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Bacterial two-component systems as sensors for synthetic biology applications

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Abstract

Two-component systems (TCSs) are a ubiquitous family of signal transduction pathways that enable bacteria to sense and respond to physical, chemical, and biological stimuli outside and inside the cell. Synthetic biologists have begun to repurpose TCSs for applications in optogenetics, materials science, gut microbiome engineering, and soil nutrient biosensing, among others. New engineering methods including genetic refactoring, DNA-binding domain swapping, detection threshold tuning, and phosphorylation cross-talk insulation are being used to increase the reliability of TCS sensor performance and tailor TCS signaling properties to the requirements of specific applications. There is now potential to combine these methods with large-scale gene synthesis and laboratory screening to discover the inputs sensed by many uncharacterized TCSs and develop a large new family of genetically-encoded sensors that respond to an unrivaled breadth of stimuli.

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Introduction

Synthetic biologists program cells to sense and respond to extracellular and intracellular stimuli for applications in medicine [1], agriculture [2], chemical synthesis [3], and many other areas. For example, a probiotic strain of *Escherichia coli* that naturally colonizes tumors was recently engineered to lyse and release immune checkpoint-inhibiting nanobodies upon reaching high intra-tumoral density [4]. By enabling local delivery of these potent biologic drugs, this approach could improve the efficacy of cancer immunotherapy while decreasing side effects. In other work, researchers are engineering bacteria to sense stresses imposed by heterologous metabolic and genetic pathways and activate the expression of enzymes or stress-response systems that ameliorate these effects [5]. Such 'host-aware' design strategies could increase the stabilities of engineered genetic systems and improve the yields of industrial fermentations, among other benefits.

To program cells to sense and respond to stimuli, synthetic biologists use genetically-encoded sensors. The canonical synthetic biological sensor is an RNA or protein that binds to a ligand or perceives a biochemical or physical property of the environment and responds by altering gene expression. Riboswitches are a well-studied family of RNA-based sensors. The prototypical riboswitch is encoded on the 5' end of a messenger RNA and comprises an aptamer domain linked to a gene regulatory domain. In the presence of a cognate stimulus (a.k.a. input), the aptamer domain undergoes a conformational rearrangement that is transmitted to the gene regulatory domain, resulting in a change in the level of expression of the gene encoded on the mRNA *in cis* [6]. Riboswitch inputs are often involved in fundamental biological processes and include metal ions, molecules involved in RNA metabolism, and amino acids [7]. The gene regulatory domains of riboswitches most often modulate transcription of the downstream mRNA by exposing or obscuring transcriptional terminators, translation of the downstream open reading frame by exposing or obscuring a ribosome binding site, or mRNA splicing [8]. Synthetic riboswitches that sense inputs not known to be detected by their natural counterparts have been engineered by rational design [9–11], directed evolution [12], and machine learning [13]. One notable recent study used a physics-based model of riboswitch structure and function to engineer translation-regulating sensors of theophylline, tetramethylrosamine, fluoride, dopamine, thyroxine, and 2,4 dinitrotoluene with activation ratios up to several hundred-fold [14]. Though good progress is being made on riboswitch design, the repertoire of inputs that these RNA-based sensors can detect remains small. On the

other hand, nature has provided a wealth of protein-based sensors that sense diverse inputs relevant to synthetic biology applications.

One-component systems (OCSs) are the largest family of bacterial signal transduction pathways [15,16] and the most frequently used family of genetically-encoded sensors. The typical OCS comprises an allosteric transcription factor (aTF) and a target (a.k.a. output) promoter. The aTF usually consists of an N-terminal sensor domain linked to a C-terminal DNA-binding domain (DBD). In the presence of the input, the aTF sensor domain allosterically modulates the activity of the DBD, and thus the rate of transcription from the output promoter. A handful of well-characterized OCSs (e.g. LacI, TetR, AraC, LuxR) are often used as model sensors in synthetic biology studies. Recent efforts based on genome mining, directed evolution, protein engineering, and computational protein design have generated several dozen new OCS sensors, including those that detect inputs linked to bacterial or host physiology [17–21]. It is likely that many new OCSs will be characterized and engineered for synthetic biology applications going forward.

Two-component systems (TCSs) are the largest class of multi-step signal transduction pathways in nature [22] and an important family of sensors for synthetic biology. The classical TCS sensor comprises a sensor histidine kinase (SHK), a response regulator (RR), and an output promoter (Figure 1). A given SHK contains an N-terminal sensor domain linked to a C-terminal transmitter domain, often by a transmembrane region [23]. The presence of the input causes the SHK sensor domain to undergo a conformational rearrangement that is relayed to the transmitter domain, typically increasing kinase activity. In the activated state, the transmitter domain phosphorylates the partner RR on a conserved aspartate residue within an N-terminal receiver (REC) domain. Similar to aTFs, most RRs contain C-terminal DBDs that regulate transcription [24]. Phosphorylation induces a conformational switch that activates the DBD, often via REC-mediated homodimerization [25–27]. Most SHK transmitter domains also dephosphorylate their cognate RRs in the absence of input, thus deactivating the TCS response. A spectrum of signal transduction pathways that elaborate upon the core TCS architecture are present in bacteria, archaea, and non-animal eukaryotes [28–30]. However, the prototypical TCS is predominantly found in bacteria.

TCS performance features

Though OCSs are simpler and more abundant, TCSs offer several advantages for synthetic biology. First, SHKs can be membrane-bound or cytoplasmic while OCSs are almost exclusively cytoplasmic [15]. Accordingly, TCSs can sense extracellular, intramembrane, or intracellular inputs while OCSs typically sense intracellular inputs. From a sensor design perspective, a transporter must be co-expressed alongside an OCS if the input does not naturally diffuse or is not naturally transported across the membrane [31]. As transporters are not readily available for many compounds (e.g. large molecular weight species), OCSs tend to sense a more restricted range of inputs than TCSs. Second, the bi-functional kinase/phosphatase activity of SHKs makes TCS output signals (phosphorylated RR and thus transcription rate) relatively insensitive to changes in the expression levels of SHKs and RRs [32,33]. This built-in robustness can buffer TCS responses against gene expression noise or fluctuations in SHK and RR expression that arise from changing growth conditions. Third, we recently demonstrated that SHK phosphatase activity acts as a built-in knob for tuning TCS detection threshold [34]. In particular, by introducing transmitter domain mutations that specifically reduce SHK phosphatase activity, TCSs can be made to respond to their inputs at up to two orders of magnitude lower concentrations. Furthermore, we demonstrated that the first variable residue in the transmitter domain GXGXG motif, which is present in 64% of SHKs, can be mutated to different hydrophobic residues to tune the detection thresholds of TCSs even in the absence of well-characterized phosphatase-altering mutations. This phosphatase tuning method is simpler to implement than computational design and directed evolution, which are frequently used to tune OCS sensitivity.

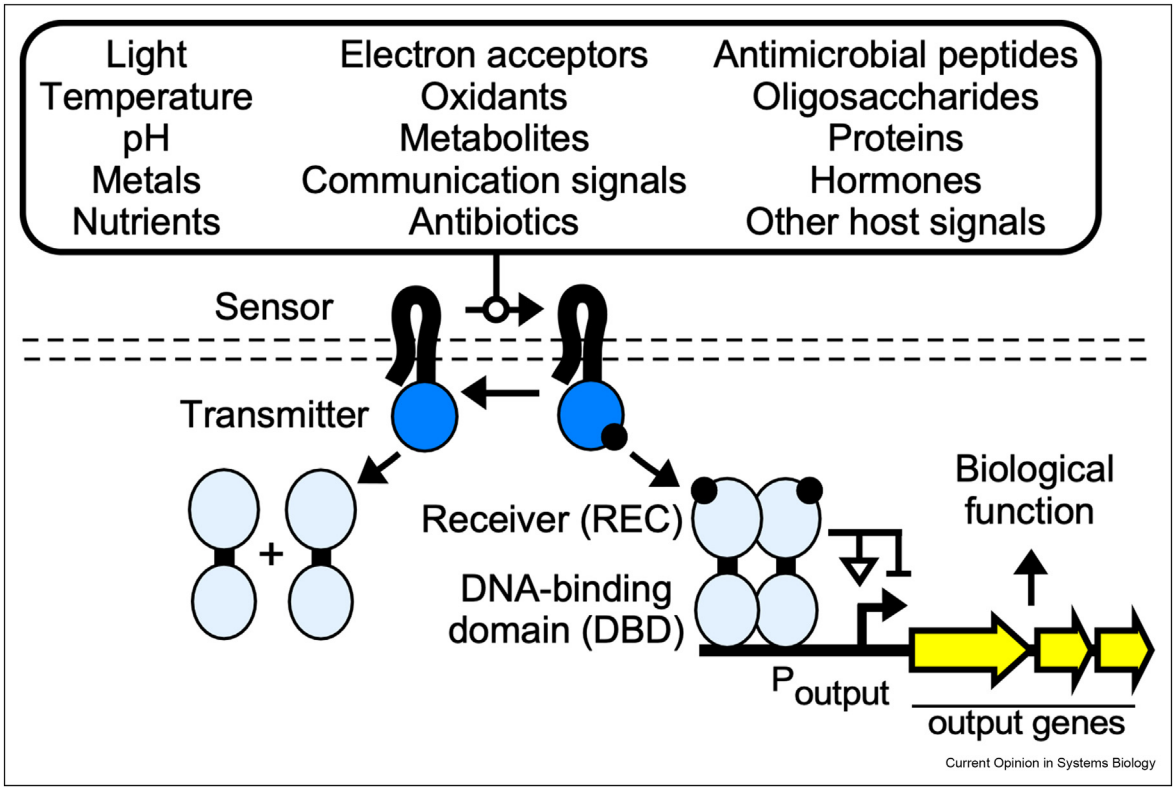
TCSs have evolved to sense a remarkable assortment of inputs. Classes of known TCS inputs include light, temperature, pH, metals, nutrient availability, respiratory electron acceptors, oxidizing agents, small-molecule metabolites, inter-bacterial communication signals, antibiotics, antimicrobial peptides, oligosaccharides, proteins, hormones, and other host-derived signals (Table 1). Some SHKs are highly specific for a single input. For example, *E. coli* NarX and *Shewanella halifaxensis* ThsS are activated by the terminal electron acceptors nitrate (NO_3^-) and thiosulfate ($S_2O_3^{2-}$) but discriminate against closely-related compounds such as nitrite (NO_2^-) and tetrathionate ($S_4O_6^{2-}$), respectively [35,36]. Other SHKs sense multiple inputs characteristic of a specific environment. For example, *Salmonella* Typhimurium PhoQ is activated by low divalent cation concentrations, acidic pH, and antimicrobial peptides; three distinct stimuli that are likely to be encountered by the bacterium during the infection of a host [37]. SHKs can detect multiple inputs via multiple binding sites in a single sensor domain [37], or multiple sensor domains [38]. Finally, many SHKs sense general phenomena such as membrane disruption that occur in the presence of a wide range of inputs [39].

Repurposing TCSs as sensors for synthetic biology

Researchers have begun to utilize TCSs as sensors for synthetic biology applications. For example, light-responsive TCSs (Table 1) have been used to spatially manipulate gene expression across two-dimensional bacterial lawns [40–42], program bacteria to perform the image processing function of edge detection [43], pattern biofilm deposition on ceramics, polystyrene, and cotton [44], introduce single base pair edits in genomic DNA [45], characterize the input/output dynamics of transcriptional regulatory circuits [46], dynamically control metabolic pathway flux [47–49], and characterize how gut bacterial metabolite secretion can impact host health and longevity [50]. In another series of studies, TCSs activated by thiosulfate, tetrathionate, and acidic pH (Table 1) have been used to program bacteria to sense and report intestinal inflammation in mouse models of colitis and Crohn's Disease [36,51,52]. Such diagnostic gut bacteria could be advanced to enable long-term monitoring and treatment of inflammatory bowel diseases with less invasiveness and fewer side effects than current standards of care.

It is often necessary to replace the evolved gene regulatory systems that govern TCS function before using them as sensors (Figure 2a). First, *shk* and *rr* genes are generally regulated by multiple interacting pathways [53]. Evolved TCS regulatory networks can cause problems such as silencing [54] or transient input responses [55] that can compromise TCS sensor function. These challenges can often be overcome using genetic refactoring (Figure 2b). Here, evolved promoters, ribosome binding sites (RBSs), and terminators are replaced with well-characterized alternatives that function reliably in a bacterium of interest. The open reading frames of *shk*, *rr*, and any required auxiliary genes can also be computationally redesigned to eliminate known and unknown regulation while increasing translational efficiency in a heterologous host [56]. Though TCSs are relatively insensitive to the levels of their component proteins, it is still important to optimize *shk* and *rr* expression levels during refactoring. TCSs are generally more sensitive to RR than SHK levels. If total RR abundance is too low, there will be too few phosphorylated RRs to bind to output promoters and activate transcription in response to the input. Conversely, if total RR abundance is too high, output

Figure 1



Bacterial two-component systems. Inputs are shown inside of the rounded box. Sensor histidine kinase (SHK): Black/dark blue. Phosphoryl group: black circle. Response regulator (RR): light blue. P_{output}: output promoter.

Table 1

Available two-component system (TCS) sensors. Often, numerous TCSs sense a given input and a given response regulator regulates numerous output promoters. In these cases, one well-characterized TCS or output promoter is listed for simplicity.

Input	Native organism(s)	SHK	RR	Output promoter	Performance notes	Reference
Light						
UV-violet light	*Synechocystis* PCC6803	UirS	UirR	P_{csiR1}	5-fold activation. Also reported to sense ethylene	[54]
Blue light	*B. subtilis*, *B. japonicum*	YF1	FixJ	P_{fixK2}	Chimeric YtvA-FixL SHK. 460-fold activation when coupled to transcriptional inverter (pDusk system)	[80]
Green light	*Synechocystis* PCC6803	CcaSmini#10	CcaR	$P_{cpcG2\text{-}172}$	600-fold activation	[81]
Red light	*Synechocystis* PCC6803, *E. coli*	Cph8*	OmpR	$P_{ompF112}$	80-fold de-activation	[60]
Near infrared light	*Rps. palustris*, *B. japonicum* BTAi1	BphP1	PpsR2	P_{Br_crtE}	Signaling based on RR sequestration. 2-fold activation	[62]
Temperature						
25°C	*B. subtilis*	DesK	DesR	P_{des}	Fully activated and repressed at 25°C and 37°C, respectively	[82]
pH						
Acidic pH (<6.2)	*S. oneidensis*, *B. subtilis*	SO_4387	SO_4388_{REC}-$PsdR_{DBD}$ 137	$P_{psdA110}$	Used to sense small intestinal inflammation in mice	[52,61]
Acidic pH (extracellular)	*H. pylori*	ArsS	ArsR	P_{amiE}, P_{amiF}	Activated in the human stomach	[83,84]
Metals						
As^{3+} (extracellular)	*A. tumefaciens*	AioS	AioR	P_{aioB}	Requires AioX inner membrane accessory protein	[85]
Ca^{2+} (extracellular)	*P. aeruginosa* PAO1	CarS	CarR	P_{carO}	CarS is related to PhoQ but senses the presence not absence of the divalent cation	[86]
Cu^{+} (extracellular)	*E. coli*	CusS	CusR	P_{cusC}	Also activated by Ag (I)	[87–89]
Cu^{2+} (extra- and intracellular)	*Synechocystis* PCC 6803	CopS	CopR	P_{copM}	CopS partially localizes to thylakoid membranes	[90]
Cu^{2+} (extracellular)	*M. xanthus*	CorS	CorR	P_{cuoA}		[91]
Fe^{2+}, Fe^{3+} (extracellular)	*S. marcescens*	RssA	RssB	P_{pvcA}	Activity can be tuned with the natural product 2-isocyano-6,7-dihydroxycoumarin	[92]
K^{+} (extracellular, intracellular)	*E. coli*	KdpD	KdpE	P_{kdpF}	Inactivated by K^{+}	[93,94]
Ni^{2+}	*Synechocystis* PCC6803	NrsS	NrsR	P_{nrsB}	10-fold activation. Also weakly activated by Co^{2+}	[95]
U	*C. crescentus*	UzcS	UzcR	P_{urcA}	Sensitivity and specificity improved by coupling to UrpRS via AND gate	[96]
Zn^{2+}	*E. coli*	ZraS	ZraR	P_{zraP}	P_{zraP} is σ^{54}-dependent	[97]
Nutrient availability						
Nitrogen limitation	*E. coli*	NtrB	NtrC	P_{ddp}		[98]
Respiratory electron acceptors						
O_2	*S. meliloti*	FixL	FixJ	P_{nifA}	O_2 inhibits the pathway. O_2 binds FixL via covalently attached heme.	[99]
Thiosulfate	*S. halifaxensis*	ThsS	ThsR	$P_{phsA342}$	Activated proportional to DSS-induced inflammation in mouse colon	[36]
Tetrathionate	*S. baltica*	TtrS	TtrR	$P_{ttrB185\text{-}269}$	100-fold dynamic range	[36]
Nitrate	*E. coli*	NarX	$NarL_{REC}$-$YdfI_{DBD}$ 131	$P_{ydfJ115}$	1300-fold activation in *B. subtilis*.	[61]

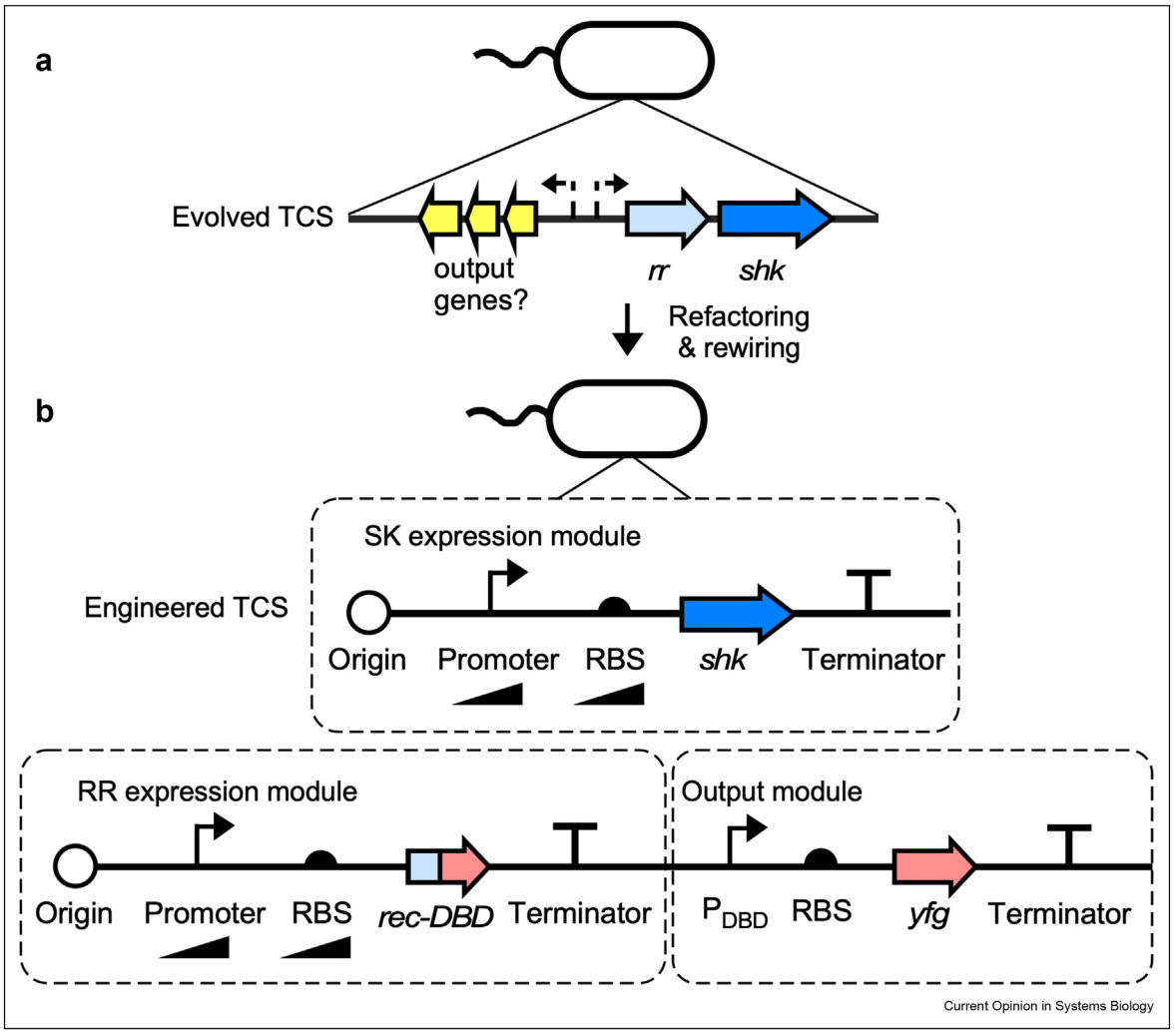
Nitrate OR Nitrite	*E. coli*	NarQ	NarP	P_{nrfA}	NarX cross-phosphorylates NarP. NarL cross-regulates NarP output promoters.	[100]
Trimethyl amine *N*-oxide (TMAO)	*E. coli*	TorS	$TorR_{REC}$-$PsdR_{DBD}$ 137	$P_{psdA110}$	DBD swapping eliminates O_2 cross repression at native output promoter. Requires periplasmic TorT accessory protein	[61]
Oxidizing agents						
O_2, H_2O_2, NO	*S. aureus*	AirS	AirR	P_{crtO}	AirS requires a $[2Fe-2S]^{2+}$ cluster	[101,102]
Small molecule metabolites						
α-ketoglutarate (extracellular)	*P. aeruginosa PAO1*	MifS	MifR	P_{PA5530}	10-fold activation. Weak response to glutarate	[103]
Butanol	*C. acetobutylicum*	BtrK	BtrR	P_{btrT}	Involved in butanol tolerance	[104]
C4-dicarboxylates (extracellular)	*E. coli*	DcuS	DcuR	P_{frdA}	22-fold activation	[105]
Citrate	*K. pneumoniae*	CitA	CitB	P_{citC}	Requires anaerobic conditions	[106]
Fucose	*E. coli*	FusK	FusR	P_{z0461}	Fucose represses transcriptional output	[107]
Fumarate	*E. coli*	DcuSZ	OmpR	P_{ompC}	Chimeric DcuS-EnvZ SHK. < 5-fold activation	[66]
Glucose-6-phosphate	*E. coli*	UhpB	UhpA	P_{uhpT99}	Requires UhpC inner-membrane accessory protein	[61,108]
L-Glutamate	*P. aeruginosa PAO1*	AauS	AauR	P_{aatJ}	Very weakly activated by aspartate, glutamine, and asparagine	[109]
Heme (extracellular)	*S. aureus*	HssS	HssR	P_{hrtA}	>100-fold activation	[110]
Indole	*E. coli*	BaeS	BaeR	P_{acrD}	Presence of CpxAR TCS amplifies the indole response	[111]
Malate	*B. subtilis*	YufL	YufM	$P_{maeN381}$	100-fold activation	[112]
Methanol	*P. denitrificans/E. coli*	FlhS-EnvZ	OmpR	P_{ompC}	2-fold activation	[65]
Pyruvate (extracellular)	*E. coli*	BtsS	BtsR	P_{yjiY}	Active in uropathogenic *E. coli* during urinary-tract infections	[113]
Ribose	*E. coli*	Trg-EnvZ	OmpR	P_{ompC}	Chimeric SHK. 20-fold activation	[71]
D-xylose (extracellular)	*C. beijerinckii*	LytS	YesN	P_{xylF}	D-xylose recognized by outer membrane transporter-like protein XylFII	[114]
Styrene	*Pseudomonas* sp.*strain Y2*	StyS	StyR	P_{styA}	StyS has non-canonical HK-REC-HK structure	[115]
Inter-bacterial communication signals						
AIP-I (autoinducer peptide)	*S. aureus*	AgrC	AgrA	P_{agrB}	AIP-II inhibits AgrC kinase activity	[116]
CAI-1 ((*S*)-3-hydroxytridecan-4-one)	*V. cholerae*	CqsS	LuxO	P_{tpqrr4}	Requires intermediate phosphotransfer protein LuxU	[117]
CSP (Competence Stimulating Peptide)	*S. gordonii*	ComD	ComE	P_{comC}		[118]
ComX (extracellular pheromone)	*B. subtilis*	ComP	ComA	P_{srfA}		[119]
Antibiotics						
β-lactams	*V. cholerae*	VxrA	VxrB	P_{murJ}	Generally activated by cell envelope damage	[120]
Linearmycins (intra-membrane)	*B. subtilis*	LnrJ	LnrK	P_{lnrL}	Can also detect the antifungal polyene amphotericin B.	[121,122]
Vancomycin (extracellular)	*S. coelicolor*	VanS	VanR	P_{vanJ}	Phosphorylated VanR additionally activates the VanSR operon	[123]
Antimicrobial peptides						
Antimicrobial peptides produced by the oral pathogen *S. mutans* (extracellular)	*Streptococcus* sp.*A12*	PcfK	PcfR	P_{pcfF}	Nearly 100-fold activation	[124]

(*continued on next page*)

Table 1. (*continued*)

Input	Native organism(s)	SHK	RR	Output promoter	Performance notes	Reference
Bacitracin (extracellular)	*B. subtilis*	LiaS	LiaR	$P_{liaI(opt)}$	1000-fold activation. Activation occurs in a 1–2 h pulse. Requires accessory membrane protein LiaF.	[125]
Nisin (extracellular)	*L. lactis*	NisK	NisR	P_{nisA}	1000-fold activation.	[126]
Subtilin (extracellular)	*B. subtilis*	SpaK	SpaR	P_{spaS}	110-fold induction	[126,127]
Oligosaccharides						
Arabinogalactan	*B. thetaiotaomicron*	BT0267	BT0267	P_{BT0268}	BT0267 is a hybrid TCS wherein the SHK and RR are fused	[128]
Chondroitin sulfate	*B. thetaiotaomicron*	BT3334	BT3334	P_{BT3324}	BT3334 is a hybrid TCS	[128]
Mucin glycans	*P. aeruginosa*	GacS	GacA	P_{rsmY}	Mucin glycans are sensed via the accessory histidine kinase RetS	[129]
Oligo-Arabinose	*B. thetaiotaomicron*	BT0366	BT0366	P_{BT0365}	BT0366 is a hybrid TCS	[130]
Proteins						
PilA	*P. aeruginosa*	PilS	PilR	P_{pilA}	PilA is the major Type IV pilin protein	[131]
Host signals						
Antimicrobial peptides, divalent cation limitation, and acidic pH produced by mammalian hosts during infection	*S. Typhimurium*	PhoQ	PhoP	P_{virK}		[132]
Epinephrine, norepinephrine	*E. coli O157:H7*	QseC	QseB	P_{flhD}	2-fold activation by epinephrine. 1.5-fold repression by norepinephrine. Also activated by bacterial autoinducer 3.	[133]
Indole-3-acetic acid (auxin)	*P. phytofirmans PsJN*	IacS	IacR1	P_{iacA}	Dioxindole-3-acetic acid amplifies the signal	[134]
2-isopentenyladenine (cytokinin)	*X. campestris*	PcrK	PcrR	P_{ctrA}	3-fold activation	[135]
Phenolics, monosaccharides, and acidic pH present at plant wounds	*A. tumefaciens*	VirA	VirG	P_{vir}	Monosaccharide sensing requires the periplasmic accessory protein ChvE.	[136]
Trans-zeatin (cytokinin)	*A. thaliana, E. coli*	AQ_4^*	$PhoP_4^*$	P_{mgrB}	AQ_4^* is a fusion of the sensing domain of *A. thaliana* AHK4 and *E. coli* PhoQ. This TCS is insulated against phosphorylation cross-talk with all *E. coli* TCSs	[70]

Figure 2



Refactoring and rewiring TCSs. (**a**) Evolved TCSs can be computationally identified in bacterial genomes due to the conservation of SHK and RR domain architectures and the fact that the genes encoding interacting SHK-RR pairs often reside adjacent to one another. However, the promoters (dashed bent lines) and RBSs driving the expression of *shk* and *rr* genes can be difficult to predict and characterize from sequence information. This problem is exacerbated for output promoters, which may or may not reside adjacent to *shk* and *rr* genes. (**b**) TCSs can be engineered to function more reliably by replacing native promoters and RBSs with well-characterized synthetic versions. Promoters and RBSs of different strengths should be screened to achieve optimal SHK and RR expression levels. If an evolved output promoter is unknown or has undesirable features, it can be replaced by DBD swapping, wherein the native *rr* gene is replaced by a chimeric *rec-DBD* gene and the native output promoter is replaced by a well-characterized promoter that responds to the Rec-DBD protein (P_{DBD}). *yfg*: your favorite gene. DBD, DNA-binding domain; RBS, ribosome binding site; RR, response regulator; SHK, sensor histidine kinase; TCS, two-component system.

promoters will often exhibit strong activity in the absence of any input. This problem can arise because of promoter binding by non-phosphorylated RRs, which exist in an equilibrium between inactive and active conformations [24,57]. It can also arise due to residual SHK kinase activity in the absence of input, or alternative sources of RR phosphorylation, such as small molecule donors [58] or non-cognate SHKs [59], the effects of which become pronounced at high total RR expression. Very low and very high SHK expression often degrade TCS responses [34,36,52,60,61] and should be avoided. SHK and RR abundances can be co-optimized using orthogonal gene expression inducers (e.g. IPTG, aTc) or libraries of constitutive promoters or RBSs of different strengths [34,36,52,54,60–62].

Unfortunately, many evolved TCS output promoters can only be activated to a small extent. Furthermore, TCS output promoters are often cross-regulated by alternative pathways or silent in heterologous hosts [36,54,61,62]. These challenges can be addressed by introducing mutations or truncations into output promoters to remove unwanted regulatory sites or nested constitutive promoters that generate leaky transcription

[36,54,60,61]. TCSs can also be rewired to non-cognate output promoters with superior performance features. The traditional approach to TCS rewiring is to make a chimera between an SHK sensor domain of interest and a non-native transmitter domain. Here, the chimeric SHK controls the phosphorylation of a non-cognate RR and thereby transcription from a non-cognate output promoter. Though sensor domain swapping has been used to engineer numerous TCS sensors [40,63–71], the allosteric mechanisms that enable communication between sensor and transmitter domains are intricate and incompletely understood. As a result, general strategies for TCS sensor domain swapping remain elusive. Approaches based on SHK-RR interface swapping [72] and protein scaffolds that redirect SHK phosphorylation to non-cognate RRs [73] have also shown promise for TCS rewiring. However, these approaches tend to yield TCSs whose activity is not dependent on the presence of the input.

Recently, we developed a general method for rewiring TCSs to well-characterized output promoters by modularly swapping RR DBDs [61]. In particular, we identified standard amino acids at which the DBDs of RRs from the OmpR/PhoB or NarL/FixJ sub-families can be removed and replaced with those from structurally related but functionally unrelated RRs. In addition, we developed standard output modules (e.g. the CcaR DBD and its $P_{cpcG2\text{-}172}$ output promoter for OmpR/PhoB family RRs, and the YdfI DBD and its $P_{ydfJ115}$ output promoter for NarL/FixJ RRs) to which TCSs from those families can be rewired with high rates of success. OmpR/PhoB and NarL/FixJ constitute over 70% of all transcription-regulating RRs [24], suggesting that thousands of TCSs could be characterized and potentially deployed as sensors using this approach.

Due to similarities in the sequences and structures of their interaction interfaces, SHKs and RRs from different TCSs may cross-talk with one another in the same cell. Such phospho-signaling cross-talk could compromise the fidelity of a given TCS sensor in the complex cellular environment. However, work by McClune et al. [70] has demonstrated that SHKs generally exhibit a high degree of specificity toward their cognate RRs in the cell because of a large and relatively unoccupied interaction sequence space. This result suggests that synthetic biologists can use multiple TCS sensors in a single cell without a high risk of phospho-signaling cross-talk. In the event that phospho-signaling cross-talk is a problem, this group also demonstrated that the evolved interaction interfaces can be replaced with insulated versions that have been shown not to cross-talk with other TCSs. The authors used this approach to engineer a system based on a sensor domain-swapped version of PhoQ that responds to the plant cytokinin trans-zeatin and does not cross-talk with any native TCS in *E. coli* [70] (Table 1).

Porting TCSs into eukaryotes

There is substantial interest in using TCSs to endow eukaryotic cells with novel sensing capabilities. In early work, an engineered *E. coli* Trg-PhoR system was used to sense 2,4,6-trinitrotoluene via an interaction with the computationally designed extracellular accessory protein TNT.R3 in *A. thaliana* [74,75]. To achieve transcription regulation in plants, the RR PhoB was fused to a VP64 transactivation domain and used to activate a minimal plant promoter engineered to contain multiple *Pho* operator sites. Porting TCSs into plants more broadly remains a challenge. In other work, Hansen et al. [76] expressed the *E. coli* TCSs EnvZ-OmpR, NarX-NarL, and DcuS-DcuR in mammalian cells. Similar RR:transactivator domain fusions and synthetic eukaryotic output promoter design strategies were used to enable control of transcription. While these three TCSs were capable of phospho-signaling and transcriptional activation, they did not respond to their cognate inputs. The reasons for sensing failure are unclear and warrant further investigation. One likely source of the problem is an incompatibility between bacterial SHK transmembrane regions and eukaryotic membranes. In an interesting recent follow-up study, this group fused two NarX transmitter domain mutants that must be brought into close physical proximity to phosphorylate NarL to a G-protein coupled receptor-β arrestin pair that heterodimerize in the presence of ligands, including procaterol [77]. This engineered pathway generates large transcriptional responses to these ligands in mammalian cells. This approach may be useful for engineering synthetic mammalian signaling pathways that do not cross-talk with endogenous systems.

Harnessing nature's treasure trove of TCS sensors

Despite exciting progress, TCSs remain a largely untapped source of sensors for synthetic biology. The number of TCSs with well-characterized inputs is on the order of one hundred (Table 1). However, many thousands of TCSs are present in bacterial genomes [34,78], and the inputs of most of these systems cannot currently be predicted. A major impediment to identifying the inputs of these orphan TCSs is that most bacteria are intractable; they can neither be cultured nor genetically-manipulated in the laboratory. Synthetic biology methods are helping to overcome this challenge. If an output promoter of an orphan TCS is known or can be inferred from the genomic context, the system can be introduced into a tractable organism such as *E. coli* or *Bacillus subtilis* [36]. If an output promoter is not known or does not function well in laboratory conditions, sensor domain swapping or DBD-swapping can be used to replace it with an alternative that functions reliably. In either case, the orphan TCS can then be screened against targeted input panels designed by analyzing the

function of genes residing adjacent to the TCS or the environment in which the native organism lives [36], complex samples representative of those environments, or even large panels of untargeted chemicals. If inputs of interest are known, commercial gene synthesis can be used to apply this process to hundreds or thousands of orphan TCS pathways to identify novel sensors. For example, libraries of orphan TCSs from the human gut microbiome could be synthesized and screened against disease biomarkers found in the gut. If a TCS responsive to the biomarker is found, it can be converted into a high-performance sensor using genetic refactoring and phospho-signaling insulation. Finally, the detection threshold of the new sensor can be matched to the needs of applications such as engineering bacteria that diagnose and treat disease [79]. Similar approaches are being taken to engineer bacteria to detect soil nitrate levels [34], which could eventually be coupled with engineered nitrogen fixation pathways [2] to maintain nitrogen homeostasis in soil without fertilizer.

Conclusions

Though they are more complex than alternatives such as riboswitches and OCSs, TCSs offer a number of benefits as sensors for synthetic biology. First, because of the transmembrane architecture of most SHKs, TCSs can sense inputs that are both accessible and inaccessible to cytoplasmic sensors. Second, TCS phospho-signaling increases the reliability of sensor function in the face of variable protein expression levels while also providing a built-in knob for tuning TCSs to respond to different input concentrations. It is likely that bacteria have exploited these and other features of TCSs to better adapt to diverse environmental conditions. Synthetic biologists are increasingly taking advantage of these same properties to endow bacteria with artificial sense and respond capabilities for new engineering applications. However, we have only reached the tip of the iceberg. Bacterial genomes host a huge number of uncharacterized TCSs that likely sense inputs of agricultural, biotechnological, environmental, medical, physiological, and scientific relevance for which no biosensors are currently available. Recent methods for porting TCSs into laboratory bacteria are accelerating the pace at which their inputs can be discovered. Recapitulating TCS function in eukaryotes remains an important challenge that will likely require both new biological insights and new engineering approaches to solve. Overall, the breadth of inputs that they sense combined with the robustness and programmability of their performance will make TCSs an important family of sensors for synthetic biology in the future.

Conflict of interest statement

JJT is a founder of PanaBio, a company that aims to commercialize diagnostic and therapeutic bacteria.

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References

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

* of special interest
** of outstanding interest

1. McNerney MP, Doiron KE, Ng TL, Chang TZ, Silver PA: **Theranostic cells: emerging clinical applications of synthetic biology**. *Nat Rev Genet* 2021, https://doi.org/10.1038/s41576-021-00383-3.

2. Ryu M-H, Zhang J, Toth T, Khokhani D, Geddes BA, Mus F, Garcia-Costas A, Peters JW, Poole PS, Ané J-M, *et al.*: **Control of nitrogen fixation in bacteria that associate with cereals**. *Nat Microbiol* 2020, **5**:314–330.

3. Ni C, Dinh CV, Prather KLJ: **Dynamic control of metabolism**. *Annu Rev Chem Biomol* 2021, **12**:1–23.

4. Gurbatri CR, Lia I, Vincent R, Coker C, Castro S, Treuting PM, Hinchliffe TE, Arpaia N, Danino T: **Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies**. *Sci Transl Med* 2020, **12**, eaax0876.

5. Boo A, Ellis T, Stan G-B: **Host-aware synthetic biology**. *Curr Opin Syst Biol* 2019, **14**:66–72.

6. Roth A, Breaker RR: **The structural and functional diversity of metabolite-binding riboswitches**. *Annu Rev Biochem* 2009, **78**:305–334.

7. McCown PJ, Corbino KA, Stav S, Sherlock ME, Breaker RR: **Riboswitch diversity and distribution**. *RNA* 2017, **23**:995–1011.

8. Breaker RR: **Riboswitches and translation control**. *Csh Perspect Biol* 2018, **10**:a032797.

9. Berens C, Suess B: **Riboswitch engineering — making the all-important second and third steps**. *Curr Opin Biotechnol* 2015, **31**:10–15.

10. Wu MJ, Andreasson JOL, Kladwang W, Greenleaf W, Das R: **Automated design of diverse stand-alone riboswitches**. *ACS Synth Biol* 2019, **8**:1838–1846.

11. Werstuck G, Green MR: **Controlling gene expression in living cells through small molecule-RNA interactions**. *Science* 1998, **282**:296–298.

12. Boussebayle A, Torka D, Ollivaud S, Braun J, Bofill-Bosch C, Dombrowski M, Groher F, Hamacher K, Suess B: **Next-level riboswitch development—implementation of Capture-SELEX facilitates identification of a new synthetic riboswitch**. *Nucleic Acids Res* 2019, **47**:4883–4895.

13. Schmidt CM, Smolke CD: **A convolutional neural network for the prediction and forward design of ribozyme-based gene-control elements**. *Elife* 2021, **10**, e59697.

14. Espah Borujeni A, Mishler DM, Wang J, Huso W, Salis HM: **Automated physics-based design of synthetic riboswitches from diverse RNA aptamers**. *Nucleic Acids Res* 2016, **44**:1–13.

15. Ulrich LE, Koonin EV, Zhulin IB: **One-component systems dominate signal transduction in prokaryotes**. *Trends Microbiol* 2005, **13**:52–56.

16. Eckweiler D, Dudek C-A, Hartlich J, Brötje D, Jahn D: **PRODORIC2: the bacterial gene regulation database in 2018**. *Nucleic Acids Res* 2017, **46**:gkx1091.

17. Meyer AJ, Segall-Shapiro TH, Glassey E, Zhang J, Voigt CA: **Escherichia coli "Marionette" strains with 12 highly optimized small-molecule sensors**. *Nat Chem Biol* 2019, **15**: 196–204.

18. Hanko EKR, Paiva AC, Jonczyk M, Abbott M, Minton NP, Malys N: **A genome-wide approach for identification and characterisation of metabolite-inducible systems**. *Nat Commun* 2020, **11**:1213.

19. Feng J, Jester BW, Tinberg CE, Mandell DJ, Antunes MS, Chari R, Morey KJ, Rios X, Medford JI, Church GM, *et al.*: **A general strategy to construct small molecule biosensors in eukaryotes**. *Elife* 2015, **4**, e10606.

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

21. Juárez JF, Lecube-Azpeitia B, Brown SL, Johnston CD, Church GM: **Biosensor libraries harness large classes of binding domains for construction of allosteric transcriptional regulators**. *Nat Commun* 2018, **9**:3101.

22. Gao R, Stock AM: **Biological insights from structures of two-component proteins**. *Annu Rev Microbiol* 2009, **63**:133–154.

23. Jacob-Dubuisson F, Mechaly A, Betton J-M, Antoine R: **Structural insights into the signalling mechanisms of two-component systems**. *Nat Rev Microbiol* 2018, **16**:585–593.

24. Gao R, Bouillet S, Stock AM: **Structural basis of response regulator function**. *Annu Rev Microbiol* 2019, **73**:1–23.

25. Menon S, Wang S: **Structure of the response regulator PhoP from Mycobacterium tuberculosis reveals a dimer through the receiver domain**. *Biochemistry-us* 2011, **50**:5948–5957.

26. Barbieri CM, Wu T, Stock AM: **Comprehensive analysis of OmpR phosphorylation, dimerization, and DNA binding supports a canonical model for activation**. *J Mol Biol* 2013, **425**:1612–1626.

27. Leonard PG, Golemi-Kotra D, Stock AM: **Phosphorylation-dependent conformational changes and domain rearrangements in Staphylococcus aureus VraR activation**. *Proc Natl Acad Sci USA* 2013, **110**:8525–8530.

28. Papon N, Stock AM: **What do archaeal and eukaryotic histidine kinases sense?** *F1000research* 2019, **8**:2145.

29. Bourret RB, Kennedy EN, Foster CA, Sepúlveda VE, Goldman WE: **A radical reimagining of fungal two-component regulatory systems**. *Trends Microbiol* 2021, https://doi.org/10.1016/j.tim.2021.03.005.

30. Grefen C, Harter K: **Plant two-component systems: principles, functions, complexity and cross talk**. *Planta* 2004, **219**: 733–742.

31. Mimee M, Nadeau P, Hayward A, Carim S, Flanagan S, Jerger L, Collins J, McDonnell S, Swartwout R, Citorik RJ, *et al.*: **An ingestible bacterial-electronic system to monitor gastrointestinal health**. *Science* 2018, **360**:915–918.

32. Batchelor E, Goulian M: **Robustness and the cycle of phosphorylation and dephosphorylation in a two-component regulatory system**. *Proc Natl Acad Sci USA* 2003, **100**: 691–696.

33. Shinar G, Milo R, Martínez MR, Alon U: **Input–output robustness in simple bacterial signaling systems**. *Proc Natl Acad Sci USA* 2007, **104**:19931–19935.

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

Here, we show that the phosphatase activity of an SHK affects the detection threshold of a TCS sensor. We identified the first X in the GXGXG motif as a general mutation target for tuning TCS detection threshold. Finally, we express unmodified and sensitivity-enhanced engineered versions of NarX-NarL in *B. subtilis* to sense 3 orders of magnitude nitrate levels in soil.

35. Rabin RS, Stewart V: **Dual response regulators (NarL and NarP) interact with dual sensors (NarX and NarQ) to control nitrate- and nitrite-regulated gene expression in Escherichia coli K-12**. *J Bacteriol* 1993, **175**:3259–3268.

36. Daeffler KN, Galley JD, Sheth RU, Ortiz-Velez LC, Bibb CO, Shroyer NF, Britton RA, Tabor JJ: **Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation**. *Mol Syst Biol* 2017, **13**:923.

37. Prost LR, Miller SI: **The Salmonellae PhoQ sensor: mechanisms of detection of phagosome signals**. *Cell Microbiol* 2008, **10**:576–582.

38. Song J-Y, Cho HS, Cho J-I, Jeon J-S, Lagarias JC, Park Y-I: **Near-UV cyanobacteriochrome signaling system elicits negative phototaxis in the cyanobacterium Synechocystis sp. PCC 6803**. *Proc Natl Acad Sci USA* 2011, **108**:10780–10785.

39. Mascher T, Helmann JD, Unden G: **Stimulus perception in bacterial signal-transducing histidine kinases**. *Microbiol Mol Biol Rev* 2006, **70**:910–938.

40. Levskaya A, Chevalier AA, Tabor JJ, Simpson ZB, Lavery LA, Levy M, Davidson EA, Scouras A, Ellington AD, Marcotte EM, *et al.*: **Engineering Escherichia coli to see light**. *Nature* 2005, **438**:441–442.

41. Tabor JJ, Levskaya A, Voigt CA: **Multichromatic control of gene expression in Escherichia coli**. *J Mol Biol* 2010, **405**: 315–324.

42. Fernandez-Rodriguez J, Moser F, Song M, Voigt CA: **Engineering RGB color vision into Escherichia coli**. *Nat Chem Biol* 2017, **13**:706–708.

43. Tabor JJ, Salis HM, Simpson ZB, Chevalier AA, Levskaya A, Marcotte EM, Voigt CA, Ellington AD: **A synthetic genetic edge detection program**. *Cell* 2009, **137**:1272–1281.

44. * Moser F, Tham E, González LM, Lu TK, Voigt CA, Light-Controlled: **High-resolution patterning of living engineered bacteria onto textiles, ceramics, and plastic**. *Adv Funct Mater* 2019, **29**:1901788.

This group utilized 3 light-responsive TCSs (CcaS-CcaR, Cph8-OmpR, Yf1-FixJ) to control three separate genes involved in curli biofilm formation in order to pattern bacteria onto commercially-relevant materials.

45. Tang W, Liu DR: **Rewritable multi-event analog recording in bacterial and mammalian cells**. *Science* 2018, **360**, eaap8992.

46. Olson EJ, Hartsough LA, Landry BP, Shroff R, Tabor JJ: **Characterizing bacterial gene circuit dynamics with optically programmed gene expression signals**. *Nat Methods* 2014, **11**: 449–455.

47. Davidson EA, Basu AS, Bayer TS: **Programming microbes using pulse width modulation of optical signals**. *J Mol Biol* 2013, **425**:4161–4166.

48. * Tandar ST, Senoo S, Toya Y, Shimizu H: **Optogenetic switch for controlling the central metabolic flux of Escherichia coli**. *Metab Eng* 2019, **55**:68–75.

This group utilized the green/red photoreversible sensor CcaS-CcaR to control the direction of metabolic flux at the *pgi* glycolytic branchpoint with green and red light.

49. * Senoo S, Tandar ST, Kitamura S, Toya Y, Shimizu H: **Light-inducible flux control of triosephosphate isomerase on glycolysis in Escherichia coli**. *Biotechnol Bioeng* 2019, **116**: 3292–3300.

These authors placed the *tpiA* gene under control of CcaS-CcaR to control flux distribution between the glycolysis and the methylglyoxal pathway.

50. ** Hartsough LA, Park M, Kotlajich MV, Lazar JT, Han B, Lin C-CJ, Musteata E, Gambill L, Wang MC, Tabor JJ: **Optogenetic control of gut bacterial metabolism to promote longevity**. *Elife* 2020, **9**, e56849.

This paper is the first report of using optogenetics to manipulate the gut microbiome. CcaS-CcaR was used to control colanic acid production in the gut of *C. elegans* with an external green light stimuli in order to study its impact on mitochondrial health and longevity.

51. Riglar DT, Giessen TW, Baym M, Kerns SJ, Niederhuber MJ, Bronson RT, Kotula JW, Gerber GK, Way JC, Silver PA: **Engineered bacteria can function in the mammalian gut long-term**

as live diagnostics of inflammation. *Nat Biotechnol* 2017, **35**: 653–658.

52. * Cartwright IM, Dowdell AS, Lanis JM, Brink KR, Mu A, Kostelecky RE, Schaefer REM, Welch N, Onyiah JC, Hall CHT, *et al.*: **Mucosal acidosis elicits a unique molecular signature in epithelia and intestinal tissue mediated by GPR31-induced CREB phosphorylation**. *Proc Natl Acad Sci USA* 2021, **118**, e2023871118.
In this work, the acidic pH-activated DBD-swapped TCS SO_4387-SO_4388$_{REC}$-PsdR$_{DBD}$ 137 was used to detect mucosal acidification in inflamed intestinal tissue in mice.

53. Bijlsma JJE, Groisman EA: **Making informed decisions: regulatory interactions between two-component systems**. *Trends Microbiol* 2003, **11**:359–366.

54. Ramakrishnan P, Tabor JJ: **Repurposing synechocystis PCC6803 UirS-UirR as a UV-Violet/Green photoreversible transcriptional regulatory tool in E. coli**. *ACS Synth Biol* 2016, **5**: 733–740.

55. Salazar ME, Laub MT: **Temporal and evolutionary dynamics of two-component signaling pathways**. *Curr Opin Microbiol* 2015, **24**:7–14.

56. Song M, Sukovich DJ, Ciccarelli L, Mayr J, Fernandez-Rodriguez J, Mirsky EA, Tucker AC, Gordon DB, Marlovits TC, Voigt CA: **Control of type III protein secretion using a minimal genetic system**. *Nat Commun* 2017, **8**:14737.

57. Volkman BF, Lipson D, Wemmer DE, Kern D: **Two-state allosteric behavior in a single-domain signaling protein**. *Science* 2001, **291**:2429–2433.

58. Lukat GS, McCleary WR, Stock AM, Stock JB: **Phosphorylation of bacterial response regulator proteins by low molecular weight phospho-donors**. *Proc Natl Acad Sci USA* 1992, **89**: 718–722.

59. Podgornaia AI, Laub MT: **Determinants of specificity in two-component signal transduction**. *Curr Opin Microbiol* 2013, **16**: 156–162.

60. Schmidl SR, Sheth RU, Wu A, Tabor JJ: **Refactoring and optimization of light-switchable Escherichia coli two-component systems**. *ACS Synth Biol* 2014, **3**:820–831.

61. ** Schmidl SR, Ekness F, Sofjan K, Daeffler KN-M, Brink KR, Landry BP, Gerhardt KP, Dyulgyarov N, Sheth RU, Tabor JJ: **Rewiring bacterial two-component systems by modular DNA-binding domain swapping**. *Nat Chem Biol* 2019, **15**: 690–698.
Here we demonstrate that RR DBDs can be modularly interchanged and develop standard output modules for rewiring TCSs with OmpR/PhoB and NarL/FixJ family DBDs to well-characterized output promoters. This method can increase TCS dynamic range, eliminate cross-regulation at output promoters, enabling TCS porting between distant species, and facilitate TCS input discovery.

62. Ong NTX, Olson EJ, Tabor JJ: **Engineering an E. coli near-infrared light sensor**. *ACS Synth Biol* 2017, **7**:240–248.

63. Utsumi R, Brissette R, Rampersaud A, Forst S, Oosawa K, Inouye M: **Activation of bacterial porin gene expression by a chimeric signal transducer in response to aspartate**. *Science* 1989, **245**:1246–1249.

64. Hori M, Oka S, Sugie Y, Ohtsuka H, Aiba H: **Construction of a photo-responsive chimeric histidine kinase in Escherichia coli**. *J Gen Appl Microbiol* 2017, **63**:2016. 07.005.

65. Selvamani V, Ganesh I, kannan Maruthamuthu M, Eom GT, Hong SH: **Engineering chimeric two-component system into Escherichia coli from Paracoccus denitrificans to sense methanol**. *Biotechnol Bioproc E* 2017, **22**:225–230.

66. Ganesh I, Ravikumar S, Lee SH, Park SJ, Hong SH: **Engineered fumarate sensing Escherichia coli based on novel chimeric two-component system**. *J Biotechnol* 2013, **168**: 560–566.

67. Ganesh I, Ravikumar S, Yoo I, Hong SH: **Construction of malate-sensing Escherichia coli by introduction of a novel chimeric two-component system**. *Bioproc Biosyst Eng* 2015, **38**:797–804.

68. Möglich A, Ayers RA, Moffat K: **Design and signaling mechanism of light-regulated histidine kinases**. *J Mol Biol* 2008, **385**: 1433–1444.

69. Lehning CE, Heidelberger JB, Reinhard J, Nørholm MHH, Draheim RR: **A modular high-throughput in vivo screening platform based on chimeric bacterial receptors**. *ACS Synth Biol* 2017, **6**:1315–1326.

70. ** McClune CJ, Alvarez-Buylla A, Voigt CA, Laub MT: **Engineering orthogonal signalling pathways reveals the sparse occupancy of sequence space**. *Nature* 2019, **574**:702–706.
This study demonstrates that the sequence space of SHK-RR interaction domains is sparsely occupied. This result implies that TCS sensors will generally not cross-talk with one another or native TCSs in a cell. A new trans-zeatin sensing TCS is engineered and given a synthetic SHK-RR interaction interface shown to be insulated from cross-talk with all *E. coli* TCSs.

71. Baumgartner JW, Kim C, Brissette RE, Inouye M, Park C, Hazelbauer GL: **Transmembrane signalling by a hybrid protein: communication from the domain of chemoreceptor Trg that recognizes sugar-binding proteins to the kinase/phosphatase domain of osmosensor EnvZ**. *J Bacteriol* 1994, **176**: 1157–1163.

72. Skerker JM, Perchuk BS, Siryaporn A, Lubin EA, Ashenberg O, Goulian M, Laub MT: **Rewiring the specificity of two-component signal transduction systems**. *Cell* 2008, **133**: 1043–1054.

73. Whitaker WR, Davis SA, Arkin AP, Dueber JE: **Engineering robust control of two-component system phosphotransfer using modular scaffolds**. *Proc Natl Acad Sci USA* 2012, **109**: 18090–18095.

74. Antunes MS, Morey KJ, Tewari-Singh N, Bowen TA, Smith JJ, Webb CT, Hellinga HW, Medford JI: **Engineering key components in a synthetic eukaryotic signal transduction pathway**. *Mol Syst Biol* 2009, **5**:270.

75. Antunes MS, Morey KJ, Smith JJ, Albrecht KD, Bowen TA, Zdunek JK, Troupe JF, Cuneo MJ, Webb CT, Hellinga HW, *et al.*: **Programmable ligand detection system in plants through a synthetic signal transduction pathway**. *PLoS One* 2011, **6**, e16292.

76. Hansen J, Mailand E, Swaminathan KK, Schreiber J, Angelici B, Benenson Y: **Transplantation of prokaryotic two-component signaling pathways into mammalian cells**. *Proc Natl Acad Sci USA* 2014, **111**:15705–15710.

77. * Mazé A, Benenson Y: **Artificial signaling in mammalian cells enabled by prokaryotic two-component system**. *Nat Chem Biol* 2020, **16**:179–187.
This study demonstrates artificial NarXL TCS signaling in HEK293 cells by fusing the GPCR beta-arrestin to the sensor kinase NarX, resulting in a transcriptional output in the presence of epinephrine/procaterol.

78. Salvado B, Vilaprinyo E, Sorribas A, Alves R: **A survey of HK, HPt, and RR domains and their organization in two-component systems and phosphorelay proteins of organisms with fully sequenced genomes**. *Peer J* 2015, **3**:e1183.

79. Landry BP, Tabor JJ: **Engineering diagnostic and therapeutic gut bacteria**. *Microbiol Spectr* 2017, **5**.

80. Ohlendorf R, Vidavski RR, Eldar A, Moffat K, Möglich A: **From dusk till dawn: one-plasmid systems for light-regulated gene expression**. *J Mol Biol* 2012, **416**:534–542.

81. Ong NT, Tabor JJ: **A miniaturized Escherichia coli green light sensor with high dynamic range**. *Chembiochem* 2018, **19**: 1255–1258.

82. Aguilar PS, Hernandez-Arriaga AM, Cybulski LE, Erazo AC, Mendoza D de: **Molecular basis of thermosensing: a two-component signal transduction thermometer in Bacillus subtilis**. *EMBO J* 2001, **20**:1681–1691.

83. Wen Y, Marcus EA, Matrubutham U, Gleeson MA, Scott DR, Sachs G: **Acid-adaptive genes of Helicobacter pylori**. *Infect Immun* 2003, **71**:5921–5939.

84. Wen Y, Feng J, Scott DR, Marcus EA, Sachs G: **The HP0165-HP0166 two-component system (ArsRS) regulates acid-**

induced expression of HP1186 α-carbonic anhydrase in Helicobacter pylori by activating the pH-dependent Promoter ▽. *J Bacteriol* 2007, **189**:2426–2434.

85. Liu G, Liu M, Kim E, Maaty WS, Bothner B, Lei B, Rensing C, Wang G, McDermott TR: **A periplasmic arsenite-binding protein involved in regulating arsenite oxidation**. *Environ Microbiol* 2012, **14**:1624–1634.

86. Guragain M, King MM, Williamson KS, Pérez-Osorio AC, Akiyama T, Khanam S, Patrauchan MA, Franklin MJ: **The Pseudomonas aeruginosa PAO1 two-component regulator CarSR regulates calcium homeostasis and calcium-induced virulence factor production through its regulatory targets CarO and CarP**. *J Bacteriol* 2016, **198**:951–963.

87. Ravikumar S, Pham VD, Lee SH, Yoo I, Hong SH: **Modification of CusSR bacterial two-component systems by the introduction of an inducible positive feedback loop**. *J Ind Microbiol Biotechnol* 2012, **39**:861–868.

88. Gudipaty SA, Larsen AS, Rensing C, McEvoy MM: **Regulation of Cu(I)/Ag(I) efflux genes in Escherichia coli by the sensor kinase CusS**. *FEMS Microbiol Lett* 2012, **330**:30–37.

89. Yamamoto K, Ishihama A: **Transcriptional response of Escherichia coli to external copper**. *Mol Microbiol* 2005, **56**: 215–227.

90. Giner-Lamia J, López-Maury L, Reyes JC, Florencio FJ: **The CopRS two-component system is responsible for resistance to copper in the cyanobacterium synechocystis sp. PCC 6803**. *Plant Physiol* 2012, **159**:1806–1818.

91. Sánchez-Sutil MC, Marcos-Torres FJ, Pérez J, Ruiz-González M, García-Bravo E, Martínez-Cayuela M, Gómez-Santos N, Moral-eda-Muñoz A, Muñoz-Dorado J: **Dissection of the sensor domain of the copper-responsive histidine kinase CorS from Myxococcus xanthus**. *Env Microbiol Rep* 2016, **8**:363–370.

92. Lin C-S, Tsai Y-H, Chang C-J, Tseng S-F, Wu T-R, Lu C-C, Wu T-S, Lu J-J, Horng J-T, Martel J, *et al.*: **An iron detection system determines bacterial swarming initiation and biofilm formation**. *Sci Rep* 2016, **6**:36747.

93. Heermann R, Jung K: **The complexity of the 'simple' two-component system KdpD/KdpE in Escherichia coli**. *FEMS Microbiol Lett* 2010, **304**:97–106.

94. Laermann V, Ćudić E, Kipschull K, Zimmann P, Altendorf K: **The sensor kinase KdpD of Escherichia coli senses external K+**. *Mol Microbiol* 2013, **88**:1194–1204.

95. López-Maury L, García-Domínguez M, Florencio FJ, Reyes JC: **A two-component signal transduction system involved in nickel sensing in the cyanobacterium Synechocystis sp. PCC 6803**. *Mol Microbiol* 2002, **43**:247–256.

96. * Park DM, Taffet MJ: **Combinatorial sensor design in Caulobacter crescentus for selective environmental uranium detection**. *ACS Synth Biol* 2019, **8**:807–817.
Here, UzcR-UzcS and UrpR-UrpS were used to detect uranium in the presence of other elemental metals.

97. Petit-Härtlein I, Rome K, Rosny E de, Molton F, Duboc C, Gueguen E, Rodrigue A, Covès J: **Biophysical and physiological characterization of ZraP from Escherichia coli, the periplasmic accessory protein of the atypical ZraSR two-component system**. *Biochem J* 2015, **472**:205–216.

98. Zimmer DP, Soupene E, Lee HL, Wendisch VF, Khodursky AB, Peter BJ, Bender RA, Kustu S: **Nitrogen regulatory protein C-controlled genes of Escherichia coli: scavenging as a defense against nitrogen limitation**. *Proc Natl Acad Sci USA* 2000, **97**:14674–14679.

99. David M, Daveran M-L, Batut J, Dedieu A, Domergue O, Ghai J, Hertig C, Boistard P, Kahn D: **Cascade regulation of nif gene expression in Rhizobium meliloti**. *Cell* 1988, **54**:671–683.

100. Darwin AJ, Tyson KL, Busby SJW, Stewart V: **Differential regulation by the homologous response regulators NarL and NarP of Escherichia coli K-12 depends on DNA binding site arrangement**. *Mol Microbiol* 1997, **25**:583–595.

101. Sun F, Ji Q, Jones MB, Deng X, Liang H, Frank B, Telser J, Peterson SN, Bae T, He C: **AirSR, a [2Fe-2S] cluster-containing two-component system, mediates global oxygen sensing and redox signaling in Staphylococcus aureus**. *J Am Chem Soc* 2012, **134**:305–314.

102. Hall JW, Yang J, Guo H, Ji Y: **The Staphylococcus aureus AirSR two-component system mediates reactive oxygen species resistance via transcriptional regulation of staphyloxanthin production**. *Infect Immun* 2017, **85**. e00838-16.

103. Sarwar Z, Wang MX, Lundgren BR, Nomura CT: **MifS, a DctB family histidine kinase, is a specific regulator of α-ketoglutarate response in Pseudomonas aeruginosa PAO1**. *Microbiology* 2020, **166**:867–879.

104. * Yang Y, Lang N, Zhang L, Wu H, Jiang W, Gu Y: **A novel regulatory pathway consisting of a two-component system and an ABC-type transporter contributes to butanol tolerance in Clostridium acetobutylicum**. *Appl Microbiol Biotechnol* 2020, **104**:5011–5023.
This study reports the characterization of the novel butanol-sensing TCS BtrK-BtrR that can be used for *in vivo* biosensing in the process of butanol bioproduction.

105. Golby P, Davies S, Kelly DJ, Guest JR, Andrews SC: **Identification and characterization of a two-component sensor-kinase and response-regulator system (DcuS-DcuR) controlling gene expression in response to C4-dicarboxylates in Escherichia coli**. *J Bacteriol* 1999, **181**:1238–1248.

106. Yamamoto K, Matsumoto F, Oshima T, Fujita N, Ogasawara N, Ishihama A: **Anaerobic regulation of citrate fermentation by CitAB in Escherichia coli**. *Biosci Biotechnol Biochem* 2008, **72**: 3011–3014.

107. Pacheco AR, Curtis MM, Ritchie JM, Munera D, Waldor MK, Moreira CG, Sperandio V: **Fucose sensing regulates bacterial intestinal colonization**. *Nature* 2012, **492**:113–117.

108. Olekhnovich IN, Kadner RJ: **Mutational scanning and affinity cleavage analysis of UhpA-binding sites in the Escherichia coli uhpT promoter**. *J Bacteriol* 2002, **184**:2682–2691.

109. Lundgren BR, Shoytush JM, Scheel RA, Sain S, Sarwar Z, Nomura CT: **Utilization of L-glutamate as a preferred or sole nutrient in Pseudomonas aeruginosa PAO1 depends on genes encoding for the enhancer-binding protein AauR, the sigma factor RpoN and the transporter complex AatJQMP**. *BMC Microbiol* 2021, **21**:83.

110. Stauff DL, Torres VJ, Skaar EP: **Signaling and DNA-binding activities of the Staphylococcus aureus HssR-HssS two-component system required for heme sensing**. *J Biol Chem* 2007, **282**:26111–26121.

111. Hirakawa H, Inazumi Y, Masaki T, Hirata T, Yamaguchi A: **Indole induces the expression of multidrug exporter genes in Escherichia coli**. *Mol Microbiol* 2005, **55**:1113–1126.

112. Tanaka K, Kobayashi K, Ogasawara N: **The Bacillus subtilis YufLM two-component system regulates the expression of the malate transporters MaeN (YufR) and YflS, and is essential for utilization of malate in minimal medium**. *Microbiology* 2003, **149**:2317–2329.

113. Behr S, Kristoficova I, Witting M, Breland EJ, Eberly AR, Sachs C, Schmitt-Kopplin P, Hadjifrangiskou M, Jung K: **Identification of a high-affinity pyruvate receptor in Escherichia coli**. *Sci Rep* 2017, **7**:1388.

114. Sun Z, Chen Y, Yang C, Yang S, Gu Y, Jiang W: **A novel three-component system-based regulatory model for d-xylose sensing and transport in Clostridium beijerinckii**. *Mol Microbiol* 2015, **95**:576–589.

115. Velasco A, Alonso S, García JL, Perera J, Díaz E: **Genetic and functional analysis of the styrene catabolic cluster of Pseudomonas sp. strain Y2**. *J Bacteriol* 1998, **180**: 1063–1071.

116. Wang B, Zhao A, Novick RP, Muir TW: **Activation and inhibition of the receptor histidine kinase AgrC occurs through opposite helical transduction motions**. *Mol Cell* 2014, **53**: 929–940.

117. Jayaraman P, Holowko MB, Yeoh JW, Lim S, Poh CL: **Repurposing a two-component system-based biosensor for the killing of Vibrio cholerae**. *ACS Synth Biol* 2017, **6**:1403–1415.

118. Davey L, Halperin SA, Lee SF: **Mutation of the thiol-disulfide oxidoreductase SdbA activates the CiaRH two-component system, leading to bacteriocin expression shutdown in Streptococcus gordonii**. *J Bacteriol* 2016, **198**:321–331.

119. Guan C, Cui W, Cheng J, Zhou L, Guo J, Hu X, Xiao G, Zhou Z: **Construction and development of an auto-regulatory gene expression system in Bacillus subtilis**. *Microb Cell Factories* 2015, **14**:150.

120. Shin J, Choe D, Ransegnola B, Hong H, Onyekwere I, Cross T, Shi Q, Cho B, Westblade LF, Brito IL, *et al.*: **A multifaceted cellular damage repair and prevention pathway promotes high-level tolerance to β-lactam antibiotics**. *EMBO Rep* 2021, **22**, e51790.

121. Stubbendieck RM, Straight PD: **Linearmycins activate a two-component signaling system involved in bacterial competition and biofilm morphology**. *J Bacteriol* 2017, **199**. e00186-17.

122. * Revilla-Guarinos A, Dürr F, Popp PF, Döring M, Mascher T: **Amphotericin B specifically induces the two-component system LnrJK: development of a novel whole-cell biosensor for the detection of amphotericin-like polyenes**. *Front Microbiol* 2020, **11**:2022.

Here, LnrJ-LnrK is refactored and engineered to enable amphotericin B detection in *B. subtilis*.

123. Lockey C, Edwards RJ, Roper DI, Dixon AM: **The extracellular domain of two-component system sensor kinase VanS from streptomyces coelicolor binds vancomycin at a newly identified binding site**. *Sci Rep* 2020, **10**:5727.

124. * Lee K, Kaspar JR, Rojas-Carreño G, Walker AR, Burne RA: **A single system detects and protects the beneficial oral bacterium Streptococcus sp. A12 from a spectrum of antimicrobial peptides**. *Mol Microbiol* 2021, https://doi.org/10.1111/mmi.14703.

This group demonstrates that the TCS PcfK-PcfR is activated by oral antimicrobial peptides.

125. Toymentseva AA, Schrecke K, Sharipova MR, Mascher T: **The LIKE system, a novel protein expression toolbox for Bacillus subtilis based on the lial promoter**. *Microb Cell Factories* 2012, **11**:143.

126. Wolf D, Mascher T: **The applied side of antimicrobial peptide-inducible promoters from Firmicutes bacteria: expression systems and whole-cell biosensors**. *Appl Microbiol Biotechnol* 2016, **100**:4817–4829.

127. Bongers RS, Veening J-W, Wieringen MV, Kuipers OP, Kleerebezem M: **Development and characterization of a subtilin-regulated expression system in Bacillus subtilis: strict control of gene expression by addition of subtilin**. *Appl Environ Microbiol* 2005, **71**:8818–8824.

128. Mimee M, Tucker AC, Voigt CA, Lu TK: **Programming a human commensal bacterium, Bacteroides thetaiotaomicron, to sense and respond to stimuli in the murine gut microbiota**. *Cell Syst* 2015, **1**:62–71.

129. Wang BX, Wheeler KM, Cady KC, Lehoux S, Cummings RD, Laub MT, Ribbeck K: **Mucin glycans signal through the sensor kinase RetS to inhibit virulence-associated traits in Pseudomonas aeruginosa**. *Curr Biol* 2021, **31**:90–102.e7.

130. Schwalm ND, Townsend GE, Groisman EA: **Multiple signals govern utilization of a polysaccharide in the gut bacterium Bacteroides thetaiotaomicron**. *mBio* 2016, **7**. e01342-16.

131. Kilmury SLN, Burrows LL: **Type IV pilins regulate their own expression via direct intramembrane interactions with the sensor kinase PilS**. *Proc Natl Acad Sci USA* 2016, **113**:6017–6022.

132. Richards SM, Strandberg KL, Conroy M, Gunn JS: **Cationic antimicrobial peptides serve as activation signals for the Salmonella Typhimurium PhoPQ and PmrAB regulons in vitro and in vivo**. *Front Cell Infect Microbiol* 2012, **2**:102.

133. Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V: **The QseC sensor kinase: a bacterial adrenergic receptor**. *Proc Natl Acad Sci USA* 2006, **103**:10420–10425.

134. Donoso R, Leiva-Novoa P, Zúñiga A, Timmermann T, Recabarren-Gajardo G, González B: **Biochemical and genetic bases of indole-3-acetic acid (auxin phytohormone) degradation by the plant-growth-promoting rhizobacterium paraburkholderia phytofirmans PsJN**. *Appl Environ Microbiol* 2017, **83**. e01991-16.

135. Wang F-F, Cheng S-T, Wu Y, Ren B-Z, Qian W: **A bacterial receptor PcrK senses the plant hormone cytokinin to promote adaptation to oxidative stress**. *Cell Rep* 2017, **21**:2940–2951.

136. Lin Y-H, Pierce BD, Fang F, Wise A, Binns AN, Lynn DG: **Role of the VirA histidine autokinase of Agrobacterium tumefaciens in the initial steps of pathogenesis**. *Front Plant Sci* 2014, **5**:195.