Transplantation of prokaryotic two-component signaling pathways into mammalian cells

Jonathan Hansen, Erik Mailand, Krishna Kumar Swaminathan, Joerg Schreiber, Bartolomeo Angelici and Yaakov Benenson

Department of Biosystems Science and Engineering, Eidgenössische Technische Hochschule Zürich (ETH Zurich), CH-4058 Basel, Switzerland.

Supplementary Information

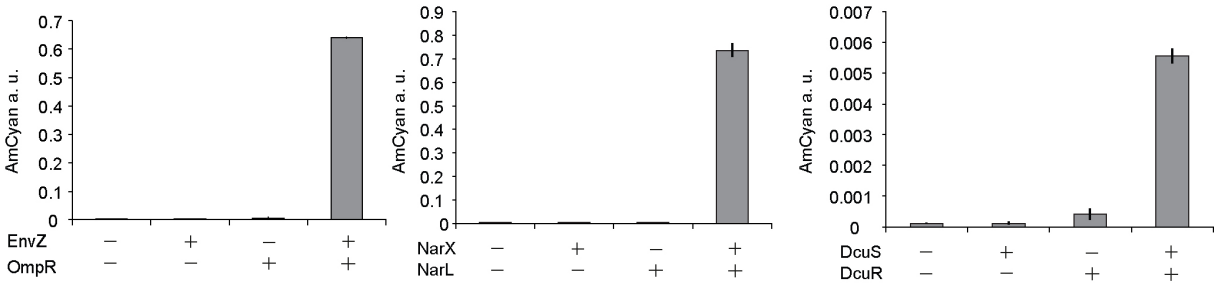

Fig. S1. Operation of two-component pathways in HeLa cells. The plasmid mixtures are the same as used in Fig. 2A. Presence or absence of different components is indicated. The response genes are present in all transfections. Note that for DcuRS pathway, the conditions are suboptimal resulting in reproducible, but low, induction.

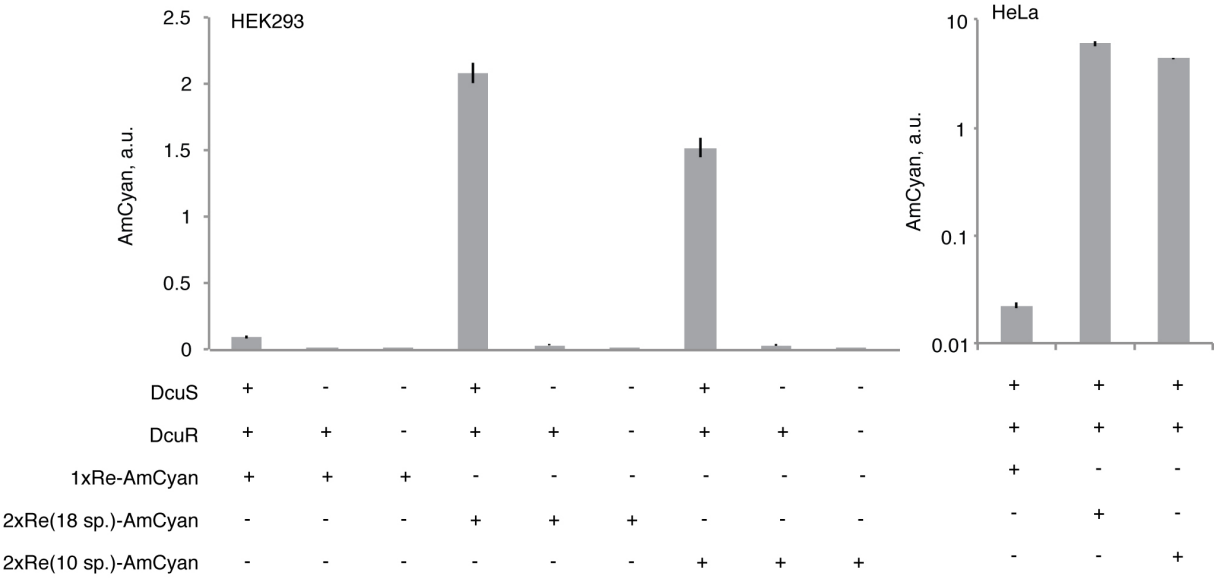

Fig. S2. Comparison between one and two repeats of DcuR response element in the inducible promoter. The measurement is made with optimal amount of DcuS (15 ng) found in titration (Fig. 2G). The induced signal with 1x response element is shown for comparison. In HEK293 cells, for each RE we also show the reporter expression in the absence of the HK as well as in the absence of either TCS gene. In HeLa cells, we only show the fully-induced signal for every RE. Each bar represents an average of three biological replicas and different transfected components are indicated. Cell line where the measurement has been performed is shown on top of each chart. Where applicable, we use 15 ng of DcuS-encoding plasmid, 300 ng of the DcuR-encoding plasmid, and 450 ng of the reporter plasmid.

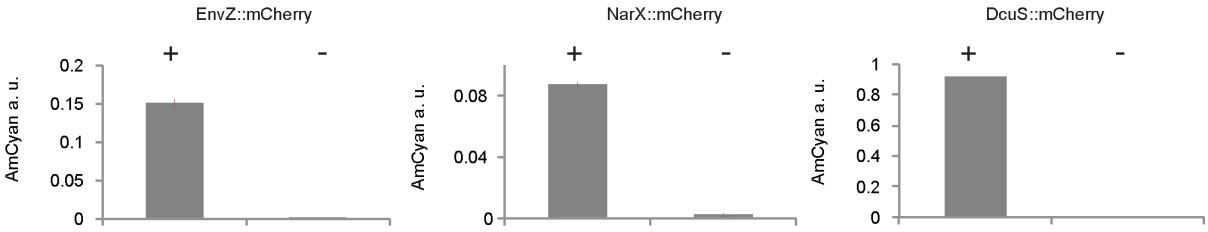

Fig. S3. mCherry HK fusions retain their activity. In this experiment, 300 ng of each RR, 400 ng of the inducible AmCyan reporter (using 2xDcuS_RE (18bp)-AmCyan for DcuSR) and 200 ng of CMV-iRFP transfection marker were transfected either with or without the respective HK::mCherry fusion. The AmCyan signal is normalized to iRFP fluorescence.

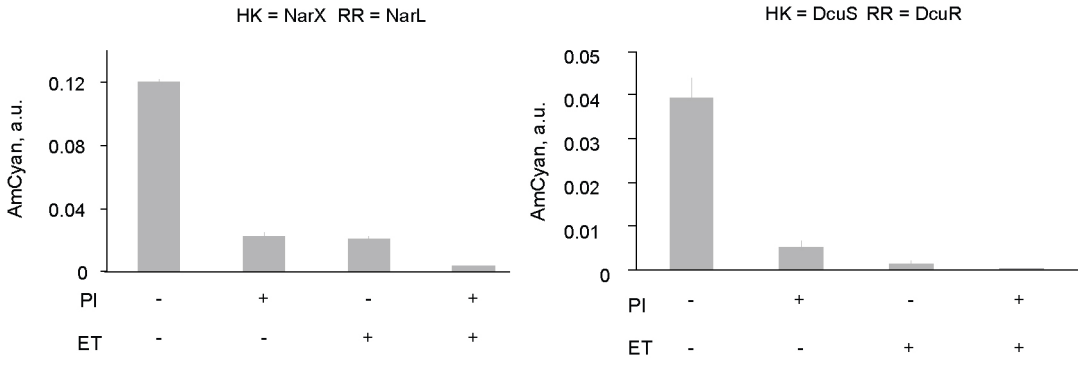

Fig. S4. Logic gates using inducible genes encoding for HKs and RRs, characterized in HeLa cells in a manner similar to the experiment in Fig. 3A-C

Table S1. Design of promoter sequences induced by phosphorylated RRs. Red-colored sequences indicates the RR DNA binding sites in each respective construct. Green color indicates the TATA Box. Blue bold sequence indicates regions of the minimal promoter that flank the TATA box. Start codon is shown in black bold. For OmpR RE, we also underline restriction enzyme sites (left to right, NdeI, SpeI, NheI, BmtI, AfeI and AgeI, with the last four overlapping) and double-underline the Kozak sequence. These sequences appear in all regulated promoters in the table.

OmpR RE	ATTTACATTTTGAAACATCTATAGCGCCGGCATTTACATTTTGAAACATCTA TCCATATGC**TCTAGAGGG**TATATAA**TGGGGGCCA**CTAGTCTACTACCAGA GCTCATCGCTAGCGCTACCGGTCGCCACC**ATG**
NarL RE	TACCCCTATAGGGGTATAGCGCCGGCTACCCCTATAGGGGTATCCATATG C**TCTAGAGGG**TATATAA**TGGGGGCCA**CTAGTCTACTACCAGAGCTCATCG CTAGCGCTACCGGTCGCCACC**ATG**
DcuR RE	TGATTACAAAACTTTAAAAAGTGCTGCACCATATGC**TCTAGAGGG**TATATA A**TGGGGGCCA**CTAGTCTACTACCAGAGCTCATCGCTAGCGCTACCGGTC GCCACC**ATG**
DcuR 2xRE (18 bp)	TGATTACAAAACTTTAAAAAGTGCTGCATAGCGCCGGCCGCGCCTGATTA CAAAACTTTAAAAAGTGCTGCACCATATGC**TCTAGAGGG**TATATAA**TGGG GGCCA**CTAGTCTACTACCAGAGCTCATCGCTAGCGCTACCGGTCGCCAC C**ATG**
DcuR 2xRE (10 bp)	TGATTACAAAACTTTAAAAAGTGCTGTAGCGCCGGCTGATTACAAAACTTT AAAAAGTGCTGTCCATATGC**TCTAGAGGG**TATATAA**TGGGGGCCA**CTAGT CTACTACCAGAGCTCATCGCTAGCGCTACCGGTCGCCACC**ATG**

Table S2. Transfection setup of the experiments in Fig. 2A. Each respective plasmid's amount is listed in nanogram (ng) per transfection sample in a 12-well plate setup.

EnvZ – OmpR – OmpR RE						
Histidine Kinase	Response Regulator	CMV-EnvZ (pJH001)	CMV-OmpR (pJH003)	OmpR_RE-AmCyan (pJH008)	CMV-dsRed (pBH0015)	pUC19 (pKH008)
-	-	0	0	200	200	400
+	-	200	0	200	200	200
-	+	0	200	200	200	200
+	+	200	200	200	200	0
NarX - NarL - NarL RE						
Histidine Kinase	Response Regulator	CMV-NarX (pJH002)	CMV-NarL (pJH004)	NarL_RE-AmCyan (pJH007)	CMV-dsRed (pBH0015)	pUC19 (pKH008)
-	-	0	0	200	200	400
+	-	200	0	200	200	200
-	+	0	200	200	200	200
+	+	200	200	200	200	0
DcuS - DcuR - DcuR RE						
Histidine Kinase	Response Regulator	CMV-DcuS (pJH019)	CMV-DcuR (pJH020)	DcuR_RE-AmCyan (pJH022)	CMV-dsRed (pBH0015)	pUC19 (pKH008)
-	-	0	0	200	200	400
+	-	200	0	200	200	200
-	+	0	200	200	200	200
+	+	200	200	200	200	0
PMT: 561 586/15: 270 V, 445 473/10: 253 V						

Table S3. Quantitative values of fluorescent outputs in experiments displayed in Fig. 2A

Figure 2A - EnvZ - OmpR - OmpR-RE			
CMV-EnvZ (pJH001)	CMV-OmpR (pJH003)	AmCyan a. u.	Standard deviation
-	-	$5.55*10^{-5}$	$1.46*10^{-6}$
+	-	$5.71*10^{-5}$	$1.46*10^{-6}$
-	+	$2.50*10^{-3}$	$3.00*10^{-4}$
+	+	$1.17*10^{-1}$	$7.80*10^{-3}$
Figure 2A - NarX - NarL - NarL-RE			
CMV-NarX (pJH002)	CMV-NarL(pJH004)	AmCyan a. u.	Standard deviation
-	-	$5.25*10^{-5}$	$2.56*10^{-6}$
+	-	$6.43*10^{-5}$	$4.37*10^{-6}$
-	+	$1.40*10^{-3}$	$3.13*10^{-5}$
+	+	$1.35*10^{-1}$	$1.23*10^{-2}$
Figure 2A - DcuS - DcuR - DcuR RE			
CMV-DcuS (pJH019)	CMV-DcuR (pJH020)	AmCyan a. u.	Standard deviation
-	-	$5.25*10^{-5}$	$2.56*10^{-6}$
+	-	$6.43*10^{-5}$	$4.37*10^{-6}$
-	+	$1.40*10^{-3}$	$3.13*10^{-5}$
+	+	$1.35*10^{-1}$	$1.23*10^{-2}$

Table S4. Transfection setup for loss-of-function experiments in Fig. 2B. The amounts are given in nanogram per 12-well plate.

CMV-EnvZ (pJH001)	150	CMV- OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	400	CMV-dsRed (pBH0015)	200
CMV-EnvZ_H243V (pEM013)	150	CMV- OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	400	CMV-dsRed (pBH0015)	200
		CMV- OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	400	CMV-dsRed (pBH0015)	200
CMV-NarX (pJH002)	150	CMV-NarL (pJH004)	300	NarL_RE-AmCyan (pJH007)	400	CMV-dsRed (pBH0015)	200
CMV-NarX_H399Q (pEM014)	150	CMV-NarL (pJH004)	300	NarL_RE-AmCyan (pJH007)	400	CMV-dsRed (pBH0015)	200
		CMV-NarL (pJH004)	300	NarL_RE-AmCyan (pJH007)	400	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	150	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS_H350L (pEM015)	150	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
		CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
PMT: 561 586/15: 270 V, 445 473/10: 253 V							

Table S5. Quantitative values of fluorescent outputs in loss-of-function experiments in Fig. 2B. Each AmCyan value is the mean of a biological triplicate (n=3).

Figure 2B - EnvZ - EnvZH243V		
HK variant	AmCyan a. u.	Standard deviation
EnvZ-wt	0.0790	0.0010
EnvzH243V	0.0061	0.0004
No HK	0.0016	0.0003
Figure 2B - NarX - NarXH399Q		
HK variant	AmCyan a. u.	Standard deviation
NarX-wt	0.238	0.007
NarXH399Q	0.024	0.004
No HK	0.027	0.002
Figure 2B - DcuS - DcuSH350L		
HK variant	AmCyan a. u.	Standard deviation
DcuS-wt	0.1564	0.0157
DcuSH350L	0.0026	0.0002
No HK	0.0035	0.0003

Table S6. Transfection setup of the TCS crosstalk displayed in Fig. 2D. Each respective plasmid's amount is listed in nanogram (ng) per sample in a 12-well plate setup (n=3) (TC = transfection control).

HK		**RR**		**RE**		**TC**	
CMV-EnvZ (pJH001)	200	CMV-OmpR (pJH003)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH0015)	200
CMV-EnvZ (pJH001)	200	CMV-OmpR (pJH003)	200	DcuR_RE-AmCyan (pJH022)	200	CMV-dsRed (pBH0015)	200
CMV-EnvZ (pJH001)	200	CMV-OmpR (pJH003)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH0015)	200
CMV-NarX (pJH002)	200	CMV-NarL(pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH0015)	200
CMV-NarX (pJH002)	200	CMV-NarL (pJH004)	200	DcuR_RE-AmCyan (pJH022)	200	CMV-dsRed (pBH0015)	200
CMV-NarX (pJH002)	200	CMV-NarL(pJH004)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-DcuR (pJH020)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-DcuR (pJH020)	200	DcuR_RE-AmCyan (pJH022)	200	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-DcuR (pJH020)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH0015)	200
PMT: 561 586/15: 270 V, 445 473/10: 253 V							

Table S7. Quantitative data corresponding to measurements in Fig. 2D. Means and standard deviations of biological triplicates are shown.

	OmpR_RE-AmCyan (pJH008)	NarL_RE-AmCyan (pJH007)	DcuR_RE-AmCyan (pJH022)
CMV-EnvZ (pJH001) + CMV-OmpR (pJH003)	0.050 ± 0.028	$0.0006 \pm 2*10^{-5}$	$0.00067 \pm 7*10^{-5}$
CMV-NarX (pJH002) + CMV-NarL (pJH004)	$0.00025 \pm 2*10^{-5}$	0.064 ± 0.007	$0.00028 \pm 4*10^{-5}$
CMV-DcuS (pJH019) + CMV-DcuR (pJH022)	$0.0008 \pm 5*10^{-5}$	$0.00079 \pm 8*10^{-5}$	0.018 ± 0.001

Table S8. Transfection setup of the TCS crosstalk displayed in Fig. 2E. Each respective plasmid's amount is listed in nanogram (ng) per sample in a 12-well plate setup.

HK		**RR**		**RE**		**TC**	
CMV-EnvZ (pJH001)	200	CMV-OmpR (pJH003)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH0015)	200
CMV-NarX (pJH002)	200	CMV-OmpR (pJH003)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-OmpR (pJH003)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH0015)	200
CMV-EnvZ (pJH001)	200	CMV-NarL (pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH0015)	200
CMV-NarX (pJH002)	200	CMV-NarL (pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-NarL (pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH0015)	200
CMV-EnvZ (pJH001)	200	CMV-DcuR (pJH020)	200	DcuR_RE-AmCyan (pJH022)	200	CMV-dsRed (pBH0015)	200
CMV-NarX (pJH002)	200	CMV-DcuR (pJH020)	200	DcuR_RE-AmCyan (pJH022)	200	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-DcuR (pJH020)	200	DcuR_RE-AmCyan (pJH022)	200	CMV-dsRed (pBH0015)	200
PMT: 561 586/15: 270 V, 445 473/10: 253 V							

Table S9. Quantitative data corresponding to measurements in Fig. 2E. Means and standard deviations of biological triplicates are shown.

	CMV-EnvZ (pJH001)	CMV-NarX (pJH002)	CMV-DcuS (pJH019)
CMV-OmpR (pJH003) + OmpR_RE-AmCyan (pJH008)	0.147 ± 0.059	0.0049 ± 0.0016	0.102 ± 0.005
CMV-NarL (pJH004) + NarL_RE-AmCyan (pJH007)	0.0020 ± 0.0007	0.115 ± 0.014	0.0026 ± 0.0010
CMV-DcuR (pJH020) + DcuR_RE-AmCyan (pJH022)	0.0016 ± 0.0005	0.00029 ± 0.00015	0.037 ± 0.008

Table S10. Transfection setup of the TCS crosstalk between two pathways DcuSR and EnvZ-OmpR shown in Fig. 2F. Each respective plasmid's amount is listed in nanogram (ng) per sample in a 12-well plate setup.

CMV-DcuS (pJH019)	0	CMV-OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	5	CMV-OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	15	CMV-OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	50	CMV-OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	150	CMV-OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-OmpR (pJH003)	300	OmpR_RE-AmCyan (pJH008)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	0	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	5	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	15	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	50	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	150	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
CMV-DcuS (pJH019)	200	CMV-DcuR (pJH020)	300	DcuR_RE-AmCyan (pJH022)	450	CMV-dsRed (pBH0015)	200
PMT: 561 586/15: 270 V, 445 473/10: 253 V							

Table S11. Quantitative data corresponding to measurements in Fig. 2F. Means and standard deviations of biological duplicates are shown.

CMV-DcuS (pJH019) + CMV-OmpR (pJH003) + OmpR_RE-AmCyan (pJH008)			CMV-DcuS (pJH019) + CMV-DcuR (pJH020) + DcuR_RE-AmCyan (pJH022)		
HK ng	AmCyan, a.u.	Standard Deviation	HK ng	AmCyan, a.u.	Standard Deviation
0 ng	0.0133	0.0005	0 ng	0.0024	0.0001
5 ng	0.0181	0.0001	5 ng	0.1053	0.0065
15 ng	0.0305	0.0011	15 ng	0.1447	0.0012
50 ng	0.1712	0.0024	50 ng	0.1205	0.0058
150 ng	0.283	0.017	150 ng	0.093	0.011
200 ng	0.278	0.027	200 ng	0.077	0.018

Table S12. Quantitative data corresponding to measurements in Fig. 2G. Means of biological duplicates are shown.

CMV-EnvZ (pJH001) + CMV-OmpR (pJH003) + OmpR_RE-AmCyan (pJH008)						
AmCyan, a.u.		RR 0 ng	RR 10 ng	RR 30 ng	RR 100 ng	RR 300 ng
	HK 0 ng	0.00007	0.00039	0.00081	0.00402	0.01126
	HK 5 ng	0.00010	0.13234	0.21395	0.22135	0.18577
	HK 15 ng	0.00012	0.11063	0.20603	0.27911	0.25401
	HK 50 ng	0.00007	0.11714	0.23829	0.37743	0.49033
	HK 150 ng	0.00007	0.08077	0.23548	0.44254	0.59634
CMV-NarX (pJH002) + CMV-NarL (pJH004) + NarL_RE-AmCyan (pJH007)						
AmCyan, a.u.		RR 0 ng	RR 10 ng	RR 30 ng	RR 100 ng	RR 300 ng
	HK 0 ng	0.00017	0.00024	0.00095	0.00318	0.00860
	HK 5 ng	0.00026	0.02729	0.12249	0.11964	0.24347
	HK 15 ng	0.00022	0.05774	0.10806	0.20194	0.37716
	HK 50 ng	0.00024	0.04239	0.13536	0.19222	0.39983
	HK 150 ng	0.00025	0.01145	0.07040	0.27141	0.45193
CMV-DcuS (pJH019) + CMV-DcuR (pJH020) + DcuR_RE-AmCyan (pJH022)						
AmCyan, a.u.		RR 0 ng	RR 10 ng	RR 30 ng	RR 100 ng	RR 300 ng
	HK 0 ng	0.000173	0.000101	0.000531	0.000574	0.001308
	HK 5 ng	0.000132	0.005843	0.022312	0.061694	0.091898
	HK 15 ng	0.000057	0.001645	0.008475	0.039896	0.106170
	HK 50 ng	0.000096	0.000715	0.002364	0.008809	0.060203
	HK 150 ng	0.000227	0.000419	0.000923	0.005172	0.017278

Table S13. Transfection setup for antibiotic-regulated logic gates in Fig. 3B,C. Each respective plasmid's amount is listed in nanogram (ng) per transfection sample in a 12-well plate setup.

CMV-PIT2 (pMF206)	pPIRtight-NarX (pJH027)	CMV-ET1 (pEL0190)	pETRtight-NarL (pJH025)	NarL_RE-AmCyan (pJH007)	CMV-dsRed (pBH0015)
15	150	15	300	400	200
CMV-PIT2 (pMF206)	pPIRtight-DcuS (pJH034)	CMV-ET1 (pEL0190)	pETRtight-DcuR (pJH035)	DcuR_RE-AmCyan (pJH022)	CMV-dsRed (pBH0015)
15	15	15	300	400	200
PMT: 561 586/15: 270 V, 445 473/10: 253 V 10 ug/ml PI, 4 ug/ml ET					

Table S14. Quantitative values of fluorescent outputs from antibiotic-regulated gates in Fig. 3B, C. Each AmCyan value is the mean of a biological triplicate.

Figure 3B: NarXL-based gate			
PI (10 µg/ml)	ET (4 µg/ml)	AmCyan, a. u.	Standard Deviation
-	-	$1.71*10^{-2}$	$9.25*10^{-4}$
+	-	$1.72*10^{-3}$	$2.14*10^{-4}$
-	+	$2.82*10^{-4}$	$1.75*10^{-5}$
+	+	$1.56*10^{-4}$	$2.10*10^{-5}$
Figure 3C: DcuS-based gate			
PI (10 µg/ml)	ET (4 µg/ml)	AmCyan, a. u.	Standard Deviation
-	-	$2.08*10^{-2}$	$6.26*10^{-4}$
+	-	$5.57*10^{-3}$	$4.61*10^{-4}$
-	+	$1.91*10^{-4}$	$1.69*10^{-5}$
+	+	$7.37*10^{-4}$	$1.27*10^{-4}$

Table S15. Transfection setup for NarXQL OR logic gate in Fig. 3D. Each respective plasmid's amount is listed in nanogram (ng) per transfection sample in a 12-well plate setup.

CMV-NarX (pJH002)	200			CMV-NarL (pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH015)	200
		CMV-NarQ (pJH018)	200	CMV-NarL (pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH015)	200
CMV-NarX (pJH002)	200	CMV-NarQ (pJH018)	200	CMV-NarL (pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH0015)	200
				CMV-NarL (pJH004)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH015)	200

Table S16. Quantitative values of fluorescent outputs from OR logic gate in Fig. 3D. Each AmCyan value is the mean of a biological triplicate.

CMV-NarX (pJH002)	CMV-NarQ (pJH018)	AmCyan a. u.	Standard deviation
-	-	$8.21*10^{-4}$	$2.17*10^{-4}$
+	-	$6.48*10^{-2}$	$1.20*10^{-3}$
-	+	$5.14*10^{-2}$	$1.06*10^{-2}$
+	+	$4.98*10^{-2}$	$8.79*10^{-3}$

Table S17. Transfection setup for mutant testing in Fig. 4. Each respective plasmid's amount is listed in nanogram (ng) per transfection sample in a 12-well plate setup.

		CMV-NarLc (pJH015)	200	NarL_RE-AmCyan (pJH007)	200	CMV-dsRed (pBH015)	200
CMV-EnvZ_cyt (pJH009)	200	CMV-OmpR (pJH003)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH015)	200
		CMV-OmpR D55E (pJH005)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH015)	200
CMV-EnvZ(pJH001)	200	CMV-OmpR (pJH003)	200	OmpR_RE-AmCyan (pJH008)	200	CMV-dsRed (pBH015)	200
PMT: 561 586/15: 270 V, 445 473/10: 253 V							

Table S18. Quantitative values of fluorescent outputs from constitutively active and truncated proteins displayed in Fig. 4. Each AmCyan value is the mean of a biological triplicate.

Components	AmCyan a.u.	Standard deviation
CMV-NarLc (pJH015)	0.3393	0.0375
CMV-EnvZ_cyt (pJH009) + CMV-OmpR (pJH003)	0.0414	0.0105
CMV-OmpR D55E (pJH005)	0.0491	0.0087
CMV-EnvZ (pJH001) + CMV-OmpR (pJH003)	0.0896	0.0183
CMV-OmpR (pJH003)	0.0014	0.0004

Table S19. Oligonucleotide sequences used for cloning of the constructs described below.

PR0376	cgcgccatttacattttgaaacatctatagcgccggcatttacattttgaaacatctatcca
PR0377	tatggatagatgtttcaaaatgtaaatgccggcgctatagatgtttcaaaatgtaaatgg
PR0378	cgcgcctacccctataggggtatagcgccggctacccctataggggtatcca
PR0379	tatggataccctataggggtagccggcgctataccctataggggtagg
PR0383	tccatctgatggtgctggaattgatgcttcccggtgag
PR0384	ctcaccgggaagcatcaattccagcaccatcagatgga
PR1020	gctgctgctctcgagtcattatcatccttcct
PR1022	gctgctaccggtcgccaccatgagaatccaaaatcggcctctg
PR1090	gctgctgctagccgccaccatgcaagaaaacta
PR1130	gctgctaccggtcgccaccatggctacgaccgagcgggacgtaaacc
PR1131	gctgctgctctcgagtcattatcaacccgggagcatatc
PR1158	cgcgcctgattacaaaactttaaaaagtgctgca
PR1159	tatgcagcacttttaaagttttgtaatcagg
PR1325	gctgctgctagccgccaccatgcttaaaagatgtc
PR1326	gctgctggatcctcattatcattcgtgagtgtc
PR1433	gctgctgatatcaccggtcgccaccatgaggca
PR1434	gctgctgatatcctcgagttatcattagcg
PR1503	gctgctgaattccgccaccatgtccaatcagg
PR1504	gctgctgtcgaccgagatagggttgagtgttg
PR1505	gctgctgctagcccggtcgccaccatgaggcactccc
PR1506	gctgctggatccctcgagttatcattagcggttg
PR1507	gctgctgaattcgaccggtcgccaccatgattaacg
PR1508	gctgctgcggccgcttattatcagccaggcagcatg
PR1520	gctgctgctagccgccaccatgcgacgacttcg
PR1521	gctgctggatcctcattatcatccttccttgg
PR1581	ccagtcacgacgttgtaaaacgacgg
PR1582	gtcacgacgttgtaaaacgacgg
PR1583	catgattacgccaagcttgcatgc
PR1861	cgcgcccgcgcctgattacaaaactttaaaaagtgctgcatagcgccggccgcgcctgattacaaaactttaaaaagtgctgcacca
PR1862	tatggtgcagcacttttaaagttttgtaatcaggcgcggccggcgctatgcagcacttttaaagttttgtaatcaggcgcggg
PR1863	ctatagggaccggtcgccaccatg
PR1864	tccttccttggtagttccctgggctcttg
PR1865	ctcactataggggaccggtcgccaccatgc
PR1866	ttcgtgagtgtcaccctgcacgtcggtgaatgtc

PR1867	ctataggggaccggtcgccaccatgaggc
PR1868	gcggttgcttctctccccatcccaagg
PR1869	ggtggcggtggctcgatggccatcatcaaggag
PR1870	gctgctctcgagtcattactacttgtacagctcgtccatgccgcc
PR1871	gtgtccgaggtcctgagagatgga
PR1872	cgctgtctggcagctgtgactc
PR1873	gtaggcgtgtacggtgggaggtct
PR1874	atctttgttaattgactccgcgaggtagc
PR1875	tggcaggctaaccgacggttg
PR1876	gaatactcttcgcacgatgcctcc
PR1964	cgcgcctgattacaaaactttaaaaagtgctgtagcgccggctgattacaaaactttaaaaagtgctgtcca
PR1965	tatggacagcacttttttaaagttttgtaatcagccggcgctacagcacttttttaaagttttgtaatcagg
EnvZ H243V gBlock	gtaggcgtgtacggtgggaggtctatataagcagagctctctggctaactagagaacccactgcttactggctt atcgaaattaatacgactcactataggggaccggtcgccaccatgcgacgacttcggttctcaccgaggtcct catttgcgaggacgctgctcctgatcgtcacacttttgttcgccagcctggtgacgacttacttggtagtactcaa cttcgcgattcttccctccttgcagcagttcaataaggtactggcgtatgaggtcagaatgctgatgacggataa gctccagctcgaagatgggacgcagcttgtcgtgcctccagcgtttcggcgcgaaatctaccgcgagctggg aatttcgctctactcgaatgaggctgcggaagaagcggggttgcgctgggcacagcactatgaatttctgtcg catcaaatggcgcaacagttgggaggtccgacagaggtcagagtagaggtcaacaaatcgtcgcccgtgg tgtggcttaagacctggctctcccctaacatctgggtcagggtgccattgaccgagatccatcagggcgattttt cgcccctgtttcgatacaccctggctatcatgttgcttgcgatcgggggagcctggcttttcatcagaatccaaa atcggcctctggtagatttggaacatgctgcgctccaagtcgggaaggggattattcctccaccgctgagaga gtacggtgcctcggaagtgaggtcagtaacacgcgcattcaatcacatggcagcgggcgtcaagcagctg gcagacgaccggactcttctcatggccggagtctcagtagatctccgcacgcccctgacacggattcgccttg cgactgagatgatgtccgagcaggacggctacctcgcggagtcaattaacaaagat
NarX H399Q gBlock	tggcaggctaaccgacggttgcactcgcgggcacccctgtgcgagaggctgagccctgtattgaacgggctt cagaatctgactctcttgcgcgacatcgaactccgagtctatgatacggatgatgaggagaatcatcaggagt tcacgtgccagccggacatgacgtgtgacgacaagggttgccagttgtgtcccaggggcgtcctccccgtgg gtgatcggggaaccactttgaagtggcgactggccgattcacacacgcagtatgggattctcctggcgaccct cccgcaaggacggcatctgagccacgaccagcaacaacttgtcgacacgttggtggaacagttgacggcc acgctcgcactcgaccgccatcaagagcggcagcagcagctcatcgtcatggaagagagggcgactattg cccgggagcttcaggattccattgcacagtcactgtcgtgcatgaagatgcaagtgagctgtctgcaaatgca gggggacgcgctccccgagtcgagccgcgaactgctgtcccagatccgaaatgagttgaatgcgagctgg gcacagctgagagagcttctgacgacattccgcctgcagctgaccgagcccggtcttaggccagctttggag gcatcgtgcgaagagtattc
DcuS H350L gBlock	gtgtccgaggtcctgagagatggaaccccacggagagatgaggaaatcacaattaaggatagactgctgct gatcaataccgtgcccgtcaggtccaacggcgtgatcattggagccatttctactttccgggataagaccgag gtgagaaaactgatgcagaggctggacggactggtcaattacgctgatgcactgcgagagcggtccctgga gttcatgaacaagctgcatgtgatcctgggcctgctgcacctgaagtcttacaaacagctggaggactatatcc tgaaaacagccaacaattatcaggaggaaattggctcactgctggggaagatcaaaagccctgtgattgctg ggttcctgatctccaagattaatagggcaacagatctgggacacactctgatcctgaacagtgagtcacagct gccagacagcg

Plasmid Construction

CMV-EnvZ (pJH001): We performed de novo synthesis of sequence via DNA2.0 with codon optimization to *Homo sapiens sapiens,* with amino acid sequence based upon *Escherichia coli* wildtype sequence. Insert was delivered in pJ609 (DNA 2.0) with CMV-immediate early promoter and BGH polyA signal sequence.

CMV-NarX (pJH002): We performed de novo synthesis of sequence via DNA2.0 with codon optimization to *Homo sapiens sapiens,* with amino acid sequence based upon *Escherichia coli O157:H7 str. EDL933* wildtype sequence. Insert was delivered in pJ609 (DNA 2.0) with CMV-immediate early promoter and BGH polyA signal sequence.

CMV-OmpR (pJH003): We performed de novo synthesis of sequence via DNA 2.0 with codon optimization to *Homo sapiens sapiens,* with amino acid sequence based upon *Escherichia coli O157:H7 str. EDL933* wildtype sequence. Insert was delivered in pJ609 (DNA 2.0) with CMV-immediate early promoter and BGH polyA signal sequence.

CMV-NarL (pJH004): We performed de novo synthesis of sequence via DNA2.0 with codon optimization to *Homo sapiens sapiens,* with amino acid sequence based upon *Escherichia coli O157:H7 str. EDL933* wildtype sequence. Insert was delivered in pJ609 (DNA 2.0) with CMV-immediate early promoter and BGH polyA signal sequence.

CMV-OmpR D55E (pJH005): OmpR insert of pJH003 was PCR amplified with PR0383 and PR0384 introducing a base pair mutation, exchanging a glutamate (E) residue at the 55th amino acid aspartate (D) using Quickchange Site-directed Mutagenesis (1).

NarL_RE-AmCyan (pJH007): Minimal response element was formed out of the annealed product of PR0378 and PR0379. The product was digested with AscI-SpeI and inserted into a respectively digested pBA001 vector containing a minimal promoter and AmCyan coding sequence interspersed with an unrelated intron (Table S1).

OmpR_RE-AmCyan (pJH008): Minimal response element was formed out of the annealed product of PR0376 and PR0377. The product was digested with AscI-SpeI and inserted into a respectively digested pBA001 vector, containing a minimal promoter and AmCyan coding sequence interspersed with an unrelated intron (Table S1).

CMV-EnvZ_cyt (pJH009): EnvZ insert (pJH001) was PCR amplified with primers PR1020 and PR1022 in order to remove the first 179 amino acids of the translated sequence and introducing XhoI and AgeI cut sites, respectively (2). The product was then cloned into the aforementioned vector pJ609 (DNA 2.0).

CMV-NarLc (pJH015): NarL insert (pJH004) was amplified with PR1130 and PR1031 (3). The PCR product was then digested with AgeI and XhoI followed by ligation into the vector pJ609 (DNA 2.0).

CMV-NarQ (pJH018): We performed de novo synthesis of sequence via Genscript with codon optimization to *Homo sapiens sapiens*. Amino acid sequence based upon *Escherichia coli* wildtype sequence. Insert was digested with AgeI/XhoI and ligated into pJ609 (DNA 2.0).

CMV-DcuS (pJH019): We performed de novo synthesis of sequence via Genscript with codon optimization to *Homo sapiens sapiens*. Amino acid sequence is based upon *Escherichia coli* wildtype sequence. Insert was delivered on pUC57 commercial plasmid. Insert was PCR amplified using PR1433 and PR1444 followed by digestion with AgeI/XhoI and ligation into an appropriately digested pJ609 vector (DNA 2.0).

CMV-DcuR (pJH020): We performed de novo synthesis of sequence via Genscript with codon optimization to *Homo sapiens sapiens*. Amino acid sequence based upon *Escherichia coli* wildtype sequence. Insert was digested with AgeI/XhoI and ligated into pJ609 (DNA 2.0) vector.

DcuR_RE-AmCyan (pJH022): Minimal response element was formed out of the annealed product of PR1158 and PR1159. The product was digested with AscI-SpeI and inserted into a respectively digested pBA001 vector containing a minimal promoter and AmCyan coding sequence interspersed with an unrelated intron (Table S1).

pETR$_{tight}$-NarL (pJH025): pJH004 was digested with AgeI - XhoI. NarL insert was cloned into the appropriately digested pEL0185 (4) containing pETR$_{tight}$ promoter.

pPIR$_{tight}$-NarX (pJH027): NarX from pJH002 was PCR amplified with the primers PR1325 and PR1326 after which the PCR product is digested with NheI and BamHI. pNL115 (5) containing pPIR$_{tight}$ promoter was in parallel digested with NheI and BamHI. The two digest products are then ligated together.

$pPIR_{tight}$-DcuS (pJH034): DcuS from pJH019 was PCR amplified with the primers PR1505 and PR1506 with the PCR product digested by NheI and BamHI-HF. pNL115 (5) was in parallel digested with NheI and BamHI-HF. The two digest products are then ligated together.

$pETR_{tight}$-DcuR (pJH035): DcuR insert from pJH020 was digested with AgeI - XhoI and cloned into pEL0185 vector (4) digested with AgeI and SalI. The two are then ligated and sequenced.

CMV-tTA (pBA166): CMV-tTA-Advanced was purchased from Clontech Laboratories, Inc (Cat # 631069)

CMV-PIT2 (pMF206): Provided by Fussenegger Lab, Weber et al. (6)

CMV-ET1 is pEL0190 (4) originally derived from pSV40-ET1 (pWW35, provided by Fussenegger lab, Weber et al. (7))

CMV-dsRed (pBH0015): pDsRed-Express-C1 from Clontech (Cat # 632430).

CMV-AmCyan (pBH0017): pAmCyan1-C1 from Clontech (Cat # 632441).

pUC19 (pKH008): pUC19 from Thermo Scientific (Cat # SD0061).

CMV-EnvZ H243V (pEM013): We performed de novo synthesis of gBlock "EnvZ H243V" (Table S19) via IDT with codon optimization to *Homo sapiens sapiens*. Amino acid sequence is based upon *Escherichia coli* wildtype sequence with a mutation of H243V (2). gBlock was PCR amplified with PR1873 and PR1874 and digested with AgeI (leaving a blunt end at 3'-terminus). pJH001 was digested with XhoI-EcoRV and the 541 bp fragment was gel-purified. In parallel, pJH001 was digested with AgeI and XhoI and the 4746 bp fragment was gel-purified and dephosphorylated. Finally, digested gBlock as well as gel-purified 541 bp XhoI-EcoRV and 4746 bp AgeI-XhoI fragments from pJH001 were ligated in a 3-way reaction to deliver the desired sequence.

CMV-NarX H399Q (pEM014): We performed de novo synthesis of gBlock "NarX H399Q" (Table S19) via IDT with codon optimization to *Homo sapiens sapiens*. Amino acid sequence is based upon *Escherichia coli* wildtype sequence with a mutation of H399Q (8). gBlock was

PCR amplified with PR1875 and PR1876, then digested with BlpI-PstI and ligated into the pJH002 plasmid digested with BlpI-PstI.

CMV-DcuS H350L (pEM015): We performed de novo synthesis of gBlock sequence "DcuS H350L" (Table S19) via IDT with codon optimization to *Homo sapiens sapiens*. Amino acid sequence is based upon *Escherichia coli* wildtype sequence with a mutation of H350L (9). gBlock was PCR amplified with PR1871 and PR1872, then digested with BmgBI-BglII and inserted into the pJH019 plasmid digested with BmgBI-BglII.

EnvZ-GGGGS-mCherry (pEM017): EnzV insert from plasmid pJH001 was PCR amplified with PR1863 and PR1864 primers and digested with AgeI, with a blunt end creating an EcoRV site. mCherry fluorescent protein from pKH015 was amplified with primers PR1869 and PR1870 and phosphorylated at both ends. The PCR product was digested with XhoI. The digested EnvZ PCR product was ligated into a pre-digested pJH001 vector between AgeI-XhoI sites along with the digested and phosphorylated mCherry PCR fragment in vector to insert ratios of 1:3 and 1:5. A GGTGGCGGTGGCTCG sequence coding for a GGGGS amino acid sequence (10) was included on the 5' primer to serve as a linker.

NarX-GGGGS-mCherry (pEM016): NarX insert from plasmid pJH002 was PCR amplified with PR1865 and PR1866 primers and digested with AgeI, with a blunt end creating an EcoRV site. mCherry fluorescent protein from pKH015 was amplified with primers PR1869 and PR1870, phosphorylated and digested with XhoI. The digested NarX PCR product was ligated into a pre-digested pJH001 vector between AgeI-XhoI sites along with the digested, phosphorylated mCherry PRC fragment in vector to insert ratios of 1:3 and 1:5. A GGTGGCGGTGGCTCG sequence coding for a GGGGS amino acid sequence (10) was included on the 5' primer to serve as a linker.

DcuS-GGGGS-mCherry (pEM018): DcuS insert from plasmid pJH019 was PCR amplified with PR1867 and PR1868 primers and digested with AgeI, with a blunt end creating an EcoRV site. mCherry fluorescent protein from pKH015 was amplified with primers PR1869 and PR1870, phosphorylated and digested with XhoI. Both digested PCR products were ligated into a pJH001 vector pre-digested between AgeI-XhoI cut sites in vector to insert ratios 1:3 and 1:4. A GGTGGCGGTGGCTCG sequence coding for a GGGGS amino acid sequence (10) was included on the 5' primer to serve as a linker.

DcuR 2xRE 18sp (pEM012): Minimal response element was formed out of the annealed product of PR1861 and PR1862. The product, with preformed AscI and SpeI-compatible

sticky ends, was ligated into a respectively digested pBA001 vector containing a minimal promoter and the AmCyan gene interspersed with an unrelated intron. The promoter sequence contains two 26 bp DcuR DNA-binding sites from the *dctA* promoter (11) separated by 18 bp in front of a minimal mammalian promoter (Table S1).

DcuR 2xRE 10sp (pEM019): Minimal response element was formed out of the annealed product of the 5' phosphorylated PR1964 and PR1965. The product, which has sticky ends compatible with AscI-NdeI restriction sites, was inserted into a respectively digested pBA001 vector containing a minimal promoter and the AmCyan gene interspersed with an unrelated intron. The promoter sequence contains two 26 bp DcuR DNA-binding sites from the *dctA* promoter (11) separated by 10-bp spacer in front of a minimal mammalian promoter (Table S1)

CMV-mCherry (pKH015): CMV-mCherry was obtained from Addgene (plasmid 30125: 6B).

Abbreviations

DsRed, *Discosoma sp*. red fluorescent protein; **AmCyan1**, *Anemonia majano* cyan fluorescent protein; **iRFP**, near-infrared fluorescent protein derived from bacteriophytochrome *Rp*BphP2; **VP16**, Herpes simplex-derived transactivation domain; **TRE_{tight}**, tetracycline responsive element promoter (7x modified tet operator sequence fused to CMVmin promoter; **ET1**, ET-dependent transactivator (MphR(A) fused to VP16); **ET**, Erythromycin; **PIT2**, Streptogramin-responsive transactivator (Pristinamycin-induced protein (Pip) fused to p65 transactivating domain; **ETR_{tight},** ET-dependent macrolide-responsive promoter; **ETR,** 35 base-pair (bp) operator sequence of the mph(A) promoter from *Escherichia coli* (12); **PIR_{tight},** PI-dependent streptogramin repsonsive promoter; **PI,** Pristinamycin IA; **PTR,** 66-bp sequence in the promoter of the PI-inducible pristinamycin resistance gene *ptr* from *Streptomyces pristinaespiralis* (13)*;* **CMV_{min}**, human cytomegalovirus immediate promoter; **CMV**, human cytomegalovirus immediate early enhancer and promoter.

Supplementary References

1. Lan CY, M.M. Igo (1998) Differential expression of the OmpF and OmpC porin proteins in Escherichia coli K-12 depends upon the level of active OmpR. *Journal of Bacteriology* 180:171-174.

2. Forst S, Delgado J, & Inouye M (1989) Phosphorylation of OmpR by the osmosensor EnvZ modulates expression of the ompF and ompC genes in Escherichia coli. *Proc Natl Acad Sci U S A* 86(16):6052-6056.
3. Xiao G, Cole DL, Gunsalus RP, Sigman DS, & Chen CH (2002) Site-specific DNA cleavage of synthetic NarL sites by an engineered Escherichia coli NarL protein-1,10-phenanthroline cleaving agent. *Protein Sci* 11(10):2427-2436.
4. Prochazka L, Angelici B, Haefliger B, & Benenson Y (2014) Highly modular bow-tie gene circuits with programmable dynamic behaviour. *Nature Communications* 5:4729.
5. Lapique N & Benenson Y (2014) Digital switching in a biosensor circuit via programmable timing of gene availability. *Nature Chemical Biology* (Accepted for publication).
6. Weber W, Kramer BP, Fux C, Keller B, & Fussenegger M (2002) Novel promoter/transactivator configurations for macrolide- and streptogramin-responsive transgene expression in mammalian cells. *J Gene Med* 4(6):676-686.
7. Weber W*, et al.* (2002) Macrolide-based transgene control in mammalian cells and mice. *Nat Biotechnol* 20(9):901-907.
8. Rabin RS & Stewart V (1992) Either of two functionally redundant sensor proteins, NarX and NarQ, is sufficient for nitrate regulation in Escherichia-Coli K-12. *Proc. Natl. Acad. Sci. U. S. A.* 89(18):8419-8423.
9. Kaspar S*, et al.* (1999) The periplasmic domain of the histidine autokinase CitA functions as a highly specific citrate receptor. *Molecular Microbiology* 33(4):858-872.
10. Zhao HL*, et al.* (2008) Increasing the homogeneity, stability and activity of human serum albumin and interferon-alpha 2b fusion protein by linker engineering. *Protein Expression and Purification* 61(1):73-77.
11. Abo-Amer AE*, et al.* (2004) DNA interaction and phosphotransfer of the C-4-dicarboxylate-responsive DcuS-DcuR two-component regulatory system from Escherichia coli. *Journal of Bacteriology* 186(6):1879-1889.
12. Noguchi N, Takada K, Katayama J, Emura A, & Sasatsu M (2000) Regulation of transcription of the mph(A) gene for macrolide 2 '-phosphotransferase I in Escherichia coli: Characterization of the regulatory gene mphR(A). *Journal of Bacteriology* 182(18):5052-5058.
13. Salahbey K & Thompson CJ (1995) Unusual regulatory mechanism for a streptomyces multidrug-resistance gene, ptr, involving 3 homologous protein-binding sites overlapping the promoter region. *Molecular Microbiology* 17(6):1109-1119.