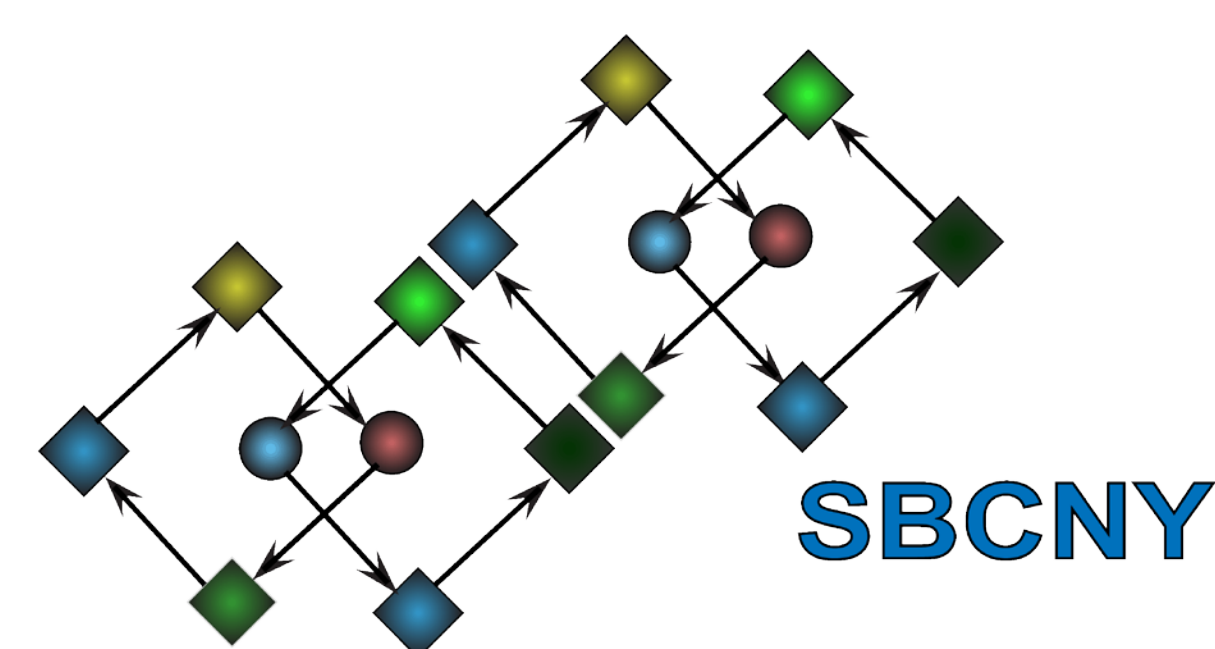




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Modeling of the Road Coloring Problem using Cell-Cell Communication and a Microfluidic Device

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Background

Synthetic Biology:

Synthetic biology uses DNA, cells and other molecular materials as basic components (parts) to build functional modules. From these modules novel biological systems are constructed [3, 5,9]. Such biological systems have either introduced new functionality by using natural parts in an unnatural configuration or environment, or using modified parts to mimic natural functions [3,5,9]. Some examples include synthetic biofilm, logic gates and switches, sensors and artificial cell-cell communication devices [4,7,9,10,13].

To illustrate the computational capability of molecular materials to implement mathematical games or problems, Adleman used DNA to implement a solution to the directed Hamiltonian path problem, more commonly known as the Traveling Salesman Problem [1]. Additionally, Stojanovic and Stefanovic built a DNA automaton that can play tic-tac-toe with a human [11, second version in 8].

The Road Coloring Problem:

The Road Coloring Problem (RCP) originated in the fields of Graph Theory, Symbolic Dynamics, and Synchronization. The problem, based on the Cerny Conjecture, was stated by Adler et al. [2] to be as follows: is it possible to create a graph where following a set of synchronized instructions, regardless of the starting point one would always arrive at the same destination node? Synchronizing instructions in this context refer to a set of instructions that would make the system converge to a single destination point regardless of the origin while following a pattern of an identical set of instructions. The RCP problem states that such a pattern can be found in any directed graph where all nodes have the same out degree. In 2007, Trahtman proved the conjecture for the general case [12]. In 2002, Jonoska and Karl proposed DNA as a synthetic biology approach to execute the Road Coloring Problem *in-vitro* providing protocols and procedures for implementation [6]. This project seeks to model a Road Coloring Problem graph using cell-cell communication and a microfluidic device.

Methods

System Design:

We propose a microfluidic device that can be used to facilitate communication among several synthetically engineered cell colonies. Cell colonies will be positioned at locations corresponding to the nodes in an RCP graph. Microfluidic directional tubes will connect the cell colonies. Gene constructs will be used to program cells in the different colonies for the purpose of cell-cell communication. Transcription is activated upon receipt of specific ligands which bind to nuclear receptors upstream of transfected genes. Recipient cells trigger the expression of genes located adjacent to promoters bound by a nuclear receptor. Gene expression triggers the production of specific extracellular signaling proteins. These proteins can exit the cell and travel to neighboring cells to induce expression of other genes that can give rise to other extracellular signaling proteins. A detailed design summary describing the interactions amongst cell colonies based on the gene construct configurations is provided in figure 1.

Design of an RCP Graph Simulated using NetLogo:

Using the agent-based modeling software NetLogo, we modeled such a system in order to identify potential barriers for a physical implementation. For this, we attempted to adapt the model to the cellular domain and align it with the rules of biology. The following describes the design of the solution implemented in NetLogo. NetLogo is a stochastic agent based modeling software. The system simulation environment is run on a grid that contains "turtles" and "patches". Patches are used to represent the diffusion of ligands, and turtles are used to represent cells. Cells are grouped in cell colonies and are plotted or stationed at fixed points on the grid as if they were contained in microfluidic compartments connected by directed tubes. The description below explains the communication mechanism that is used when six colonies communicate with each other. This mechanism is then replicated amongst the rest of the colonies. The user is also able to activate specific pathways in this solution. Each pathway is displayed on the left hand side menu (Fig. 2).

Methods (continued)

Communications module:

Each cell in each colony has counters that keep track of the levels of genes and proteins inside each cell. Once the counter for a specific gene in a specific cell colony reaches a certain threshold, the cell produces an extracellular ligand that can diffuse to other cells. The ligand then diffuses to the next cell colony. Upon reaching the second cell colony, the receiver cells' counters are triggered. Once the receiver's counter reaches a certain threshold, cells turn green to signify receipt of the message and GFP expression. This emulates the process of a cell receiving a ligand that activates the expression of GFP, a protein commonly used as a marker/reporter of transcriptional activity. Additionally, upon receipt of the ligand, expression of another gene occurs. This gene can give rise to another ligand which is eventually secreted. This communication system reuses few promoter-gene pairs as possible such that the system is relatively easy to scale up and implement experimentally.

Preliminary Results

Upon modeling this system using Netlogo, we discovered a potential difficulty. Because each cell colony has two output paths or tubes, when a ligand arrives at its intended destination and prompts the cells in that colony to secrete a new output ligand, the output will propagate down both output tubes because there is nothing blocking its passage. This is not preferred because the unintended destination might also activate genes at an unintended colony undesirably. To address this concern, we plan to allow the unintended destination colonies to "hear" the incoming ligands but not respond to them. This would require careful design in order to customize the gene constructs in each cell colony.

Fig. 1

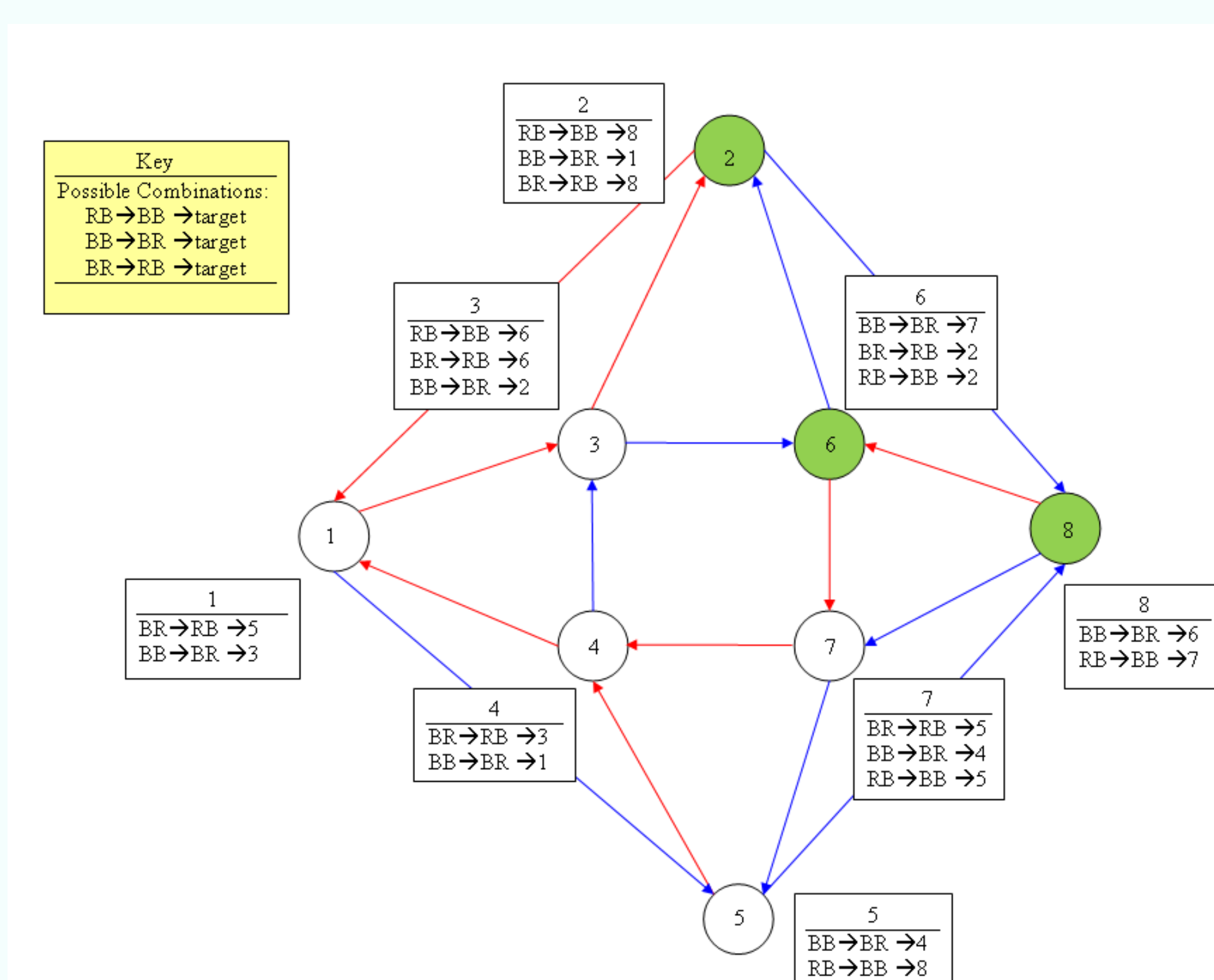


Fig. 1 An example of a Road Coloring Problem solution. Nodes represent cell colonies and the directed arrows represent communication pathways traveled by ligands. The directed graph also includes the routes possible from each node as per the synchronization word, Blue-Blue-Red, which allows the system to converge to single destination feedback loop made of nodes 2, 6, and 8.

Fig. 2

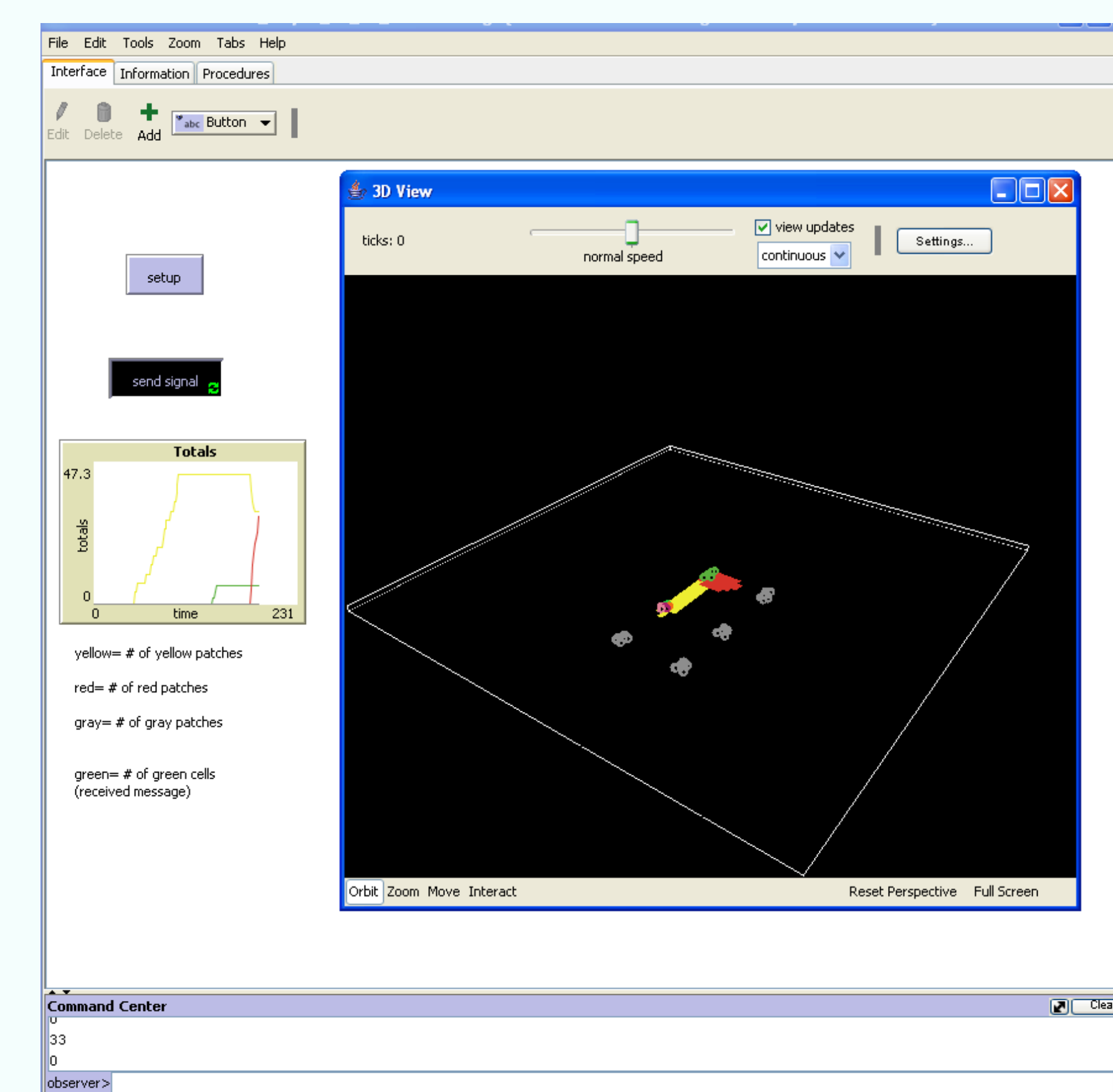


Fig. 2 The NetLogo simulation environment used to implement a cell-cell communication module. The buttons on the left allow the user to display the cell colonies and to start the simulation. A real-time graph also tracks the levels of the yellow, red, and gray ligands, as well as the number of cells that turned green.

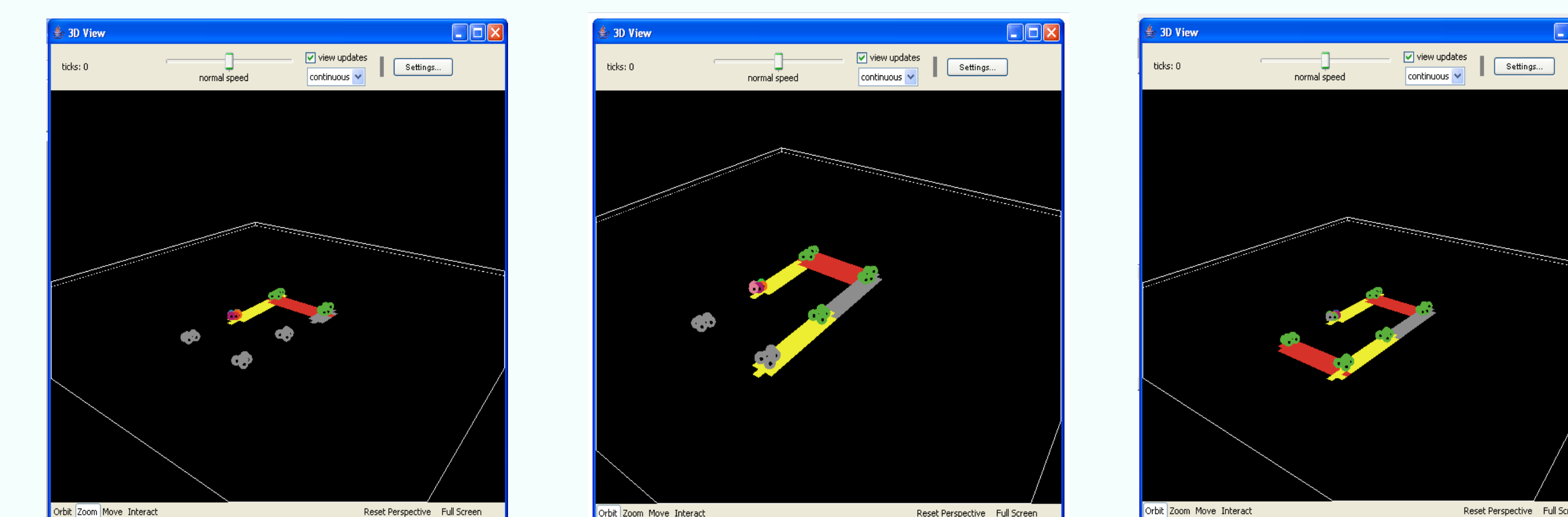


Fig. 3 The cell-cell communication mechanism between different cell colonies modeled in NetLogo. These screen shots show an initial stimulation where cells in colony 1 (middle left) stimulate the second colony; subsequently, the second colony stimulates the third colony. The stimulating ligands are represented by patch color, i.e. yellow, red, gray. Green cells signify receipt of message and GFP fluorescence.

Discussion and Future Directions

- One alternative approach that we may consider is to use inhibitors to prevent cells within a cell colony from responding to their own output. However, this would increase the construction complexity because each cell would need an additional set of inhibitors.
- Although we designed a cell-cell communication system, a RCP solution can also be implemented using a gene regulatory network within a single cell. Such system would activate a positive feedback loop gene regulatory circuit in response to any combination of multiple stimulations.
- The Standard Biological Parts Registry from the OpenWetWare project developed at MIT, which is a collection of freely available parts popular in the Synthetic Biology community, could be utilized for implementing this project's proposed design and model.

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