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Realizing Molecular Machine Learning through Communications for Biological AI: Future Directions and Challenges

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Abstract

Artificial Intelligence (AI) and Machine Learning (ML) are weaving their way into the fabric of society, where they are playing a crucial role in numerous facets of our lives. As we witness the increased deployment of AI and ML in various types of devices, we benefit from their use into energy-efficient algorithms for low powered devices. In this paper, we investigate a scale and medium that is far smaller than conventional devices as we move towards molecular systems that can be utilized to perform machine learning functions, i.e., Molecular Machine Learning (MML). Fundamental to the operation of MML is the transport, processing, and interpretation of information propagated by molecules through chemical reactions. We begin by reviewing the current approaches that have been developed for MML, before we move towards potential new directions that rely on gene regulatory networks inside biological organisms as well as their population interactions to create neural networks. We then investigate mechanisms for training machine learning structures in biological cells based on calcium signaling and demonstrate their application to build an Analog to Digital Converter (ADC). Lastly, we look at potential future directions as well as challenges that this area could solve.

Keywords

Artificial Intelligence; Machine Learning; Molecular Communications; Synthetic Biology

I. Introduction

In recent years we have started to witness the widespread development of systems to apply Artificial Intelligence (AI) and Machine Learning (ML) to very diverse application scenarios [1]. This has resulted in software-based systems for AI, such as Artificial Neural Networks

(ANN) [2] as well as hardware based systems like neuromorphic hardware [3]. In particular, within the area of ANN various algorithms have been developed, that includes Recurrent Neural Networks (RNN), Convolutional Neural Networks (CNN), amongst others, where each has its own properties and behaviour derived from specific functions of neuronal networks of the brain. While developments have been made in AI for both hardware and software, there is still a number of challenges that exists. These challenges include the ability to mimic the behaviour and realism of neurons and their internal functionalities, as well as matching their energy requirements. The former challenge is still today a major issue that continues to motivate research to ensure that new algorithms or hardware designs will resemble the properties of internal neuronal signaling (e.g., ion transfer, action potential generation and propagation). However, the more realistic we design AI algorithms to closely resemble neuronal cells, the higher the energy consumption since we are mimicking the chemical and molecular reactions that occurs internally. When making this comparison, the brain consumes approximately 20W for 100 billion neurons and 1,000 trillion synapses compared to a neuromorphic processor such as the Neurogrid with 65 thousand neurons and 500M synapses, which consumes 3.1W [4]. In order to minimize energy consumptions, alternative materials have also been proposed for artificial neural systems and one example is the use of spintronics [5].

A number of alternative solutions have also been proposed to mimic natural neuron functions, where biological neuronal cells have been used to perform AI computing to replace conventional computing systems, i.e., biological AI. Examples of this include living neurons that can play pong [6], robots integrated with neuronal cells to control their operation [7], control of a robotic arm [8], and Organoid Intelligence Biocomputing [9]. This approach has also shown that the neurons can also be taught and trained to adapt to specific applications. Besides neurons, other forms for biological systems have also been considered to perform computing functions. Examples include the use of *Physarum* to solve networking problems at the Tokyo railway network [10], and most recently the use of fungii to perform molecular computing [11]. Using these approaches can possibly result in new solutions where biological cells work in tandem with silicon technologies, i.e., bio-hybrid AI. While this may address the aforementioned challenges of including more realistic biological properties, protocols and technologies to maintain biological cell lines and keeping them alive for a long period may also invalidate the quest for higher efficiency of these systems.

Fundamental to all biological AI solutions and models that have been proposed is the exchange of molecules between cells to realize computing functions. This communication based on molecules occurs as both an intra as well as inter-cellular signaling. However, the training and computing processes within these systems can be further enhanced through modeling, optimization, and engineering of these same processes, with the help of molecular communication theory. As this field is slowly maturing, models and systems have been developed to study and engineer information encoding into molecules to be exchanged between different biological or bio-hybrid entities, also called bio-nanomachines, such as the aforementioned AIenabling cells. Examples include characterizations of channels within biological environments [15][16] [17] [18] and molecular modulation techniques (e.g., MoSK [19]). These new communication models have been applied to characterize

and engineer numerous types of molecular communication systems such as neuronal interconnections [20], multi-hop diffusion-based networks [21], and large scale systems with 3D geometry [22]. Test beds and proofs-of-concept have also been developed including table top molecular communication systems [23], as well as molecular modulators that transmit digital information between computers [24]. The engineering of molecular communication systems in biological or biohybrid AI systems can enable new design as well as efficiency and robustness. This may include the design of engineered molecules to propagate information during gene expression leading to intra-cellular signaling, as well as inter-cellular signaling that can support ANN functionalities between populations of cells. This can be achieve through the combination of molecular communication theory and the tools provided by synthetic biology, where genetic circuits are engineered to produce molecular signals communicated between cells.

In this paper, we will analyze a number of different biological AI and the types of communication that is inherent in the models, i.e., Molecular Machine Learning (MML). MML in here intended as machine learning realized with molecules and chemical reactions as building blocks, rather than computer programs to inform synthetic chemistry, as in [25]. This includes engineered cells to create perceptrons found in ANN or interconnecting engineered cells to behave as neural networks. We will then follow with alternative future directions for developing ANN using the concepts of molecular communication theory through the natural Gene Regulatory Networks (GRN), molecular communication between multi-species population of cells, as well as engineering of Ca^{2+} signaling based molecular communications to create an Analog-to-Digital Converter (ADC). Lastly we will focus on future challenges for MML.

This paper is organized as follows. Section II discusses current background on engineered cells as well as metabolic reaction models to realize ANN. In Section III we propose a new direction whereby natural GRNs and their embedded intracellular molecular communication for AI. In Section IV we introduce an idea for utilizing a multi-species cellular consortia to perform AI using inter-cellular molecular communication. In Section V we move towards engineering calcium (Ca^{2+}) signaling in cells to achieve perceptron like behaviour. In Section VI we discuss future directions and challenges, while in Section VII we conclude the paper.

II. Current Background on Biological AI

Numerous research have indicated natural intelligence that occurs within cells. From the perspective of molecular communications, this deals with initially sensing molecular signals from the environment, followed by internal signal transduction that leads to gene expressions, as well as corresponding metabolic pathways. This process is largely programmed into the cell's genome [26]. In certain cases, this intelligence and memory management can be performed with organisms that lack a brain, or non-neuronal systems as pointed out in [27]. In the case of bacteria, claims have been made the microbes contain 'minimal cognition' [28].

In [12] a single layer ANN was developed using engineered *E.Coli*, known as **Bactoneuron** (Figure 1 (a)). The developed model is able to achieve both reversible as well as irreversible computing. Each cell is engineered to receive inter-cellular diffusing molecules, and as a response, execute a log-sigmoid activation function to produce Green Fluorescent Protein (GFP) output. This execution is established through a transcriptional regulation which is undertaken by an engineered genetic circuit (also referred to as *cellular device*). The solution proposed uses established set of general rules to map the complete ANN architecture and to derive unit bactoneurons directly from the functional truth table of a complex computing function. The study produced both simulations as well as experimental validation. Example applications included a 2to-4 decoder, a 4-to-2-priority encoder, a majority function, a 1-to-2 de-multiplexer, and a 2-to-1 multiplexer and reversible logic mapping through Feynman and Fredkin gates.

Rizik et al [13] developed the **Perceptgene** (Figure 1 (b)), which is a perceptron model of an ANN. This was achieved through the genetic circuit engineering in *E. Coli* bacteria. The perceptron behaviour is established through a logarithmic input-output relationship that fits to the non-linear biochemical reactions that occur in the genetic circuits. The implementation is based on engineered genetic circuits whose input-output behavior includes both the power-law as well as a multiplication function. The power-law function encodes the weighted chemical inputs, while the multiplication function aggregates all the inputs that will determine the activation. The weight of each input is determined by the Hill coefficient. The two inputs used are *isopropyl Beta-D-1-thiogalactopyranoside* (*IPTG*) and *anhydrotetracycline* (*aTc*) molecular signals and results in a repression process that in turn regulates their own production using an auto-negative feedback loop. Similar to the perceptrons of an ANN, the perceptgene also contains a bias component for the sigmoidal activation function. The bias input is set by the ratio of the maximum transcription process to the binding affinities of the protein-protein/protein-DNA reactions. The applications of the perceptgene include weighted multi-input functions, classification, as well as an offline gradient descent learning algorithms.

In [28], an offline trained perceptron neural network is used to program a population of bacteria and it is simulated *in silico*. Through the diffusion of inter-cellular molecular communication within a population, the cells were able to have social interactions and form complex communities. The programmed perceptron was also used to solve an optimization problem. The work was based on an in-silico model, where the plasmid encoded perceptron was designed using *Cello*, while the simulation of the bacterial communication was developed through the *Gro* simulation tool. A particular aspect of the study is the use of programmed ANN into the genetic circuit to control signaling between cells in the population to perform functions. The input are natural molecules (e.g., galactose), which in turn control a downstream behaviour. This includes (i) emitting molecular signals proportional to the concentration of oxygen that is used for metabolic purposes, (ii) inducing chemotaxis for cell movement, (iii) commensalism, where the cells emit a signal that degrades the waste products from other bacteria in the population, and (iv) controlling of cell growth when the environment is harsh.

In [29], a consortia-based bacterial ANN was developed and proved experimentally. An interesting feedback process is developed between the receiver and the sender, which are the perceptron nodes for decision making and this is achieved using quorum sensing. The sender bacteria are able to emit varying molecular signals (*OHC14 - acyl-homoserine lactone 3OHC14:1-HSL*), which represent the weights. These molecular signals are induced by an external signal (*OC6* (*acyl-homoserine lactone 3OC6-HSL*)). The application was specific to 4-bit pattern recognition, where varying levels of the *OC6* inducers are applied to sender bacterial populations and once the molecular signals diffuse to the receiver, they will activate a genetic circuit to produce an output signal. A novel gradient descent algorithm was also developed to optimize the weights of molecular signals to suit the pattern recognition application.

A cell-free perceptron model was proposed in [14] using the metabolic circuit illustrated in Figure 1 (c). The latter was designed with a focus on biochemical retrosynthesis to predict the pathways, which was achieved using the *Retropath* and *Sensipath* computational design tools. The circuit was then embedded into a cell-free system in order to create the **Metabolic Perceptron.** The metabolic perceptron was able to perform binary classification based on metabolite molecular signals that leads to a classification process. The example application was here a four-input binary classifier.

III. Genetic Regulatory AI

While the previous section focused on the genetic engineering of living cells to create machine learning systems, in this section we will look at an alternative approach that is based on computing structures naturally present in biological cells, i.e., GRNs. This approach is based on essential similarities between a GRN and its structure to an ANN. While a number of different works have investigated neural-like properties in GRNs, our investigation focuses on how molecular communication properties can be exploited to perform computing functions as well as training by externally manipulating the weight connections between gene relationships.

A. Background on Gene Regulatory Networks

A GRN is a highly complex network of multi-layered interactions between genes. Each individual cell carries a GRN specific to its species and strain, giving an unique behavioural pattern as well as functionalities. A cell can sense a range of external stimuli using membrane receptors, perform computing through the GRN and express genes accordingly, thus resembling an input-process-output sequence found in conventional computing. A typical process of gene expression starts with the transcription process of converting the genes into mRNA and this depending on the gene can be followed by the translation process that coverts the information contained in the mRNA into proteins. However, during gene expression within the GRN, molecular communication patterns can be identified in gene-gene interactions, which are complex processes that occur at multiple layers. For example, while these interactions in prokaryotes contribute to the regulation of the aforementioned transcription process, for eukaryotes they can be post-transcriptional, i.e., contributing to, among other things, mRNA (or other transcript) and/or protein functionalities.

Moreover, the regulation in the post-transcription layer contributes to specific dynamics in the behavior of GNRs. In this context, proteins plays a crucial role complementing the regulation mechanism by integrating sensing, transfer, storage, and processing of information. As an example, proteins can perform computational tasks such as amplification, Boolean logic functions, and information storage through mechanisms of allosteric regulation [30]. In addition, the inter-conversions between phosphorylated and non-phosphorylated states of proteins act as switches enabling them to exhibit sigmoidal behaviours over a limited concentration range.

In the following, we show how these complex molecular signaling processes that involve multiple layers of chemical reactions as well as components during gene expressions, combined with the network structure of genome relationships, can allow us to identify and exploit natural ANN within GRNs, i.e., Genetic Regulatory AI (GRAI).

B. ANN Learning and Training Models in a Simple Gene Regulatory Network

The transcription of a particular gene in a GRN is combinatorial action of products of other genes as well as its own. Subsequently, the state of the cell is an action based on a combination of diverse translated gene products. When we observe these properties, we see a resemblance to the dynamics of an ANN, specifically a Recurrent Neural Network (RNN), where the current state depends on the previous. This means that there is a potential to create MML from manipulating the gene expression patterns.

To describe our concept, we will focus on a simple communication pattern found in the GRN of a bacterial cell. Bacteria uses signal transduction pathways to sense the environment by processing input signals. *Two-Component Systems* (*TCS*) are among the most widespread signal transduction mechanisms, which contain a *Sensor Histidine Kinase* (*SHK*) that receives external signals and a response regulator that accordingly initiates the expression of a set of genes. On average, a bacterial cell contains 30 TCSs that are essential for their virulence, growth and survival. Approximately, 87% of the known response regulators of TCS involve gene expression regulation at the transcription layer. Based on this, 96% of SHKs are capable of sensing small-molecule-binding from the extra-cellular space. Hence, the combination of TCSs can be considered a viable example of a natural GRN pattern that can be modeled and characterized as an ANN, where the input layer is represented by the SHKs, and multiple hidden layers as well as an output layer consist of genes and their mutual interactions. There are several advantages in using the TCS sub-network of the GRN as an ANN for MML. This includes availability of experimental data that offer validation and quantification of the relationships between gene expressions for both input and output layers. In a number of cases, the direct mapping of a GRN sub-network to an ANN is not feasible. The reason is because sometimes the number of gene interactions (network hops) from the input layer to the output layer can vary for different gene expression paths, resulting in the corresponding ANN to be asymmetric, which leads to less computational efficiency. There are wellknown approaches to address this problem, such as introducing phantom nodes that do not alter the overall behavior or treat the network as asymmetric ANN structure. Another alternative is to introduce missing gene interactions

through engineered genetic circuits, which can further align the sub-network closer to a typical ANN structure.

Figure 2 illustrates how we recognize an ANN structure from a TCS sub-network of a GRN. As shown in the figure, the cell is able to combine multiple input signals and accordingly express downstream genes through the network. Gene expression products from one gene reach the non-coding region of another via intra-cellular diffusion [31]. The relationship of genes to be expressed in the network can be associated to a set of weights. The values of the weights are a result of several factors that include the transcription factors, affinity of the transcription factor binding site, thermoregulation, enhancers [32] as well as the noise due to the diffusive motion of regulatory molecules [33], [34]. Here, we focus mainly on two TCSs: *PhoB-PhoR* and *BqsR-BqsS* systems, which are associated with phosphate and iron uptake of the *P. aeruginosa* species. Further, we target the inter-cellular molecular communications by considering three QS systems, namely, *Las*, *Rhl* and *PQS* genes where *Las* uses *3O-C12-HSL* and *Rhl* uses *C4-HSL*, while the *PQS* relies on *2-heptyl-3-hydroxy-4(1H)-quinolone*. To identify the corresponding ANN structure, we first modeled the GRNs as graphs using the interaction structural data from publicly available database [35]. This is followed by extracting the TCS sub-network related to the phosphate intakes iron along with the quorum sensing process. The obtained ANN model contains various numbers of hops from the input layer to the output layer, which require the introduction of phantom nodes that do not have an impact on the interaction dynamics of the network. The weights of the ANN represented by the TCS are estimated relatively using the interaction dynamics as well as transcriptomic data [36], [37]. The performance accuracy of this model is then evaluated based the pyocyanin production and gene expression levels in low and high phosphate conditions with the data from wet-lab experiments in similar setups [38]. A typical ANN will require modification of weights as its being trained to serve for a specific purpose. Here, we investigated how the weights of the ANN related to the TCS can be changed with a specific focus on changes that can be operated externally to the biological cell, from the environment. Previous research has demonstrated how the temperature can impact the cellular functions of *P. aeruginosa*. This usually results in the modulation of one specific gene expression interaction of the *Rhl* QS system [32]. As highlighted in Figure 2b, with the reception of *C4-RhlR* at 37°C temperature, the weight of *hn21 - rhlR* is significantly higher compared to the same at 30°C, as shown in Figure 2c. This corresponds to a higher expression rate of *RhlR* at 37°C. This demonstrates that updating and training of GRAIs is possible through changes in the environmental conditions, such as temperature.

C. Mining ANN in GRNs

Our previous section has shown that certain sub-networks of the GRN exhibit natural neural networks. In this section we want to investigate if other sub-networks that exhibit ANN structures can be extracted from the GRN. We perform this through a search algorithm that mines the GRN for specific types of structures. During the search process, if we need a structure with i number of input nodes and j number of output nodes, the algorithm first mines j number of nodes that have a common predecessor. The j number of nodes will have a number of different predecessors and will be put together into the same group. Within the same group, the nodes will be put together to create different combination, where the

combinations must have *i* number of input nodes that re the predecessor as well as *j* output nodes. These combination will reflect the different number of sub-network for nodes input nodes *i* and output nodes *j*.

Figure 3a illustrates examples of a Feed-Forward neural network with different structures of fully connected ANN subnetworks extracted from the GRN. Figure 3b shows the number of perceptron and Feed-Forward neural network structures we obtained from the GRN using our mining algorithm. We are able to discover a significant number of perceptron structures with the highest recorded for one output node and two input nodes. As we increase the number of inputs, the number of fully connected Feed-Forward networks becomes harder to discover. In particular, Feed-Forward networks with five output nodes and higher than three input nodes are very rare.

Since these Feed-Forward neural networks are pre-trained with defined weights, the question now rises as to how we can use this for applications. One approach towards using the ANN found in the GRN is to match it to an application's requirement. This will require a mining algorithm that matches to the problems that require an ANN with the same structure as well as weight combination. While this can create challenges in terms of finding the right problem to suit the ANN found in a GRN, there is an opportunity to engineer the circuit with addition of genes that will increase the diversity of the network as well as integrate hidden layers.

IV. Bacterial Multi-species Diffusion-Based Neural Network

In this section, we look at an alternative model for MML, where we investigate how multiple species of bacteria with symbiotic relationships, such as those found in a bacteriome, i.e., bacteria living in endosymbiosis with a host organisms, can be modeled and exploited as an ANN. In general, bacteria of the same species receive specific types of molecular signals from other populations and process them to produce a set of molecules that can influence other species or host cells. These multi-species bacterial populations can be considered the nodes of a network, where the molecular signals that diffuse between population are the link/edges, based on diffusion-based molecular communications. As the molecular signal cascades through the network from layer to layer, this resembles a feed forward neural network (layer in this instance are bacterial species that receive the same type of signals). The relationship structure of the bacteria and signaling weights depend on factors such as the diversity of the species, population sizes, cross-feeding/inter-cellular communications and molecular signal diffusion dynamics. The population sizes determine the rate of molecular signal reception and production and this reflects the weight of the edges of the corresponding ANN model. If a larger population produces a signal and another population that has higher relative abundance consumes that signal, the weight corresponding to the link between these larger populations will be modeled with an ANN edge with a larger weight. On the other hand, if the population sizes of the two different species are smaller, the interaction between them is comparatively weaker and will result in a smaller weight value of the corresponding edge.

One of the well-studied bacterial ecosystems is the Human Gut Bacteriome (HGB), which constitutes up to 1000 species [39] and it suggests a relevant use case for the aforementioned concept. The reliability of the molecular signal flow between the different species is vital in modeling and exploiting the ecosystem as an ANN. In our previous study, the structural derivation of a network of multi-bacterial species using graph theory was analyzed, where input of glucose is received by certain species to produce various Short Chain Fatty Acid (SCFA) communicated between the cells [40]. The study revealed that the weights of the edges, which are the lactate and acetate signals exchanged between the populations, can be modified and adapted based on external inputs (e.g., glucose). Using this concept, we believe we could design a Bacterial Multi-species ANN from the SCFA molecular communication network within the HGB. Figure 4(a) illustrates an example of multi-species bacteria population that are organized into an ANN structure. The arrangement of the structure is based on the input-output relationship of molecular production. For example, when input glucose is consumed, it produces lactate and two SCFA (acetate and proprionate) by six species to produce butyrate for other species, then the six species will be the first layer of a corresponding ANN of our NN, and the species that produce butyrate will be the ANN's second layer. Figure 4a shows the ANN with the relative weights of each edge shown with different color shades. Our aim is to train the ANN in Figure 4a into an ANN with a specific functionality, shown in Figure 4b. Our training is based on the external input of glucose, where we can see in Figure 4c that as the species are consuming and producing molecules, their weight is slowly being modulated by changing the population sizes, Figure 4d (as the Mean Squared Error (MSE) of the population converges, similarly the molecular production error).

Further, we show how significant the impact of the population size variation is on the overall gut metabolic performance by altering the abundance of each species relative to a healthy HGB composition. Figure 4e shows the network outputs in terms of acetate, propionate and butyrate when the abundance of *Bacteroides* is changed from zero cells in the environment to a population size of 200% as in the healthy HGB. Figure 4f and Figure 4g present the behaviors of the same outputs when altering the population sizes of *Alistipes* and *Faecalibacterium*, respectively. These results indicate the possibility of altering weights of Bacterial Multi-species ANN to modify the network outputs significantly, which can be used in applications such as personalized treatment of metabolic disorders.

V. Ca^{2+} Signaling Perceptron Based on Molecular Communications

In this section we discuss a perceptron that can be trained by controlling the ion flow as well as the basal reactions of Ca^{2+} Signaling between biological cells. As an example, we demonstrate the design of a multi-cell ADC realized by modulating the cell's Ca^{2+} influx as well as through the engineering of genetic circuits.

A. Calcium Signaling

Communication through Ca^{2+} ions is one of the essential signaling processes at the basis of numerous cell functions. While a few mathematical models for Ca^{2+} signaling have been proposed, the model by Korngren et al. for Ca^{2+} ion transients in electrically non-excitable

cells is one of the most recognized and is at the basis of the concepts we present in the following [41]. According to this well-regarded model, this communication process is based on Ca^{2+} ion influx into the cytoplasm from the extracellular medium, where ion-conducting channels are established through the membrane and controlled by receptors. The receptor in the model is designed in terms of a linear activation instead of complicated non-linear agonist binding curve [41]. As the influx of ions increases the Ca^{2+} signaling reaction is activated, where the Ca^{2+} ion pumps allow the outflow of ions from the cytoplasm to the external medium as well as its store. Eventually, the Ca^{2+} ions concentration in the cytoplasm reaches a saturated level. Based on this sequence of events, numerous Ca^{2+} signaling based molecular communications systems, models, and their characterization have been investigated and proposed over the years [42] [43] [44][20].

B. Obtaining a Perceptron from Ca^{2+} Signaling

We adapt the Korngreen et al. model to exploit a Ca^{2+} signaling system as a perceptron. As illustrated in Figure 5a, the input (x) will be the Ca^{2+} ion concentration in the extracellular medium and the weight (w) is the Ca^{2+} ions influx rate through the plasma membrane channels. Therefore, x^*w represents the amount of Ca^{2+} ion influx (y) into the cytoplasm, representing its transient. As described earlier, the Ca^{2+} ion transients are multi-stage signaling processes that involve the transition of ions within the cytoplasm, store, buffer, as well as the extracellular medium and regulate the concentration in the cytoplasm. In order to train the Ca^{2+} signaling process into a perception, the cell needs to be the incorporation of an engineered genetic circuit to modify its basal fractional activity to trigger the Ca^{2+} signaling reaction or to modulate the influx channel. In the case of a multiple-cell system to realize an ANN multi-perceptron network, the engineered genetic circuits are required to enable dynamic activation and deactivation of the Ca^{2+} channel.

C. Two-bit Analog to Digital Converter

1) Architecture—We adapted the Ca^{2+} ion signaling model to create interacting perceptrons in multiple cells that altogether realize a two-bit ADC through a simulation model. The architecture of a conventional ADC is illustrated in Figure 5b. The equivalent model based on Ca^{2+} signaling, where made clear the essential role of ion flow between two cells (the blue arrows in the Figure 5c indicate Ca^{2+} ions reactions to facilitate this). The input x is the incoming extracellular Ca^{2+} concentration into the two cells, where the range of input considered in the simulation is set between 500μM to 2500μM and sampled according to an interval of 500μM. By dividing this range into four intervals, each interval will produce different Ca^{2+} signals from two cells, i.e., *Cell* 1 and *Cell* 2, which map to different digital bits. Based on this, the *Cell* 1 and *Cell* 2 produce the Most Significant Bit (MSB) and the least significant bit (LSB), respectively. Ca^{2+} ions in the extracellular medium(x) flow into the cytoplasm through the Ca^{2+} channel with an influx rate w_0 and w_1 for *Cell* 1 and *Cell* 2, respectively. A bias to the Ca^{2+} ions influx for each of the two cells (y_0, y_1) is randomly selected and applied (in this example this is b_0 =0.169255μM and b_1 = 0.287264μM, respectively). Through the Ca^{2+} transients, the ion concentrations in the cytoplasm that are set to C_0 and C_1, respectively. By setting a threshold, in our case, 1μM, the Ca^{2+} concentration in the cytoplasm can be converted into digital bit (Z_0, Z_1), which are the MSB and LSB. In order to make an ADC, *Cell* 1 is genetically engineered to

produce molecules when enough Ca^{2+} ions (1μM) are present in the cytoplasm. The output molecules temporally deactivate the calcium channel in *Cell* 2 plasma. This deactivation rate is indicated as d_0.

2) Training process—The flow chart for training the Ca^{2+} signaling perceptron is presented in Figure 5d. The two cells have to be trained to obtain optimal Ca^{2+} influx rates (w_0, w_1) as well as the correct *Cell* 1's calcium channel deactivation rate for *Cell* 2 (d_0) so that *Cell* 1 and *Cell* 2 can produce the aforementioned MSB and LSB, respectively. *Cell* 1 is trained first to find an optimal w_0, then *Cell* 2 to obtain w_1 and d_0. With initial w_0, Ca^{2+} flows into *Cell* 1 and is regulated in the cytoplasm (C_0) for a certain period. Based on the amount of input from the extracellular medium (x), the concentration at saturation will represent an MSB digital bit (Z_0). When Z_0 is bit 0, but the expected output is bit 1, an activation chemical from the engineered circuit is injected to elevate the basal activity of the calcium channel in *Cell* 1 plasma. Due to the increased activity of the channel, an increased amount of Ca^{2+} ions will flow into *Cell* 1, which means the influx rate (w_0) is also increased. For the opposite case, when Z_0 is bit 1 and the expected value is bit 0, a different deactivation chemical signal is expressed by the engineered genetic circuit to reduce the basal activity of the Ca^{2+} channel. Then w_0 is updated to a lower value. Based on this sequential training process, the optimal w_0 will be found. The same training process is performed on *Cell* 2, except for one case. This exception case is when Z_0 and Z_1 are bits 1, but the expected Z_1 is bit 0, which will require manual intervention to modify the rate of *Cell* 1 output chemical production instead of injecting chemicals. Figure 5e shows how the perceptron behaves for different levels of Ca^{2+} within the cytoplasm based on varying extracellular influx. Figure 5f illustrates an example of convergence of weight w_0 during training with respect to the error for varying levels of extracellular input (x). Finally, Figure 5g shows the variations of output from the two cells that represent digital bits from *Cell* 1 and *Cell* 2. For example, an input between 1000μM and 1500μM results in '01', where the 0 bit is from *Cell* 1 and 1 bit is from *Cell* 2.

VI. Challenges

While we have identified solutions that enable non-neural cells to develop perceptron properties, or the exploitation of gene regulations to obtain ANN functionalities, there is still a number of challenges that needs to be addressed to move towards practical applications in the future, some important ones are discussed next.

A. Controlling Molecular Communications in Molecular Machine Learning

The MML that we have discussed so far are based on training and computing operations that stem from communications of molecules and chemical reactions. To develop MML systems processes matching the computational capabilities of silicon-based technologies, we will eventually need to consider multi-layer perceptron architectures. While the genetic engineering will possibly be the main enabling technology, specific challenges are as follows. Firstly, since the training of the edge weights of molecular signals, which in our case is based on population control. Therefore, a mechanism is required to ensure that parallel changes in the bacteriome can be performed to modify the relative population of

different species/strains in the system. This becomes more challenging when we consider Ca^{2+} signaling between cells and in particular controlling the flow of ions through the gap junction of cells. Secondly, while GRAI might be inherently including multi-layer perceptrons, the question is how do we determine appropriate chemical inputs to express genes of the input nodes and at the same time detect expressions on specific output nodes. From a multi-bacterial species perspective, this will require engineering of cells with different receptors to detect diverse molecular signals from the previous layers. The cells will, therefore, need to have the ability to detect signals efficiently and operate in noisy environments. The other challenge is the ability to synchronize all transmissions as signals propagate between different layers. The latter challenge, can have an immense impact on the reliability of the resulting ANN. Since we have shown that multiple ANNs are embedded in a GRN through a sub-network, the question is whether multiple parallel processing can be achieved through different gene expression paths.

B. Bio-Hybrid AI

The paradigm of the Internet of Bio-Nano Things [45] includes the need to interconnect molecular communication systems to connect to the cyber-Internet by propagating information between the molecular and the electrical domains. This can be realized through an electro-chemical based Bio-cyber interfaces. While this can allow to detect chemical outputs from the MML, an issue arises when we want to actively interact and reconfigure the MML system from the electrical domain. In particular, the challenge lies in the mechanism to reconfigure the weights.

C. Responsible AI in Molecular Machine Learning

As AI continues to spread and weave into our everyday lives, besides developing sophisticated hardware and software, we are facing new and emerging ethical concerns has risen, which altogether call for the notion of responsible AI. Responsible AI aims to address the ethical and legal issues in regards to deployment as well as utilization of AI. This is already a major challenge in conventional AI, which is necessary to address to provide trust for the public in using the technology. This challenge will deepen further when AI is extended in living machines. This is particularly true, when we consider the potential applications of learning-based living machines for treating diseases, where they can potentially be deployed into the body or the environment. Another challenge is also the security aspect, in the similar manner that this is a challenge in conventional AI.

VII. Conclusion

As our society embraces AI to play a part in our everyday lives, we are starting to witness various forms and algorithms that are embedded into devices with different computational capabilites. In this paper we investigate MML for Biological AI, where AI occurs in living systems and is based on information propagation through chemical reaction and molecule transport, i.e., molecular communications. We reviewed the current background in Biological AI. This is followed by our proposed directions of MML through the GRN, bacterial multi-species communication, as well as Ca^{2+} signaling. We then discuss future possible directions for the molecular communications research.

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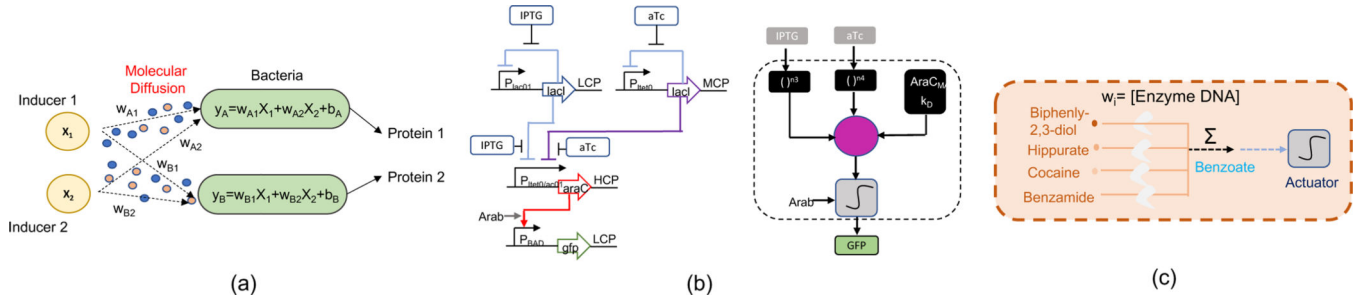

Fig. 1:

Proposed solutions to develop neural networks from engineering cells, (a) Bactoneuron [12], (b) Perceptgene [13], and (c) metabolic perceptron [14].

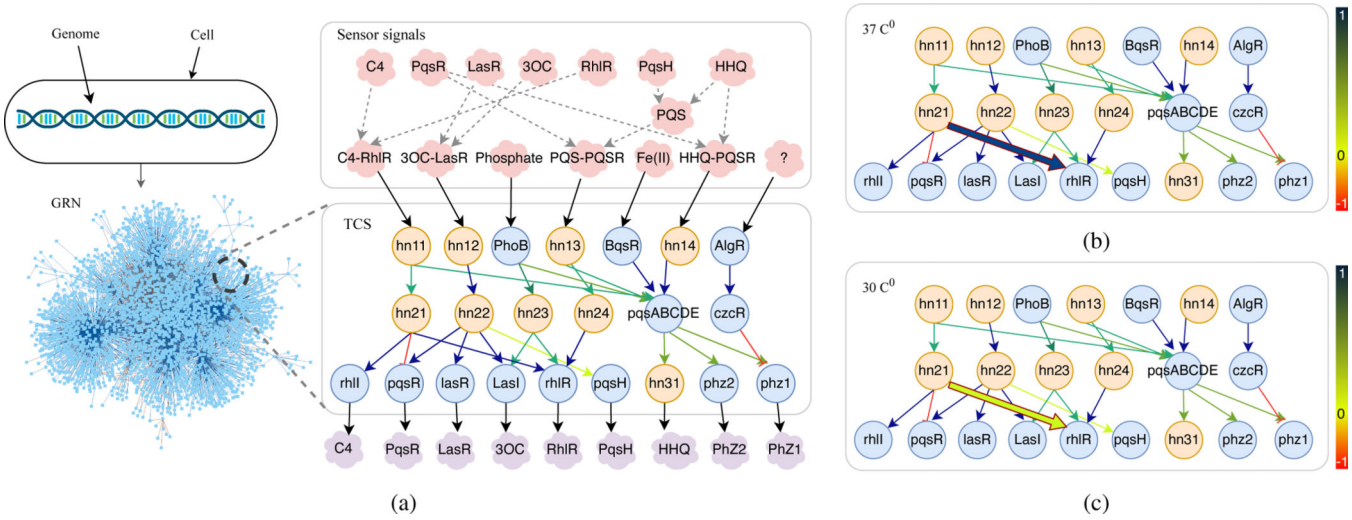

Fig. 2:

Illustration of inherited GRAI where, a) shows the extraction of a subnetwork that resembles an ANN with relative weights, b) set of relative weights in one environment condition (temperature at 37°*C*), and c) modified weight in a different environment condition (temperature at 30°*C*).

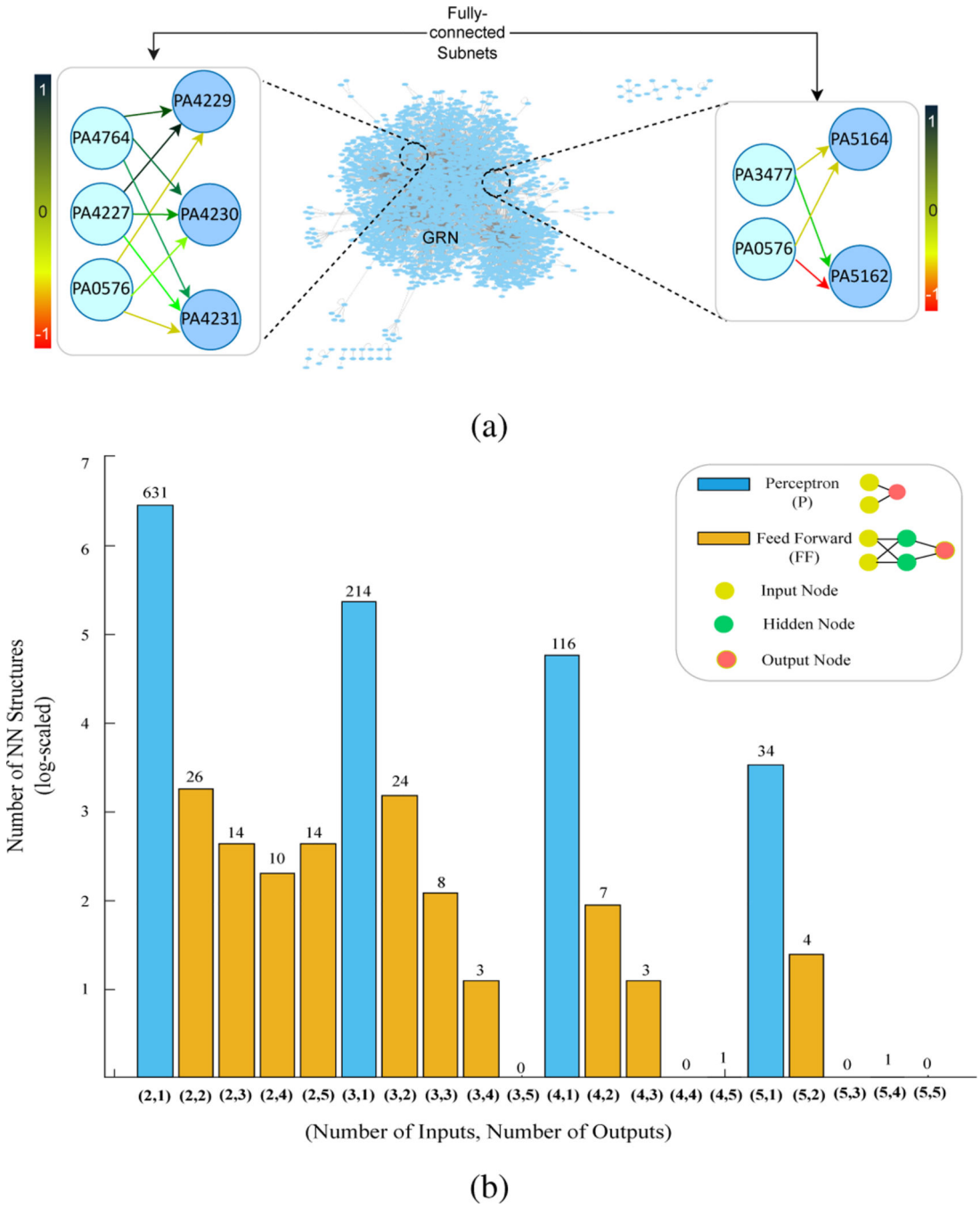

Fig. 3:

Two fully-connected ANN sub-networks extracted from the full GRN is shown in (a) and number of different sub-network structures that can extracted from the GRN is illustrated in (b)

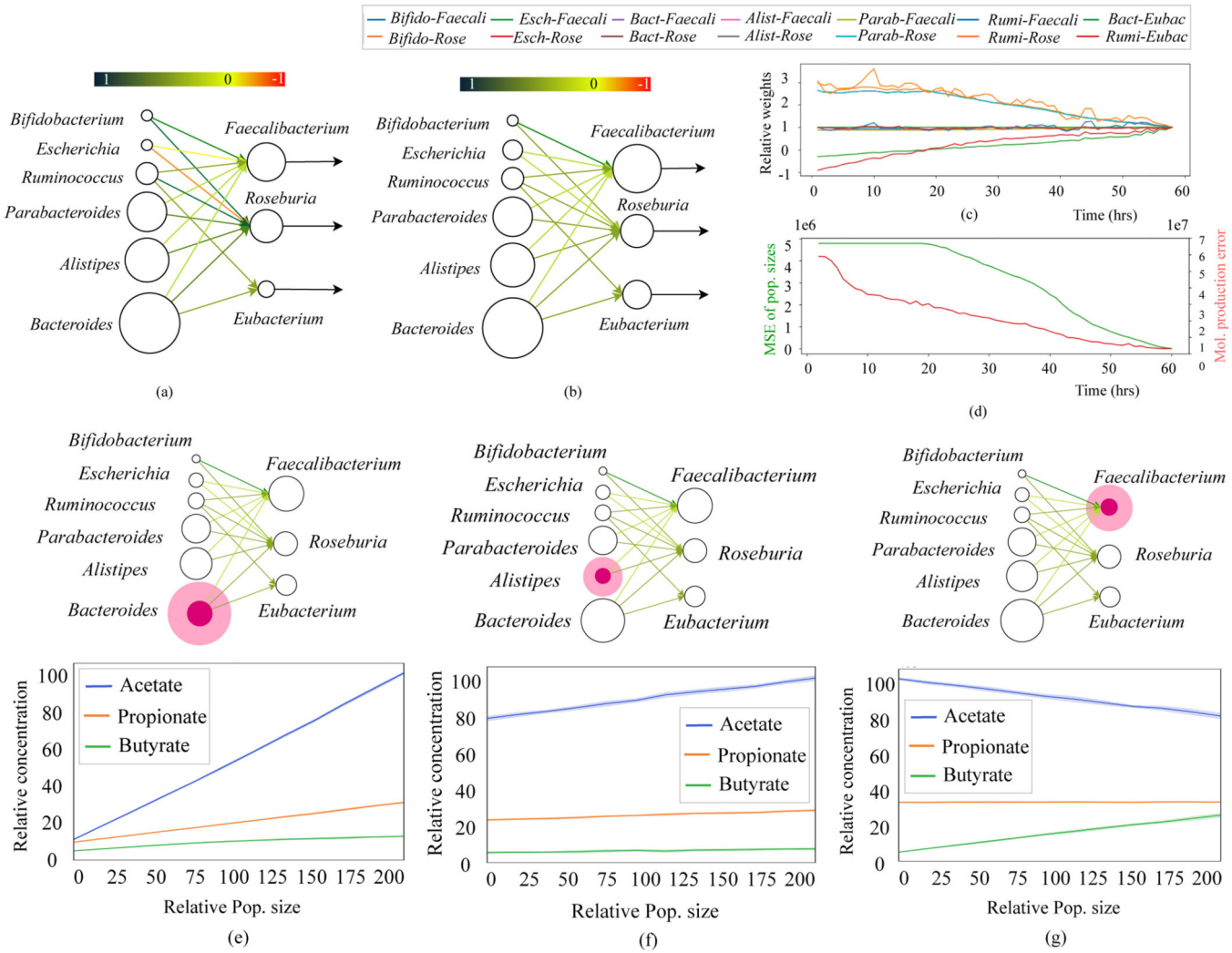

Fig. 4:

Illustration of population-based ANN weight alteration and its impact on the network outputs is shown here, where a) is the initial ANN setup, b) is the ANN with the preferred network weights, b) is the convergence of weights of all the edges relative to the preferred ANN over the transformation period and d) is the MSE behaviors of molecular production relative to the preferred ANN weights. Further, the output signal behaviors due to variations in weights caused by network structural changes are shown in e), f) and g) by changing the population sizes of *Bacteroides*, *Alistipes*, and *Faecalibacterium*, respectively.

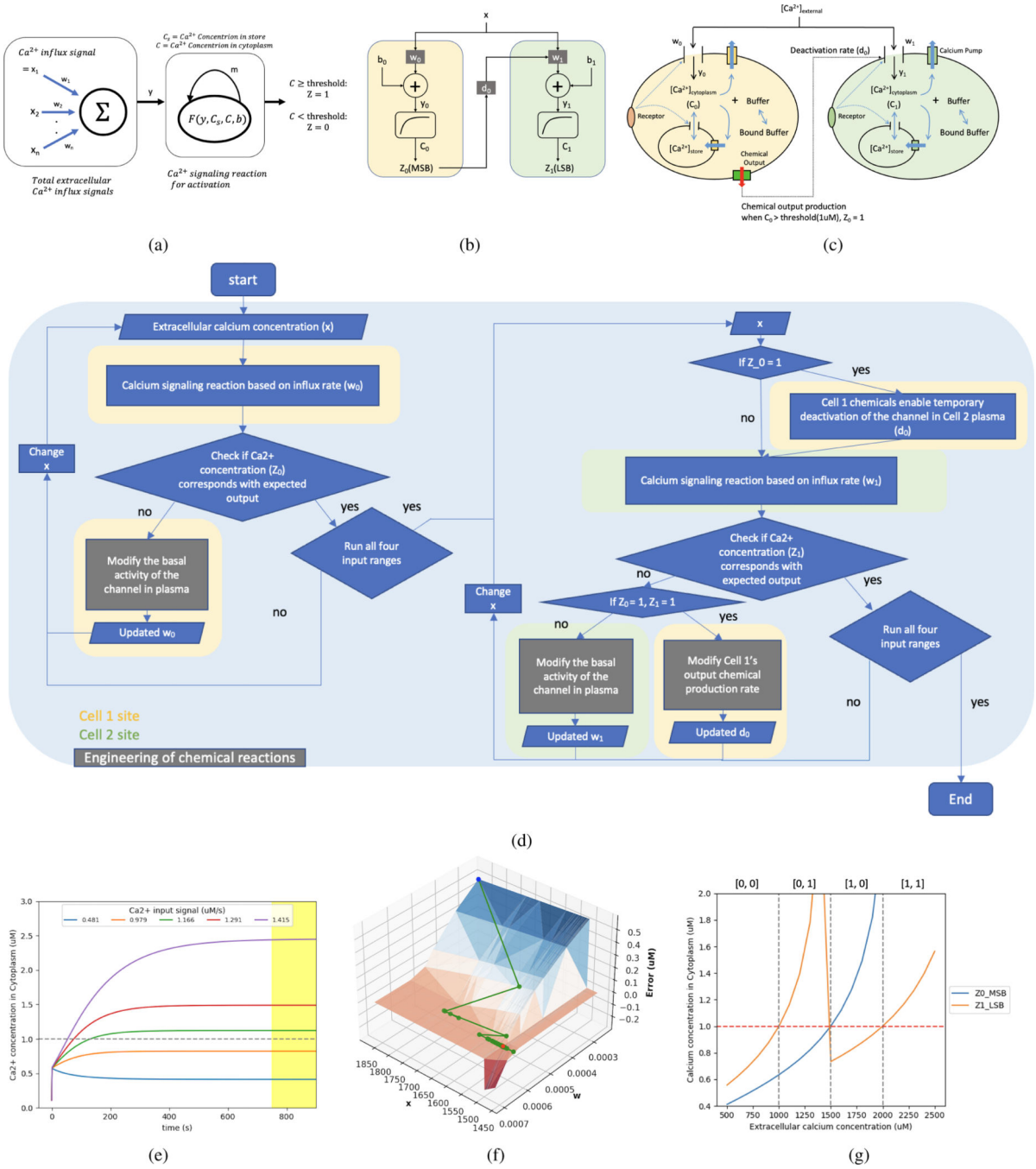

Fig. 5:

Transforming Ca^{2+} ions molecular communication into a perceptron. (a)A conventional perceptron model, (b)a Two-bit ADC architecture, (c) engineering Ca^{2+} signaling into an ADC between two cells, (d) Ca^{2+} signaling training process to modify the basal functional activity and communication channel flowchart, (e) Trained Ca^{2+} ions transients in the cytoplasm, (f) Dynamics of Cell 1 weight w_0 through the training process with respect to

the input extracellular Ca^{2+} input (x), (g) variations in output Ca^{2+} ions for the two cells to represent the ADC digital bits.