Supplementary Information for "Rapid engineering of versatile molecular logic gates using heterologous genetic transcriptional modules"

Baojun Wang,*[a] and Martin Buck[b]

[a] *Centre for Synthetic and Systems Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, UK. Fax: +44 131 6508650; Tel: +44 131 6505527; E-mail: Baojun.Wang@ed.ac.uk*

[b] *Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, London, SW7 2AZ, UK. Fax: +44 20 75945419; Tel: +44 20 75945442; E-mail: m.buck@imperial.ac.uk*

Supplementary Table (S1), Figures (S1-S4) and Methods

Table S1 Gene regulatory sequences used for characterising sensor dose responses

Identifier[a]	Type	DNA sequence (5'– 3')	Reported strength[1]
rbs30	RBS[b]	TCTAGAGATTAAAGAGGAGAAATACTAG**ATG**	Very strong
rbs31	RBS[b]	TCTAGAGTCACACAGGAAACCTACTAG**ATG**	Middle strong
rbs32	RBS[b]	TCTAGAGTCACACAGGAAAGTACTAG**ATG**	Weak
rbs33	RBS[b]	TCTAGAGTCACACAGGACTACTAG**ATG**	Very weak
rbs34	RBS[b]	TCTAGAGAAAGAGGAGAAATACTAG**ATG**	Very strong
J115	Promoter	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC	– (constitutive)
J101	Promoter	TTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGC	– (constitutive)

[a]The regulatory sequences are from the Registry of Standard Biological Parts (http://partsregistry.org).
[b]Sequence of RBS (ribosome binding site) is underlined and start codon is in bold.

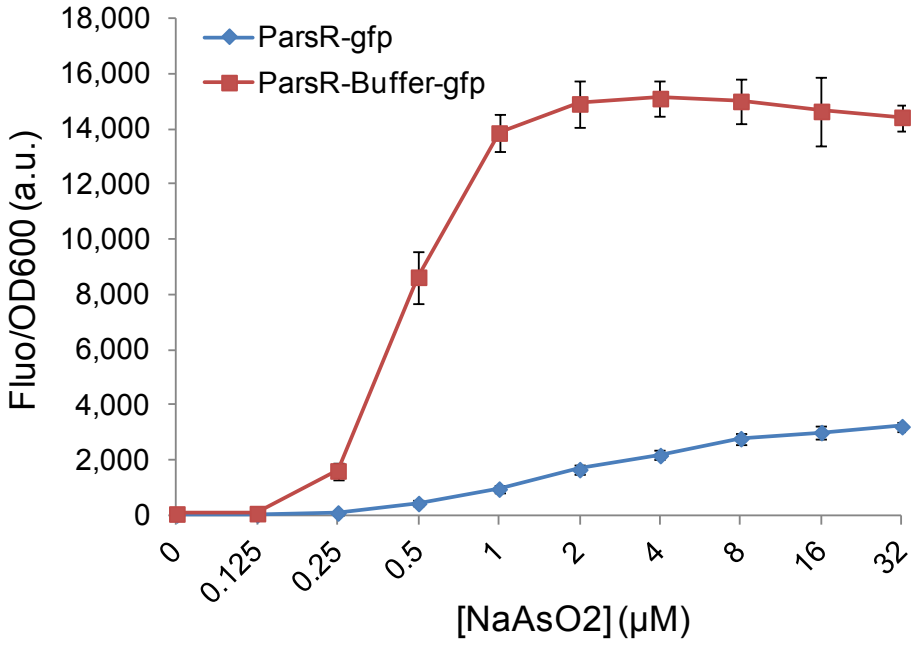

Figure S1. The dose responses of the arsenic responsive promoter input without (blue line) and with the Buffer gate (red line), as shown in Fig. 2 in main text. rbs30-*gfp* is used as the output reporter. Data shown are means and s.d. from three biological replicates.

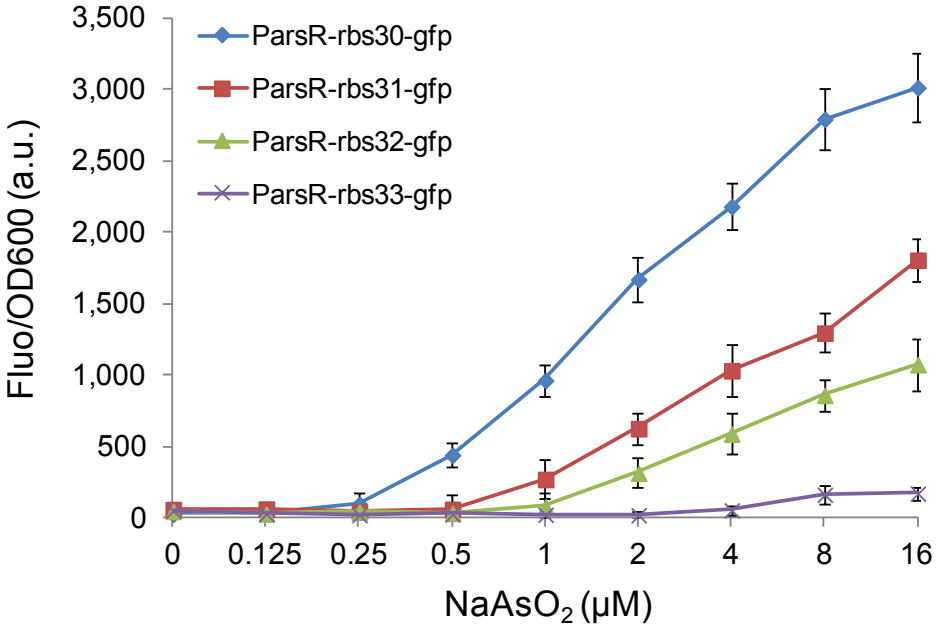

Figure S2. The dose responses of the arsenic responsive promoter input (P_{arsR}). The promoter was characterised with *gfp* as the output using 4 versions of RBS under varying induction conditions (0, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16 μM $NaAsO_2$). Data shown are means and s.d. from three biological replicates.

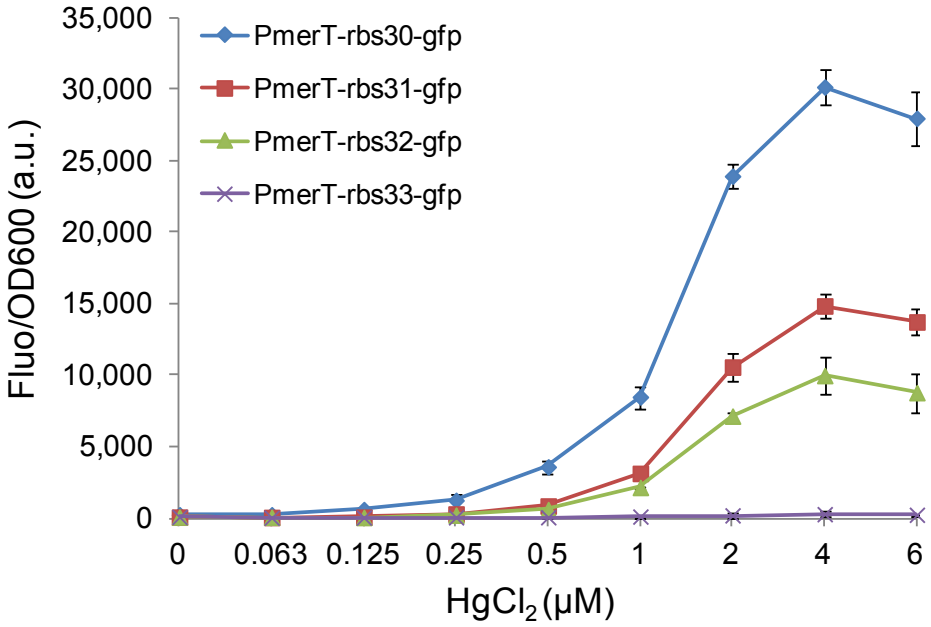

Figure S3. The dose responses of the mercury responsive promoter input (P_{merT}). The promoter was characterised with *gfp* as the output r using 4 versions of RBS under varying induction conditions (0, 0.063, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0 μM $HgCl_2$). Data shown are means and s.d. from three biological replicates.

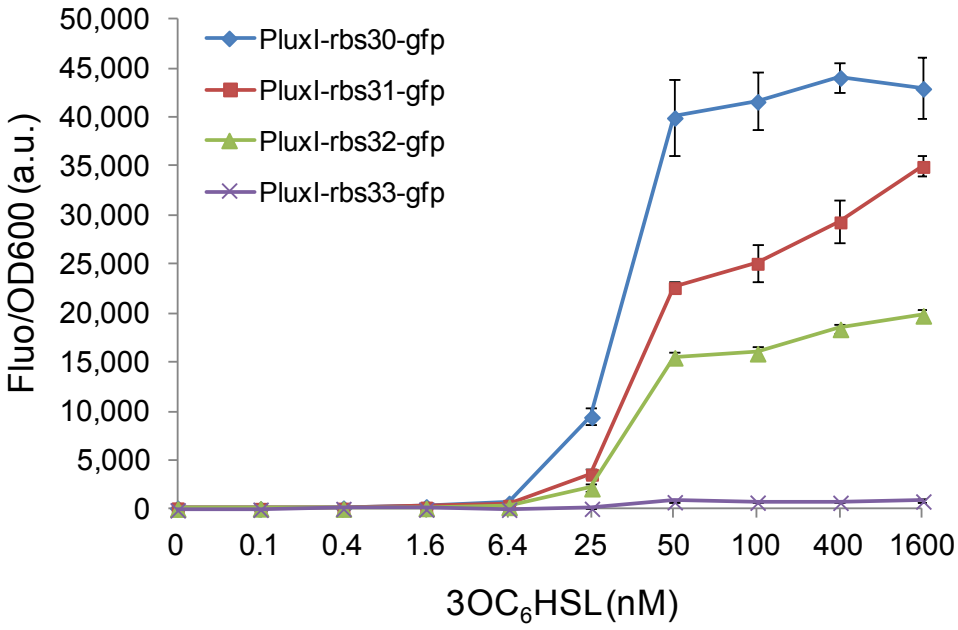

Figure S4. The dose responses of the AHL responsive promoter input (P_{luxI}). The promoter was characterised with *gfp* as the output using 4 versions of RBS under varying inducing conditions (0, 0.1, 0.4, 1.6, 6.4, 25, 50, 100, 400, 1600 nM $HgCl_2$). Data shown are means and s.d. from three biological replicates.

Supplementary Methods

Plasmid circuit construction

Plasmid construction and DNA manipulations were performed following standard molecular biology techniques. The *hrpR*, *hrpS*, *hrpV* genes, *hrpL* promoter, arsenic responsive sensor construct *arsR*-P_{arsR}, mercury responsive *merR*-P_{merT} and AHL responsive *luxR*-P_{luxI} were synthesised by GENEART following the BioBrick standard (http://biobricks.org), by eliminating the four restriction sites (EcoRI, XbaI, SpeI and PstI) for the BioBrick standard[2] via synonymous codon exchange and flanking with prefix and suffix sequences containing the appropriate restriction sites and RBS sequences. The double terminator BBa_B0015 (http://partsregistry.org) was used to terminate gene transcription in all cases. pSB3K3[2] (p15A ori, Kan^r) was used to clone and characterize all the genetic constructs in this study. The GFP (*gfpmut3b*, BBa_E0840) reporter was from the Registry of Standard Biological Parts (http://partsregistry.org). The various RBS sequences (Supplementary Table S1) for each gene construct were introduced by PCR amplification (using Phusion high-fidelity DNA polymerase from New England Biolabs and an Eppendorf Mastercycler gradient thermal cycler) with primers containing the corresponding RBS sequences and appropriate restriction sites. The constitutive promoters were assembled from two annealed single stranded primers flanked with appropriate restriction sites. All circuit constructs were assembled following the BioBrick DNA assembly method and verified by DNA sequencing (Beckman Coulter Genomics) prior to their use. Primers were synthesised by Sigma Aldrich.

Strains, media, chemicals and growth conditions

Plasmid cloning work and circuit construct characterization were all performed in *E. coli* TOP10 strain. Cells were cultured in LB (Luria-Bertani Broth) media (10 g/L peptone, 5 g/L NaCl, 5 g/L yeast extract). Kanamycin was present at 50 μg/ml. Cells inoculated from single colonies on freshly streaked plates were grown overnight in 5 ml LB in sterile 20 ml universal tubes at 37 °C with shaking (200 rpm). Overnight cultures were diluted into pre-warmed LB media at OD_{600} = 0.025 for the day cultures, which were induced (see below) and grown for 5 hours at 37 °C prior to analysis. For fluorescence assay, diluted day cultures were loaded into a 96 well microplate (Bio-Greiner, chimney black, flat clear bottom) using a repetitive pipette and induced with 5 μl (for single input induction), 10 μl (for double input induction) or 15 μl (for triple input induction) inducers of varying concentrations to a final growth volume of 200 μl per well, using a multichannel pipette. The microplate was covered

by a UV transparent lid to counteract evaporation and incubated in a BMG FLUOstar fluorometer with continuous shaking (200 rpm, linear mode, 37 °C) between each cycle of repetitive measurements. Chemical reagents and inducers (sodium arsenite, mercury chloride, and acyl homoserine lactone) used were analytical grade from Sigma Aldrich.

Assay of gene expression

Fluorescence levels as estimates of *gfp* gene expression were determined by fluorometry at the cell population level. Cells grown in 96-well plates were monitored and assayed using a BMG FLUOstar fluorometer for repeated absorbance (OD_{600}) and fluorescence (485 nm for excitation, 520 ± 10 nm for emission, Gain = 1000, bottom reading) readings (20 min/cycle). The fluorometry data of gene expression were first processed in BMG Omega Data Analysis Software (v1.10) and were analysed in Microsoft Excel 2007 and Matlab after being exported. The growth medium backgrounds (absorbance and fluorescence) were determined from wells loaded with LB media and were subtracted from the readings of other wells. The fluorescence/OD_{600} (Fluo/OD_{600}) at a specific time for a sample culture was determined after subtracting its triplicate-averaged counterpart of the negative control cultures (GFP-free) at the same time. For population-averaged assay by fluorometry, the fluorescence/OD_{600} after 5 hours growth post initial day dilution and induction was used as the output response of the cells in the steady state when cells were in the exponential growth phase and the steady state assumption for protein expression can be applied.

Supplementary notes and references

1 B. Wang, R. I. Kitney, N. Joly and M. Buck, *Nat. Commun.*, 2011, **2**, 508.

2 R. Shetty, D. Endy and T. Knight, *J. Biol. Eng.*, 2008, **2**, 5.