Systematic evaluation of genetic and environmental factors affecting performance of translational riboswitches

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Site Directed Mutagenesis

Site directed mutagenesis of plasmids was carried out using the Q5 site direct mutagenesis protocol (NEB #E0554S). Template DNA was amplified by PCR with the required primers (Supplementary Table 4). The PCR reaction was electrophoresed to confirm the size and quality of the PCR product, before column purification and KLD treatment. 5 ul of the reaction was then heat shock transformed into DH5α. Correct mutagenesis was confirmed by Sanger sequencing.

Construction of Delta Riboswitch and modified RBS plasmids

The ΔRS plasmid pTAC-ΔRS-eGFP from a previous study was used in this work (Kent et al. 2018), termed here pTAC_ΔRS_WT_RBS_L30_eGFP. The NF_RBS-L30 and Con_RBS-L30 plasmids where then generated by isothermal assembly. The pTAC_ORS_L30_eGFP termed plasmid from a previous study (Kent et al. 2018) was miniprepped from E. coli Δ*dam*$^{-}$*/dcm*$^{-}$ (C2925H, NEB) digested with NdeI and XbaI, and column purified. The synthesised (IDT) ssDNA oligonucleotides (rwk001, rwk002) were then assembled into the linearized backbone by isothermal assembly (HiFi NEBuilder, NEB).

Orthogonal riboswitch ribosome binding site modification Anti-RBS library construct

The ORS con_RBS control (rwk003) was synthesised as ssDNA (IDT) and amplified with rwk004/ rwk005. This part was initially synthesised with a T7 promoter and so this was removed during PCR, to allow regulation by P_{tac}. This PCR product was column purified (Qiagen), and combined with pTAC_ORS_Linker-30_eGFP which had been linearized by PCR (rwk007, rwk008), DpnI treated (2 hours, 37 °C) and column purified. These parts were then assembled by isothermal assembly according to the manufacturers protocol (HiFi NEBuilder, NEB). Similarly ORS anti-con_RBS (rwk006) was cloned into pTAC_ORS_Linker-30_eGFP which had been linearized by PCR (rwk007, rwk008) by isothermal assembly.

The anti-RBS ssDNA library (rwk009) was amplified with rwk005 and rwk006 and into the linearized pTAC_ORS_A6_His_eGFP (linearized by rwk010 and rwk011). These two parts were then assembled according to HiFi assembly manufacturer's protocol (NEB). Briefly, 25 fmol of linear plasmid was combined with 250 fmol of insert and incubated at 50 °C for 15 minutes. Following assembly of the anti RBS library, two separate 5 ul aliquots of the assembly reaction were transformed into *E. coli* DH5α (NEB #C2987) by heat shock. Following recovery these transformations were pooled and a 50 ul aliquot was serially diluted and plated onto LB + carbenicillin 100 μg ml^{-1} for CFU quantification, of the library size (~110,000). The remaining cells were plated on multiple LB + carbenicillin 100 μg ml^{-1} agar plates and incubated for 16 hours at 37 °C. Colonies were re-suspended in Qiagen P1 buffer directly from the agar plates. The cell suspension was split in two, one half was cryopreserved in 15 % glycerol, whilst the remaining volume was used for the purification of plasmid DNA. The purified anti-RBS variant library was transformed into *E. coli* DH10 β Top10 F' in duplicate. Following heat shock, cells were recovered in 5 ml of LB + carbenicillin 100 μg ml^{-1}, pooled and a 50 ul aliquot was serially diluted and plated for CFU counts as before. The remaining culture was cryopreserved in 15 % glycerol at -80 °C.

Constructing plasmids according to the DoE structured data table

The remaining combinatorial DoE plasmids, not isolated from the anti-RBS library by FACS; i.e. with alternate combinations of RBS, anti-RBS and N-terminal codon variance, were constructed using isothermal assembly of ssDNA parts. These parts were synthesised (IDT) according to the structure of the D-optimal DoE design (Supplementary Table 5) and assembled, by isothermal assembly (HiFi NEBuilder, NEB), into the linearized pTAC_ORS_Linker-30_eGFP, which had been digested with NdeI and XbaI, gel extracted following fragment separation and column purified (Qiagen).

FACS analysis

Single colonies of the ΔRS-conRBS-L35 and D4-ORS-L35 were inoculated into LB medium Liquid cultures were incubated at 37 °C, shaking at 200 RPM for overnight 16 hours. The cultures were then diluted 100x in LB supplemented with 0.2 % glucose and carbenicillin 100 μg ml^{-1}, and incubated at 37 °C, shaking at 180 rpm for 2 hours (O.D600 ~ 0.3). Cultures were then aliquoted (500 μl) into deep well plates (Amgen 1.4 ml 96 well plates) with the designated final inducer concentrations (10 μM IPTG, 100 μM IPTG, 8, 40, 200 and 1000 μM PPDA). Following incubation for 24 hours cells harvested were harvested by centrifugation (2250 g, 10 minutes at 4 °C). Cell pellets were then washed with PBS, diluted 1:10 and fluorescence intensity (Exλ/Emλ = 488/530 nm) was analysed by flow cytometry. All FACS analysis was carried out using a Sony SH800, Data was analysed using FlowJo Single Cell Analysis Software version 10.

mKate2 cloning

The mKate2 plasmids were assembled by restriction cloning. mKate2 was synthesized (rwk012) as detailed in Supplementary Table 4. The pTAC-ORS constructs (WT RBS_ORS, con_RBS-ORS and D4-ORS-L32/L35/L36) and mKate2 dsDNA was then incubated with NdeI-HF and BamHI-HF (NEB) at 37 °C for 2 hours. Following gel electrophoresis of the digested backbone and column purification of backbone, the synthesised mKate2 insert (was ligated into the backbone with T4 DNA ligase (incubated at room temperature for 15 minutes. The assembled plasmid was then transformed into *E. coli* DH5α by heat shock transformation, and correct assembly confirmed by sequencing.

Genome integration

Initially Riboswitch eGFP cassettes were cloned into the pKIKO LacZ vector (Sabri et al. 2013) and inserted into the LacZ locus using λ red mediated recombination (Datta et al. 2006). Briefly, the riboswitch eGFP cassette DNA was amplified by PCR (Q5 high fidelity polymerase) using rwk013 and rwk014 and inserted into the linearized pKIKO plasmid (PCR amplification using rwk015, rwk016). Following confirmation of correct assembly, by sequencing, the genome insertion cassette was amplified using rwk017 and rwk018. The size and quality of the linear donor DNA was confirmed by gel electrophoresis. The remaining PCR reaction was treated with DpnI for 2 hours at 37 μl prior to silca column purification (Qiagen). Linear DNA (300 ng) was transformed into *E. coli* DH10β TOP10 F' containing the pSIM18 vector via electroporation. Cells were then heat shocked to induce expression of λ red recombinase production (submerged in a water bath at 42°C for 15 minutes) (Sharan et al. 2009). Cells were incubated at 37 °C, with shaking (180 RPM) for 2 hours and then plated LB +

chloramphenicol 12.5 µg ml^{-1}. Integration was confirmed by colony PCR using rwk019 and rwk020 (Phire Green Pfu, ThermoFisher Scientific) and growth on chloramphenicol 12.5 µg ml^{-1}. Integrated colonies where re-streaked on LB + hygromycin 150 µg ml^{-1} and incubated at 37 °C for 16 hours to confirm curing of pSIM18. Those strains where pSIM18 was not successfully cured were re-streaked onto LB + hygromycin 150 µg ml^{-1} and incubated at 37 °C for 16 hours. Finally, those isolates which had cured pSIM18 were inoculated into LB media containing either carbenicillin 100 µg ml^{-1} or chloramphenicol 25 µg ml^{-1} prior to expression testing to confirm that no pKIKO plasmid been retained in the genome integrated strains. Isolates which grew in LB + chloramphenicol but not LB + carbenicillin 100 µg ml^{-1} were cryo-stocked in 15 % glycerol and stored at -80 °C prior to expression testing.

Site direct mutagenesis to restore the *addA* aptamer

The ORS aptamer was also converted to the ARS by site directed mutagenesis of 4 nucleotides within the ligand binding region (C at -68 to T, C at -72 to T, C at -77 to G, G at -91 to T). the primers used were rwk021 and rwk022. For details of all primers used in site directed mutagenesis see Supplementary Table 4.

Ptrp construct generation via LacO deletion

In order to generate the Ptrp promoter the LacI operator site (de Boer et al. 1983) ((LacO) 5'-AATTGTGAGCGGATAACAATT-3') from Ptac was deleted by inverse PCR (rwk023 and rwk024), thus removing any allosteric regulation by LacI. LacO deletion was carried out using site directed mutagenesis. However, the LacO site of Ptac overlaps the transcription start site (TSS) of the promoter. Therefore LacO deletion means modifiying the TSS and shortening the transcript sequence upstream of the riboswitch (Figure. 8A). The upstream linker was extended with site directed mutagenesis as previously described using primers rwk025 and rwk026. This extended linker was rationally designed with minimised secondary structure, as predicted by RNAfold. For details of primers used in site directed mutagenesis see Supplementary Table 4.

Anderson promoter cloning

Anderson promoters were derived from BBa_J23114 (relative strength = 0.1), BBa_J23118 (relative strength = 0.56), andBBa_J23100, (relative strength = 1). These sequences were modified by the addition of an upstream insulator sequence, previously selected by the Densmore lab (Carr et al. 2017) for use with BBa_J23100. Two versions of each promoter part were synthesised, each containing an upstream linker sequence of variable length (rwk027 – rwk032). These parts were synthesised as ssDNA oligonucleotides and assembled by isothermal assembly into pTAC_D4-ORS_His_77_eGFP which had been linearized by PCR (rwk033, rwk034). See Supplementary Table 4 for details of ssDNA parts.

Stress promoter cloning

The stress responsive promoter seqeunces were cloned into the PCR linearized (rwk033, rwk034) pET_15b_D4-ORS_His_77_eGFP plasmid, replacing the P_{tac} promoter and upstream linker region. Sequences for the plasmids were derived from the respective standard parts: BBa_K1139200

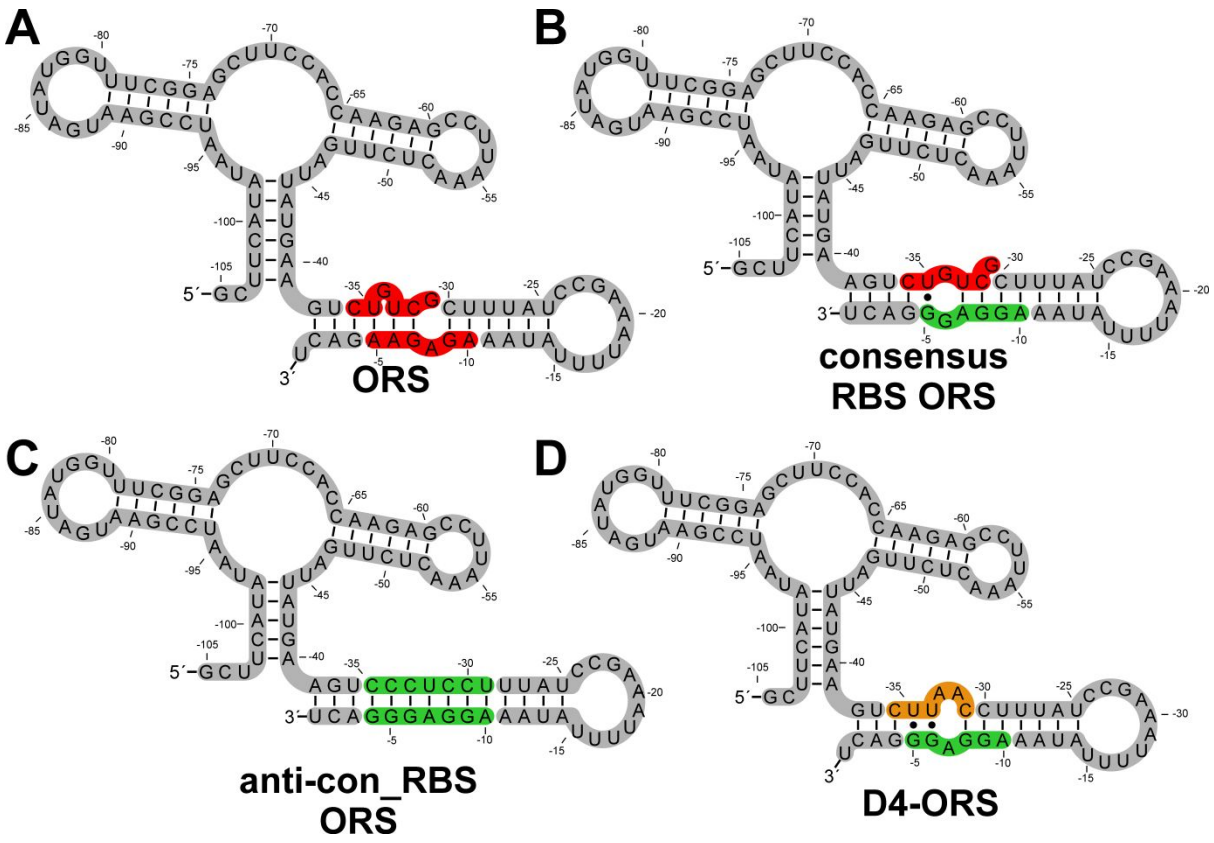
(rwk035), BBa_J45993 (rwk036), BBa_K223041 (rwk037), , and BBa_K118011 (rwk038). For details of the synthesised ssDNA and primers used to linearize the plasmid vector see Supplementary Table 4.

Data processing

Normalised expression was calculated (relative fluorescence units (RFU) (λEx/λEm = 488 nm/ 530 nm)/optical density (OD) (absorbance, λ 600 nm)) and used to calculate ON/OFF and ON/UI. Previous work (Kent et al. 2018) with this expression system used an alternative data processing method where UI was subtracted from OFF and ON expression to show the riboswitch dependent fold change only. This was modified here to allow consistent data processing of expression for both inducible and constitutive promoter systems; where no UI (un-induced promoter activity) reading can be taken.

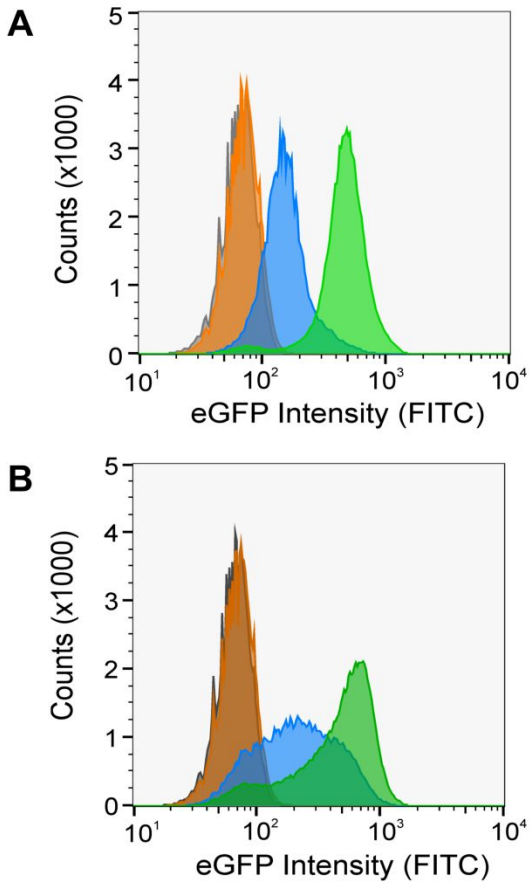
Statistics and modelling

Design of Experiments, Standard Least Squares and associated statistical analysis was carried out using JMP Pro 12 and GraphPad PRISM 7.



Supplementary Figure 1: Representative structural prediction and annotation of key riboswitch variants used in this study:

A) The riboswitch region of the original pTAC_ORS_His-L30_eGFP construct riboswitch, highlighting pairing of the native RBS and anti-RBS regions (red). B) Structure of the pTAC_ORS_con-RBS_His-L30_eGFP, showing predicted hybridization of the consensus RBS (green) with the native anti-RBS (red). C) Rational design of a synthetic expression platform, to reduce OFF expression level, following insertion of the consensus RBS. D) Structural prediction of the D4-ORS riboswitch, showing the selected anti-RBS region (orange) pairing with the consensus RBS. Structural predictions were generated by RNAfold and visualized with R2R. Watson-Crick paired bases are shown by -, and wobble pairing is indicated by a •.

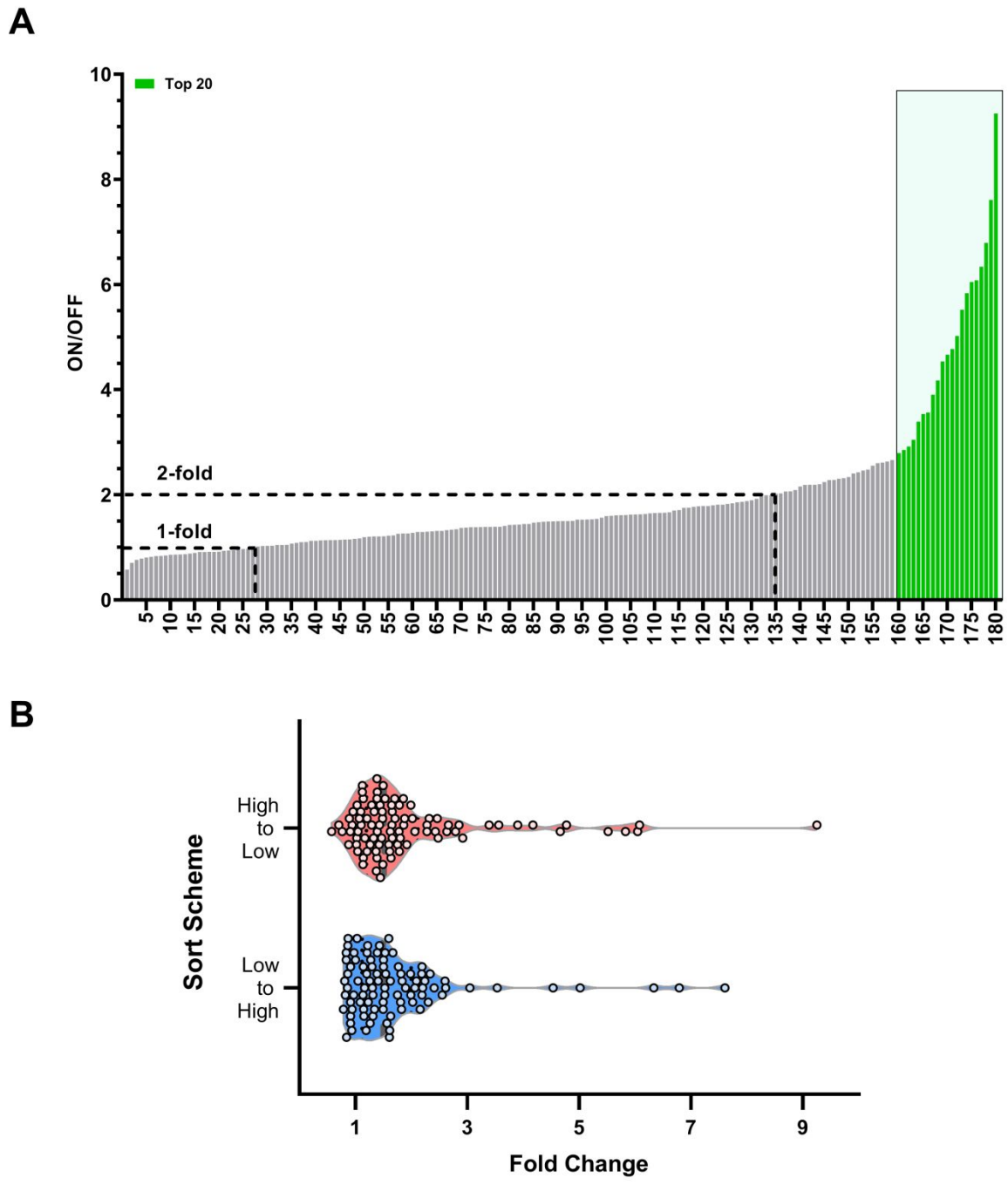


	Induction condition	Geometric mean	C.V	Median
	IPTG + PPDA	445.0	43.3	474.0
	IPTG	154.0	59.5	150.0
	PPDA	70.1	31.5	71.7
	Uninduced	65.0	41.6	67.2

	Induction condition	Geometric mean	C.V	Median
	IPTG + PPDA	406.0	57.1	513.0
	IPTG	209.0	75.3	213.0
	PPDA	70.0	37.0	71.7
	Uninduced	66.2	32.0	68.3

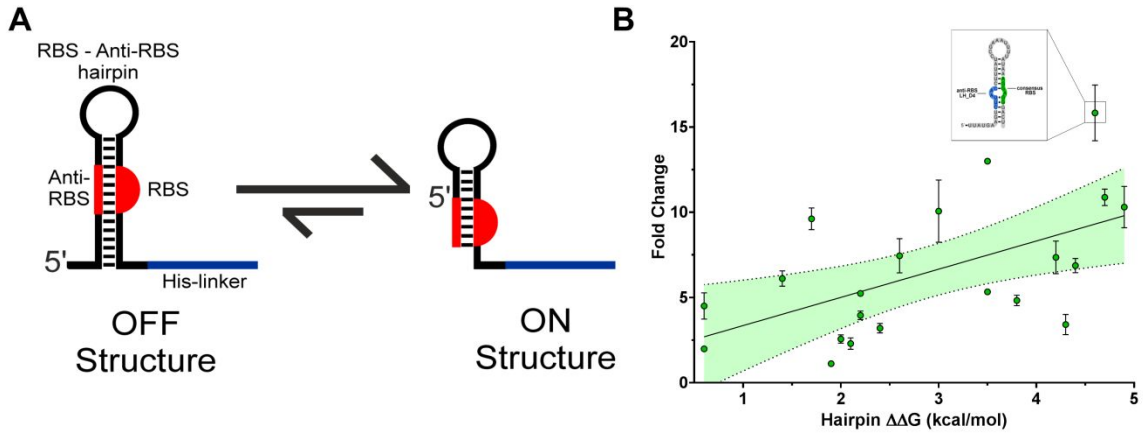
Supplementary Figure 2: Histograms showing the eGFP fluorescent intensities of the WT_ORS and anti-RBS library under vary induction conditions

Representative FACS histograms, showing the A) pTAC_WT-ORS-His-L30_eGFP and B) pTAC_ORS_anti-con_RBS_lib*_His-L30_eGFP following growth and induction (materials and methods) for 20 hours with PPDA, IPTG and IPTG + PPDA. The IPTG and IPTG + PPDA populations were then subjected to two rounds of sorting under alternating IPTG and IPTG + PPDA induction (materials and methods).



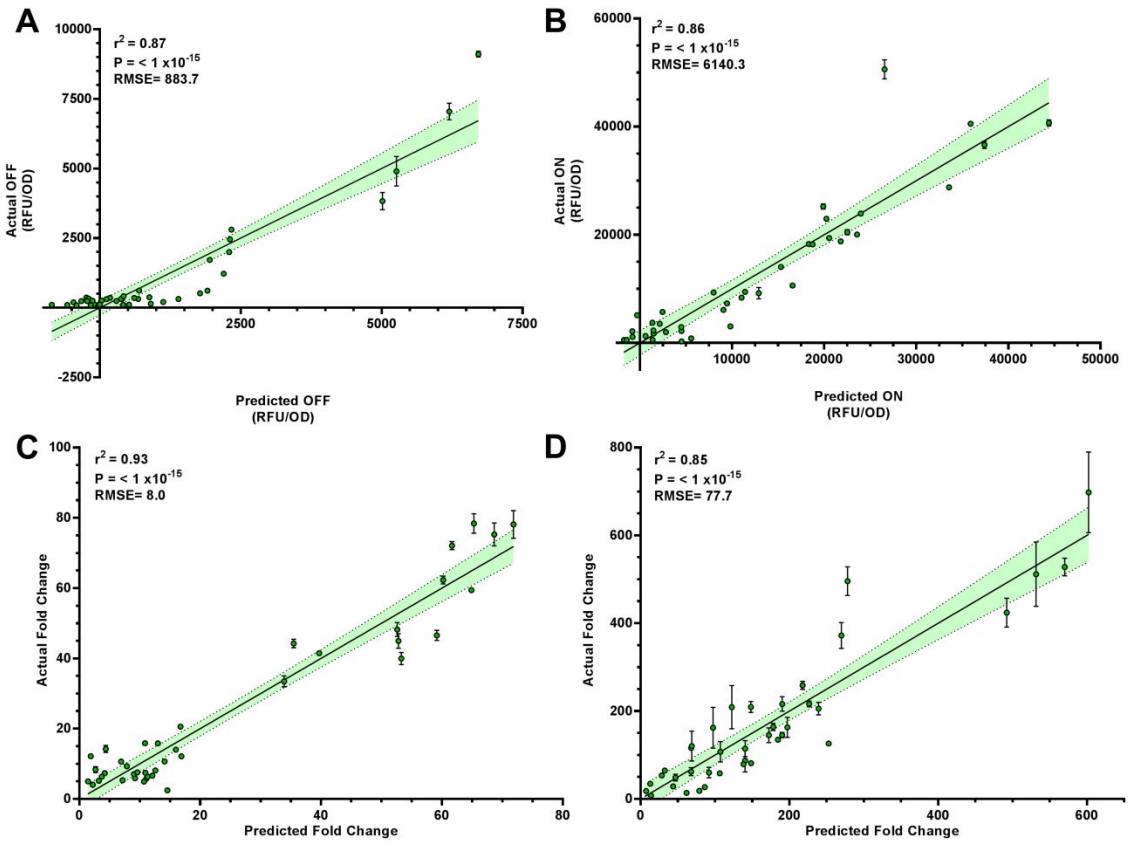
Supplementary Figure 3: Screening functionality of FACS selected anti-RBS clones

Initial screening of riboswitch function following FACS enrichment of active riboswitches. Single colonies were screened for expression +/- PPDA (1000 μM), in the presence of 100 μM IPTG. The ON/OFF ratio was then calculated. From the screened colonies 20 individual clones were taken forward for further testing and sequencing (Green box).



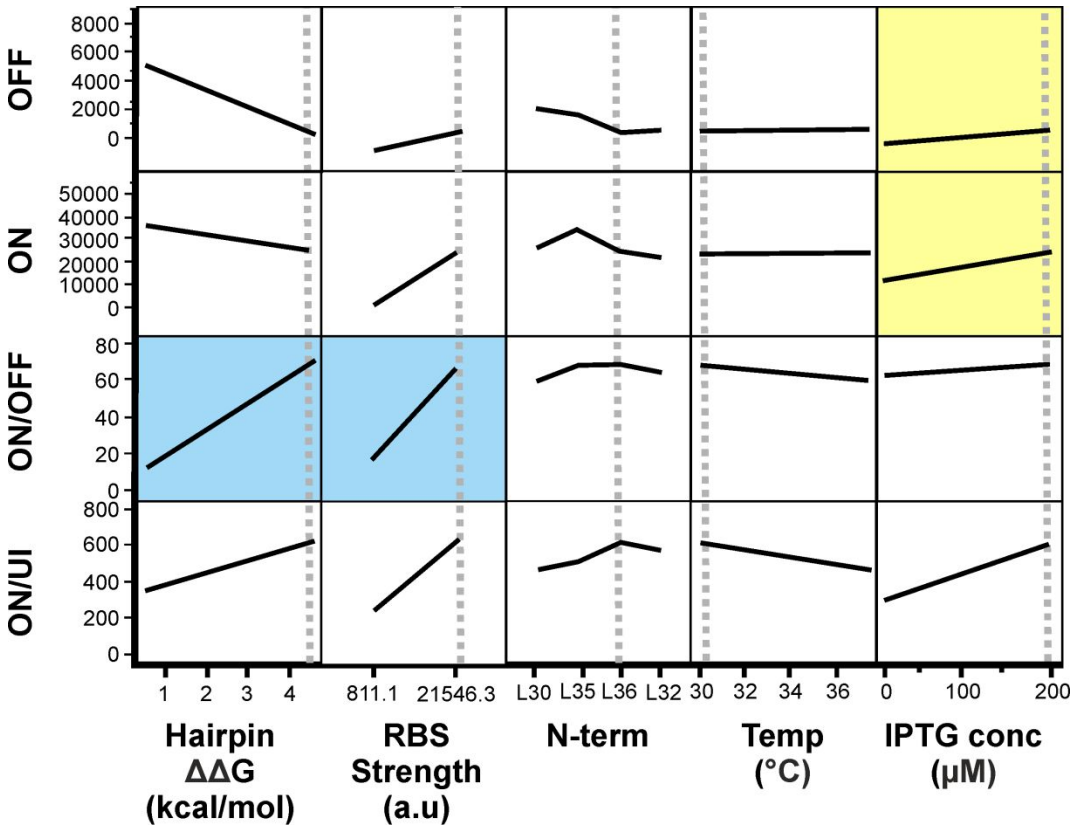
Supplementary Figure 4: Correlating Fold Change with Hairpin stability ΔΔG

A) A schematic representation of the sequence regions used for predicting the structures of the OFF and ON states of the riboswitch hairpin. The ΔΔG was then calculated from the ΔG predictions of the OFF and ON states. B) Correlation between the change in structural stability (ΔΔG) and fold change (ON/OFF) of the FACS selected, riboswitch expression platforms. This ΔΔG value was calculated from in silico generated OFF and ON states of the hairpin within the expression platform (r = 0.57, P = 7.5 x10^{-3}). Shown inset is the RNAfold predicted OFF structure of the translation regulating hairpin from the D4-ORS expression platform.



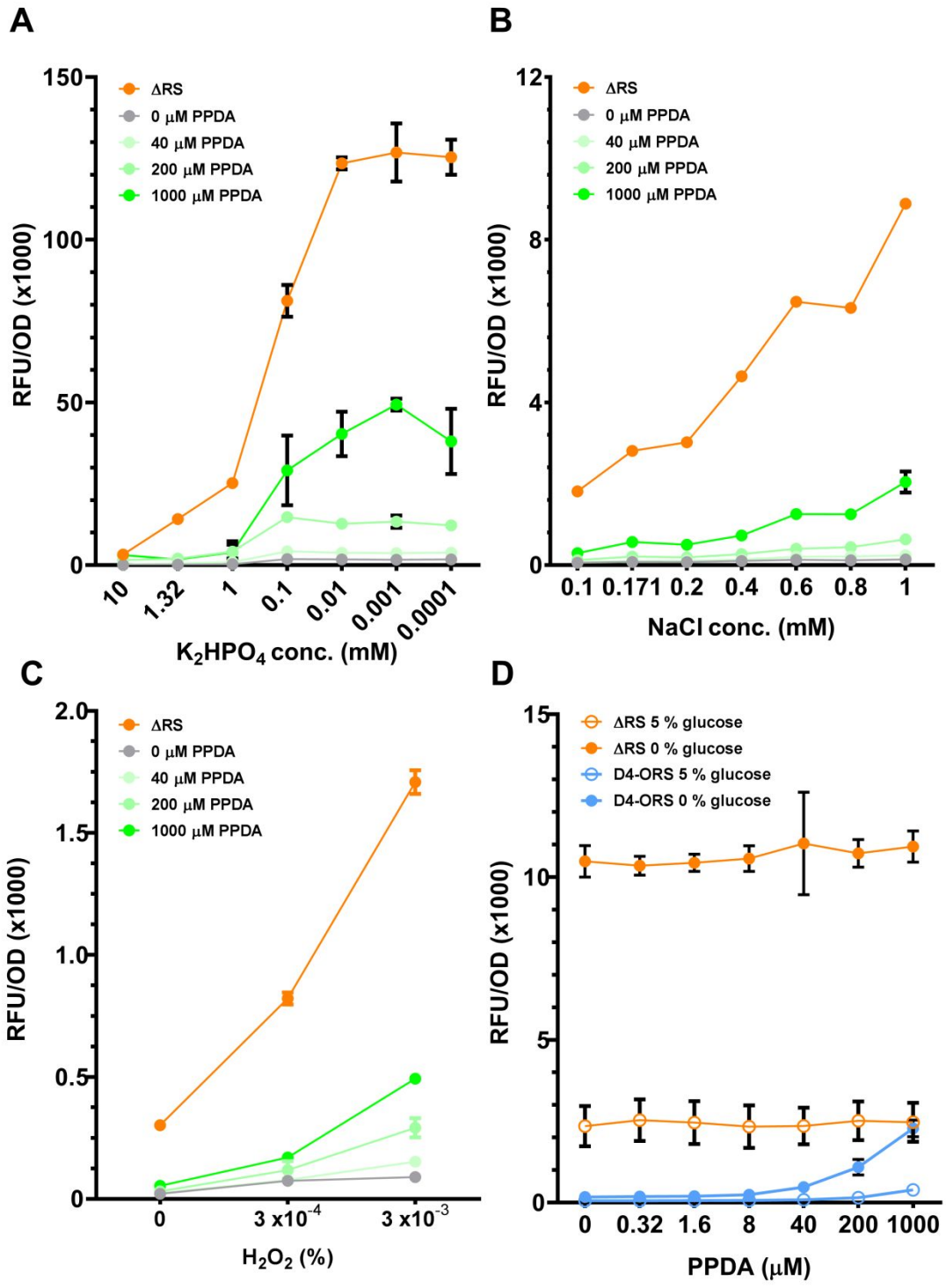
Supplementary Figure 5: Comparing experimental protein production with SLS predicted values for OFF, ON, ON/OFF and ON/UI.

Experimental vs predicted plots of the DoE data for each of the four response variables, OFF (A), ON (B), ON/OFF (C) and ON/UI (D) showing agreement of the model with the experimental dataset.



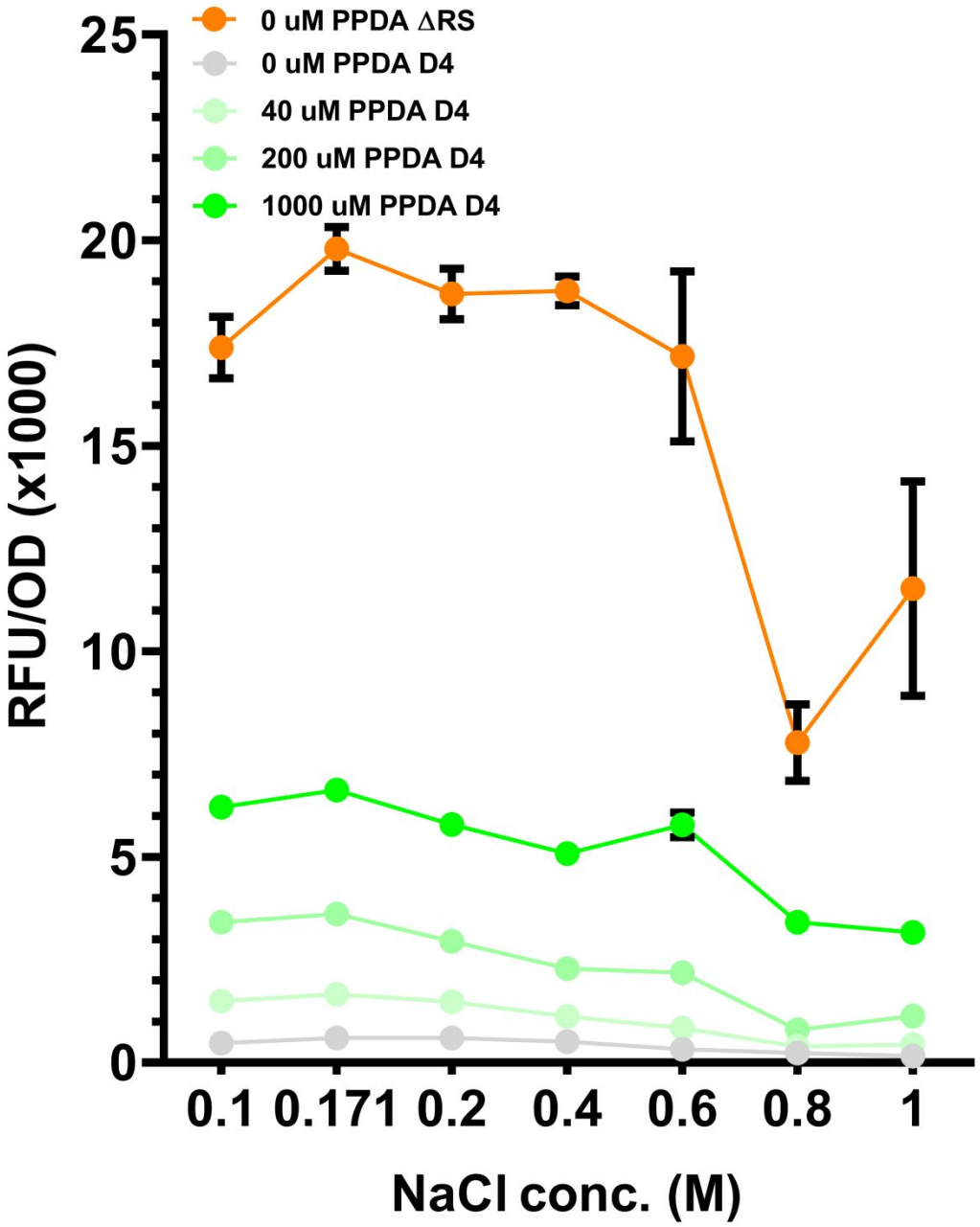
Supplementary Figure 6: Investigating factors effecting riboswitch performance through Design of Experiments

Standard Least Squares regression modelling of the five-dimensional, multi-response expression landscape of the ORS. The grey dashed lines indicate the model term parameter settings. These settings represent those of run #20, and achieve the best balance between the each of the four responses (OFF, ON, ON/OFF and ON/UI). The effect of RBS and strength and hairpin stability (ΔΔG) on ON/OFF is indicated by the blue boxes. The impact of altering IPTG concentration and its effect on OFF and ON eGFP production is highlighted in the yellow boxes.



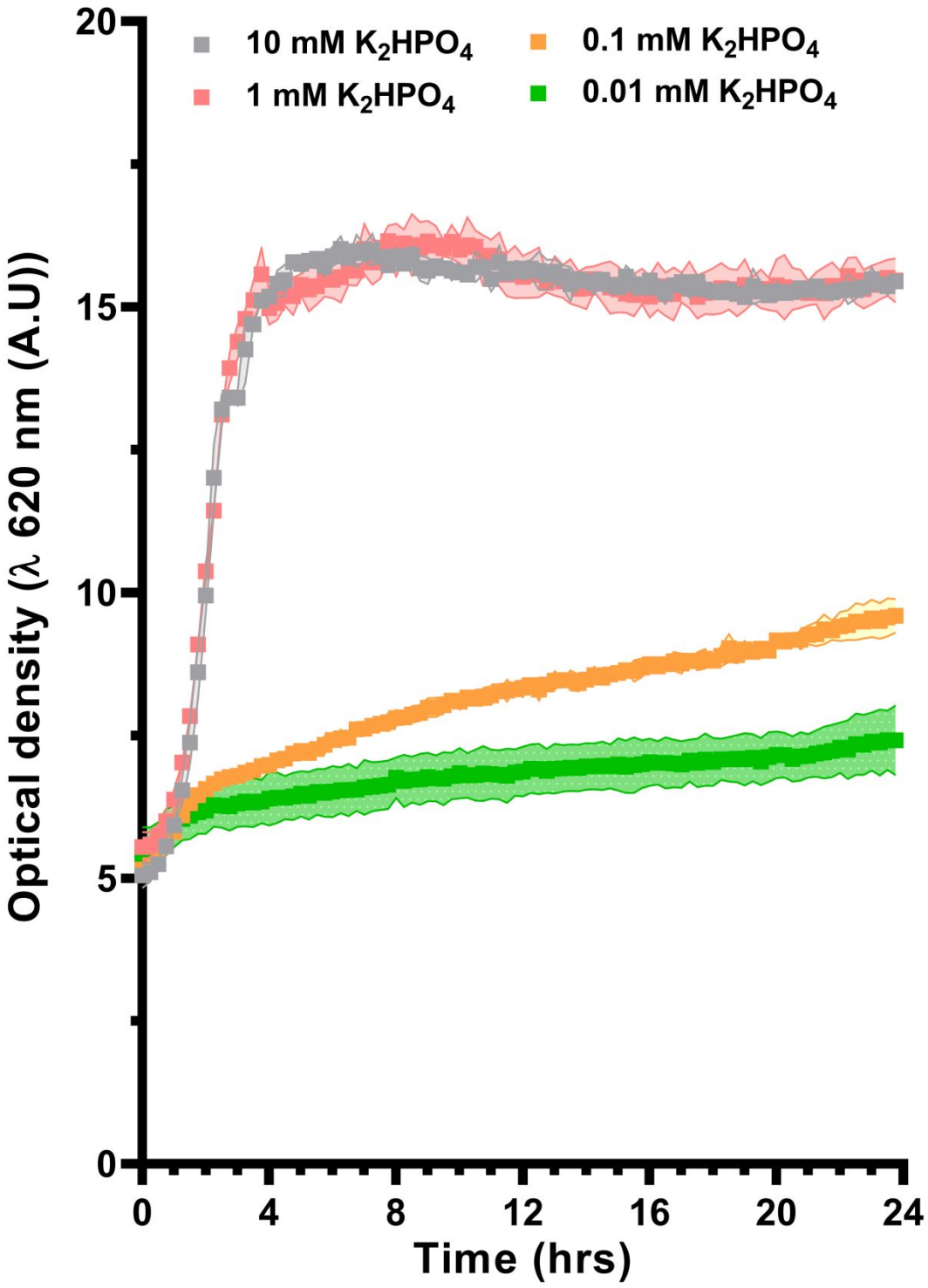
Supplementary Figure 7: Stress response promoter RFU/OD data

The production of eGFP from the stress response promoters A) P_{phoA}, B) P_{osmY}, C) P_{soxS} and D) P_{cstA} of the D4-ORS-L35 devices, and ΔRS controls (orange). This data was used to calculate the ON/OFF and ON/UI performance of each device. Error bars represent standard deviation.



Supplementary Figure 8: Stationary phase eGFP production levels of the P_{osmY}::D4-ORS, 24 hours after hyperosmotic stress.

Riboswitch mediated regulation of eGFP production, regulated by the hyperosmotic shock and stationary phase promoter, P_{osmY}; showing the absence of NaCl dependent induction of protein synthesis during stationary phase, after 24 hours of induction. Grey (0 μM PPDA) and green (40 μM, 200 μM and 1000 μM respectively) lines indicate the expression of eGFP by the P_{osmY}::D4-ORS-L35 device compared to the transcriptionally regulated control (orange).



Supplementary Figure 9: Representative growth curves from micro-fermentation analysis of the PphoA:D4-ORS-L35 constructs.

The growth of *E. coli* DH10β was measured by the change in optical density (λ = 620 nm) over 24 hours, when cultured in EZ rich media at 37 °C, shaking at 1,000 RPM. Different cultures where supplemented with varying concentrations of K_2HPO_4 to assess stress response and P_{phoA} activation**.** Data points represent the mean of three repeats carried out on separate days, with the standard deviation represented by the coloured shaded area.

Supplementary Table 1: Oligonucleotides used in this study.

See attached file.

RBS I.D	Sequence	Un-induced (UI)	IPTG 100 µM (ON)	ON/UI
WT_RBS	AGAGAA	57 ± 5.2	2436 ± 57.6	43.0 ± 3.1
NF_RBS	TCCTCC	34 ± 3.8	208 ± 20.8	6.1 ± 0.1
con_RBS	AGGAGG	1169 ± 95.1	13425 ± 149.0	11.5 ± 0.8

Supplementary Table 2: Ribosome binding sites used for translation initiation rate engineering.

I.D	Anti-RBS Sequence	Un-induced		OFF (100 µM IPTG)		ON (100 µM IPTG + 1000 µM PPDA)		ON /OFF	
		RFU/OD	S.D	RFU/OD	RFU/OD	RFU/OD	S.D	RFU/OD	S.D
ORS	CTGTCG	47.60	14.14	517.01	2309.96	2309.96	254.40	51.35	15.18
Con-ORS	CTGTCG	299.78	77.73	10607.49	21054.29	21054.29	1267.01	73.04	16.35
LH_A11	GGAAGA	112.30	15.14	2299.01	7408.32	7408.32	1734.49	66.62	16.74
LH_A9	AGCCCA	35.79	18.41	447.99	2351.74	2351.74	478.60	77.31	34.23
LH_C2	TCATTG	256.36	65.39	6190.72	21437.37	21437.37	2926.73	98.11	14.11
LH_D4	CTTAAC	59.45	32.05	964.30	15191.80	15191.80	1933.78	196.51	60.31
LH_E10	TCGATC	60.53	27.96	1826.22	8848.96	8848.96	2133.76	164.79	62.63
LH_F10	CCACCA	110.37	22.90	1283.77	13356.11	13356.11	3221.94	120.80	13.97
LH_F9	AGTCAT	366.90	81.06	12602.74	32168.38	32168.38	985.60	91.01	22.70
LH_G7	CACTTA	73.48	8.74	2115.14	27468.70	27468.70	4808.86	372.65	30.63
LH_H3	CCAGTG	252.70	21.19	8540.30	28607.16	28607.16	491.54	113.82	11.16
LH_H8	CTAACC	88.57	18.08	2202.76	16549.31	16549.31	4180.93	196.38	83.14
HL_B2	CCAACT	117.70	7.91	2621.55	28457.85	28457.85	710.26	242.49	17.12
HL_C1	CCTCTA	40.55	9.51	341.74	3472.15	3472.15	916.57	86.73	17.80
HL_D9	CTATGC	83.56	31.59	978.70	9443.91	9443.91	1583.95	127.86	65.45
HL_F1	CATCCT	60.04	12.33	1261.92	7767.42	7767.42	1645.54	131.53	27.23
HL_F7	CGTCCC	98.26	48.01	464.83	1073.33	1073.33	199.20	79.05	5.50
HL_G8	CCCCTC	107.05	119.07	130.21	489.88	489.88	559.10	4.47	0.26
HL_H2	CTGCTA	147.40	27.56	3437.00	21007.47	21007.47	690.78	155.09	43.55
HL_H3	TCATTT	119.23	7.51	2729.37	19113.69	19113.69	2348.98	166.15	28.13
HL_H7	CACTGA	122.31	19.40	3479.701	17385.99	17385.99	206.42	138.30	24.25

Supplementary Table 3: Anti-RBS sequences of functional riboswitch variants selected by in vivo selection/counter-selection.

Factors	Low Set point (-1)	Centre Point (0)		High Set point (+1)
Continuous and Numeric Factors				
IPTG Concentration (μM)	1	100.5		200
Hairpin ΔΔG (kcal/mol) (anti-RBS I.D)	0.6 (WT)	2.6 (H8)		4.6 (D4)
RBS strength (a.u.)	851	-		21546
Temperature (°C)	30	33.5		37
Categorical Factors	**(1)**	**(2)**	**(3)**	**(4)**
N- terminal Linker	A6	MFE 77	MFE 87	MFE 99

Supplementary Table 4: Selected DoE Factors and levels tested.

Run Number	N-terminal Linker	IPTG (μM)	Temperature (°C)	T.I.R (Salis a.u.)	Hairpin ΔΔG (anti-RBS I.D) (kcal/mol)	Block
1	MFE_77	1	37	21546.3	0.6 (WT)	1
2	MFE_99	200	30	851.1	0.6 (WT)	1
3	MFE_99	1	37	21546.3	4.6 (D4)	1
4	MFE_77	200	37	21546.3	4.6 (D4)	1
5	MFE_77	1	30	851.1	0.6 (WT)	1
6	MFE_77	200	30	851.1	4.6 (D4)	1
7	A6	200	30	21546.3	0.6 (WT)	1
8	A6	1	37	851.1	4.6 (D4)	1
9	MFE_99	100.5	33.5	21546.3	2.6 (H8)	2
10	MFE_99	100.5	33.5	851.1	2.6 (H8)	2
11	MFE_77	100.5	33.5	21546.3	2.6 (H8)	2
12	MFE_77	1	37	851.1	4.6 (D4)	2
13	MFE_87	100.5	33.5	21546.3	2.6 (H8)	2
14	MFE_77	200	30	851.1	0.6 (WT)	2
15	A6	200	37	851.1	0.6 (WT)	2
16	A6	1	30	21546.3	4.6 (D4)	2
17	MFE_87	1	37	851.1	0.6 (WT)	3
18	MFE_87	200	37	851.1	4.6 (D4)	3
19	MFE_99	1	30	851.1	4.6 (D4)	3
20	MFE_87	200	30	21546.3	4.6 (D4)	3
21	MFE_87	1	30	21546.3	0.6 (WT)	3
22	MFE_99	200	37	21546.3	0.6 (WT)	3
23	A6	200	30	851.1	0.6 (WT)	3
24	MFE_77	200	30	21546.3	0.6 (WT)	3
25	MFE_99	200	37	851.1	4.6 (D4)	4
26	MFE_99	200	30	21546.3	4.6 (D4)	4
27	A6	1	37	21546.3	4.6 (D4)	4
28	MFE_77	1	37	21546.3	4.6 (D4)	4
29	MFE_87	200	37	21546.3	0.6 (WT)	4
30	MFE_99	1	30	21546.3	0.6 (WT)	4
31	MFE_87	1	30	851.1	4.6 (D4)	4
32	MFE_99	1	37	851.1	0.6 (WT)	4
33	A6	200	30	851.1	4.6 (D4)	5
34	A6	1	37	21546.3	0.6 (WT)	5
35	MFE_77	1	30	21546.3	4.6 (D4)	5
36	MFE_87	1	37	21546.3	4.6 (D4)	5
37	A6	200	37	21546.3	4.6 (D4)	5
38	MFE_87	200	30	851.1	0.6 (WT)	5
39	MFE_77	200	37	851.1	0.6 (WT)	5
40	A6	1	30	851.1	0.6 (WT)	5

Supplementary Table 5: D-Optimal DoE design matrix

Source	Log Worth
hairpin RNAfold ΔΔG	5.826
RBS Strength * hairpin RNAfold ΔΔG	5.667
RBS Strength	5.541
IPTG Concentration	3.427
IPTG Concentration * hairpin RNAfold ΔΔG	2.932
IPTG Concentration * RBS Strength	2.532
Temp * RBS Strength	1.828
N-Terminal linker * Temp	1.458
N-Terminal linker * RBS Strength	1.349
Block	0.966
N-Terminal linker (#)	0.896
IPTG Concentration * Temp	0.772
Temp (#)	0.765
N-Terminal linker * hairpin RNAfold ΔΔG	0.666
Temp * hairpin RNAfold ΔΔG	0.643
N-Terminal linker * IPTG Concentration	0.251

Supplementary Table 6: Selection of significant factors for SLS fitting and refinement. Factors highlighted in grey were excluded from the model (log worth < 1.0), except for those terms contained within and significant interactions (indicated by #), which were retained.

Supplementary Table 7: Standard Least Squares refined model coefficients for OFF, ON, ON/OFF and ON/UI.

See additional file.

Supplementary Table 8: RFU/OD and standard deviation data for kinetic PphoA characterisation.

See additional file.

	PPDA (uM)						
K2HPO4 (mM)	**0**	**1.6**	**8**	**40**	**200**	**400**	**1000**
10	0.044 ± 0.003	0.049 ± 0.005	0.053 ± 0.003	0.061 ± 0.005	0.13 ± 0.008	0.19 ± 0.011	0.32 ± 0.024
1	0.46 ± 0.017	0.63 ± 0.012	0.68 ± 0.007	1.0 ± 0.025	2.7 ± 0.054	4.6 ± 0.097	8.2 ± 0.243
0.1	0.34 ± 0.013	0.30 ± 0.004	0.31 ± 0.002	0.37 ± 0.003	1.1 ± 0.006	1.6 ± 0.003	3.4 ± 0.026
0.01	0.25 ± 0.013	0.21 ± 0.004	0.21 ± 0.003	0.28 ± 0.004	0.78 ± 0.012	1.2 ± 0.032	2.2 ± 0.039

Supplementary Table 9: Expression rate coefficients (± standard error) of micro-fermentation expression testing.

Supplementary Table 10: Key plasmids generated in this study.