity. *J Mol Biol* **1998**, *276* (2), 313–318. https://doi.org/10.1006/jmbi.1997.1540.

(110) Helbl, V.; Tiebel, B.; Hillen, W. Stepwise Selection of TetR Variants Recognizing Tet Operator 6C with High Affinity and Specificity. *J Mol Biol* **1998**, *276* (2), 319–324. https://doi.org/10.1006/jmbi.1997.1539.

(111) Swint-Kruse, L.; Zhan, H.; Fairbanks, B. M.; Maheshwari, A.; Matthews, K. S. Perturbation from a Distance: Mutations That Alter LacI Function through Long-Range Effects. *Biochemistry* **2003**, *42* (47), 14004–14016. https://doi.org/10.1021/bi035116x.

(112) Scholz, O.; Henßler, E.; Bail, J.; Schubert, P.; Bogdanska-Urbaniak, J.; Sopp, S.; Reich, M.; Wisshak, S.; Köstner, M.; Bertram, R.; Hillen, W. Activity Reversal of Tet Repressor Caused by Single Amino Acid Exchanges. *Mol Microbiol* **2004**, *53* (3), 777–789. https://doi.org/10.1111/j.1365-2958.2004.04159.x.

(113) Poelwijk, F. J.; de Vos, M. G. J.; Tans, S. J. Tradeoffs and Optimality in the Evolution of Gene Regulation. *Cell* **2011**, *146* (3), 462–470. https://doi.org/10.1016/j.cell.2011.06.035.

(114) Richards, D. H.; Meyer, S.; Wilson, C. J. Fourteen Ways to Reroute Cooperative Communication in the Lactose Repressor: Engineering Regulatory Proteins with Alternate Repressive Functions. *ACS Synth Biol* **2017**, *6* (1), 6–12. https://doi.org/10.1021/acssynbio.6b00048.

(115) Meyer, S.; Ramot, R.; Kishore Inampudi, K.; Luo, B.; Lin, C.; Amere, S.; Wilson, C. J. Engineering Alternate Cooperative-Communications in the Lactose Repressor Protein Scaffold. *Protein Engineering Design and Selection* **2013**, *26* (6), 433–443. https://doi.org/10.1093/protein/gzt013.

(116) Rondon, R. E.; Wilson, C. J. Engineering a New Class of Anti-LacI Transcription Factors with Alternate DNA Recognition. *ACS Synth Biol* **2019**, *8* (2), 307–317. https://doi.org/10.1021/acssynbio.8b00324.

(117) Satya Lakshmi, O.; Rao, N. M. Evolving Lac Repressor for Enhanced Inducibility. *Protein Engineering, Design and Selection* **2009**, *22* (2), 53–58. https://doi.org/10.1093/protein/gzn069.

(118) Chong, H.; Ching, C. B. Development of Colorimetric-Based Whole-Cell Biosensor for Organophosphorus Compounds by Engineering Transcription Regulator DmpR. *ACS Synth Biol* **2016**, *5* (11), 1290–1298. https://doi.org/10.1021/acssynbio.6b00061.

(119) Tang, R.-Q.; Wagner, J. M.; Alper, H. S.; Zhao, X.-Q.; Bai, F.-W. Design, Evolution, and Characterization of a Xylose Biosensor in *Escherichia coli* Using the XylR/ *XylO* System with an Expanded Operating Range. *ACS Synth Biol* **2020**, *9* (10), 2714–2722. https://doi.org/10.1021/acssynbio.0c00225.

(120) Meyer, A. J.; Segall-Shapiro, T. H.; Voigt, C. A. Marionette: *E. coli* Containing 12 Highly-Optimized Small Molecule Sensors. *bioRxiv* **2018**, 285866. https://doi.org/10.1101/285866.

(121) Ellefson, J. W.; Ledbetter, M. P.; Ellington, A. D. Directed Evolution of a Synthetic Phylogeny of Programmable Trp Repressors. *Nature Chemical Biology 2018 14:4* **2018**, *14* (4), 361–367. https://doi.org/10.1038/s41589-018-0006-7.

(122) Galvão, T. C.; de Lorenzo, V. Transcriptional Regulators à La Carte: Engineering New Effector Specificities in Bacterial Regulatory Proteins. *Curr Opin Biotechnol* **2006**, *17* (1), 34–42. https://doi.org/10.1016/j.copbio.2005.12.002.

(123) Machado, L. F. M.; Dixon, N. Directed Evolution of Transcription Factor-Based Biosensors for Altered Effector Specificity. In *Methods in molecular biology (Clifton, N.J.)*; Humana Press Inc., 2022; Vol. 2461, pp 175–193. https://doi.org/10.1007/978-1-0716-2152-3_12.

(124) Umeno, D.; Kimura, Y.; Kawai-Noma, S. Transcription Factors as Evolvable Biosensors. *Analytical Sciences* **2021**, *37* (5), 699–703. https://doi.org/10.2116/analsci.20SCR12.

(125) Pavel, H.; Forsman, M.; Shingler, V. An Aromatic Effector Specificity Mutant of the Transcriptional Regulator DmpR Overcomes the Growth Constraints of *Pseudomonas sp.* Strain CF600 on Para-Substituted Methylphenols. *J Bacteriol* **1994**, *176* (24), 7550–7557. https://doi.org/10.1128/jb.176.24.7550-7557.1994.

(126) Skärfstad, E.; O'Neill, E.; Garmendia, J.; Shingler, V. Identification of an Effector Specificity Subregion within the Aromatic-Responsive Regulators DmpR and XylR by DNA Shuffling. *J Bacteriol* **2000**, *182* (11), 3008–3016. https://doi.org/10.1128/JB.182.11.3008-3016.2000.

(127) Gupta, S.; Saxena, M.; Saini, N.; Mahmooduzzafar; Kumar, R.; Kumar, A. An Effective Strategy for a Whole-Cell Biosensor Based on Putative Effector Interaction Site of the Regulatory DmpR Protein. *PLoS One* **2012**, *7* (8), e43527. https://doi.org/10.1371/journal.pone.0043527.

(128) Reed, B.; Blazeck, J.; Alper, H. Evolution of an Alkane-Inducible Biosensor for Increased Responsiveness to Short-Chain Alkanes. *J Biotechnol* **2012**, *158* (3), 75–79. https://doi.org/10.1016/J.JBIOTEC.2012.01.028.

(129) Salto, R.; Delgado, A.; Michán, C.; Marqués, S.; Ramos, J. L. Modulation of the Function of the Signal Receptor Domain of XylR, a Member of a Family of Prokaryotic Enhancer-Like Positive Regulators. *J Bacteriol* **1998**, *180* (3), 600. https://doi.org/10.1128/JB.180.3.600-604.1998.

(130) Galvão, T. C.; Mencía, M.; De Lorenzo, V. Emergence of Novel Functions in Transcriptional Regulators by Regression to *Stem* Protein Types. *Mol Microbiol* **2007**, *65* (4), 907–919. https://doi.org/10.1111/j.1365-2958.2007.05832.x.

(131) Ogawa, Y.; Katsuyama, Y.; Ueno, K.; Ohnishi, Y. Switching the Ligand Specificity of the Biosensor XylS from *Meta* to *Para* -Toluic Acid through Directed Evolution Exploiting a Dual Selection System. *ACS Synth Biol* **2019**, *8* (12), 2679–2689. https://doi.org/10.1021/acssynbio.9b00237.

(132) Tang, S.-Y.; Fazelinia, H.; Cirino, P. C. AraC Regulatory Protein Mutants with Altered Effector Specificity. *J Am Chem Soc* **2008**, *130* (15), 5267–5271. https://doi.org/10.1021/ja7109053.

(133) Tang, S.-Y.; Qian, S.; Akinterinwa, O.; Frei, C. S.; Gredell, J. A.; Cirino, P. C. Screening for Enhanced Triacetic Acid Lactone Production by Recombinant *Escherichia coli* Expressing a Designed Triacetic Acid Lactone Reporter. *J Am Chem Soc* **2013**, *135* (27), 10099–10103. https://doi.org/10.1021/ja402654z.

(134) Tang, S.; Cirino, P. C. Design and Application of a Mevalonate-Responsive Regulatory Protein. *Angewandte Chemie International Edition* **2011**, *50* (5), 1084–1086. https://doi.org/10.1002/anie.201006083.

(135) Chen, W.; Zhang, S.; Jiang, P.; Yao, J.; He, Y.; Chen, L.; Gui, X.; Dong, Z.; Tang, S.-Y. Design of an Ectoine-Responsive AraC Mutant and Its Application in Metabolic Engineering of Ectoine Biosynthesis. *Metab Eng* **2015**, *30*, 149–155. https://doi.org/10.1016/j.ymben.2015.05.004.

(136) Frei, C. S.; Qian, S.; Cirino, P. C. New Engineered Phenolic Biosensors Based on the AraC Regulatory Protein. *Protein Engineering, Design and Selection* **2018**, *31* (6), 213–220. https://doi.org/10.1093/protein/gzy024.

(137) Wang, Z.; Doshi, A.; Chowdhury, R.; Wang, Y.; Maranas, C. D.; Cirino, P. C. Engineering Sensitivity and Specificity of AraC-Based Biosensors Responsive to Triacetic Acid Lactone and Orsellinic Acid. *Protein Engineering, Design and Selection* **2020**, *33*, 1–9. https://doi.org/10.1093/protein/gzaa027.

(138) Scholz, O.; Köstner, M.; Reich, M.; Gastiger, S.; Hillen, W. Teaching TetR to Recognize a New Inducer. *J Mol Biol* **2003**, *329* (2), 217–227. https://doi.org/10.1016/S0022-2836(03)00427-3.

(139) Henssler, E.-M.; Scholz, O.; Lochner, S.; Gmeiner, P.; Hillen, W. Structure-Based Design of Tet Repressor To Optimize a New Inducer Specificity. *Biochemistry* **2004**, *43* (29), 9512–9518. https://doi.org/10.1021/bi049682j.

(140) Li, S.; Chen, D.; Liu, Z.; Tao, S.; Zhang, T.; Chen, Y.; Bao, L.; Ma, J.; Huang, Y.; Xu, S.; Wu, L.; Chen, S. Directed Evolution of TetR for Constructing Sensitive and Broad-Spectrum Tetracycline Antibiotics Whole-Cell Biosensor. *J Hazard Mater* **2023**, *460*, 132311. https://doi.org/10.1016/j.jhazmat.2023.132311.

(141) Cebolla, A.; Sousa, C.; De Lorenzo, V. Effector Specificity Mutants of the Transcriptional Activator NahR of Naphthalene Degrading *Pseudomonas* Define Protein Sites Involved in Binding of Aromatic Inducers. *Journal of Biological Chemistry* **1997**, *272* (7), 3986–3992. https://doi.org/10.1074/jbc.272.7.3986.

(142) Wise, A. A.; Kuske, C. R. Generation of Novel Bacterial Regulatory Proteins That Detect Priority Pollutant Phenols. *Appl Environ Microbiol* **2000**, *66* (1), 163–169. https://doi.org/10.1128/AEM.66.1.163-169.2000.

(143) Shin, H. J. Development of Highly-Sensitive Microbial Biosensors by Mutation of the NahR Regulatory Gene. *J Biotechnol* **2010**, *150* (2), 246–250. https://doi.org/10.1016/j.jbiotec.2010.09.936.

(144) Lönneborg, R.; Varga, E.; Brzezinski, P. Directed Evolution of the Transcriptional Regulator DntR: Isolation of Mutants with Improved DNT-Response. *PLoS One* **2012**, *7* (1), e29994. https://doi.org/10.1371/journal.pone.0029994.

(145) Hawkins, A. C.; Arnold, F. H.; Stuermer, R.; Hauer, B.; Leadbetter, J. R. Directed Evolution of *Vibrio fischeri* LuxR for Improved Response to Butanoyl-Homoserine Lactone. *Appl Environ Microbiol* **2007**, *73* (18), 5775–5781. https://doi.org/10.1128/aem.00060-07.

(146) Collins, C. H.; Leadbetter, J. R.; Arnold, F. H. Dual Selection Enhances the Signaling Specificity of a Variant of the Quorum-Sensing Transcriptional Activator LuxR. *Nat Biotechnol* **2006**, *24* (6), 708–712. https://doi.org/10.1038/nbt1209.

(147) Collins, C. H.; Arnold, F. H.; Leadbetter, J. R. Directed Evolution of *Vibrio Fischeri* LuxR for Increased Sensitivity to a Broad Spectrum of Acyl-homoserine Lactones. *Mol Microbiol* **2005**, *55* (3), 712–723. https://doi.org/10.1111/j.1365-2958.2004.04437.x.

(148) F. M. Machado, L.; Currin, A.; Dixon, N. Directed Evolution of the PcaV Allosteric Transcription Factor to Generate a Biosensor for Aromatic Aldehydes. *J Biol Eng* **2019**, *13* (1), 91. https://doi.org/10.1186/s13036-019-0214-z.

(149) Jia, X.; Ma, Y.; Bu, R.; Zhao, T.; Wu, K. Directed Evolution of a Transcription Factor PbrR to Improve Lead Selectivity and Reduce Zinc Interference through Dual Selection. *AMB Express* **2020**, *10* (1), 67. https://doi.org/10.1186/s13568-020-01004-8.

(150) Liang, Y.; Luo, J.; Yang, C.; Guo, S.; Zhang, B.; Chen, F.; Su, K.; Zhang, Y.; Dong, Y.; Wang, Z.; Fu, H.; Sui, G.; Wang, P. Directed Evolution of the PobR Allosteric Transcription Factor to Generate a Biosensor for 4-Hydroxymandelic Acid. *World J Microbiol Biotechnol* **2022**, *38* (6), 104. https://doi.org/10.1007/s11274-022-03286-5.

(151) Cai, Y.; Zhu, K.; Shen, L.; Ma, J.; Bao, L.; Chen, D.; Wei, L.; Wei, N.; Liu, B.; Wu, Y.; Chen, S. Evolved Biosensor with High Sensitivity and Specificity for Measuring Cadmium in Actual Environmental Samples. *Environ Sci Technol* **2022**, *56* (14), 10062–10071. https://doi.org/10.1021/acs.est.2c00627.

(152) Chai, Y.; Winans, S. C. Site-directed Mutagenesis of a LuxR-type Quorum-sensing Transcription Factor: Alteration of Autoinducer Specificity. *Mol Microbiol* **2004**, *51* (3), 765–776. https://doi.org/10.1046/j.1365-2958.2003.03857.x.

(153) Ray, S.; Gunzburg, M. J.; Wilce, M.; Panjikar, S.; Anand, R. Structural Basis of Selective Aromatic Pollutant Sensing by the Effector Binding Domain of MopR, an NtrC Family Transcriptional Regulator. *ACS Chem Biol* **2016**, *11* (8), 2357–2365. https://doi.org/10.1021/acschembio.6b00020.

(154) Taylor, N. D.; Garruss, A. S.; Moretti, R.; Chan, S.; Arbing, M. A.; Cascio, D.; Rogers, J. K.; Isaacs, F. J.; Kosuri, S.; Baker, D.; Fields, S.; Church, G. M.; Raman, S. Engineering an Allosteric Transcription Factor to Respond to New Ligands. *Nature Methods 2015 13:2* **2015**, *13* (2), 177–183. https://doi.org/10.1038/nmeth.3696.

(155) de los Santos, E. L. C.; Meyerowitz, J. T.; Mayo, S. L.; Murray, R. M. Engineering Transcriptional Regulator Effector Specificity Using Computational Design and In Vitro Rapid Prototyping: Developing a Vanillin Sensor. *ACS Synth Biol* **2016**, *5* (4), 287–295. https://doi.org/10.1021/acssynbio.5b00090.

(156) Jha, R. K.; Chakraborti, S.; Kern, T. L.; Fox, D. T.; Strauss, C. E. M. Rosetta Comparative Modeling for Library Design: Engineering Alternative Inducer Specificity in a Transcription Factor. *Proteins: Structure, Function, and Bioinformatics* **2015**, *83* (7), 1327–1340. https://doi.org/10.1002/prot.24828.

(157) Jha, R. K.; Kern, T. L.; Kim, Y.; Tesar, C.; Jedrzejczak, R.; Joachimiak, A.; Strauss, C. E. M. A Microbial Sensor for Organophosphate Hydrolysis Exploiting an Engineered Specificity Switch in a Transcription Factor. *Nucleic Acids Res* **2016**, *44* (17), 8490–8500. https://doi.org/10.1093/nar/gkw687.

(158) Simons, K. T.; Bonneau, R.; Ruczinski, I.; Baker, D. Ab Initio Protein Structure Prediction of CASP III Targets Using ROSETTA. https://doi.org/10.1002/(SICI)1097-0134(1999)37:3.

(159) Das, R.; Baker, D. Macromolecular Modeling with Rosetta. *Annu Rev Biochem* **2008**, *77* (1), 363–382. https://doi.org/10.1146/annurev.biochem.77.062906.171838.

(160) Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; Bridgland, A.; Meyer, C.; Kohl, S. A. A.; Ballard, A. J.; Cowie, A.; Romera-Paredes, B.; Nikolov, S.; Jain, R.; Adler, J.; Back, T.; Petersen, S.; Reiman, D.; Clancy, E.; Zielinski, M.; Steinegger, M.; Pacholska, M.; Berghammer, T.; Bodenstein, S.; Silver, D.; Vinyals, O.; Senior, A. W.; Kavukcuoglu, K.; Kohli, P.; Hassabis, D. Highly Accurate Protein Structure Prediction with AlphaFold. *Nature* **2021**, *596* (7873), 583. https://doi.org/10.1038/S41586-021-03819-2.

(161) Ding, N.; Yuan, Z.; Ma, Z.; Wu, Y.; Yin, L. AI-Assisted Rational Design and Activity Prediction of Biological Elements for Optimizing Transcription-Factor-Based Biosensors. *Molecules* **2024**, *29* (15). https://doi.org/10.3390/MOLECULES29153512.

(162) Della Corte, D.; van Beek, H. L.; Syberg, F.; Schallmey, M.; Tobola, F.; Cormann, K. U.; Schlicker, C.; Baumann, P. T.; Krumbach, K.; Sokolowsky, S.; Morris, C. J.; Grünberger, A.; Hofmann, E.; Schröder, G. F.; Marienhagen, J. Engineering and Application of a Biosensor with Focused Ligand Specificity. *Nat Commun* **2020**, *11* (1), 4851. https://doi.org/10.1038/s41467-020-18400-0.

(163) Flachbart, L. K.; Gertzen, C. G. W.; Gohlke, H.; Marienhagen, J. Development of a Biosensor Platform for Phenolic Compounds Using a Transition Ligand Strategy. *ACS Synth Biol* **2021**, *10* (8), 2002–2014. https://doi.org/10.1021/acssynbio.1c00165.

(164) d'Oelsnitz, S.; Kim, W.; Burkholder, N. T.; Javanmardi, K.; Thyer, R.; Zhang, Y.; Alper, H. S.; Ellington, A. D. Using Fungible Biosensors to Evolve Improved Alkaloid Biosyntheses. *Nat Chem Biol* **2022**, *18* (9), 981–989. https://doi.org/10.1038/s41589-022-01072-w.

(165) Cole, H. O.; Kosonocky, C. W.; Schechter, M.; Gollihar, J.; Ellington, A. D.; d'Oelsnitz, S. Directed Evolution of Generalist Biosensors for Single Ring Aromatics. *bioRxiv* **2022**, 2022.12.11.519963. https://doi.org/https://doi.org/10.1101/2022.12.11.519963.

(166) Pham, C.; Stogios, P. J.; Savchenko, A.; Mahadevan, R. Computation-Guided Transcription Factor Biosensor Specificity Engineering for Adipic Acid Detection. *Comput Struct Biotechnol J* **2024**, *23*, 2211–2219. https://doi.org/10.1016/J.CSBJ.2024.05.002.

(167) Dimas, R. P.; Jiang, X.-L.; Alberto de la Paz, J.; Morcos, F.; Chan, C. T. Y. Engineering Repressors with Coevolutionary Cues Facilitates Toggle Switches with a Master Reset. *Nucleic Acids Res* **2019**, *47* (10), 5449–5463. https://doi.org/10.1093/nar/gkz280.

(168) Meinhardt, S.; Swint-Kruse, L. Experimental Identification of Specificity Determinants in the Domain Linker of a LacI/GalR Protein: Bioinformatics-Based Predictions Generate True Positives and False Negatives. *Proteins* **2008**, *73* (4), 941–957. https://doi.org/10.1002/PROT.22121.

(169) Meinhardt, S.; Manley, M. W.; Becker, N. A.; Hessman, J. A.; Maher, L. J.; Swint-Kruse, L. Novel Insights from Hybrid LacI/GalR Proteins: Family-Wide Functional Attributes and Biologically Significant Variation in Transcription Repression. *Nucleic Acids Res* **2012**, *40* (21), 11139–11154. https://doi.org/10.1093/NAR/GKS806.

(170) Shis, D. L.; Hussain, F.; Meinhardt, S.; Swint-Kruse, L.; Bennett, M. R. Modular, Multi-Input Transcriptional Logic Gating with Orthogonal LacI/GalR Family Chimeras. *ACS Synth Biol* **2014**, *3* (9), 645–651. https://doi.org/10.1021/sb500262f.

(171) Garmendia, J.; Devos, D.; Valencia, A.; De Lorenzo, V. *À La Carte* Transcriptional Regulators: Unlocking Responses of the Prokaryotic Enhancer-binding Protein XylR to Non-natural Effectors. *Mol Microbiol* **2001**, *42* (1), 47–59. https://doi.org/10.1046/j.1365-2958.2001.02633.x.

(172) Brocklehurst, K. R.; Hobman, J. L.; Lawley, B.; Blank, L.; Marshall, S. J.; Brown, N. L.; Morby, A. P. ZntR Is a Zn(II)-responsive MerR-like Transcriptional Regulator of *ZntA* in *Escherichia coli*. *Mol Microbiol* **1999**, *31* (3), 893–902. https://doi.org/10.1046/j.1365-2958.1999.01229.x.

(173) Mohn, W. W.; Garmendia, J.; Galvao, T. C.; De Lorenzo, V. Surveying Biotransformations with *à La Carte* Genetic Traps: Translating Dehydrochlorination of Lindane (Gamma-hexachlorocyclohexane) into *LacZ* -based Phenotypes. *Environ Microbiol* **2006**, *8* (3), 546–555. https://doi.org/10.1111/j.1462-2920.2006.00983.x.

(174) Tungtur, S.; Egan, S. M.; Swint-Kruse, L. Functional Consequences of Exchanging Domains between LacI and PurR Are Mediated by the Intervening Linker Sequence. *Proteins: Structure, Function, and Bioinformatics* **2007**, *68* (1), 375–388. https://doi.org/10.1002/prot.21412.

(175) Chan, C. T. Y.; Lee, J. W.; Cameron, D. E.; Bashor, C. J.; Collins, J. J. "Deadman" and "Passcode" Microbial Kill Switches for Bacterial Containment. *Nat Chem Biol* **2016**, *12* (2), 82–86. https://doi.org/10.1038/nchembio.1979.

(176) Dimas, R. P.; Jordan, B. R.; Jiang, X.-L.; Martini, C.; Glavy, J. S.; Patterson, D. P.; Morcos, F.; Chan, C. T. Y. Engineering DNA Recognition and Allosteric Response Properties of TetR Family Proteins by

Using a Module-Swapping Strategy. *Nucleic Acids Res* **2019**, *47* (16), 8913–8925. https://doi.org/10.1093/nar/gkz666.

(177) Mukherji, R.; Zhang, S.; Chowdhury, S.; Stallforth, P. Chimeric LuxR Transcription Factors Rewire Natural Product Regulation. *Angewandte Chemie International Edition* **2020**, *59* (15), 6192–6195. https://doi.org/10.1002/anie.201914449.

(178) Ghataora, J. S.; Gebhard, S.; Reeksting, B. J. Chimeric MerR-Family Regulators and Logic Elements for the Design of Metal-Sensitive Genetic Circuits in *Bacillus subtilis*. *ACS Synth Biol* **2023**, *12* (3), 735–749. https://doi.org/10.1021/acssynbio.2c00545.

(179) Snoek, T.; Chaberski, E. K.; Ambri, F.; Kol, S.; Bjørn, S. P.; Pang, B.; Barajas, J. F.; Welner, D. H.; Jensen, M. K.; Keasling, J. D. Evolution-Guided Engineering of Small-Molecule Biosensors. *Nucleic Acids Res* **2020**, *48* (1), e3–e3. https://doi.org/10.1093/nar/gkz954.

(180) Solomon, M. J.; Larsen, P. L.; Varshavsky, A. Mapping ProteinDNA Interactions in Vivo with Formaldehyde: Evidence That Histone H4 Is Retained on a Highly Transcribed Gene. *Cell* **1988**, *53* (6), 937–947. https://doi.org/10.1016/S0092-8674(88)90469-2.

(181) Mikkelsen, T. S.; Ku, M.; Jaffe, D. B.; Issac, B.; Lieberman, E.; Giannoukos, G.; Alvarez, P.; Brockman, W.; Kim, T. K.; Koche, R. P.; Lee, W.; Mendenhall, E.; O'Donovan, A.; Presser, A.; Russ, C.; Xie, X.; Meissner, A.; Wernig, M.; Jaenisch, R.; Nusbaum, C.; Lander, E. S.; Bernstein, B. E. Genome-Wide Maps of Chromatin State in Pluripotent and Lineage-Committed Cells. *Nature 2007 448:7153* **2007**, *448* (7153), 553–560. https://doi.org/10.1038/nature06008.

(182) Barski, A.; Cuddapah, S.; Cui, K.; Roh, T.-Y.; Schones, D. E.; Wang, Z.; Wei, G.; Chepelev, I.; Zhao, K. High-Resolution Profiling of Histone Methylations in the Human Genome. *Cell* **2007**, *129* (4), 823–837. https://doi.org/10.1016/j.cell.2007.05.009.

(183) Fried, M.; Crothers, D. M. Equilibria and Kinetics of Lac Repressor-Operator Interactions by Polyacrylamide Gel Electrophoresis. *Nucleic Acids Res* **1981**, *9* (23), 6505–6525. https://doi.org/10.1093/nar/9.23.6505.

(184) Garner, M. M.; Revzin, A. A Gel Electrophoresis Method for Quantifying the Binding of Proteins to Specific DNA Regions: Application to Components of the *Escherichia coli* Lactose Operon Regulatory System. *Nucleic Acids Res* **1981**, *9* (13), 3047–3060. https://doi.org/10.1093/nar/9.13.3047.

(185) Smith, A. J. P.; Humphries, S. E. Characterization of DNA-Binding Proteins Using Multiplexed Competitor EMSA. *J Mol Biol* **2009**, *385* (3), 714–717. https://doi.org/10.1016/J.JMB.2008.11.035.

(186) Pan, Y.; Duncombe, T. A.; Kellenberger, C. A.; Hammond, M. C.; Herr, A. E. High-Throughput Electrophoretic Mobility Shift Assays for Quantitative Analysis of Molecular Binding Reactions. *Anal Chem* **2014**, *86* (20), 10357. https://doi.org/10.1021/AC502700B.

(187) Jolma, A.; Taipale, J. Methods for Analysis of Transcription Factor DNA-Binding Specificity In Vitro. *Subcell Biochem* **2011**, *52*, 155–173. https://doi.org/10.1007/978-90-481-9069-0_7.

(188) Jolma, A.; Kivioja, T.; Toivonen, J.; Cheng, L.; Wei, G.; Enge, M.; Taipale, M.; Vaquerizas, J. M.; Yan, J.; Sillanpää, M. J.; Bonke, M.; Palin, K.; Talukder, S.; Hughes, T. R.; Luscombe, N. M.; Ukkonen, E.;

Taipale, J. Multiplexed Massively Parallel SELEX for Characterization of Human Transcription Factor Binding Specificities. *Genome Res* **2010**, *20* (6), 861–873. https://doi.org/10.1101/GR.100552.109.

(189) Wang, T.; Sun, W.; Fan, L.; Hua, C.; Wu, N.; Fan, S.; Zhang, J.; Deng, X.; Yan, J. An Atlas of the Binding Specificities of Transcription Factors in *Pseudomonas aeruginosa* Directs Prediction of Novel Regulators in Virulence. *Elife* **2021**, *10*. https://doi.org/10.7554/ELIFE.61885.

(190) Inukai, S.; Kock, K. H.; Bulyk, M. L. Transcription Factor–DNA Binding: Beyond Binding Site Motifs. *Curr Opin Genet Dev* **2017**, *43*, 110. https://doi.org/10.1016/J.GDE.2017.02.007.

(191) Bray, D. Protein Molecules as Computational Elements in Living Cells. *Nature 1995 376:6538* **1995**, *376* (6538), 307–312. https://doi.org/10.1038/376307a0.

(192) Gao, Y.; Wang, L.; Wang, B. Customizing Cellular Signal Processing by Synthetic Multi-Level Regulatory Circuits. *Nat Commun* **2023**, *14* (1), 8415. https://doi.org/10.1038/s41467-023-44256-1.

(193) Brophy, J. A. N.; Voigt, C. A. Principles of Genetic Circuit Design. *Nat Methods* **2014**, *11* (5), 508–520. https://doi.org/10.1038/nmeth.2926.

(194) Gardner, T. S.; Cantor, C. R.; Collins, J. J. Construction of a Genetic Toggle Switch in *Escherichia coli*. *Nature* **2000**, *403* (6767), 339–342. https://doi.org/10.1038/35002131.

(195) Elowitz, M. B.; Leibier, S. A Synthetic Oscillatory Network of Transcriptional Regulators. *Nature 2000 403:6767* **2000**, *403* (6767), 335–338. https://doi.org/10.1038/35002125.

(196) Becskel, A.; Serrano, L. Engineering Stability in Gene Networks by Autoregulation. *Nature 2000 405:6786* **2000**, *405* (6786), 590–593. https://doi.org/10.1038/35014651.

(197) Hooshangi, S.; Thiberge, S.; Weiss, R. Ultrasensitivity and Noise Propagation in a Synthetic Transcriptional Cascade. *Proceedings of the National Academy of Sciences* **2005**, *102* (10), 3581–3586. https://doi.org/10.1073/pnas.0408507102.

(198) Nistala, G. J.; Wu, K.; Rao, C. V; Bhalerao, K. D. A Modular Positive Feedback-Based Gene Amplifier. *J Biol Eng* **2010**, *4* (1), 4. https://doi.org/10.1186/1754-1611-4-4.

(199) Wang, B.; Barahona, M.; Buck, M. Engineering Modular and Tunable Genetic Amplifiers for Scaling Transcriptional Signals in Cascaded Gene Networks. *Nucleic Acids Res* **2014**, *42* (14), 9484–9492. https://doi.org/10.1093/nar/gku593.

(200) Strasser, B. J. Collecting, Comparing, and Computing Sequences: The Making of Margaret O. Dayhoff's Atlas of Protein Sequence and Structure, 1954-1965. *J Hist Biol* **2010**, *43* (4), 623–660. https://doi.org/10.1007/S10739-009-9221-0.

(201) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res* **2000**, *28* (1), 235–242. https://doi.org/10.1093/NAR/28.1.235.

(202) Escape from Lysosomes. *Nat New Biol* **1971**, *233* (42), 223–223. https://doi.org/10.1038/NEWBIO233223A0.

(203) Benson, D. A.; Cavanaugh, M.; Clark, K.; Karsch-Mizrachi, I.; Lipman, D. J.; Ostell, J.; Sayers, E. W. GenBank. *Nucleic Acids Res* **2013**, *41* (Database issue), D36. https://doi.org/10.1093/NAR/GKS1195.

(204) *GenBank and WGS Statistics*. https://www.ncbi.nlm.nih.gov/genbank/statistics/ (accessed 2024-10-02).

(205) Choudhuri, S. Data, Databases, Data Format, Database Search, Data Retrieval Systems, and Genome Browsers. *Bioinformatics for Beginners* **2014**, 77–131. https://doi.org/10.1016/B978-0-12-410471-6.00005-0.

(206) Eckweiler, D.; Dudek, C. A.; Hartlich, J.; Brötje, D.; Jahn, D. PRODORIC2: The Bacterial Gene Regulation Database in 2018. *Nucleic Acids Res* **2018**, *46* (D1), D320–D326. https://doi.org/10.1093/NAR/GKX1091.

(207) Cipriano, M. J.; Novichkov, P. N.; Kazakov, A. E.; Rodionov, D. A.; Arkin, A. P.; Gelfand, M. S.; Dubchak, I. RegTransBase - a Database of Regulatory Sequences and Interactions Based on Literature: A Resource for Investigating Transcriptional Regulation in Prokaryotes. *BMC Genomics* **2013**, *14* (1), 1–8. https://doi.org/10.1186/1471-2164-14-213/FIGURES/3.

(208) Karp, P. D.; Paley, S.; Caspi, R.; Kothari, A.; Krummenacker, M.; Midford, P. E.; Moore, L. R.; Subhraveti, P.; Gama-Castro, S.; Tierrafria, V. H.; Lara, P.; Muñiz-Rascado, L.; Bonavides-Martinez, C.; Santos-Zavaleta, A.; Mackie, A.; Sun, G.; Ahn-Horst, T. A.; Choi, H.; Covert, M. W.; Collado-Vides, J.; Paulsen, I. The EcoCyc Database (2023). *EcoSal Plus* **2023**, *11* (1), eesp00022023. https://doi.org/10.1128/ecosalplus.esp-0002-2023.

(209) Karp, P. D.; Riley, M.; Paley, S. M.; Pelligrini-Toole, A. EcoCyc: An Encyclopedia of *Escherichia coli* Genes and Metabolism. *Nucleic Acids Res* **1996**, *24* (1), 32. https://doi.org/10.1093/NAR/24.1.32.

(210) Salgado, H.; Gama-Castro, S.; Lara, P.; Mejia-Almonte, C.; Alarcón-Carranza, G.; López-Almazo, A. G.; Betancourt-Figueroa, F.; Peña-Loredo, P.; Alquicira-Hernández, S.; Ledezma-Tejeida, D.; Arizmendi-Zagal, L.; Mendez-Hernandez, F.; Diaz-Gomez, A. K.; Ochoa-Praxedis, E.; Muñiz-Rascado, L. J.; García-Sotelo, J. S.; Flores-Gallegos, F. A.; Gómez, L.; Bonavides-Martínez, C.; del Moral-Chávez, V. M.; Hernández-Alvarez, A. J.; Santos-Zavaleta, A.; Capella-Gutierrez, S.; Gelpi, J. L.; Collado-Vides, J. RegulonDB V12.0: A Comprehensive Resource of Transcriptional Regulation in *E. coli* K-12. *Nucleic Acids Res* **2024**, *52* (D1), D255–D264. https://doi.org/10.1093/nar/gkad1072.

(211) Ishii, T.; Yoshida, K. I.; Terai, G.; Fujita, Y.; Nakai, K. DBTBS: A Database of *Bacillus subtilis* Promoters and Transcription Factors. *Nucleic Acids Res* **2001**, *29* (1), 278–280. https://doi.org/10.1093/NAR/29.1.278.

(212) Parise, M. T. D.; Parise, D.; Kato, R. B.; Pauling, J. K.; Tauch, A.; Azevedo, V. A. de C.; Baumbach, J. CoryneRegNet 7, the Reference Database and Analysis Platform for Corynebacterial Gene Regulatory Networks. *Scientific Data 2020 7:1* **2020**, *7* (1), 1–9. https://doi.org/10.1038/s41597-020-0484-9.

(213) Baumbach, J.; Brinkrolf, K.; Czaja, L. F.; Rahmann, S.; Tauch, A. CoryneRegNet: An Ontology-Based Data Warehouse of Corynebacterial Transcription Factors and Regulatory Networks. *BMC Genomics* **2006**, *7* (1), 1–12. https://doi.org/10.1186/1471-2164-7-24/FIGURES/6.

(214) Novichkov, P. S.; Kazakov, A. E.; Ravcheev, D. A.; Leyn, S. A.; Kovaleva, G. Y.; Sutormin, R. A.; Kazanov, M. D.; Riehl, W.; Arkin, A. P.; Dubchak, I.; Rodionov, D. A. RegPrecise 3.0 – A Resource for Genome-Scale Exploration of Transcriptional Regulation in Bacteria. *BMC Genomics* **2013**, *14* (1), 745. https://doi.org/10.1186/1471-2164-14-745.

(215) Novichkov, P. S.; Laikova, O. N.; Novichkova, E. S.; Gelfand, M. S.; Arkin, A. P.; Dubchak, I.; Rodionov, D. A. RegPrecise: A Database of Curated Genomic Inferences of Transcriptional Regulatory Interactions in Prokaryotes. *Nucleic Acids Res* **2010**, *38* (suppl_1), D111–D118. https://doi.org/10.1093/NAR/GKP894.

(216) Ortet, P.; De Luca, G.; Whitworth, D. E.; Barakat, M. P2TF: A Comprehensive Resource for Analysis of Prokaryotic Transcription Factors. *BMC Genomics* **2012**, *13* (1), 628. https://doi.org/10.1186/1471-2164-13-628.

(217) Kiliç, S.; Sagitova, D. M.; Wolfish, S.; Bely, B.; Courtot, M.; Ciufo, S.; Tatusova, T.; O'Donovan, C.; Chibucos, M. C.; Martin, M. J.; Erill, I. From Data Repositories to Submission Portals: Rethinking the Role of Domain-Specific Databases in CollecTF. *Database (Oxford)* **2016**, *2016*. https://doi.org/10.1093/DATABASE/BAW055.

(218) Kiliç, S.; White, E. R.; Sagitova, D. M.; Cornish, J. P.; Erill, I. CollecTF: A Database of Experimentally Validated Transcription Factor-Binding Sites in Bacteria. *Nucleic Acids Res* **2014**, *42* (Database issue). https://doi.org/10.1093/NAR/GKT1123.

(219) Rychel, K.; Decker, K.; Sastry, A. V; Phaneuf, P. V; Poudel, S.; Palsson, B. O. IModulonDB: A Knowledgebase of Microbial Transcriptional Regulation Derived from Machine Learning. *Nucleic Acids Res* **2021**, *49* (D1), D112–D120. https://doi.org/10.1093/nar/gkaa810.

(220) d'Oelsnitz, S.; Love, J. D.; Diaz, D. J.; Ellington, A. D. GroovDB: A Database of Ligand-Inducible Transcription Factors. *ACS Synth Biol* **2022**, *11* (10), 3534–3537. https://doi.org/10.1021/acssynbio.2c00382.

(221) Needleman, S. B.; Wunsch, C. D. A General Method Applicable to the Search for Similarities in the Amino Acid Sequence of Two Proteins. *J Mol Biol* **1970**, *48* (3), 443–453. https://doi.org/10.1016/0022-2836(70)90057-4.

(222) Smith, T. F.; Waterman, M. S. Identification of Common Molecular Subsequences. *J Mol Biol* **1981**, *147* (1), 195–197. https://doi.org/10.1016/0022-2836(81)90087-5.

(223) Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W.; Lipman, D. J. Basic Local Alignment Search Tool. *J Mol Biol* **1990**, *215* (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

(224) Cai, P.; Liu, S.; Zhang, D.; Xing, H.; Han, M.; Liu, D.; Gong, L.; Hu, Q. N. SynBioTools: A One-Stop Facility for Searching and Selecting Synthetic Biology Tools. *BMC Bioinformatics* **2023**, *24* (1), 1–11. https://doi.org/10.1186/s12859-023-05281-5.

(225) Gauthier, J.; Vincent, A. T.; Charette, S. J.; Derome, N. A Brief History of Bioinformatics. *Brief Bioinform* **2019**, *20* (6), 1981–1996. https://doi.org/10.1093/BIB/BBY063.

(226) Delépine, B.; Libis, V.; Carbonell, P.; Faulon, J. L. SensiPath: Computer-Aided Design of Sensing-Enabling Metabolic Pathways. *Nucleic Acids Res* **2016**, *44* (W1), W226–W231. https://doi.org/10.1093/NAR/GKW305.

(227) Libis, V.; Delépine, B.; Faulon, J. L. Expanding Biosensing Abilities through Computer-Aided Design of Metabolic Pathways. *ACS Synth Biol* **2016**, *5* (10), 1076–1085. https://doi.org/10.1021/acssynbio.5b00225.

(228) Lázaro, H. M.; Bautista, R. M.; Carbonell, P. DetSpace: A Web Server for Engineering Detectable Pathways for Bio-Based Chemical Production. *Nucleic Acids Res* **2024**, *52* (W1), W476–W480. https://doi.org/10.1093/NAR/GKAE287.

(229) Hanko, E. K. R.; Joosab Noor Mahomed, T. A.; Stoney, R. A.; Breitling, R. TFBMiner: A User-Friendly Command Line Tool for the Rapid Mining of Transcription Factor-Based Biosensors. *ACS Synth Biol* **2023**, *12* (5), 1497–1507. https://doi.org/10.1021/acssynbio.2c00679.

(230) Tellechea-Luzardo, J.; Martín Lázaro, H.; Moreno López, R.; Carbonell, P. Sensbio: An Online Server for Biosensor Design. *BMC Bioinformatics* **2023**, *24* (1), 71. https://doi.org/10.1186/s12859-023-05201-7.

(231) d'Oelsnitz, S.; Stofel, S. K.; Love, J. D.; Ellington, A. D. Snowprint: A Predictive Tool for Genetic Biosensor Discovery. *Communications Biology 2024 7:1* **2024**, *7* (1), 1–9. https://doi.org/10.1038/s42003-024-05849-8.

(232) d'Oelsnitz, S.; Love, J. D.; Ellington, A. D.; Ross, D. Ligify: Automated Genome Mining for Ligand-Inducible Transcription Factors. *ACS Synth Biol* **2024**. https://doi.org/10.1021/acssynbio.4c00372.

(233) Kim, G. B.; Gao, Y.; Palsson, B. O.; Lee, S. Y. DeepTFactor: A Deep Learning-Based Tool for the Prediction of Transcription Factors. *Proc Natl Acad Sci U S A* **2021**, *118* (2), e2021171118. https://doi.org/10.1073/pnas.2021171118.

(234) De Wannemaeker, L.; Mey, F.; Bervoets, I.; Ver Cruysse, M.; Baldwin, G. S.; De Mey, M. Standardization of Fluorescent Reporter Assays in Synthetic Biology across the Visible Light Spectrum. *ACS Synth Biol* **2023**, *12* (12), 3591–3607. https://doi.org/10.1021/acssynbio.3c00386.

(235) Lucks, J. B.; Qi, L.; Whitaker, W. R.; Arkin, A. P. Toward Scalable Parts Families for Predictable Design of Biological Circuits. *Curr Opin Microbiol* **2008**, *11* (6), 567–573. https://doi.org/10.1016/J.MIB.2008.10.002.

(236) Shetty, R. P.; Endy, D.; Knight, T. F. Engineering BioBrick Vectors from BioBrick Parts. *J Biol Eng* **2008**, *2* (1), 1–12. https://doi.org/10.1186/1754-1611-2-5/TABLES/4.

(237) Knight, T. Idempotent Vector Design for Standard Assembly of Biobricks. **2003**.

(238) Silva-Rocha, R.; Martínez-García, E.; Calles, B.; Chavarría, M.; Arce-Rodríguez, A.; De Las Heras, A.; Páez-Espino, A. D.; Durante-Rodríguez, G.; Kim, J.; Nikel, P. I.; Platero, R.; De Lorenzo, V. The Standard European Vector Architecture (SEVA): A Coherent Platform for the Analysis and

Deployment of Complex Prokaryotic Phenotypes. *Nucleic Acids Res* **2013**, *41* (Database issue), D666. https://doi.org/10.1093/NAR/GKS1119.

(239) Martínez-Garćía, E.; Aparicio, T.; Goñi-Moreno, A.; Fraile, S.; De Lorenzo, V. SEVA 2.0: An Update of the Standard European Vector Architecture for de-/Re-Construction of Bacterial Functionalities. *Nucleic Acids Res* **2015**, *43* (Database issue), D1183–D1189. https://doi.org/10.1093/NAR/GKU1114.

(240) Martínez-García, E.; Goñi-Moreno, A.; Bartley, B.; McLaughlin, J.; Sánchez-Sampedro, L.; Pascual Del Pozo, H.; Prieto Hernández, C.; Marletta, A. S.; De Lucrezia, D.; Sánchez-Fernández, G.; Fraile, S.; De Lorenzo, V. SEVA 3.0: An Update of the Standard European Vector Architecture for Enabling Portability of Genetic Constructs among Diverse Bacterial Hosts. *Nucleic Acids Res* **2020**, *48* (D1), D1164–D1170. https://doi.org/10.1093/NAR/GKZ1024.

(241) Galdzicki, M.; Clancy, K. P.; Oberortner, E.; Pocock, M.; Quinn, J. Y.; Rodriguez, C. A.; Roehner, N.; Wilson, M. L.; Adam, L.; Anderson, J. C.; Bartley, B. A.; Beal, J.; Chandran, D.; Chen, J.; Densmore, D.; Endy, D.; Grünberg, R.; Hallinan, J.; Hillson, N. J.; Johnson, J. D.; Kuchinsky, A.; Lux, M.; Misirli, G.; Peccoud, J.; Plahar, H. A.; Sirin, E.; Stan, G. B.; Villalobos, A.; Wipat, A.; Gennari, J. H.; Myers, C. J.; Sauro, H. M. The Synthetic Biology Open Language (SBOL) Provides a Community Standard for Communicating Designs in Synthetic Biology. *Nature Biotechnology 2014 32:6* **2014**, *32* (6), 545–550. https://doi.org/10.1038/nbt.2891.

(242) Rondon, R. E.; Groseclose, T. M.; Short, A. E.; Wilson, C. J. Transcriptional Programming Using Engineered Systems of Transcription Factors and Genetic Architectures. *Nature Communications 2019 10:1* **2019**, *10* (1), 1–13. https://doi.org/10.1038/s41467-019-12706-4.

(243) Deng, C.; Wu, Y.; Lv, X.; Li, J.; Liu, Y.; Du, G.; Chen, J.; Liu, L. Refactoring Transcription Factors for Metabolic Engineering. *Biotechnol Adv* **2022**, *57*, 107935. https://doi.org/10.1016/J.BIOTECHADV.2022.107935.

(244) Pham, C.; Stogios, P. J.; Savchenko, A.; Mahadevan, R. Advances in Engineering and Optimization of Transcription Factor-Based Biosensors for Plug-and-Play Small Molecule Detection. *Curr Opin Biotechnol* **2022**, *76*, 102753. https://doi.org/10.1016/J.COPBIO.2022.102753.

(245) Kim, N. M.; Sinnott, R. W.; Sandoval, N. R. Transcription Factor-Based Biosensors and Inducible Systems in Non-Model Bacteria: Current Progress and Future Directions. *Curr Opin Biotechnol* **2020**, *64*, 39–46. https://doi.org/10.1016/J.COPBIO.2019.09.009.

(246) De Wannemaeker, L.; Bervoets, I.; De Mey, M. Unlocking the Bacterial Domain for Industrial Biotechnology Applications Using Universal Parts and Tools. *Biotechnol Adv* **2022**, *60*. https://doi.org/10.1016/J.BIOTECHADV.2022.108028.

(247) Wang, K.; Sybers, D.; Maklad, H. R.; Lemmens, L.; Lewyllie, C.; Zhou, X.; Schult, F.; Bräsen, C.; Siebers, B.; Valegård, K.; Lindås, A. C.; Peeters, E. A TetR-Family Transcription Factor Regulates Fatty Acid Metabolism in the Archaeal Model Organism *Sulfolobus acidocaldarius*. *Nature Communications 2019 10:1* **2019**, *10* (1), 1–16. https://doi.org/10.1038/s41467-019-09479-1.

(248) D'Ambrosio, V.; Pramanik, S.; Goroncy, K.; Jakočiūnas, T.; Schönauer, D.; Davari, M. D.; Schwaneberg, U.; Keasling, J. D.; Jensen, M. K. Directed Evolution of VanR Biosensor Specificity in Yeast. *Biotechnology Notes* **2020**, *1*, 9–15. https://doi.org/10.1016/J.BIOTNO.2020.01.002.

(249) Wei, W.; Shang, Y.; Zhang, P.; Liu, Y.; You, D.; Yin, B.; Ye, B. Engineering Prokaryotic Transcriptional Activator Xylr as a Xylose-Inducible Biosensor for Transcription Activation in Yeast. *ACS Synth Biol* **2020**, *9* (5), 1022–1029. https://doi.org/10.1021/acssynbio.0c00122.

(250) Pandi, A.; Grigoras, I.; Borkowski, O.; Faulon, J. L. Optimizing Cell-Free Biosensors to Monitor Enzymatic Production. *ACS Synth Biol* **2019**, *8* (8), 1952–1957. https://doi.org/10.1021/acssynbio.9b00160.

(251) Wang, T.; Lu, Y. Advances, Challenges and Future Trends of Cell-Free Transcription-Translation Biosensors. *Biosensors 2022, Vol. 12, Page 318* **2022**, *12* (5), 318. https://doi.org/10.3390/BIOS12050318.

(252) Kim, W.; Lee, S.; Sung, B. H.; Na, J. G.; Lee, J. W. Cell-Free Systems and Genetic Biosensors for Accelerating Enzyme and Pathway Prototyping. *Curr Opin Syst Biol* **2024**, *37*, 100501. https://doi.org/10.1016/J.COISB.2023.100501.

(253) Silverman, A. D.; Akova, U.; Alam, K. K.; Jewett, M. C.; Lucks, J. B. Design and Optimization of a Cell-Free Atrazine Biosensor. *ACS Synth Biol* **2020**, *9* (3), 671–677. https://doi.org/10.1021/acssynbio.9b00388.

(254) Jung, J. K.; Alam, K. K.; Verosloff, M. S.; Capdevila, D. A.; Desmau, M.; Clauer, P. R.; Lee, J. W.; Nguyen, P. Q.; Pastén, P. A.; Matiasek, S. J.; Gaillard, J. F.; Giedroc, D. P.; Collins, J. J.; Lucks, J. B. Cell-Free Biosensors for Rapid Detection of Water Contaminants. *Nat Biotechnol* **2020**, *38* (12), 1451. https://doi.org/10.1038/S41587-020-0571-7.

(255) Lopreside, A.; Wan, X.; Michelini, E.; Roda, A.; Wang, B. Comprehensive Profiling of Diverse Genetic Reporters with Application to Whole-Cell and Cell-Free Biosensors. *Anal Chem* **2019**, *91* (23), 15284–15292. https://doi.org/10.1021/acs.analchem.9b04444.

(256) Soudier, P.; Faure, L.; Kushwaha, M.; Faulon, J. L. Cell-Free Biosensors and AI Integration. *Methods in Molecular Biology* **2022**, *2433*, 303–323. https://doi.org/10.1007/978-1-0716-1998-8_19.

(257) Karig, D. K. Cell-Free Synthetic Biology for Environmental Sensing and Remediation. *Curr Opin Biotechnol* **2017**, *45*, 69–75. https://doi.org/10.1016/J.COPBIO.2017.01.010.

(258) Grazon, C.; Baer, R. C.; Kuzmanović, U.; Nguyen, T.; Chen, M.; Zamani, M.; Chern, M.; Aquino, P.; Zhang, X.; Lecommandoux, S.; Fan, A.; Cabodi, M.; Klapperich, C.; Grinstaff, M. W.; Dennis, A. M.; Galagan, J. E. A Progesterone Biosensor Derived from Microbial Screening. *Nature Communications 2020 11:1* **2020**, *11* (1), 1–10. https://doi.org/10.1038/s41467-020-14942-5.

(259) Voyvodic, P. L.; Pandi, A.; Koch, M.; Conejero, I.; Valjent, E.; Courtet, P.; Renard, E.; Faulon, J. L.; Bonnet, J. Plug-and-Play Metabolic Transducers Expand the Chemical Detection Space of Cell-Free Biosensors. *Nature Communications 2019 10:1* **2019**, *10* (1), 1–8. https://doi.org/10.1038/s41467-019-09722-9.

(260) Nguyen, P. Q.; Soenksen, L. R.; Donghia, N. M.; Angenent-Mari, N. M.; de Puig, H.; Huang, A.; Lee, R.; Slomovic, S.; Galbersanini, T.; Lansberry, G.; Sallum, H. M.; Zhao, E. M.; Niemi, J. B.; Collins, J. J. Wearable Materials with Embedded Synthetic Biology Sensors for Biomolecule Detection. *Nat Biotechnol* **2021**, *39* (11), 1366–1374. https://doi.org/10.1038/s41587-021-00950-3.

(261) Choi, S. Electrogenic Bacteria Promise New Opportunities for Powering, Sensing, and Synthesizing. *Small* **2022**, *18* (18), 2107902. https://doi.org/10.1002/SMLL.202107902.

(262) Webster, D. P.; TerAvest, M. A.; Doud, D. F. R.; Chakravorty, A.; Holmes, E. C.; Radens, C. M.; Sureka, S.; Gralnick, J. A.; Angenent, L. T. An Arsenic-Specific Biosensor with Genetically Engineered *Shewanella oneidensis* in a Bioelectrochemical System. *Biosens Bioelectron* **2014**, *62*, 320–324. https://doi.org/10.1016/J.BIOS.2014.07.003.

(263) Zhou, A. Y.; Baruch, M.; Ajo-Franklin, C. M.; Maharbiz, M. M. A Portable Bioelectronic Sensing System (BESSY) for Environmental Deployment Incorporating Differential Microbial Sensing in Miniaturized Reactors. *PLoS One* **2017**, *12* (9). https://doi.org/10.1371/JOURNAL.PONE.0184994.

(264) Atkinson, J. T.; Su, L.; Zhang, X.; Bennett, G. N.; Silberg, J. J.; Ajo-Franklin, C. M. Real-Time Bioelectronic Sensing of Environmental Contaminants. *Nature* **2022**, *611* (7936), 548–553. https://doi.org/10.1038/S41586-022-05356-Y.

(265) Kwok, R. Five Hard Truths for Synthetic Biology. *Nature* **2010**, *463* (7279), 288–290. https://doi.org/10.1038/463288A.

(266) Moraskie, M., Roshid, M. H. O., O'Connor, G., Dikici, E., Zingg, J. M., Deo, S., & Daunert, S. Microbial whole-cell biosensors: Current applications, challenges, and future perspectives. *Biosensors and Bioelectronics* **2021**, *191*, 113359. https://doi.org/10.1016/J.BIOS.2021.113359