

ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Rapid engineering of versatile molecular logic gates using heterologous genetic transcriptional modules

Baojun Wang,^{*a} and Martin Buck^b

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

We designed and constructed versatile modular genetic logic gates in bacterial cells. These function as digital logic 1-input Buffer gate, 2-input and 3-input AND gates with one inverted input and integrate multiple chemical input signals in customised logic manners. Such rapidly engineered devices serve to achieve increased sensing signal selectivity.

Biomolecular computing is an emerging computing paradigm that has the potential of combining both the sensing and processing of biochemical signals to generate autonomous programmed output readout and action.^{1,2} Great progress has been seen in this area which typically applies enzymes,^{3,4} DNA^{5,6} or RNA⁷ in an *in vitro* biochemical system to execute complex logic computing tasks. For example, a DNA and restriction enzyme-based *in vitro* diagnostic automaton detected multiple biochemical disease indicators where the final positive state only forms under a pre-designed logic combination of the inputs⁸. More recently, live cells have hosted various *in vivo* logic computing tasks such as AND^{9,10,11} and XOR^{12,13} gates functionalities owing to the vast array of functions available to a living cell.¹⁴ However, the present capabilities for implementing complex logic computing functions in live cells are rather limited, since few well-characterised and versatile genetic building blocks are available.¹⁴ In addition, most of the devices constructed so far are designed to use specific inputs to drive specific outputs, which make them difficult to incorporate and reuse in other cellular circuit programs.

To address these bottlenecks, we present the rapid engineering of versatile modular molecular logic gates in model *Escherichia coli* bacteria via repurposing some heterologous genetic transcriptional modules. These modules exist in vast numbers amongst many different bacterial species, offering a largely untapped resource. As an example, we designed various modular logic devices using transcriptional modules from the *hrp* (hypersensitive response and pathogenicity) gene regulation system for Type III secretion in *Pseudomonas syringae* (Fig. 1).^{15,16} In the *hrp* gene regulatory architecture (Fig. 1), the activator proteins HrpR and HrpS form a high-order co-complex which binds the upstream activator sequence of the σ^{54} -dependent *hrpL* promoter to remodel the closed σ^{54} -RNAP-*hrpL* transcription complex to an open one through ATP hydrolysis, while negative regulation by the HrpV occurs through its direct interaction with HrpS¹⁷.

Since the *hrp* system comprises multiple components that interact and regulate each other in a defined logic manner, we considered that multiple-input combinational logic devices should be constructed based on their existing regulatory relationships. Accordingly three modular logic devices, including the digital logic 1-input Buffer gate, 2-input and 3-input AND with one inverted input gates, were designed using subsets of the *hrp* regulatory network (Fig. 1). The device inputs and outputs are promoters. The output promoter is only turned on when a specific logic combination of input promoters is active. One distinct advantage of the device design is the modularity (reusability), i.e. the inputs are exchangeable and can be connected to different environment-responsive promoters whilst the output may be wired to drive various other useful genes. Moreover, the genetic components used are heterologous to *E. coli* and thus less likely to interfere with the host endogenous genetic programs which will enable these logic devices to behave more robustly in their working contexts.⁹

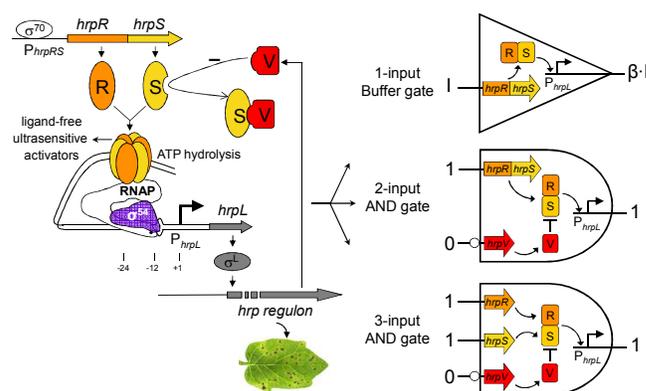


Fig. 1 Versatile modular molecular logic gates (1-input Buffer, 2-input and 3-input AND) engineered in *Escherichia coli* using the heterologous *hrp* transcriptional modules from *Pseudomonas syringae* plant pathogen.

We first designed the single-input digital Buffer gate which functions as an amplifier for the input signal to achieve increased output expression capability for downstream connected logic modules.¹⁸ The Buffer gate does not change the logic relationship between the input and output, but instead acts as an isolator and enhancer that can transform the input signal to a higher amplitude signal to match the input requirement of the aligned output modules.

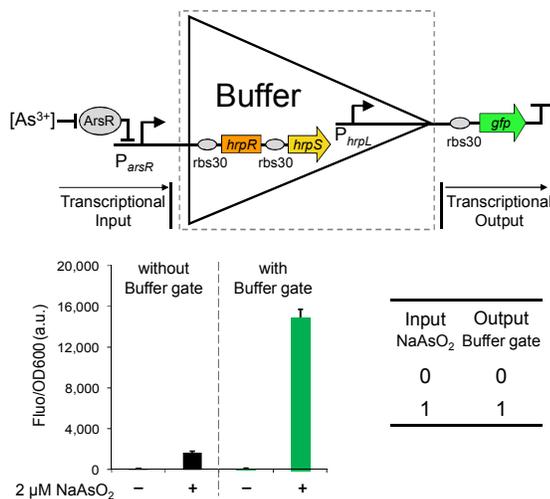


Fig. 2 Design and characterisation of the single-input Buffer gate. Data shown (fluorescence/OD₆₀₀) were the average of three independent biological repeats in *E. coli* TOP10 in LB media at 37 °C, 5 hrs post chemical induction. Error bars, s.d. (n = 3). a.u., arbitrary units.

The Buffer gate was built to express from an operon the cooperative activator proteins HrpR and HrpS with *hrpL* as the output promoter (Fig. 2). The high order HrpRS assemblies synergistically activate the downstream tightly controlled σ^{54} -dependent¹⁹ *hrpL* promoter, thus assisting amplification²⁰ of the transcriptional input signal. To characterise the device, the input was connected to an arsenic responsive promoter²¹ and the output to a *gfp* (green fluorescent protein) reporter. The input promoter alone was first systematically characterised with *gfp* as its output, in the presence of arsenite and produced a mild output response (Fig. S1-S2). In contrast, when the Buffer gate was applied, the output significantly increased to a much higher level (~ 9 fold) under the same 2 μ M NaAsO₂ input induction (Fig. 2). The characterised responses under different arsenite levels help establish that the device behaves well as a digital logic Buffer gate (Fig. S1). The Buffer gate can be used to increase the sensitivity of transcription-based biosensors (e.g. the arsenic sensor in this study) as well as to modulate the amplitude of an input signal to facilitate the coupling and matching of genetic modules with vastly different input-output strengths²².

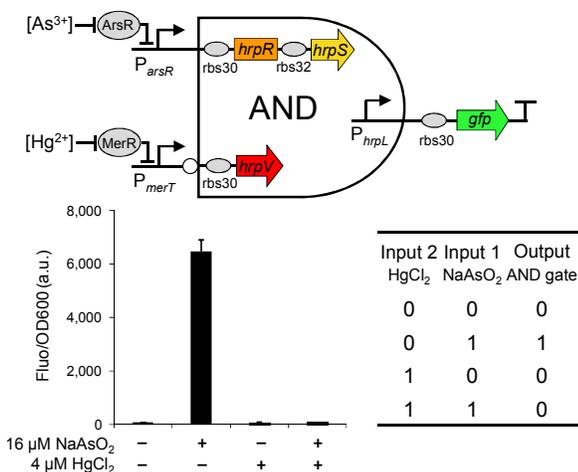


Fig. 3 The design and characterisation of the double-input modular AND gate with one inverted input. Error bars, s.d. (n = 3).

We next designed the double-input AND gate with one inverted input, a digital logic device that only produced an observable output when the 1st input is present and the 2nd input is absent (Fig. 3). The function is equivalent to the combination of connecting a NOT gate to one input of a canonical AND gate. The device can be used to improve the selectivity of biochemical sensors such that they will then only generate a valid readout under the specific logic combination of the two chosen sensing chemicals.

The double-input AND gate above was built by expressing from an operon HrpR and HrpS, under the control of the non-inverted input and expressing separately the inhibitor HrpV under the inverted input (Fig. 1). To characterise the device, we connected the non-inverted input to the arsenic responsive promoter (Fig. S2) and the inverted input to a mercury responsive promoter²¹ (Fig. S3) whilst the final output was the *gfp* reporter. To achieve a close to zero off-state behaviour, we used a weak RBS⁹ (ribosome binding site, rbs32, Table S1) ahead of the activator *hrpS* gene but a strong RBS (rbs30) ahead of the inhibitor *hrpV* gene. As a result, HrpV inhibitor will be expressed in excess over the activator HrpRS complex when both inputs are activated, leading to complete inhibition of the output *hrpL* promoter. The characterisation result shows that the device only produced an observable output when NaAsO₂ (16 μ M) is present and HgCl₂ (4 μ M) is absent among the four possible logic combinations of the two chemical inputs (Fig. 3). These outcomes establish the effectiveness of the digital logic AND gate having one inverted input function.

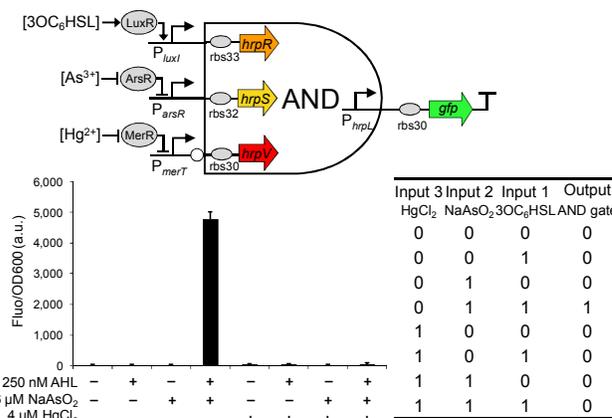


Fig. 4 The design and characterisation of the triple-input modular AND gate with one inverted input. Error bars, s.d. (n = 3).

Extending from the double-input AND gate, we designed the triple-input AND gate with one inverted input, a combinational digital logic device that should only produce an output when the first two inputs are present and the third input is absent (Fig. 4). The predicted function is equivalent to that of connecting the output of a canonical AND gate to the non-inverted input of the above built double-input AND gate. Similarly, the device can be used to increase the selectivity of biochemical sensors to only produce a significant readout under the specific logic combination of the three target sensing chemicals.

The triple-input AND gate was then built by expressing the two cooperative activator proteins HrpR and HrpS under the control of the two non-inverted inputs while expressing the

inhibitor HrpV under the control of the third inverted input (Fig. 1). To characterise the device, we connected the two non-inverted inputs to the arsenic (Fig. S2) and AHL²¹ (Fig. S4) responsive promoters respectively whereas the inverted input connected to the mercury responsive promoter (Fig. S3). Similarly, to achieve a close to zero off-state behaviour, we used two weak RBSs (rbs33 and rbs32, Table S1)⁹ ahead of the activator *hrpR* and *hrpS* gene but a strong RBS (rbs30) ahead of the inhibitory *hrpV* gene. Characterisation shows that the device only produced an observable output when both 3OC₆HSL (250 nM) and NaAsO₂ (16 μM) are present and HgCl₂ (4 μM) is absent among the eight possible logic combinations of the three chemical inputs (Fig. 4), proving the correct logic function of this triple-input AND gate with one inverted input.

Here, we have constructed a set of modular genetic logic gates with versatile digital logic functions and demonstrated that use of heterologous genetic transcriptional modules in different bacterial species can guide the design and rapid engineering of novel biological devices. The method presented here should enable future design and construction of many orthogonal genetic logic devices from diverse heterologous building blocks to expand the currently limited parts kit for gene circuit engineering¹⁴.

The genetic logic devices described here have particular advantages including the tight unactivated off state attributed to the underlying σ⁵⁴-dependent strict transcriptional activation requirement.¹⁹ The high-order co-dependent HrpRS complexes employed in the system contribute to the sharp switching between the off and on states of the devices, resulting in digital-like response behaviour. Moreover, these devices are modular and can, potentially, be easily connected to other modules to construct larger and more complex systems with advanced functions.

This work was supported by a BBSRC project grant [BB/K016288/1] and a Royal Society research grant award [RG120527] to B.W.

Notes and references

^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

† Electronic Supplementary Information (ESI) available: Gene regulatory sequences used, the characterised dose responses of the single-input Buffer gate and the three promoter inputs under various cognate chemical induction levels. See DOI: 10.1039/b000000x/

- 1 Y. Benenson, *Nat. Rev. Genet.*, 2012, **13**, 455.
- 2 J. Wang and E. Katz, *Anal. Bioanal. Chem.*, 2010, **398**, 1591.
- 3 J. Zhou, M. A. Arugula, J. Halánek, M. Pita and E. Katz, *The Journal of Physical Chemistry B*, 2009, **113**, 16065.
- 4 E. Katz and V. Privman, *Chem. Soc. Rev.*, 2010, **39**, 1835.
- 5 G. Seelig, D. Soloveichik, D. Y. Zhang and E. Winfree, *Science*, 2006, **314**, 1585.
- 6 L. Qian and E. Winfree, *Science*, 2011, **332**, 1196.
- 7 D. Faulhammer, A. R. Cukras, R. J. Lipton and L. F. Landweber, *Proceedings of the National Academy of Sciences*, 2000, **97**, 1385.
- 8 B. Gil, M. Kahan-Hanum, N. Skirtenko, R. Adar and E. Shapiro, *Nano Lett.*, 2011, **11**, 2989.
- 9 B. Wang, R. I. Kitney, N. Joly and M. Buck, *Nat. Commun.*, 2011, **2**, 508.
- 10 Z. Li, M. A. Rosenbaum, A. Venkataraman, T. K. Tam, E. Katz and L. T. Angenent, *Chem. Commun.*, 2011, **47**, 3060.
- 11 M. A. Arugula, N. Shroff, E. Katz and Z. He, *Chem. Commun.*, 2012, **48**, 10174.
- 12 J. Bonnet, P. Yin, M. E. Ortiz, P. Subsoontorn and D. Endy, *Science*, 2013, **340**, 599.
- 13 A. Tamsir, J. J. Tabor and C. A. Voigt, *Nature*, 2011, **469**, 212.
- 14 B. Wang and M. Buck, *Trends Microbiol.*, 2012, **20**, 376.
- 15 Q. Jin, R. Thilmoney, J. Zwiesler-Vollick and S.-Y. He, *Microbes Infect.*, 2003, **5**, 301.
- 16 D. Buttner and U. Bonas, *Curr. Opin. Microbiol.*, 2006, **9**, 193.
- 17 M. Jovanovic, E. H. James, P. C. Burrows, F. G. M. Rego, M. Buck and J. Schumacher, *Nat. Commun.*, 2011, **2**, 177.
- 18 C.-H. Chuang and C.-L. Lin, *BMC Syst. Biol.*, 2014, **8**, 63.
- 19 M. Buck, D. Bose, P. Burrows, W. Cannon, N. Joly, T. Pape, M. Rappas, J. Schumacher, S. Wigneshweraraj and X. Zhang, *Biochem. Soc. Trans.*, 2006, **34**, 1067.
- 20 Q. Zhang, S. Bhattacharya and M. E. Andersen, *Open Biology*, 2013, **3**, 130031.
- 21 B. Wang, M. Barahona and M. Buck, *Biosens. Bioelectron.*, 2013, **40**, 368.
- 22 D. Del Vecchio, A. J. Ninfa and E. D. Sontag, *Mol. Syst. Biol.*, 2008, **4**, 161.