A single layer artificial neural network type architecture with molecular engineered bacteria for complex conventional and reversible computing

Kathakali Sarkar^{1,2}, Deepro Bonnerjee^{1,2}, Rajkamal Srivastava^{1,2}, Sangram Bagh^{1,*}

¹Biophysics and Structural Genomics Division, Saha Institute of Nuclear Physics, Homi Bhabha National Institute (HBNI), Block A/F, Sector-I, Bidhannagar, Kolkata 700064 INDIA

²These authors contributed equally to the work.

* Corresponding author E-mail: sangram.bagh@saha.ac.in

Abstract: Here, we adapted the basic concept of artificial neural networks (ANN) and experimentally demonstrate a broadly applicable single layer ANN type architecture with molecular engineered bacteria to perform complex irreversible computing like multiplexing, de-multiplexing, encoding, decoding, majority functions, and reversible computing like Feynman and Fredkin gates. The encoder and majority functions and reversible computing were experimentally implemented within living cells for the first time. We created molecular-devices, which worked as artificial neuro-synapses in bacteria, where input chemical signals were linearly combined and processed through a non-linear activation function to produce fluorescent protein outputs. To create such molecular devices, we established a set of rules by correlating truth tables, mathematical equations of ANN, and molecular-device design, which unlike molecular computing, does not require circuit diagram and the equation directly correlates the design of the molecular-device. To our knowledge this is the first adaptation of ANN type architecture with engineered cells. This work may have significance in new platform for biomolecular computing, reversible computing and in transforming living cells as ANN-enabled hardware.

Introduction

Hardware implementation of artificial neural network (ANN) through neuro-synapse type architectures¹⁻⁴ have been developed and it has important implications in creating intelligent autonomous systems^{5,6}. However, ANN hardware is mainly limited to various neuromorphic chips made out of various inorganic materials¹⁻¹⁰, photonics⁸, spintronics⁹, and in-vitro DNA computation^{10,11}. The physical mechanism of ANN hardware operation is strikingly simpler than the biological neurons¹² and their networks in the brain¹³. Though, our understanding of neural networks in the brain is far from complete¹³, our understanding of ANN is from the first principle¹². This may allow adaptation of the basic ANN type architecture with living cells by engineering interactions at the molecular level.

In this study, we experimentally created a broadly applicable single layer ANN type framework using engineered molecular devices in living bacteria for performing complex conventional irreversible and nonconventional reversible computation. Here, the molecular devices inside bacteria work as artificial neuro-synapses and we named it as ‘bactoneuron’ (BNeu). The molecular devices, linearly combine the chemical inputs and transforms it nonlinearly to a fluorescent protein output. We experimentally demonstrated that single-layer neural network type architectures stemmed from those bactoneurons were general, flexible and perform complex irreversible (conventional) computation through a 2-to-4 decoder, a 4-to-2-priority encoder, a majority function, a 1-to-2 de-multiplexer, and a 2-to-1 multiplexer^{14,15} and reversible logic mapping through Feynman and Fredkin Gate. To our knowledge, the encoder and majority function have not been demonstrated and reversible computing is never explored in living biological systems. We established a set of molecular principles by correlating truth tables, mathematical equations of ANN, and molecular-device design, such that unlike invitro and in-vivo molecular computation, our ANN framework showed a complementary way to design biomolecular computation without following the electronic hierarchical logic circuit design principles

and a single mathematical equation based on its sign of the parameters directed the design of the molecular device.

Results and Discussions

Principles of mapping functional truth table to single layer molecular engineered bacterial ANN

First, we hypothesized that an abstract ANN model can be mapped into an engineered cellular model (Fig.1a), where engineered molecular devices inside bacterial cell work as artificial neuro-synapse (bactoneurons). The bactoneurons combined the inputs in the form of environmental chemical inputs and executed appropriate log-sigmoid activation functions (equation 1) through engineered molecular devices. Equation 1 is a conventional activation function for characterization of an artificial neuro-synapse in ANN¹² with two inputs and can be applied to a wide range of functional behaviors, based on its sign and magnitudes of the weight and bias terms.

Figure 1: Single layer artificial neural network (ANN) type architecture with engineered bacteria and schematic design rules of building complex computing function. **a)** Schematic representation of an ideal single layer ANN with two weighted inputs (X_1 and X_2) and outputs (O_1 and O_2) along with their corresponding weights (w_{ij}), the biases (b_i) and summation function (y_i). This abstract ANN is mapped with the proposed artificial bacterial neurons (Bactoneurons or BNeus). **b)** Relation between signs of weights in the activation function and molecular engineering principles for a bactoneuron associated with a input. **c)** Making of bacteria-based single layer ANN type architecture from truth table of a given function.

In this equation, each bactoneuron had two weight values of varying signs and magnitude corresponding to its two chemical inducer inputs (X_1 and X_2) and a bias with its value in accordance with the functional response of the neuron. For a given bactoneuron j ,

$$O_j = \frac{1}{(1 + e^{-(X_1 \cdot w_{j1} + X_2 \cdot w_{j2} + b_j)})} \dots\dots\dots \text{equation 1}$$

where,

O_j is the output from neuron j ,

X_1 and X_2 represent two input inducer concentration,

w_{j1} represents the weight of input X_1 for the neuron j ,

w_{j2} represents the weight of input X_2 for the neuron j ,

b_j represents the bias for the neuron j .

Equation 1 suggests that if w_{j1} is positive, output O_i would increase with X_1 . In terms of molecular device, it is similar to a molecular activation (Fig.1b). Similarly, negative weight would suggest a repression and ‘zero’ weight suggests the insensitivity of the input with the output (Fig.1b). This simple correlation between the sign of a weight, w_{ji} within an activation function of a ‘unit’ bactoneuron and molecular engineering principle, guided the physical design of the molecular device.

Next, we devised a way to map a complex computing function through a single layer ANN type architecture directly from its functional truth table, without considering its hierarchical electronic design principle (Fig.1c). We built a set of rules to derive bactoneurons from the functional truth table by dividing the bigger truth tables into smaller ones and to connect them with bactoneuron design (Fig.1c). We demonstrated this process considering a random functional truth table (Fig.1c). First, we considered a single output within a functional

truth table and then looked at its relation with all the input combinations. We grouped those input combinations in the form of a smaller truth tables, in such a way, that each input corresponding to that particular output possessed a weight with only one type of sign (+, - or 0) (step 1). Such individual bactoneurons in this step were named as ‘functional bactoneurons’, which in appropriate ANN combinations would give rise to the actual function. Next, we ignored the weight(s) with ‘zero’ values, if any, from functional bactoneurons and mapped them back with smaller truth tables (step 2). Further, we looked at the output of the smaller truth table from step2. If the output value 1 (true) appeared only once in the smaller truth tables, we defined them as ‘unit bactoneurons’. Otherwise, we kept dividing the truth table (step 3), until the above condition appeared. This way we identified the unit bactoneurons, which is the smallest unit required to be constructed as molecular device. Once those unit bactoneurons are combined appropriately, they would operate as the functional bactoneurons. Thus, when the unit bactoneurons were assembled according to ANN structure, the actual function was physically realized (Fig.1c).

Now, we chose a range of computing functions with varying complexities (Fig.2) and derived their functional bactoneurons (Fig.2) from their functional truth table following the principle stated above (Fig.1c). The chosen functions included 1-to-2 demultiplexer¹⁴ (Fig.2a), 2-to-1 multiplexer¹⁴ (Fig.2b), majority functions¹⁵ (Fig.2c), 2-to-4 decoder¹⁴ (Fig.2d), and 4-to-2 priority encoder¹⁴ (Fig.2e). A de-multiplexer performs as an output selector where it takes input from just one source and the logical state(s) of selector line(s) direct(s) to select only one among multiple output channels to process the signal and interpret it. Multiplexer performs the complementary function where the logical state(s) of selector line(s) directs which input is to be received for generating an output. A majority function suggests that in a ternary system if more than 50% of the inputs are true then the output is true, otherwise false. A N:2N decoder converts N bit binary-coded inputs into 2N coded outputs in a one-to one mapping fashion and an encoder encodes input signals to fewer bits and transforms them into encoded outputs. The details of deriving functional and unit bactoneurons from the truth tables of all

those functions without considering its integrated circuit design are shown in figure 2 and supplementary figure S1. The specific activation function equations for all functional bactoneurons are shown in table S1.

Figure 2: Derivation of functional bactoneurons and abstraction of single layer ANN type architectures from functional truth tables of complex computing functions. a) 1-to-2 de-multiplexer, b) 2-to-1 multiplexer, c) 3-input majority function, d) 2-to-4 decoder and e) 4-to-2 priority encoder.

Design and construction of molecular devices and unit bactoneurons.

Next, we developed molecular devices to construct the unit bactoneurons (Table-S1) in our chassis organism, *E. coli* DH5 α Z1¹⁶. The molecular devices are engineered molecular networks incorporated in plasmid vectors, which are 25 nm circular DNAs replicate inside the bacteria¹⁷. In our design, the abstract inputs (X_i) were replaced by extracellular chemical inducers like Isopropyl β -D-1 thiogalactopyranoside (IPTG), anhydrotetracycline (aTc), N-Acyl homoserine lactone (AHL), and arabinose. The abstract outputs (O_i) were changed to fluorescent proteins like EGFP, mKO2, E2 Crimson, mTFP1, and mVenus as appropriate. The device design of the unit bactoneurons were based on the molecular engineering principle we stated in figure 1b.

We started with the construction and characterization of unit bactoneuron BNeu 1 (Fig.3a-d), where both the weights in the activation function are positive with respect to the inputs (X_1 and X_2). In BNeu 1, two inducer chemicals IPTG and aTc were used as the inputs X_1 and X_2 respectively while enhanced green fluorescence protein (EGFP) was used as the output O_1 . The molecular device (Fig.3a) for BNeu 1 consists of a synthetic hybrid promoter, which combines the chemical signals aTc and IPTG and processes them through a log-sigmoid function (equation 1) and according to the principle, both aTc and IPTG should work as activator for the system. TetR and LacI, two transcription factors, which are constitutively and endogenously expressed in *E. coli* DH5 α Z1, bind the hybrid promoter thereby hindering it from expressing EGFP¹⁶. Both IPTG and aTc, which bind with LacI and TetR respectively and change their conformation such that they cannot bind to the promoter anymore, the promoter is free to recruit RNA polymerase for EGFP expression.

Figure 3: Design, experimental characterization, and weight and bias adjustments of molecular devices for unit bactoneurons BNeu 1, BNeu 2 and BNeu 6. a) Neural architecture of the bactoneuron BNeu 1 where w_{11} and w_{1A} are weights of inputs IPTG (I_P : X_1) and aTc (A_T : X_2) and b_1 is the bias. Activation function generates output EGFP (E_G : O). Truth table and biological circuit design of the BNeu 1 activation function. $P_{1AA1-11}$ are synthetic promoters designed for BNeu 1 activation function and regulated by IPTG and aTc as these promoters contain binding sites for LacI and TetR proteins. **b)** Schematic representation of weight and bias adjustment of BNeu 1 through constructs IAA1-11. IAA1-11 were generated in sets by changing hybrid promoter design with

varied number and positions of the operating sites for both TetR and LacI and by simultaneously changing the origin of replication (Ori) of the plasmids carrying those promoters. Promoter maps of P_{IAA}1-11 are also shown. Positions of -10 and -35 hexamers, transcription start site, ribosome binding sites (RBS) and LacI & TetR binding sites are depicted in individual promoter maps. Weights and bias associated with IPTG and aTc of the BNeu 1 were adjusted through a two-step modification of molecular interactions. Constructs corresponding to the selected promoter from each set are shown in magenta box. **c)** Heatmaps showing percentage highest leakage ($L_{\max}(\%)$), percentage sum of leakage excluding highest leakage ($\Sigma L - L_{\max}(\%)$), modulus of weight associated with IPTG ($|w_{II}|$) and modulus of weight associated with aTc ($|w_{IA}|$) for each out of 12 constructs (constructs IAA1-11). Construct IAA7B (coloured in red) is selected as the best performer. **d)** Expression characterization, simulated behavior (3D plot), and experimental validation of unit bactoneuron BNeu 1 carrying construct IAA7B are shown. All of these experimental data were collected after 10h induction followed by resuspension and 6h induction. **e)** Neural architecture, truth table and biological circuit design of the bactoneuron BNeu 2 activation function. **f)** Weight and bias adjustment of BNeu 2 through constructs INA1A-7C, which are a two-plasmid system. Here, we re-engineered the synthetic promoter of BNeu 1 and replaced the aTc gene-activation function (+w) with aTc gene repression function (-w), such that in the presence of aTc, transcription from the promoter gets turned off. In this promoter we introduced an operating site for λ repressor CI proteins and the amount of CI was under the control of an aTc-inducible promoter. Promoter maps, Ori, RBS, and its various combinations in constructs are shown. **g)** Heat maps of weight, leakage and hence bias adjustment for BNeu 2. **h)** Characterization, simulation and validation of BNeu 2 (INA1A-7C). **i)** Neural architecture, truth table and biological design of BNeu 6. **j)** Details of the constructs (ANI1A-4D) for weight and bias adjustment of BNeu 6 and **k)** corresponding heat maps are also shown. Construct ANI2C was chosen as best performing construct. **l)** Characterization, simulation and validation of BNeu 6 construct ANI2C.

To start with random weights and biases, as in any ANN design¹², we constructed and characterized an initial set (Set 1) of molecular constructs (IAA1-5), (Fig. 3b, Table-S2) containing synthetic promoters P_{IAA1} - P_{IAA5} respectively (Table-S3). For this, we measured the EGFP expression at various combinations of ‘zero’ and ‘saturated’ concentration of IPTG and aTc (Fig.S2a) and performed dose responses (Fig.S2b) of EGFP expression as a function of IPTG/aTc, by varying the concentration of one chemical, while keeping the other at saturated concentration. The dose-response behaviors were fitted to a modified form of equation 1 (equation-2, supplementary note-1). The fitting parameters gave the values of weights for each input and biases (Table S4). This starting set (Set 1) showed high leakage and lower weight values (Fig. 3c, Fig. S2a, Fig. S2b, table-S4 and table-S5).

Higher values for w_{II} , w_{IA} would signify sharper transition from OFF to ON state and more negative bias might signify reduced intercept in a dose-response curve, which could get reflected as less ‘leakage’, which was defined by the basal EGFP expression.

Guided by the least leakage and reasonably high weight values for both w_{II} and w_{IA} , we chose construct IAA4 where promoter P_{IAA4} served as a platform and adjusted the weights and bias by tweaking the molecular design and further creating new constructs (Fig.3b, Table-S2, S3). We iteratively performed this adjustment process a few times (Fig.3c). Clearly, the weight ‘w’ of a bactoneuron was a strong function of types and degree of molecular interactions, as evident from the fact that the weights of the initial bactoneuron were adjusted to a new one in each iteration. We found w_{II} as the limiting weight as this had a lower value than w_{IA} (Fig.S2b, table-S4). In addition, we focused on the highest leakage value (L_{max}), and $(\Sigma L - L_{max})$ of each construct, where

ΣL is the total leakage (Table-S5). Our goal was to reduce it. Constructs IAA4 and IAA5 from the first set carried similar weight but IAA5 showed significantly high $\Sigma L-L_{\max}$. Thus, IAA4 was chosen for further adjustment. Figure 3c shows the adjustment of values for weights and leakage. The constructs for further adjustment in each step are boxed. Now, the construct IAA7A (for BNeu 1) from the second set of adjustment, was taken for further weight adjustment either by engineering the promoter or by altering the relative numbers of the promoters per cell by changing the copy number of the plasmids (Fig.3b). Construct IAA7B (Table-S2) had higher weight values and least leakage with respect to construct IAA7A (Fig. 3c, table-S3 and table-S4). Others (IAA8-11) from the same iteration showed comparatively poor behavior. Such scenario could be compared with overshooting of weight adjustment, as happens in ANN¹². Thus, molecular device IAA7B was selected as the unit bactoneuron BNeu 1. We performed a simulation and experimentally tested the behavior of the BNeu 1 by simultaneously changing the concentration of the IPTG and aTc (Fig.3d). The results show a close topological match with the simulation (Fig.3d).

In an ANN framework, bias may determine the intercept¹². The leakage in bactoneuron determines the intercept in the dose-response curves. A moderate correlation ($R^2=0.76$) between the bias ‘ b_1 ’ and $L_{\max}$ was found (Fig. S3a) within the experimental range for BNeu 1 construct IAA7B. However, in this case, adjustments of weight values were linked to that of the bias during iteration and was difficult to distinguish the exact molecular reasoning. We performed a simulation by varying the bias but keeping the weights constant and it suggested that, ‘bias’ manifested as leakage in BNeu 1 (Fig.S3b). We performed similar simulations (Fig. S3) for all the unit bactoneurons (Table-S1) and the results suggested that the bias value in bactoneuron indicated the leakage from the molecular devices within a parameter range.

We further illustrate the construction of two other bactoneurons namely BNeu 2 and BNeu 6, which showed positive weight for one inducer and negative for the other (Fig.3e-h, and 3i-l). The development of BNeu 1

indicated that, $L_{\max}$ could be the first parameter to look for during molecular engineering. Therefore, for BNeu 2 and BNeu 6, we first checked if the fold change between the highest signal (output logic level “1”) and the highest leakage was more than an arbitrarily threshold 8 times (Table S5). If it was so, we proceeded to adjust the weight values and linked-biases for optimal behavior of the corresponding unit bactoneurons either by engineering the cis-trans element interaction on the promoter, or by reducing the translation rate of CI via RBS designing (Table-S6) or by modulating the relative number of synthetic promoters per cell through modification of the plasmid copy number. We followed this processing pipeline to characterize, fit and adjust the weights and biases in iterations to get the unit bactoneurons BNeu 2 and BNeu 6 (Fig.3e-h and Fig.3i-l), executed by constructs INA7A and ANI2C respectively (Table-S2). The gene expression characterizations, dose-response and fitting for all constructs for BNeu 2 and BNeu 6 were shown in figure S2c,d and S2e,f respectively. The design, gene expression characterizations, dose-response, fitting, simulation, and experimental validation of all other unit bactoneurons are shown in the figure S4. In many cases the unit bactoneurons were equivalent to the functional bactoneurons which did not have any ‘0’ weight inducer input (insensitive to a certain inducer input). Therefore, for such bactoneurons, we experimentally validated the weight ‘zero’ characteristics with respect to the appropriate inputs (Fig.S5).

ANN created from molecular engineered bactoneurons generate complex computing function.

For unit bactoneuron construction, we used EGFP as an output. We changed the EGFP with mKO2, E2-Crimson, mTFP1, and mVenus (Table-S1) as appropriate. Next, the unit bactoneurons were mixed, cocultured and exposed to various combinations of input chemicals following relevant ANN designs (Fig.2). The experimental results are shown in figure 4 and figure S6 for 1-to-2 de-multiplexer (Fig.4a,b, Fig.S6a), 2-to-4 multiplexer (Fig.4c,d, Fig.S6 b), 3-input majority function (Fig.4e,f, Fig.S6c), 2-to-4 decoder (Fig.4g,h, Fig.S6 d), and 4-to-2 priority encoder (Fig.4i,j, Fig.S6e). The results showed the expected truth table behavior (Fig.4).

Figure 4: Experimental demonstration of complex computations with single layer ANN type architectures with molecular engineered bactoneurons. Truth tables of the **a)** 1-to-2 de-multiplexer, **c)** 2 to-1 multiplexer, **e)** 3-input majority function, **g)** 2-to-4 decoder and **i)** 4-to-2 priority encoder are shown. Inputs and outputs are written as X_i ($i=1$ to n) and O_i ($i=1$ to n) respectively. Experimental behavior of the bacteria-based single layer ANN type architectures corresponding to the **b)** 1-to-2 de multiplexer, **d)** 2-to-1 multiplexer, **f)** 3-input majority function, **h)** 2-to-4 decoder and **j)** 4-to-2 priority encoder, studied with fluorescence microscope. Unit bactoneuorns of a function were cultured in a mixed population and treated with all possible combinations of inputs. The resultant expression of fluorescent proteins are represented by separate output channels.

Mapping logical reversibility with molecular engineered bactoneurons: Feynman and Fredkin Gates

Reversible computing is the heart of quantum computing¹⁸ and it can map the previous state of the computation from the current state in a one-to-one basis^{18,19}. This is called logical reversibility, which was demonstrated in by implementing logically reversible Fredkin and Toffoli gates through in-vitro DNA computation²⁰. However, no logically reversible gate is implemented in living cells. Though, the thermodynamic reversibility of reversible computing, which gives lowest energy cost in computation, is not possible in living systems, the potential of logical reversibility in biological systems is yet to explore. We showed that ANN with bactoneurons had the flexibility to create reversible computing and we demonstrated the universal reversible Feynman gate (Fig.5a-b, Fig.S1f, S6f) and Fredkin gate (Fig.5c-d, Fig.S1g, S6g), which may create any linear reversible logic gate. First we derived the functional and unit bactoneurons for Feynman (Fig.5a, fig.S1f, table-S1) and Fredkin gate (Fig.5c, Fig.S1g, table-S1). Feynman gate was represented by 3 unit bactoneurons (BNeu2, BNeu6, BNeu8), which we already developed. The ANN created from the corresponding functional bactoneurons (FBNeus 16-18) of these unit bactoneurons (Fig.5a) showed successful Feynman gate (Fig.5b). Fredkin gate was represented by 5 unit bactoneurons (BNeu 3, 4, 7, 9, 10), where BNeu 3, 4, 7 were already developed and BNeu 9 and BNeu 10 were created (Fig.S4p-u). The ‘zero’ weights of the bactoneurons, where appropriate, was validated in figure S5. The ANN, created from the corresponding functional bactoneurons (FBNeus 19-23) (Fig.5c), showed successful Fredkin gate (Fig.5d).

Figure 5: ANN Architecture and experimental demonstration of reversible Feynman and Fredkin gate with molecular engineered bactoneurons. Derivation of the functional bactoneurons from truth tables for **a)** Feynman gate and **c)** Fredkin gate. Experimental behavior of **b)** Feynman gate and **d)** Fredkin gate.

Plausible correlations between ANN parameters and molecular mechanism

The abstract architectures and mathematical equations of an ANN are bridged with the physical bactoneurons through the molecular devices. Within the molecular devices, it may be possible to envisage the events of

different inducers binding to the cognate transcription factors and causing conformational changes in them as a “summing unit” (linear combinations). The sensitivity of the transcription factors to their respective inducers can be seen in parallel to the synaptic strength of a neuron that is weights. The “summed” changes in the relevant transcription factors of a promoter directly affects their promoter binding capabilities and thereby modulates the activation of the promoter to express a gene. This can be perceived as the “activation unit”. In this work we manually adjusted the weights and bias of a neuron by changing the numbers and positions of various promoter operator sites, relative molecular amounts of transcription factors and promoter copy numbers through different plasmid copy numbers or altering RBS strength. We further showed a direct relation between ‘bias’ of an artificial neuron and leakage from the genetic devices. In this way, we established a clear correlation between ANN parameters and molecular mechanism of an intracellular molecular device. The weight adjustment was done manually by molecular engineering and the inference network was ‘uploaded’ in the final bactoneural networks. This might be compared with the systems, where the weight adjustments were done in the server and the inference network was sent to the mobile device²¹. It is important to note that the mathematical models for conventional bio-circuit design is different and highly specific for different functions and in fact, the mathematical model of a computing function may not guide the actual genetic circuit design²². Whereas, our ANN based frameworks required only single type of mathematical equation (Table S1) for creating different computing functions and we established a direct correlation between the sign of the weights in the activation function equation and nature of molecular mechanism (Fig. 1B). Thus, this allowed a simple and streamlined way to design molecular device for bactoneurons of any function directly guided by the mathematical equation of the activation function.

Conclusions

In summary, we showed that the basic concept of ANN could be adapted in living bacteria with the help of molecular devices. The ANN type architecture with molecular engineered bacteria works as a flexible and

general framework in its design and architecture for performing complex bio-computation, both conventional and reversible. Unlike, the conventional way of designing in-vitro small molecule computing²³, enzyme based computing²⁴, DNA computing²⁵ and in-vivo synthetic genetic computing^{26,27}, which followed the hierarchical logic circuit approach^{28,29}, we showed that the ANN type framework can adapt a new design path to create complex computing function like encoder, majority function, mux, demux, Feynman gate and Fredkin gate, where encoder, majority function and reversible gates were demonstrated for the first time in living cells. Reversible computing is a new class of computing for biological cells and our work might pave the way in that direction. In ANN, any function can be designed and simulated just by adjusting the weights and bias values of a single mathematical equation and we established a direct relation between signs of the weights and nature of the interaction in the molecular devices. Thus, our approach established a new streamlined design and construction platform complementary to the conventional bio-circuit design²² and may have implications in complex reversible and irreversible biocomputing, bacteria-based ANN hardware, and synthetic biology.

Materials and Methods

Promoters and genes, plasmids, RBS's & primers

The genetic devices were made according to the designs. The bioparts (promoter, ribosome binding sites (RBS), gene and transcription terminators) were arranged within appropriate plasmids using standard molecular biology protocols. The designed promoters were obtained by either PCR (KOD Hot-Start DNA polymerase, Merck-Millipore). PCR amplification was performed by KOD Hot Start DNA polymerase (Merck Millipore). All enzymes, ligase, and ladders were from New England BioLabs. Plasmid isolation, gel extraction, and PCR purification kits were from QIAGEN. Translation initiation rate for EGFP and CI under the control of promoter P_{AAH} and $P_{LtetO-1}/P_{LlacO-1}$ respectively was calculated and weak RBSs (RBSs RH and RC1-3) were designed using RBS Calculator v2.0⁴², considering RBS R (BBa_B0034)⁴³ along with linker GGTACC (*KpnI* site) as degenerate RBS sequence and *E. coli*-MG1655 as the organism. All promoter sequences, RBS sequences,

primers, and plasmids are shown in supplementary tables S3, S6, S8, and S9 respectively. All primers, oligos and gene products were synthesized from IDT and Invitrogen. All cloned genes, promoters, RBS's in plasmid constructs were sequence verified by Eurofins Genomics India Pvt. Ltd., Bangalore, India.

Bacterial cell culture for characterization

Chemically competent *Escherichia coli* DH5 α strain was used for cloning and DH5 α Z1 strain was used for the experimental characterization. Working concentrations of the antibiotics in LB-Agar, Miller (Difco, Beckton Dickinson) plates as well as in LB broth, Miller (Difco, Beckton Dickinson) were: 100 μ g/ml for Ampicillin (Himedia), 34 μ g/ml for Chloramphenicol (Himedia) and 50 μ g/ml for Kanamycin (Sigma Aldrich). DH5 α Z1 cells were transformed with appropriate Sequence verified plasmid constructs. Well-isolated single colonies were picked from LB agar-plates, inoculated to fresh LB-liquid media, and grown overnight in presence of antibiotics. Next, the overnight culture was re-diluted 100 times in fresh LB media with antibiotics and with or without inducers as per the design of the gene circuit, and grown at 37 $^{\circ}$ C, $\sim$ 250 rpm. Engineered cells for weight and bias adjustment steps (expression characterization and dose response experiments) were grown for 16 hours and cells with final constructs were grown for 10 hours with inducers, resuspended and grown for another 6 hours. These 10+6 hours growth was performed for expression characterization, dose response experiments, validation experiments and for full ANN microscopy experiments.

Dose-response experiments and validation experiments

All dose-response experiments were performed by varying one inducer across 9 or more concentration points while the other inducer was kept constant ("0" state or "1" as the case may be). Here, for the linear combinations of input signals, we converted the concentration range of each input chemical from '0' to '1', where 0 signifies zero concentration and 1 signifies the saturating concentration of the chemical. Any concentration higher than the saturation concentration was treated as 1. For the validation experiments, the corresponding two inducers of the relevant constructs were simultaneously varied across 10+ concentration points. For, validation experiments, we used different concentration points compare to the concentration used in

dose response. For intermediate experiments during weight and bias adjustments of the constructs, single colony was used. For the final constructs, all experiments data from minimum 3 independent colonies were collected.

Measurement of fluorescence and optical density, Normalization, and Scaling

For fluorescence and optical density (OD) measurements, Synergy HTX Multi-Mode reader (Biotek Instruments, USA) was used. For this purpose, cells were diluted in PBS (pH 7.4) to reach around OD600 as 0.8, loaded onto 96-well multi-well plate (black, Greiner Bio-One), and both EGFP fluorescence with appropriate gain and OD600 was measured. For EGFP fluorescence measurements, we used 485/20 nm excitation filter and 516/20 nm emission bandpass filter. At least 3 biological replicates had been considered for each condition to collect the fluorescence and OD data. The raw fluorescence values were divided by respective OD600 values and thus normalized to the number of cells. Auto-fluorescence was measured as average normalized fluorescence of the untransformed DH5αZ1 set (no plasmid set) and subtracted from the normalized fluorescence value of the experimental set. The above normalization can be mathematically represented as follows: -

Unscaled normalized fluorescence

$$= \left[\frac{\left(\begin{array}{c} \text{Absolute fluorescence value} \\ \text{from experimental cell population} \end{array} \right)}{\left(\text{OD of experimental cell population} \right)} \right] - \left[\frac{\left(\begin{array}{c} \text{Absolute fluorescence value} \\ \text{from no plasmid cell population} \end{array} \right)}{\left(\text{OD of no plasmid cell population} \right)} \right]$$

The values thus obtained were then scaled down between 0 and 1-considering the normalized fluorescence value at the induction point of maximum expected fluorescence to be 1,

$$\text{Scaled normalized fluorescence} = \left[\frac{\left(\begin{array}{c} \text{Unscaled normalized fluorescence} \\ \text{at any induction point} \end{array} \right)}{\left(\begin{array}{c} \text{Unscaled normalized fluorescence} \\ \text{at induction point of} \\ \text{maximum expected fluorescence} \end{array} \right)} \right]$$

Data Analysis, Fitting, Mathematical Modelling and Simulation

The fittings of the dose response curves were done by appropriate equations (Supplementary Table S10).

However, all the equations could be brought down in a general form,

$$O_i = \frac{1}{1+e^{-(w_{jn} \cdot X_n + b)}} \dots \text{equation 2}$$

where,

O_i is the output signal from the artificial neuron j ,

X_n corresponds to the magnitude of the varying input (n) to the artificial neuron j ,

w_n corresponds to the weight of the n^{th} input to the artificial neuron j ,

and, b is given by:

$$b = X_m \cdot w_{jm} + b_j \dots \text{Equation (3)}$$

where,

X_m corresponds to the logical state of the constant input (m) to the artificial neuron j (0 or 1),

w_m corresponds to the weight of the m^{th} input to the artificial neuron j ,

b_j corresponds to the bias of the artificial neuron j .

The scaled output fluorescence values obtained from the dose-response experiments were plotted against the varying inducer concentration and fitted against equation 2. All data analysis and fitting were performed in OriginPro 2018 (OriginLab Corporation, USA) and was performed using built-in Levenberg Marquardt algorithm, a damped least squares (DLS) method. The parameter " w_{jn} " of the fitting function (equation 2) gives the "weight" of the varying input in the summation function of the corresponding neuron j . The b value obtained includes the bias plus the product of the input logic state of the second input and its weight as explained above. Upon similarly fitting the dose-response of the neuron to the second input, the weight " w_j " and " b " for input 2 is obtained. Solving equation 2 and 3 for both the inputs, " $w_{jinput1}$ ", " $w_{jinput2}$ " and " b_j " of the complete summation function of the corresponding neuron j was obtained. All parameter values for all constructs were shown in supplementary table S4. The simulations were performed by generating matrices of calculated normalized output fluorescence values against simultaneously varying concentrations of the

corresponding two inputs across 65X65 or more points following the parameterized activation function. For single-input systems, simulation for a given activation function was carried out across 19 varying input concentration points.

Microscopy

DH5 α Z1 cells were transformed with the appropriate sequence-verified plasmid construct(s). Following an 10 hours induction followed by re-suspension and 6 hour induction step, cells were washed thrice in PBS. Cell pellets were finally resuspended in fresh PBS (pH 7.2-7.4) and this re-suspension was used to prepare fresh slides. A Laser Scanning Microscope Zeiss LSM 710/ ConfoCor 3 operating on ZEN 2008 software was used for imaging of the de-multiplexer, multiplexer, majority function, decoder and encoder. The cell suspension slides were subjected to excitation by appropriate laser channels (458 nm Ar Laser for mTFP1, 488 nm Ar Laser for EGFP, 514 nm Ar Laser for mVenus, 543 nm He-Ne Laser for mKO2 and 633 nm He-Ne Laser for E2-Crimson) and fluorescence emissions were captured through proper emission filters (BP484-504 nm for mTFP1, BP500-520 nm for EGFP, BP521-541 nm for mVenus, BP 561-591 nm for mKO2, BP641-670nm (2-to-4 decoder)/BP630-650 nm (1-to-2 de-multiplexer) for E2-Crimson) with a 63x Oil immersion objective and were detected through a T-PMT. The pin hole was completely open. For reversible Fredkin gate imaging, Nikon AIR si confocal microscope along with resonant scanner and coherent CUBE diode laser system was used. The mixed cell populations, washed and resuspended in PBS (pH 7.2-7.4), was added on the top of the 1% molten agarose pad which was placed upon a cleaned glass slide. The sample field was then covered with clear cover slip, placed under 60 X water immersion and subjected to excitation by laser channels (488 nm Laser for EGFP, 561 nm for td-Tomato and 640 nm for E2-Crimson). Three different emission filters (BP 525/50 nm for EGFP, BP 585/65 for td-Tomato and BP 700/75 nm for E2-Crimson) were used for collecting fluorescence and differential interference contrast (DIC) images were captured for all samples as well. Microscopic images were processed through ImageJ software for better visualization.

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Author Contributions

SB conceived and designed the study. KS, DB, and RS performed all the experiments. KS, DB, and SB designed the experiments, analyzed and interpreted the data, and wrote the paper.

Competing Interests statement

We have no competing interest.

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Supplementary Information

A single layer artificial neural network type architecture with molecular engineered bacteria for complex conventional and reversible computing

Kathakali Sarkar^{1,2}, Deepro Bonnerjee^{1,2}, Rajkamal Srivastava^{1,2}, Sangram Bagh^{1,*}

¹Biophysics and Structural Genomics Division, Saha Institute of Nuclear Physics, Homi Bhabha National Institute (HBNI), Block A/F, Sector-I, Bidhannagar, Kolkata 700064 INDIA

² Equal contributions

* Corresponding author

Figure S1: Derivation of functional and unit bactoneurons for a) de-multiplexing function, b) multiplexing function, c) majority function, d) decoding function, e) encoding function, f) Feynman gate function and g) Fredkin gate function. In each case, combination of unit bactoneurons gives rise to single layer ANN architecture where, individual unit bactoneurons come back to their corresponding functional bactoneuron states while they get associated with ‘0’ weighted inputs (If any). In the network level, parts of the summation function of each functional bactoneuron, contributed by ‘0’ weighted input, are not shown.

Figure S2: Details of characterization and dose responses of BNeu 1, 2 and 6. Expression characterization of **a)** BNeu 1, **c)** BNeu 2 and **e)** BNeu 6 and dose responses of **b)** BNeu 1, **d)** BNeu 2 and **f)** BNeu 6.

Figure S3: Correlation between bias and leakage of a Unit bactoneuron. a) Correlation between bias (b_1) and the percentage highest leakage ($L_{\max}(\%)$) for all BNeu 1 cellular devices obtained from weight and bias adjustment steps.

Simulated output behaviors of **b)** BNeu 1, **c)** BNeu 2 **d)** BNeu 3 **e)** BNeu 4, **f)** BNeu 5, **g)** BNeu 6, **h)** BNeu 7, **i)** BNeu 8, **j)** BNeu 9 and **k)** BNeu 10. Simulation corresponding to the bias value obtained experimentally for each bactoneuron is shown in red box.

Figure S4: Characterization of unit bactoneurons BNeu 3, BNeu 4, BNeu 5, BNeu 7, BNeu 8, BNeu 9 and BNeu 10. Neural architectures, truth tables and biological circuit designs of unit bactoneurons **a)** BNeu 3, **d)** BNeu 4, **g)** BNeu 5, **j)** BNeu 7, **m)** BNeu 8, **p)** BNeu 9 and **s)** BNeu 10 are shown. Details of plasmids carrying bioparts of the biological circuit designs of **b)** BNeu 3, **e)** BNeu 4, **h)** BNeu 5, **k)** BNeu 7, **n)** BNeu 8, **q)** BNeu 9 and **t)** BNeu 10 are illustrated. Expression characterization, dose responses, 3D simulations and experimental 3D behavior of **c)** BNeu 3, **f)** BNeu 4, **i)** BNeu 5, **l)** BNeu 7, **o)** BNeu 8, **r)** BNeu 9 and **u)** BNeu 10 in terms of EGFP expression are also shown.

Figure S5: Characterizations of functional bactoneurons associated with weight=0 towards specific inducers. Each functional bactoneuron population was subjected to 10h+6h induction with a set of inducers which was chosen based on the neural architecture of individual functional bactoneurons, and then characterized in terms of EGFP expression. If the presence or absence of a specific inducer didn't change the output of the functional bactoneuron, then only we considered that, the inducer was associated with zero weight. Neural architectures and validation for '0' weighted input(s) of functional bactoneurons **a)** FBNeu 5, **b)** FBNeu 6, **c)** FBNeu 7, **d)** FBNeu 12, **e)** FBNeu 13, **f)** FBNeu 14, **g)** FBNeu 15, **h)** FBNeu 21 and **i)** FBNeu 23 are shown.

23 are shown. FBNeu 20 and FBNeu 22 from Fredkin gate function are equivalent to FBNeu 7 and FBNeu 6 respectively except their different outputs. Therefore, individual weight '0' input validation for FBNeus 6 and 7 justifies the same for FBNeus 22 and 20 as well. FBNeu 12 and FBNeu 14 are similar except their outputs. Here, individual functional bactoneurons are characterized in terms of EGFP output. Therefore, both FBNeu 12 and FBNeu 14 produce EGFP output and hence, they become identical. Thus, they share common '0' weighted input validation data (Shown with magenta arrow). FBNeu 16 from Feynman gate function is a sub-set of FBNeu 13 as it operates on lesser number of inputs whereas, their corresponding unit bactoneuron is common. Therefore, weight '0' input of FBNeu 16 can be validated from the characterization result of FBNeu 13. Similarly, weight '0' inputs of Fredkin gate functional bactoneuron FBNeu 19 can be validated by characterization result of FBNeu 12/14.

Figure S6: Microscopy images with corresponding differential interference contrast (DIC) and merged channels. Populations of different combinations of bactoneurons, depending on the complex functions they constitute, were co-cultured with appropriate inductions where, they together formed a bactoneural layer. They were viewed under relevant laser channels and emission filters. DIC images show a heterogenous population of cells in the field with each sub-population responding uniquely to the induction conditions. A bactoneuron's activation is reported by fluorescence from its respective output protein whereas inactive bactoneurons show no fluorescence. Microscopic images for the bactoneuron-based single layer

ANN type architectures for **a)** de-multiplexing function, **b)** multiplexing function, **c)** majority function, **d)** decoding function, **e)** encoding function **f)** reversible Feynman gate and **g)** reversible Fredkin gate are shown.

Table S1: Details of functional bactoneurons and corresponding unit bactoneurons associated with the computing functions developed in this study. Output fluorescent proteins and activation function equations corresponding to unit bactoneurons are also described.

Serial number	Function	Functional bactoneurons (FBNeus)	Unit bactoneurons (BNeus)	Output of unit bactoneuron	Activation function Equation
1	De-multiplexing			E2-Crimson (E2)	$O_1 = \frac{1}{1 + e^{-(w_{11}A_1 + w_{12}A_2 + b_1)}}$
				EGFP (E3)	$O_2 = \frac{1}{1 + e^{-(w_{21}A_1 + w_{22}A_2 + b_2)}}$
				EGFP (E4)	$O_3 = \frac{1}{1 + e^{-(w_{31}A_1 + w_{32}A_2 + b_3)}}$
2	Multiplexing			EGFP (E5)	$O_4 = \frac{1}{1 + e^{-(w_{41}A_1 + w_{42}A_2 + b_4)}}$
				EGFP (E6)	$O_5 = \frac{1}{1 + e^{-(w_{51}A_1 + w_{52}A_2 + b_5)}}$
				EGFP (E7)	$O_6 = \frac{1}{1 + e^{-(w_{61}A_1 + w_{62}A_2 + b_6)}}$
3	Majority function			EGFP (E8)	$O_7 = \frac{1}{1 + e^{-(w_{71}A_1 + w_{72}A_2 + b_7)}}$
				EGFP (E9)	$O_8 = \frac{1}{1 + e^{-(w_{81}A_1 + w_{82}A_2 + b_8)}}$
				EGFP (E10)	$O_9 = \frac{1}{1 + e^{-(w_{91}A_1 + w_{92}A_2 + b_9)}}$
4	Decoding			mCherry (M1)	$O_{10} = \frac{1}{1 + e^{-(w_{101}A_1 + w_{102}A_2 + b_{10})}}$
				mCherry (M2)	$O_{11} = \frac{1}{1 + e^{-(w_{111}A_1 + w_{112}A_2 + b_{11})}}$
				mCherry (M3)	$O_{12} = \frac{1}{1 + e^{-(w_{121}A_1 + w_{122}A_2 + b_{12})}}$
5	Encoding			E2-Crimson (E2)	$O_{13} = \frac{1}{1 + e^{-(w_{131}A_1 + w_{132}A_2 + b_{13})}}$
				E2-Crimson (E2)	$O_{14} = \frac{1}{1 + e^{-(w_{141}A_1 + w_{142}A_2 + b_{14})}}$
				E2-Crimson (E2)	$O_{15} = \frac{1}{1 + e^{-(w_{151}A_1 + w_{152}A_2 + b_{15})}}$
6	Feynman gate			E2-Crimson (E2)	$O_{16} = \frac{1}{1 + e^{-(w_{161}A_1 + w_{162}A_2 + b_{16})}}$
				EGFP (E3)	$O_{17} = \frac{1}{1 + e^{-(w_{171}A_1 + w_{172}A_2 + b_{17})}}$
				EGFP (E3)	$O_{18} = \frac{1}{1 + e^{-(w_{181}A_1 + w_{182}A_2 + b_{18})}}$
7	Freudian gate			EGFP (E3)	$O_{19} = \frac{1}{1 + e^{-(w_{191}A_1 + w_{192}A_2 + b_{19})}}$
				tdTomato (T1)	$O_{20} = \frac{1}{1 + e^{-(w_{201}A_1 + w_{202}A_2 + b_{20})}}$
				tdTomato (T1)	$O_{21} = \frac{1}{1 + e^{-(w_{211}A_1 + w_{212}A_2 + b_{21})}}$
8	Freudian gate			E2-Crimson (E2)	$O_{22} = \frac{1}{1 + e^{-(w_{221}A_1 + w_{222}A_2 + b_{22})}}$
				E2-Crimson (E2)	$O_{23} = \frac{1}{1 + e^{-(w_{231}A_1 + w_{232}A_2 + b_{23})}}$
				E2-Crimson (E2)	$O_{24} = \frac{1}{1 + e^{-(w_{241}A_1 + w_{242}A_2 + b_{24})}}$

Table S2: List of cellular devices constructed in this study.

Unit bactoneuron	Cellular device	Output cassette components				Regulatory cassette components			
		Promoter –gene cassette	RBS	Ori	Antibiotic selection	Promoter-regulator cassette	RBS	Ori	Antibiotic selection
BNeu 1	IAA1	P _{IAA1} -EGFP	R	pUC	Amp	-	-	-	-
	IAA2	P _{IAA2} -EGFP	R	pUC	Amp	-	-	-	-
	IAA3	P _{IAA3} -EGFP	R	pUC	Amp	-	-	-	-
	IAA4	P _{IAA4} -EGFP	R	pUC	Amp	-	-	-	-
	IAA5	P _{IAA5} -EGFP	R	pUC	Amp	-	-	-	-
	IAA6	P _{IAA6} -EGFP	R	pUC	Amp	-	-	-	-
	IAA7A	P _{IAA7} -EGFP	R	pUC	Amp	-	-	-	-
	IAA7B	P _{IAA7} -EGFP	R	p15A	Cm	-	-	-	-
	IAA8	P _{IAA8} -EGFP	R	pUC	Amp	-	-	-	-
	IAA9	P _{IAA9} -EGFP	R	pUC	Amp	-	-	-	-
	IAA10	P _{IAA10} -EGFP	R	pUC	Amp	-	-	-	-
	IAA11	P _{IAA11} -EGFP	R	pUC	Amp	-	-	-	-
BNeu 2	IAA7B.A	P _{IAA7} -E2-Crimson	R	p15A	Cm	-	-	-	-
	IAA7B.B	P _{IAA7} -mVenus	R	p15A	Cm	-	-	-	-
	INA1A	P _{INA1} -EGFP	R	pUC	Amp	P _{LtetO-1} -CI	R	p15A	Cm
	INA1B		R	pUC	Amp	P _{LtetO-1} -CI	RC1	p15A	Cm
	INA1C		R	pUC	Amp	P _{LtetO-1} -CI	RC2	p15A	Cm
	INA1D		R	pUC	Amp	P _{LtetO-1} -CI	RC3	p15A	Cm
	INA2A	P _{INA2} -EGFP	R	pUC	Amp	P _{LtetO-1} -CI	R	p15A	Cm
	INA2B		R	pUC	Amp	P _{LtetO-1} -CI	RC1	p15A	Cm
	INA2C		R	pUC	Amp	P _{LtetO-1} -CI	RC2	p15A	Cm
	INA2D		R	pUC	Amp	P _{LtetO-1} -CI	RC3	p15A	Cm
	INA3A	P _{INA3} -EGFP	R	pUC	Amp	P _{LtetO-1} -CI	R	p15A	Cm
	INA3B		R	pUC	Amp	P _{LtetO-1} -CI	RC1	p15A	Cm
	INA3C		R	pUC	Amp	P _{LtetO-1} -CI	RC2	p15A	Cm
	INA3D		R	pUC	Amp	P _{LtetO-1} -CI	RC3	p15A	Cm
	INA4	P _{INA4} -EGFP	R	pUC	Amp	P _{LtetO-1} -CI	R	p15A	Cm
	INA5A	P _{INA5} -EGFP	R	ColE1	Amp	P _{LtetO-1} -CI	RC1	p15A	Cm
	INA5B		R	ColE1	Amp	P _{LtetO-1} -CI	RC2	p15A	Cm
	INA5C		R	ColE1	Amp	P _{LtetO-1} -CI	RC3	p15A	Cm
	INA6A	P _{INA6} -EGFP	R	ColE1	Amp	P _{LtetO-1} -CI	RC1	p15A	Cm
	INA6B		R	ColE1	Amp	P _{LtetO-1} -CI	RC2	p15A	Cm
	INA6C		R	ColE1	Amp	P _{LtetO-1} -CI	RC3	p15A	Cm
	INA7A	P _{INA7} -EGFP	R	ColE1	Amp	P _{LtetO-1} -CI	RC1	p15A	Cm
	INA7B		R	ColE1	Amp	P _{LtetO-1} -CI	RC2	p15A	Cm
	INA7C		R	ColE1	Amp	P _{LtetO-1} -CI	RC3	p15A	Cm
	INA7A.A	P _{INA7} -mTFP1	R	ColE1	Amp	P _{LtetO-1} -CI	RC1	p15A	Cm
BNeu 3	AAH1	P _{AAH} -EGFP	RBS H	p15A	Cm	BBa_J23102-LuxR	R	ColE1	Amp
	AAH2	P _{AAH} -E2-Crimson	RBS H	p15A	Cm	BBa_J23102-LuxR	R	ColE1	Amp
BNeu 4	IAH1	P _{IAH} -EGFP	R	p15A	Cm	BBa_J23102-LuxR	R	ColE1	Amp
	IAH2	P _{IAH} -tdTomato	R	p15A	Cm	BBa_J23102-LuxR	R	ColE1	Amp
BNeu 5	N1	P _R -EGFP	R	p15A	Cm	P _{LlacO-1} . Frame-shifted CI* and P _{LtetO-1} . Frame- shifted CI*	R	ColE1	Amp
	N2	P _R -mKO2	R	p15A	Cm	P _{LlacO-1} . Frame-shifted CI* and P _{LtetO-1} . Frame- shifted CI*	R	ColE1	Amp
	ANI1A	P _{ANI1} -EGFP	R	ColE1	Amp	P _{LlacO-1} -CI	R	p15A	Cm
	ANI1B		R	ColE1	Amp	P _{LlacO-1} -CI	RC1	p15A	Cm
	ANI1C		R	ColE1	Amp	P _{LlacO-1} -CI	RC2	p15A	Cm
	ANI1D		R	ColE1	Amp	P _{LlacO-1} -CI	RC3	p15A	Cm
	ANI2A	P _{ANI2} -EGFP	R	ColE1	Amp	P _{LlacO-1} -CI	R	p15A	Cm
	ANI2B		R	ColE1	Amp	P _{LlacO-1} -CI	RC1	p15A	Cm

BNeu 6	ANI2C		R	ColE1	Amp	P _{LlacO-1} -CI	RC2	p15A	Cm
	ANI2D		R	ColE1	Amp	P _{LlacO-1} -CI	RC3	p15A	Cm
	ANI3A	P _{ANI3} -EGFP	R	ColE1	Amp	P _{LlacO-1} -CI	R	p15A	Cm
	ANI3B		R	ColE1	Amp	P _{LlacO-1} -CI	RC1	p15A	Cm
	ANI3C		R	ColE1	Amp	P _{LlacO-1} -CI	RC2	p15A	Cm
	ANI3D		R	ColE1	Amp	P _{LlacO-1} -CI	RC3	p15A	Cm
	ANI4A	P _{ANI4} EGFP	R	ColE1	Amp	P _{LlacO-1} -CI	R	p15A	Cm
	ANI4B		R	ColE1	Amp	P _{LlacO-1} -CI	RC1	p15A	Cm
	ANI4C		R	ColE1	Amp	P _{LlacO-1} -CI	RC2	p15A	Cm
	ANI4D		R	ColE1	Amp	P _{LlacO-1} -CI	RC3	p15A	Cm
	ANI2C.A	P _{ANI2} -E2-Crimson	R	ColE1	Amp	P _{LlacO-1} -CI	RC2	p15A	Cm
BNeu 7	AHLB1	P _{Lux} -EGFP	R	p15A	Cm	BBa J23102-LuxR	R	ColE1	Amp
	AHLB2	P _{Lux} -E2-Crimson	R	p15A	Cm	BBa J23102-LuxR	R	ColE1	Amp
BNeu 8	ATCB1	P _{LtetO-1} -E2-EGFP	R	ColE1	Amp	-	-	-	-
	ATCB2	P _{LtetO-1} -E2-Crimson	R	ColE1	Amp	-	-	-	-
BNeu 9	ANH1	P _{ANH} -EGFP	R	ColE1	Amp	BBa J23102-LuxR	R	ColE1	Amp
						P _{Lux} -Frame-shifted CI	R	p15A	Cm
	ANH2	P _{ANH} -tdTomato	R	ColE1	Amp	BBa J23102-LuxR	R	ColE1	Amp
						P _{Lux} -Frame-shifted CI	R	p15A	Cm
BNeu 10	INH1	P _{INH} -EGFP	R	ColE1	Amp	BBa J23102-LuxR	R	ColE1	Amp
						P _{Lux} -Frame-shifted CI	R	p15A	Cm
	INH2	P _{INH} -E2-Crimson	R	ColE1	Amp	BBa J23102-LuxR	R	ColE1	Amp
						P _{Lux} -Frame-shifted CI	R	p15A	Cm

*Frame-shifted CI is a mutant form of λ repressor CI [Supplementary reference 1].

Table S3: List of Promoters, primers, oligos and RBSs. *lacO1*, *tetO2*, Lux box, *OR1* and *OR2* operator sites are colored in red, brown, green, yellow and blue respectively. Transcription start site is shown in bold. -10 and -35 hexamers are underlined. Each promoter is flanked by *XhoI* and *EcoRI* restriction sites (marked in italics).

Name	Sequence (5' – 3')	Purpose	Source
P _{IAA1}	<i>CTCGAGTCCCTATCAGTGATAGAGATTGAC</i> <i>ATTGTGAGCGGATAACAAGATACTGAGCAC</i> <i>AATTGTGAGCGGATAACAATGAATTC</i>	Construction and weight & bias adjustment of BNeu 1	This study
P _{IAA2}	<i>CTCGAGTCCCTATCAGTGATAGAGATTTC</i> <i>CCTATCAGTGATAGAGATTGACATTGTGAG</i> <i>CGGATAACAAGATACTGAGCACAAATTGTGA</i> <i>GCGGATAACAATGAATTC</i>		This study
P _{IAA3}	<i>CTCGAGAATTGTGAGCGGATAACAATTGAC</i> <i>ATCCCTATCAGTGATAGAGATACTGAGCAC</i> <i>ATCCCTATCAGTGATAGAGAGAATTC</i>		This study
P _{IAA4}	<i>CTCGAGTCCCTATCAGTGATAGAGATTGAC</i> <i>ATTGTGAGCGGATAACAAGATACTGAGCAC</i> <i>ATCCCTATCAGTGATAGAGAGAATTC</i>		This study
P _{IAA5}	<i>CTCGAGTCCCTATCAGTGATAGAGATTGAC</i> <i>ATCCCTATCAGTGATAGAGATACTGAGCAC</i> <i>AATTGTGAGCGGATAACAATGAATTC</i>		This study
P _{IAA6}	<i>CTCGAGTCCCTATCAGTGATAGAGATTGAC</i> <i>ATTGTGAGCGGATAACAAGATACTGAGCAC</i> <i>AATTGTGAGCGGATAACAATGATTCCCTAT</i> <i>CAGTGATAGAGAGAATTC</i>		This study
P _{IAA7}	<i>CTCGAGTCCCTATCAGTGATAGAGATTGAC</i> <i>ATTGTGAGCGGATAACAAGATACTGAGCAC</i> <i>ATCCCTATCAGTGATAGAGAGATAATTGTG</i> <i>AGCGGATAACAATGAATTC</i>		This study
P _{IAA8}	<i>CTCGAGTCCCTATCAGTGATAGAGATTGAC</i> <i>ATTGTGAGCGGATAACAAGATACTGAGCAC</i>		[Supplementary

	ATCCCTATCAGTGATAGAGAGATAAATTGTG AGCGGATAACAATTGATAAATTGTGAGCGGA TAACAATTGAATTC		reference 1]
P _{IAA9}	CTCGAGTCCCTATCAGTGATAGAGATTGAC ATTGTGAGCGGATAACAAGATACTGAGCAC ATCCCTATCAGTGATAGAGAGATAAATTGTG AGCGGATAACAATTGATTCCCTATCAGTGA TAGAGAGAATTC		This study
P _{IAA10}	CTCGAGTCCCTATCAGTGATAGAGATTGAC ATTGTGAGCGGATAACAAGATACTGAGCAC ATCCCTATCAGTGATAGAGAGATGATAAATT GTGAGCGGATAACAATTGATGATTCCCTAT CAGTGATAGAGAGATGATAAATTGTGAGCGG ATAACAATTGAATTC		This study
P _{IAA11}	CTCGAGTCCCTATCAGTGATAGAGATTGAC ATTGTGAGCGGATAACAAGATACTGAGCAC ATCCCTATCAGTGATAGAGAGATGATAAATT GTGAGCGGATAACAATTGATGATTCCCTAT CAGTGATAGAGAGATGATAAATTGTGAGCGG ATAACAATTGATGATTCCCTATCAGTGATA GAGAGATGATAAATTGTGAGCGGATAACAAT TGAATTC		This study
P _{INA1}	CCTCGAGTACCTCTGGCGGTGATATTGACAT TGTGAGCGGATAACAAGATACTGAGCACAA TTGTGAGCGGATAACAATTGAATTC		This study
P _{INA2}	CTCGAGTACCTCTGGCGGTGATAGATTACCT CTGGCGGTGATATTGACATTGTGAGCGGAT AACAAGATACTGAGCACAAATTGTGAGCGGA TAACAATTGAATTC		This study
P _{INA3}	CTCGAGTAACACCGTGCGTGTGACTATTTT ACCTCTGGCGGTGATAATGGTTGCAATTGT GAGCGGATAACAATTGAATTC		This study
P _{INA4}	CTCGAGTAACACCGTGCGTGTGACTATTTT ACCTCTGGCGGTGATAATGGTTGCAATTGT GAGCGGATAACAATTGATAAATTGTGAGCGGA TAACAATTGAATTC	Construction and weight & bias adjustment of BNeu 2	This study
P _{INA5}	CTCGAGAAATTGTGAGCGGATAACAATTGAC ATTGTGAGCGGATAACAAGATACTGAGCAC ATCTACCTCTGGCGGTGATAGAATTC		This study
P _{INA6}	CTCGAGAAATTGTGAGCGGATAACAATTGAC ATTGTGAGCGGATAACAAGATACTGAGCAC ATCTACCTCTGGCGGTGATAGAATTC		This study
P _{INA7}	CTCGAGAAATTGTGAGCGGATAACAATTGAC ATTGTGAGCGGATAACAAGATACTGAGCAC ATCTACCTCTGGCGGTGATAGAATTC		This study
P _{ANI1}	CTCGAGTACCTCTGGCGGTGATATTGACATC CCTATCAGTGATAGAGATACTGAGCACATC CCTATCAGTGATAGAGAGAATTC	Construction and weight & bias adjustment of BNeu 6	This study
P _{ANI2}	CCTCGAGTACCTCTGGCGGTGATAGATTACC TCTGGCGGTGATATTGACATCCCTATCAGT GATAGAGATACTGAGCACATCCCTATCAGT GATAGAGAGAATTC		This study
P _{ANI3}	CTCGAGTACCTCTGGCGGTGATATTGACATC CCTATCAGTGATAGAGATACTGAGCACATC TACCTCTGGCGGTGATAGAATTC		This study
P _{ANI4}	CTCGAGTCCCTATCAGTGATAGAGATTGAC TACCTCTGGCGGTGATAGATACTGAGCAC ATCCCTATCAGTGATAGAGAGAATTC		This study

P_{AAH}	<i>CTCGAGACCTGTAGGATCGTACAGGTTTAC GTCCCTATCAGTGATAGAGTATAGTCGAAT AAATCCCTATCAGTGATAGAGAGAATTC</i>	Construction of BNeu 3	This study
P_{IAH}	<i>CTCGAGACCTGTAGGATCGTACAGGTTTAC GTTTGTGAGCGGATAACAATATAGTCGAAT AAATTGTGAGCGGATAACAATTGAATTC</i>	Construction of BNeu 4	This study
P_R	<i>CTCGAGTAACACCGTGCGTGTGACTATTTT ACCTCTGGCGGTGATAATGGTTGCATGTAC GAATTC</i>	Construction of BNeu 5	[Supplementary reference 1]
BBa_J23102	<i>CTCGAGTTGACAGCTAGCTCAGTCCTAGGT ACTGTGCTAGCGAATTC</i>	Construction of BNeus 3, 4, 7, 9 and 10	[Supplementary reference 2]
P_{Lux}	<i>CTCGAGACCTGTAGGATCGTACAGGTTTAC GCAAGAAAATGGTTTGTATAGTCGAATAA AGAATTC</i>	Construction of BNeus 9 and 10	This study
P_{Lux}*	<i>CTCGAGACCTGTAGGATCGTACAGGTTTAC GCAAGAAAATGGTTTGTACTTTTGAATAA AGAATTC</i>	Construction of BNeu 7	[20]
P_{LietO-1}	<i>CTCGAGTCCCTATCAGTGATAGAGATTGAC ATCCCTATCAGTGATAGAGATACTGAGCAC ATCAGCAGGACGCACTGACCGAATTC</i>	Construction of BNeus 5 and 8	[31]
P_{LacO-1}	<i>CTCGAGAAATTGTGAGCGGATAACAATTGAC ATTGTGAGCGGATAACAAGATACTGAGCAC ATCAGCAGGACGCACTGACCGAATTC</i>	Construction of BNeu 5	[31]
Primer 1	GCCCTTCGTCCTTCACCTC	Amplification of promoters P _{IAA} 1, P _{IAA} 2, P _{INA} 1, P _{INA} 2, P _{ANI} 1, P _{ANI} 2, P _{ANI} 3 and P _{ANI} 4: forward primer	This study
Primer 2	ATGTTTTTGGCGTCTTCCAT	Amplification of promoters P _{IAA} 1, P _{IAA} 2, P _{INA} 1, P _{INA} 2, P _{ANI} 1, P _{ANI} 2, P _{ANI} 3 and P _{ANI} 4: reverse primer	This study
Primer 3	CGAGGCCCTTCGTCCTTCACCTCGAGAATTG TGAGCGGATAACAATTGACATCCCTATCAG TGATAGAGATACTGAGCACA	Amplification of promoter P _{IAA} 3: forward primer	This study
Primer 4	ATGTTTTTGGCGTCTTCCATGGTACCTTTCT CCTCTTAATGAATTCTCTCTATCACTGATA GGGATGTGCTCAGTATCTCTATCA	Amplification of promoter P _{IAA} 3: reverse primer	This study
Primer 5	CGAGGCCCTTCGTCCTTCACCTCGAGTCCCT ATCAGTGATAGAGATTGACATTGTGAGCGG ATAACAAGATACTGAGCACATC	Amplification of promoter P _{IAA} 4: forward primer	This study
Primer 6	ATGTTTTTGGCGTCTTCCATGGTACCTTTCT CCTCTTAATGAATTCTCTCTATCACTGATA GGGATGTGCTCAGTATCTTGT	Amplification of promoter P _{IAA} 4: reverse primer	This study
Primer 7	CAATTCTTTATGCCGGTGTTG	Amplification of promoters P _{IAA} 5 and P _{IAA} 10: forward primer	This study
Primer 8	GTCGAAGATGTTGGGGTGTT	Amplification of promoters P _{IAA} 5 and P _{IAA} 10: reverse primer	This study
Primer 9	CAGAATCGTCGTATGCAGTGA	Amplification of promoters P _{IAA} 6 and P _{IAA} 11: forward primer	This study
Primer 10	TTTTCCGTCATCGTCTTTCC	Amplification of promoters P _{IAA} 6 and P _{IAA} 11: reverse primer	This study
Primer 11	TTGGCAGAAGCTATGAAACGA	Amplification of promoters P _{IAA} 7, P _{INA} 5 and P _{AAH} : forward primer	This study
Primer 12	CTTGACTGGCGACGTAATCC	Amplification of promoters P _{IAA} 7, P _{INA} 5 and P _{AAH} : reverse primer	This study
Primer 13	GCCCTTCGTCCTTCACCTC	Amplification of promoters P _{IAA} 8 and P _{IAA} 9: forward primer	This study
Primer 14	CTTGACTGGAATTCAATTGTTATCCGCTCAC AATTATCAATGTTATCCGCTCACAATTATC TCTCTATCACTGATAGGGATGTGCTCAG	Amplification of promoter P _{IAA} 8: reverse primer	This study
Primer 15	CTTGACTGGAATTCTCTCTATCACTGATAGG GAATCAATTGTTATCCGCTCACAATTATCTC	Amplification of promoter P _{IAA} 9: reverse primer	This study

	TCTATCACTGATAGGGATGTGCTCAG		
Primer 16	CAGATGCACATATCGAGGTGA	Amplification of promoters P _{INA3} and P _{INA6} : forward primer	This study
Primer 17	GCAACTTTTGGCGGTTG	Amplification of promoters P _{INA3} and P _{INA6} : reverse primer	This study
Primer 20	TGAAGAGATACGCCCTGGTT	Amplification of promoter P _{INA4} , P _{INA7} and P _{IAH} : forward primer	This study
Primer 21	TCTGATTTTCTGCGTCGAG	Amplification of promoter P _{INA4} , P _{INA7} and P _{IAH} : reverse primer	This study
Primer 22	ATCCGTGCAACTCGAGTTGACAGCTAGCTCAGTCCTAGGTAC	Amplification of promoter BBa_J23102: forward primer	This study
Primer 23	GTTCAAGACTGAATTCGCTAGCACAGTACC TAGGACTGAGCTAGC	Amplification of promoter BBa_J23102: reverse primer	This study
Primer 24	CTTCACTCGACTCGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGG	Amplification of promoters P _{Lux} and P _{Lux*} : forward primer	This study
Primer 25	CTGATTATGTGAATTCCTTATTGAAAAGTAA CAAACCATTCTTCTGCGTAAACCTG	Amplification of promoter P _{Lux*} : reverse primer	This study
Primer 26	GAGACCACAATGGGCGTAAT	Amplification of fluorescent protein mTFP1: forward primer (1 st round)	This study
Primer 27	CGTAAACGGTCACCTTGTGTGA	Amplification of fluorescent protein mTFP1: reverse primer (1 st round)	This study
Primer 28	GTCCAGTCGAGGTACCATGGTGAGCAAGGG CGAGGAGACCACAATGGGCGTAAT	Amplification of fluorescent protein mTFP1: forward primer (2 nd round)	This study
Primer 29	GCTTATGCTCTAGATTACTTGTACAGCTCGT CCATGCCGTCGGTGGAGTTGCGGGCCACGG CGCTCTCGTAAACGGTCACCTTGTGTGA	Amplification of fluorescent protein mTFP1: reverse primer (2 nd round)	This study
Primer 30	CAAGGGCGAGGAGCTGTT	Amplification of fluorescent protein EGFP and mVenus: forward primer (1 st round)	This study
Primer 31	CCATGCCGAGAGTGATCC	Amplification of fluorescent protein EGFP and mVenus: reverse primer (1 st round)	This study
Primer 32	CTTCAGTCGAGGTACCATGGTGAGCAAGGG CGAGGAGCTGTT	Amplification of fluorescent protein EGFP and mVenus: forward primer (2 nd round)	This study
Primer 33	CTGATTATGATCTAGATTACTTGTACAGCTC GTCCATGCCGAGAGTGATCC	Amplification of fluorescent protein EGFP and mVenus: reverse primer (2 nd round)	This study
Primer 34	TGGTGAGTGTGATTAAACCAGAGA	Amplification of fluorescent protein mKO2: forward primer (1 st round)	This study
Primer 35	AATGTTGCCTTCGGTTTTCC	Amplification of fluorescent protein mKO2: reverse primer (1 st round)	This study
Primer 36	GTCCAGTCGAGGTACCATGGTGAGTGTGAT TAAACCAGAGA	Amplification of fluorescent protein mKO2: forward primer (2 nd round)	This study
Primer 37	GTGATTATGATCTAGATTAGCTATGAGCTA CTGCATCTTCTACCTGCTCAGTAATGTTGCC TTCGTTTTCC	Amplification of fluorescent protein mKO2: reverse primer (2 nd round)	This study
Primer 38	TGGATAGCACTGAGAACGTCAT	Amplification of fluorescent protein E2-Crimson: forward primer (1 st round)	This study
Primer 39	ACCACGGTGTAGTCCTCGTT	Amplification of fluorescent protein E2-Crimson: reverse primer (1 st round)	This study
Primer 40	GTCCAGTCGAGGTACCATGGATAGCACTGA GAACGTCAT	Amplification of fluorescent protein E2-Crimson: forward primer (2 nd round)	This study
Primer 41	GATTATGATCTAGActaCTGGAACAGGTGGT GGCGGGCCTCGGCGCTCGTACTGCTCCA CCACGGTGTAGTCCTCGTT	Amplification of fluorescent protein E2-Crimson: reverse primer (2 nd round)	This study
Primer 42	ATGCCGACGACACATACAGA	Amplification of LuxR gene: forward primer (1 st round)	This study
Primer 43	TGATGCCTGGCTCTAGTAGTGA	Amplification of LuxR gene: reverse primer (1 st round)	This study

Primer 44	CTCCGTGGAAGGTACCATGAAAAACATAAA TGCCGACGACACATACAGA	Amplification of LuxR gene: forward primer (2 nd round)	This study
Primer 45	GTTCAAGACTTCTAGATGATGCCTGGCTCT AGTAGTGA	Amplification of LuxR gene: reverse primer (2 nd round)	This study
Primer 46	CGAAAAGTGCCACCTGAC	Amplification of gene cassette starting with promoters P _{LtetO-1} , P _{IAA1-2} , P _{IAA4-11} and P _{ANI4} : forward sequencing primer	This study
Primer 47	GTCTGATTGAGAATTCATTTTGGAGGAGTTC GGTACCATGGTGAGCAAGGGCGAGGAGCT GTT	Incorporation of RC1 upstream of EGFP gene: forward primer	This study
Primer 48	GTCTGATTGAGAATTCATTCGGGAGGAGTG CGGTACCATGGTGAGCAAGGGCGAGGAGC TGTT	Incorporation of RC2 upstream of EGFP gene: forward primer	This study
Primer 49	GTCTGATTGAGAATTCATTCGGGAGGAGTG CGGTACCATGGTGAGCAAGGGCGAGGAGC TGTT	Incorporation of RC3 upstream of EGFP gene: forward primer	This study
Primer 50	CTGATTATGTGAATTCTTTATTGACTATAACAA ACCATTTTCTTGGCTAAACCTG	Amplification of promoter P _{Lux} : reverse primer	This study
Oligo 1	AATTCATTGGAGAGGAGTCCGGTAC	RBSH: sense strand oligomer for annealing	This study
Oligo 2	CGGACTCCTCTCCAATG	RBSH: antisense strand oligomer for annealing	This study

Table S4: Weights and biases of each cellular device (construct) used for optimizing and improving corresponding unit bactoneuron (BNeu j).

Unit bactoneuron	Cellular device	w _{JR}	w _{JL}	w _{JA}	w _{JH}	b _{JR}	b _{JL}	b _{JA}	b _{JH}	b _j	S.D. of b _j
BNeu 1	IAA1	-	7.75	8.42	-	-	-1.26	-2.15	-	-9.79	0.16
	IAA2	-	10.37	9.05	-	-	-1.68	-0.77	-	-10.94	0.29
	IAA3	-	8.94	15.44	-	-	0.67	-5	-	-14.36	0.59
	IAA4	-	6.19	11.77	-	-	0.18	-5.12	-	-11.45	0.198
	IAA5	-	7.71	12.6	-	-	-1.09	-6.02	-	-13.71	0.03
	IAA6	-	7.2	7.07	-	-	-0.81	-0.64	-	-7.86	0.03
	IAA7A	-	8.5	11.73	-	-	-1.73	-4.52	-	-13.24	0.31
	IAA7B(10h+6h)	-	9.69	12.44	-	-	-2.58	-5.59	-	-15.15	0.18
	IAA8	-	8.36	11.92	-	-	-1.47	-4.8	-	-13.28	0.16
	IAA9	-	7.58	12.96	-	-	-1.15	-6.27	-	-13.98	0.18
	IAA10	-	8.84	15.91	-	-	-1.41	-8.26	-	-17.21	0.16
	IAA11	-	9.94	12.44	-	-	-1.71	-3.55	-	-13.82	0.47
BNeu 2	IAA7B.A	-	9.69	12.44	-	-	-2.58	-5.59	-	-15.15	0.18
	IAA7B.B	-	9.69	12.44	-	-	-2.58	-5.59	-	-15.15	0.18
	INA6A	-	9.59	-15.32	-	-	-2.49	7	-	-2.54	0.07
	INA6B	-	9.11	-14.87	-	-	-2.12	7	-	-2.12	0.007
	INA6C	-	9.12	-15.48	-	-	-1.71	7.5	-	-1.67	0.07
	INA7A	-	10.98	-14.57	-	-	-2.68	7.8	-	-2.93	0.35
	INA7A (10h+6h)	-	10.8	-14.89	-	-	-3.3	7.14	-	-3.48	0.25
BNeu 3	INA7B	-	9.51	-14.89	-	-	-2.12	7.5	-	-2.07	0.08
	INA7A.A	-	10.8	-14.89	-	-	-3.3	7.14	-	-3.48	0.25
BNeu 4	AAH1(10h+6h)	-	0	13.16	10.93	-	-	-4.52	-2.42	-15.52	0.09
	AAH2	-	0	13.16	10.93	-	-	-4.52	-2.42	-15.52	0.09
BNeu 5	IAH1(10h+6h)	-	10.78	0	11.98	-	-2.12	-	-3.17	-14.03	0.11
	IAH2	-	10.78	0	11.98	-	-2.12	-	-3.17	-14.03	0.11
BNeu 6	N1(10h+6h)	-	-10.03	-11.65	-	-	3.52	3.72	-	3.62	0.14
	N2	-	-10.03	-11.65	-	-	3.52	3.72	-	3.62	0.14
BNeu 7	ANI2C	-	-15.58	12.59	-	-	7.5	-4.3	-	-4.7	0.56
	ANI2C (10h+6h)	-	-16.25	13.2	-	-	7.5	-4.64	-	-5.17	0.75

	ANI2C.A	-	-16.25	13.2	-	-	7.5	-4.64	-	-5.17	0.75
BNeu 7	AHLB1(10h+6h)	0	0	0	10.84	-	-	-	-3.21	-3.21	-
	AHLB2	0	0	0	10.84	-	-	-	-3.21	-3.21	-
BNeu 8	ATCB1(10h+6h)	0	0	12.17	0	-	-	-4.85	-	-4.85	-
	ATCB2	0	0	12.17	0	-	-	-4.85	-	-4.85	-
BNeu 9	ANH1(10h+6h)	-	0	10.00	-12.94	-	-	-3.2	5.8	-3.7	0.71
	ANH2	-	0	10.00	-12.94	-	-	-3.2	5.8	-3.7	0.71
BNeu 10	INH1(10h+6h)	-	9.42	0	-13.75	-	-1.83	-	7.00	-2.13	0.42
	INH2	-	9.42	0	-13.75	-	-1.83	-	7.00	-2.13	0.42

Table S5: Leakage of each EGFP-expressing cellular device (construct) during weight and bias optimization of unit bactoneurons.

Unit bactoneuron	Cellular device	Promoter expressing Output EGFP	Total leakage (ΣL)	Highest leakage (L_{max})	Percentage total leakage ($L_{max}(\%)$)	Difference between total leakage and highest leakage ($\Sigma L - L_{max}$)	Percentage difference between total leakage and highest leakage ($\Sigma L - L_{max}(\%)$)	Fold Change between highest signal and highest leakage
BNeu 1	IAA1	P_{IAA1}	0.44075	0.27818	27.82	0.16257	16.26	3.59
	IAA2	P_{IAA2}	0.38592	0.26795	26.8	0.11797	11.8	3.73
	IAA3	P_{IAA3}	0.26577	0.23858	23.86	0.02719	2.72	4.19
	IAA4	P_{IAA4}	0.2465	0.23556	23.56	0.01094	1.09	4.25
	IAA5	P_{IAA5}	0.22367	0.17495	17.5	0.04872	4.87	5.72
	IAA6	P_{IAA6}	0.42029	0.26436	26.44	0.15593	15.59	3.78
	IAA7A	P_{IAA7}	0.12599	0.11025	11.03	0.01574	1.57	9.07
	IAA7B		0.0724	0.06992	6.99	0.00248	0.25	14.3
	IAA8	P_{IAA8}	0.11287	0.09674	9.67	0.01613	1.61	10.33
	IAA9	P_{IAA9}	0.15845	0.13822	13.82	0.02023	2.02	7.23
	IAA10	P_{IAA10}	0.23014	0.21021	21.02	0.01993	1.99	4.76
BNeu 2	IAA11	P_{IAA11}	0.52619	0.49162	49.16	0.03457	3.46	2.03
	INA1A	P_{INA1}	0.63673	0.28273	28.27	0.354	35.4	3.54
	INA1B		0.53862	0.36044	36.04	0.17819	17.82	2.77
	INA1C		0.74802	0.45623	45.62	0.29179	29.18	2.19
	INA1D		0.80989	0.45047	45.05	0.35942	35.94	2.22
	INA2A	P_{INA2}	0.72654	0.37157	37.16	0.35497	35.5	2.69
	INA2B		0.63688	0.53902	53.9	0.09787	9.79	1.86
	INA2C		0.79001	0.51294	51.29	0.27707	27.71	1.95
	INA2D		0.85405	0.4802	48.02	0.37385	37.39	2.08
	INA3A	P_{INA3}	0.77393	0.38146	38.15	0.39247	39.25	2.62
	INA3B		0.72405	0.70337	70.34	0.02067	2.07	1.42
	INA3C		0.89997	0.80164	80.16	0.09832	9.83	1.25
	INA3D		2.13488	0.8703	87.03	1.26457	126.46	1.15
	INA4	P_{INA4}	1.23277	0.80884	80.88	0.42393	42.39	1.24
	INA5A	P_{INA5}	0.40655	0.23903	23.9	0.16752	16.75	4.18
	INA5B		0.35622	0.23112	23.11	0.1251	12.51	4.33
	INA5C		0.56242	0.38104	38.1	0.18138	18.14	2.62
	INA6A	P_{INA6}	0.10504	0.06611	6.61	0.03893	3.89	15.13
	INA6B		0.0933	0.06478	6.48	0.02852	2.85	15.44
	INA6C		0.16935	0.08823	8.82	0.08112	8.11	11.33
	INA7A	P_{INA7}	0.07783	0.04258	4.26	0.03525	3.53	23.49
	INA7B		0.09163	0.04595	4.6	0.04567	4.57	21.76
	INA7C		0.40418	0.34627	34.63	0.05791	5.79	2.89

BNeu 3	AAH1	P _{AAH}	0.05393	0.0502	5.02	0.00373	0.37	19.92
BNeu 4	IAH1	P _{IAH}	0.1402	0.08861	8.86	0.05159	5.16	11.29
BNeu 5	N1	P _R	~0.00000	~0.00000	~00.00	~0.00000	~00.00	-
BNeu 6	ANI1A	P _{ANI1}	0.90311	0.74394	74.39	0.15917	15.92	1.34
	ANI1B		0.20376	0.17735	17.73	0.02642	2.64	5.64
	ANI1C		0.2262	0.20363	20.36	0.02256	2.26	4.91
	ANI1D		0.63651	0.62022	62.02	0.01629	1.63	1.61
	ANI2A	P _{ANI2}	0.60315	0.40034	40.03	0.20281	20.28	2.5
	ANI2B		0.22933	0.17476	17.48	0.05457	5.46	5.72
	ANI2C		0.15726	0.10908	10.91	0.04818	4.82	9.16
	ANI2D		0.26405	0.23224	23.22	0.03181	3.18	4.31
	ANI3A	P _{ANI3}	3.1181	2.43408	243.41	0.68398	68.4	0.41
	ANI3B		0.51524	0.3359	33.59	0.17933	17.93	2.98
	ANI3C		0.44074	0.22444	22.44	0.21629	21.63	4.46
	ANI3D		0.40381	0.30815	30.82	0.09566	9.57	3.25
	ANI4A	P _{ANI4}	0.7652	0.47934	47.93	0.28586	28.59	2.09
	ANI4B		0.18267	0.16603	16.6	0.01664	1.66	6.02
	ANI4C		0.25445	0.23556	23.56	0.0189	1.89	4.25
	ANI4D		0.60619	0.59089	59.09	0.0153	1.53	1.69
BNeu 7	AHLB1	P _{Lux} *	0.0338	0.0338	3.38	-	-	29.59
BNeu 8	ATCB1	P _{LietO-1}	0.00778	0.00778	0.78	-	-	128.53
BNeu 9	ANH1	P _{ANI2}	0.13249	0.08548	8.55	0.04701	4.7	11.7
BNeu 10	INH1	P _{INA7}	0.17488	0.09012	9.01	0.08476	8.48	11.1

Table S6: Translation initiation rate calculated from RBS calculator [Supplementary reference 3].

Name of RBS	Operating Promoter	Protein of Translational regulation	Translation initiation rate (a.u.)	Sequence (5' – 3')
R(BBa_B0034)[Supplementary reference 4]	P _{LacO-1} /P _{LietO-1}	CI	40767	GAATTCATTAAGAGGAGAAAGGTACC
RC1	P _{LacO-1} /P _{LietO-1}	CI	2091	GAATTCATTTTGGAGGAGTTCGGTACC
RC2	P _{LacO-1} /P _{LietO-1}	CI	518	GAATTCATTCGGGAGGAGTGC
RC3	P _{LacO-1} /P _{LietO-1}	CI	396	GAATTCATTCGGGAGGAGTGC
R(BBa_B0034)[Supplementary reference 4]	P _{AAH}	EGFP	46	GAATTCATTAAGAGGAGAAAGGTACC
RH	P _{AAH}	EGFP	10	GAATTCATTGGAGAGGAGTCCGGTACC

Table S7: List of bacterial strains and plasmids used in this study.

Plasmid name	Description	Ori	Antibiotic selection	Source
E. coli DH5α	-	-	-	Prof. David McMillen
E. coli DH5αZ1	-	-	-	Prof. David McMillen
pOR-EGFP-12	Source of EGFP gene and ColE1 Ori	ColE1	Amp	Prof. David McMillen
pOR-Luc-31	Source of p15A Ori	p15A	Cm	Prof. David McMillen
pmVenus-C1	Source of mVenus gene	pUC	Kan	Clontech
mTFP1-pBad	Source of mTFP1 gene	pBR322	Amp	Addgene
(Plasmid#54553)				
pUCP20T-E2Crimson	Source of E2-Crimson gene	pBR322	Amp	Addgene
(Plasmid#78473)				
mKO2-pBAD	Source of mKO2 gene	-	Amp	Addgene
(Plasmid#54555)				
pBW313lux-hrpR	Source of LuxR gene	p15A	Kan	Addgene
(Plasmid#61436)				

pXC3EGFP	EGFP gene with RBS R under P _{LUX} promoter: source of P _{LUX} promoter	p15A	Cm	This study
pTA1EGFP	EGFP gene with RBS R under P _{LtetO-1} promoter	pUC	Amp	[Supplementary reference 1]
pTA2EGFP	EGFP gene with RBS R under P _{LtetO-1} promoter	ColE1	Amp	[Supplementary reference 1]
pTA2E2-Crimson	E2-Crimson gene with RBS R under P _{LtetO-1} promoter	ColE1	Amp	This study
pRC3EGFP	EGFP gene with RBS R under P _R promoter	p15A	Cm	[Supplementary reference 1]
pRC3MKO2	mKO2 gene with RBS R under P _R promoter	p15A	Cm	This study
pTA2cl	Source of wild type CI gene	ColE1	Amp	[Supplementary reference 1]
pRA1SEGFPTclfm	Source of frame-shifted CI gene	pUC	Amp	[Supplementary reference 1]
pLA2ScIfmTclfm	Frame-shifted CI gene with RBS R under both P _{LlacO-1} promoter and P _{LtetO-1} promoter	ColE1	Amp	[Supplementary reference 1]
pP _{IAA} 1A1EGFP	EGFP gene with RBS R under P _{IAA} 1promoter	pUC	Amp	This study
pP _{IAA} 2A1EGFP	EGFP gene with RBS R under P _{IAA} 2promoter	pUC	Amp	This study
pP _{IAA} 3A1EGFP	EGFP gene with RBS R under P _{IAA} 3promoter	pUC	Amp	This study
pP _{IAA} 4A1EGFP	EGFP gene with RBS R under P _{IAA} 4promoter	pUC	Amp	This study
pP _{IAA} 5A1EGFP	EGFP gene with RBS R under P _{IAA} 5promoter	pUC	Amp	This study
pP _{IAA} 6A1EGFP	EGFP gene with RBS R under P _{IAA} 6promoter	pUC	Amp	This study
pP _{IAA} 7A1EGFP	EGFP gene with RBS R under P _{IAA} 7promoter	pUC	Amp	This study
pP _{IAA} 7C3EGFP	EGFP gene with RBS R under P _{IAA} 7promoter	p15A	Cm	This study
pP _{IAA} 7C3mVenus	mVenus gene with RBS R under P _{IAA} 7promoter	p15A	Cm	This study
pP _{IAA} 7C3E2-Crimson	E2-Crimson gene with RBS R under P _{IAA} 7promoter	p15A	Cm	This study
pP _{IAA} 8A1EGFP	EGFP gene with RBS R under P _{IAA} 8promoter	pUC	Amp	This study
pP _{IAA} 9A1EGFP	EGFP gene with RBS R under P _{IAA} 9promoter	pUC	Amp	This study
pP _{IAA} 10A1EGFP	EGFP gene with RBS R under P _{IAA} 10promoter	pUC	Amp	This study
pP _{IAA} 11A1EGFP	EGFP gene with RBS R under P _{IAA} 11promoter	pUC	Amp	This study
pP _{INA} 1A1EGFP	EGFP gene with RBS R under P _{INA} 1promoter	pUC	Amp	This study
pP _{INA} 2A1EGFP	EGFP gene with RBS R under P _{INA} 2promoter	pUC	Amp	This study
pP _{INA} 3A1EGFP	EGFP gene with RBS R under P _{INA} 3promoter	pUC	Amp	This study
pP _{INA} 4A1EGFP	EGFP gene with RBS R under P _{INA} 4promoter	pUC	Amp	This study
pP _{INA} 5A2EGFP(F)	EGFP gene with RBS R under P _{INA} 5promoter (Forward direction)	ColE1	Amp	This study
pP _{INA} 6A2EGFP(F)	EGFP gene with RBS R under P _{INA} 6promoter (Forward direction)	ColE1	Amp	This study
pP _{INA} 7A2EGFP(F)	EGFP gene with RBS R under P _{INA} 7promoter (Forward direction)	ColE1	Amp	This study
pP _{INA} 7A2mTFP1(F)	mTFP1 gene with RBS R under P _{INA} 7promoter (Forward direction)	ColE1	Amp	This study
pP _{INA} 7A2E2-Crimson(R)	E2-Crimson gene with RBS R under P _{INA} 7promoter (Reverse direction)	ColE1	Amp	This study
pP _{ANI} 1A2EGFP	EGFP gene with RBS R under P _{ANI} 1promoter	ColE1	Amp	This study
pP _{ANI} 2A2EGFP	EGFP gene with RBS R under P _{ANI} 2promoter	ColE1	Amp	This study
pP _{ANI} 2A2E2-Crimson	E2-Crimson gene with RBS R under P _{ANI} 2promoter	ColE1	Amp	This study
pP _{ANI} 2A2tdTomato(F)	tdTomato gene with RBS R under P _{ANI} 2promoter (Forward direction)	ColE1	Amp	This study
pP _{ANI} 3A2EGFP	EGFP gene with RBS R under P _{ANI} 3promoter	ColE1	Amp	This study
pP _{ANI} 4A2EGFP	EGFP gene with RBS R under P _{ANI} 4promoter	ColE1	Amp	This study
pP _{AAH} C3EGFP(R)	EGFP gene with RBS R under P _{AAH} promoter (Reverse direction)	p15A	Cm	This study
pP _{AAH} C3RBSHEGFP(R)	EGFP gene with RBS RH under P _{AAH} promoter (Reverse direction)	p15A	Cm	This study
pP _{IAH} C3EGFP(R)	EGFP gene with RBS R under P _{IAH} promoter	p15A	Cm	This study
pTA2RBSC1EGFP	EGFP gene with RBS RC1 under P _{LtetO-1} promoter	ColE1	Amp	This study
pTA2RBSC2EGFP	EGFP gene with RBS RC2 under P _{LtetO-1} promoter	ColE1	Amp	This study
pTA2RBSC3EGFP	EGFP gene with RBS RC3 under P _{LtetO-1} promoter	ColE1	Amp	This study
pTC3cl	Wild type CI gene with RBS R under P _{LtetO-1} promoter	p15A	Cm	[Supplementary reference 1]
pTC3RBSC1cl	Wild type CI gene with RBS RC1 under P _{LtetO-1} promoter	p15A	Cm	This study
pTC3RBSC2cl	Wild type CI gene with RBS RC2 under P _{LtetO-1} promoter	p15A	Cm	This study

pTC3RBSC3cI	Wild type CI gene with RBS RC3 under P _{LtetO-1} promoter	p15A	Cm	This study
pLC3cI	Wild type CI gene with RBS R under P _{LlacO-1} promoter	p15A	Cm	This study
pLC3RBSC1cI	Wild type CI gene with RBS RC1 under P _{LlacO-1} promoter	p15A	Cm	This study
pLC3RBSC2cI	Wild type CI gene with RBS RC2 under P _{LlacO-1} promoter	p15A	Cm	This study
pLC3RBSC3cI	Wild type CI gene with RBS RC3 under P _{LlacO-1} promoter	p15A	Cm	This study
pTA2LuxR	LuxR gene with RBS R under P _{LtetO-1} promoter	ColE1	Amp	[Supplementary reference 2]
pJA2LuxR(F)	LuxR gene with RBS R under BBa_J23102 promoter (Forward direction)	ColE1	Amp	This study
pJA2LuxR(R)	LuxR gene with RBS R under BBa_J23102 promoter (Reverse direction)	ColE1	Amp	This study
pJA2LuxR(F)P _{INA7} E2-Crimson(R)	LuxR gene with RBS R under BBa_J23102 promoter (Forward direction) with E2-Crimson gene with RBS R under P _{INA7} promoter (Reverse direction)	ColE1	Amp	This study
pP _{ANI2} A2tdTomato(F)JLuxR(R)	tdTomato gene with RBS R under P _{ANI2} promoter (Forward direction) with LuxR gene with RBS R under BBa_J23102 promoter (Reverse direction)	ColE1	Amp	This study
pXC3cIfm	Frame-shifted CI gene with RBS R under P _{LUX} promoter	p15A	Cm	This study
pX ⁺ C3EGFP	EGFP gene with RBS R under P _{LUX} promoter	p15A	Cm	This study
pX ⁺ C3E2-Crimson	E2-Crimson gene with RBS R under P _{LUX} promoter	p15A	Cm	This study
pA2MCS	Only MCS	ColE1	Amp	This study
pC3MCS	Only MCS	p15A	Cm	This study

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