Teaching bacteria how to dance

P. King, V. Lavrovsky, S. von Mammen and C. Jacob

Abstract: The motivational design idea for our iGEM 2006 team was to use bacteria to create swarm paintings. We designed two types of *E. coli* cells: senders and receivers. Stationary senders would act as attraction points; receivers would follow nutrition gradients towards the senders. In addition to the first steps of the actual *in vitro* designs, we developed a computer simulation of our bacterial swarm painters.

1 A great start, but then...

Since May 2006, the iGEM idea started to spark interest among students within the Faculties of Science and Medicine at the University of Calgary. Initially, ten students joined our iGEM team. Many aspiring projects were proposed, discussed and evaluated regarding their feasibility. Eventually, it turned out that most of our initial members had to leave the team as they were not able to commit on a full-time basis. Unfortunately, we had received our invitation to join the iGEM competition slightly too late to give our students enough opportunity to attain funding. This left three dedicated students to work on the actual implementation of the project (Fig. 1). So, what was the project idea?

2 Aims of our project

The key inspiration for our project came from SwarmArt.com [1], a collaboration between the Faculties of Art and Science at the University of Calgary, which has led to several interactive computer installations utilising swarm intelligence systems. Fig. 2 shows an interface through which an artist would create drawings through swarms. Painter agents, which leave trails of different colours behind, act like a simulated flock of birds (or bacteria?) which is attracted to a red dot. This target can be moved by the user and placed anywhere on the canvas. By moving the red target or leaving it at a location for a longer time, the swarms create interesting artistic compositions, in particular when obstacles are introduced into the scene. For our iGEM project, we wanted to recreate such an artistic swarm painter environment – with swarms of bacteria. Soon we realised that this endeavour is quite challenging as we wanted accurate control over a target, to have bacteria follow the target, and to produce different colours (with fluorescent proteins) to reproduce the coloured trails in the computer model.

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3 Bacterial painters

We decided to design two types of bacterial agents: senders and receivers. A Petri plate is inhabited by two strains of genetically engineered *E. coli* bacteria (Fig. 3). The first strain (the Senders) have been engineered to emit two chemical signals into the plate environment: aspartate and acyl homoserine lactone (AHSL). The senders themselves are activated by light (Fig. 4). This would allow us to simulate changing targets without having to wait for the sender bacteria to actually move. The second strain (the Receivers) have been designed to respond to each of these signals in a different way.

- The Receivers express green fluorescent protein in the vicinity of AHSL.
- The Receivers also move towards areas of greater aspartate concentration.

The same bacteria also decrease aspartate levels where they are present, as this is a nutrient and constitutes the reason for why they are attracted to it in the first place.

The goal was to utilise the Senders and Receivers to create interesting swarm drawings through bacterial interaction dynamics visualised by fluorescent patterns.

3.1 In silico *model*

In order to verify our sender-receiver system design, we implemented an *in silico* swarm-based model of interacting bacterial agents. We used the agent-based simulation environment NetLogo [2], which offers a powerful programming environment (with its own interpreted language), intuitive mechanisms to build user interfaces with sliders and buttons to control the simulation, and provides a fast visualisation engine. Fig. 5 shows the control panel and a two- and three-dimensional visualisation window.

Our bacterial sender and receiver agents are simulated within a virtual two-dimensional world. This discrete world consists of squares (so-called patches) over which the agents (so-called turtles) move. Patches can hold information, such as the concentration of AHSL and aspartate, which are visualised by different colours. The intensity of a colour reflects the actual concentration values; the higher a concentration, the darker is its representing colour patch. AHSL and aspartate are deposited onto

Fig. 1 *Our iGEM 2006 Musketeers*

Left to right: Sebastian von Mammen, Patrick King, Dr. Christian Jacob, and Vladislav Lavrovsky

patches by the sender agents. Subsequently, both AHSL and aspartate undergoes diffusion across the patches.

A light source, which illuminates the whole simulation world, can be switched on and off by a button. As shown in Fig. 6, light-activated, immobile sender cells are represented in red. Motile receiver cells are originally black, but turn white, passing through green, proportional to how much AHSL they detect on a patch. Once a receiver agent steps on a patch with a positive aspartate value, it consumes a certain amount (which can be set through a slider in the graphical user interface) of that patches aspartate.

Senders can be arranged in three different configurations: (a) along a small circle (Fig. 6), (b) in a cross-like fashion (Fig. 7), or (c) randomly distributed (Fig. 8). The visualisation window can display the senders and receivers, the AHSL or aspartate concentrations.

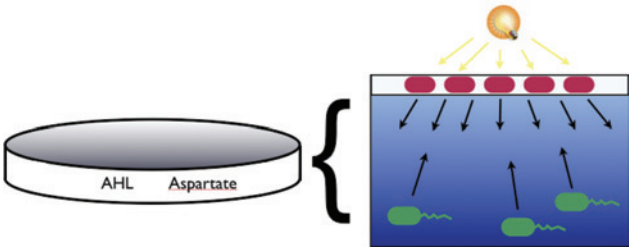

Fig. 3 *Basic idea*

Sender cells (in red) are activated by light. After activation, senders emit both AHSL and aspartate. Receiver cells (in green) move towards higher aspartate concentrations, which attracts them to the senders. After detection of AHSL, receivers express GFP

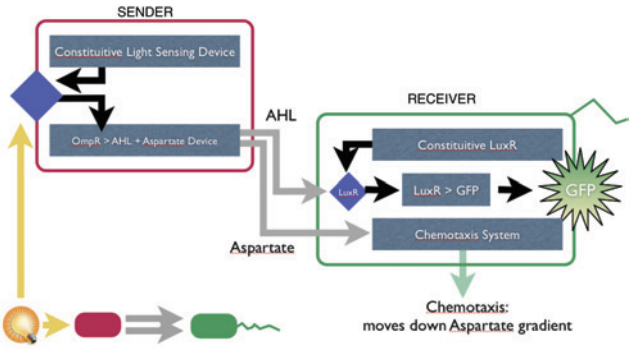

Fig. 4 *Schematic of the design for the sender and receiver cells*

3.2 The Wetware design

The final *in vitro* constructs were built using iGEM BioBricks from the MIT Registry, following our assembly plan in Fig. 9. PCR was used to produce workable quantities of the DNA as the part plasmids would often be low copy number and thus resulted in insufficient yields after plasmid purification. The BioBrick parts are contained on plasmids with ampicillin and/or kanamycin resistance

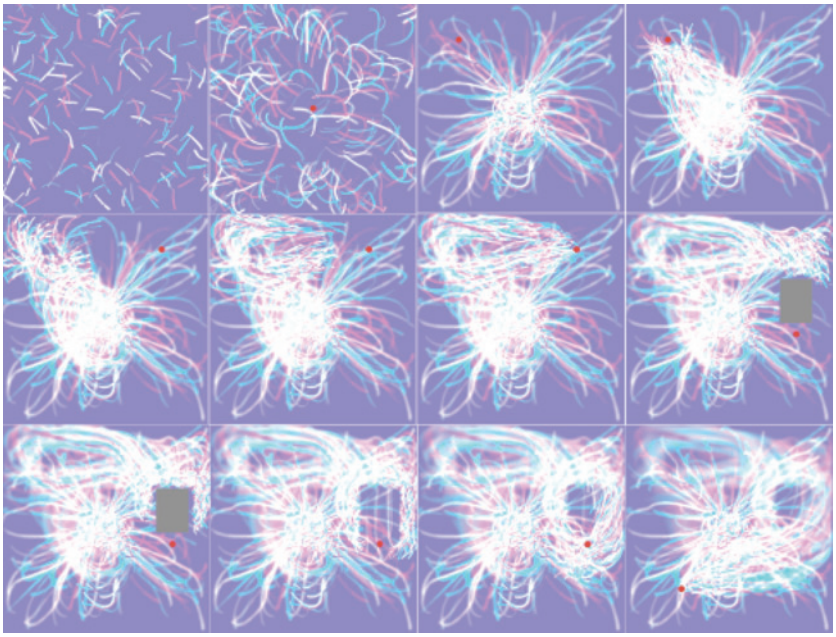

Fig. 2 *Snapshots of a computer simulation of swarm painters*

Painter agents are attracted towards the red dot, the position of which is controlled by a user. The painters leave trails of different colours behind, which eventually fade into the background. Obstacles, such as the grey rectangle, are engulfed by the swarms and reclaimed in case the object is removed

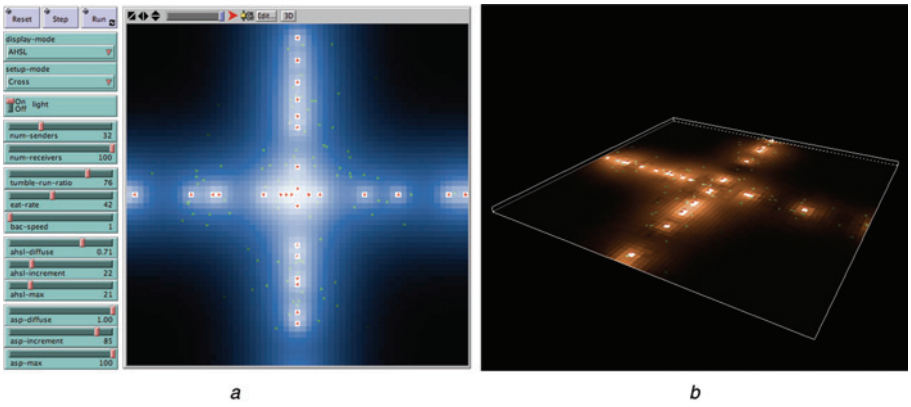

Fig. 5 *Details of the user interface components for our E. coli simulator built in NetLogo*

a Simple but effective interface to control parameter settings, as well as reset, start-stop, and step through the simulation
b 3D view of the simulation

markers and standardised upstream and downstream multiple cloning sites. We designed primers which are directly flanking these sites and thus work for any part in the registry. The primers are far enough outside the multiple cloning site to allow efficient digestion.

The typical construction went as follows. Two parts are selected and were amplified by PCR with Platinum Taq Polymerase (Invitrogen) using the generic primers. The part lengths were verified using 1% agarose electrophoresis to ensure that the correct product was produced, they were also quantified using a NanoDrop spectrophotometer; parts were diluted to equal concentrations. The left part was then digested on the right flank with Spe I and the right part was digested with Xba I. Ligating the products together results in a permanent construct which has sites for neither of the restriction endonucleases Xba I and Spe I in between the two parts. This allows the addition of parts both upstream and downstream using the same method. In this way, part

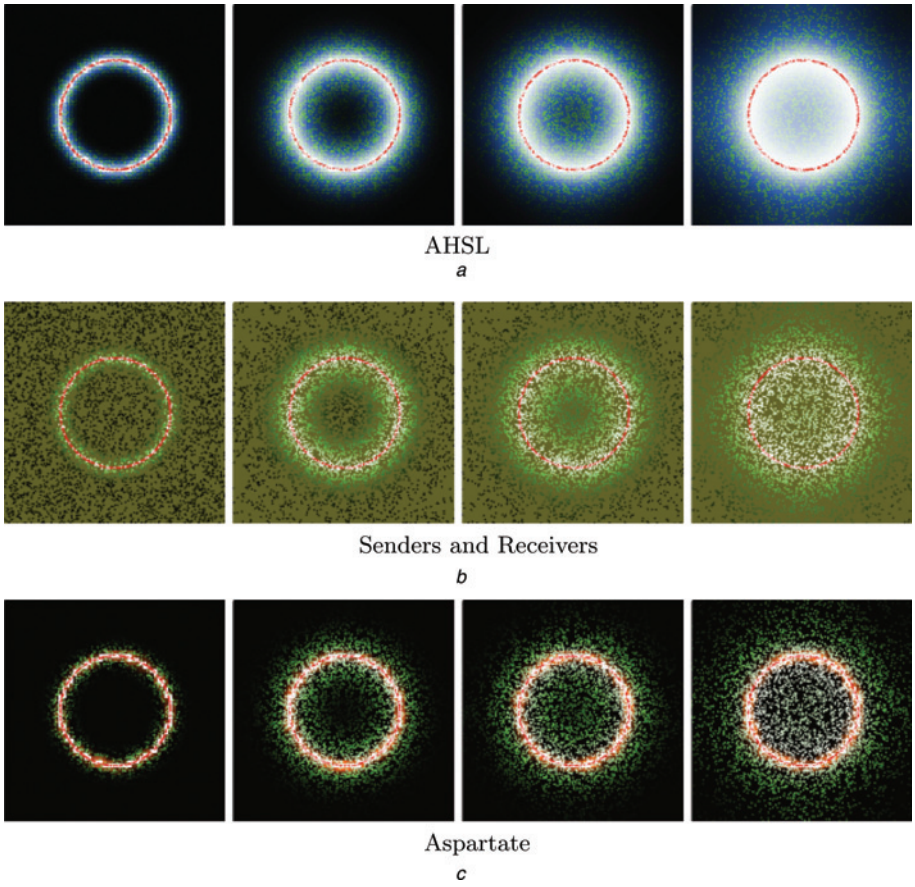

Fig. 6 *Circular arrangement of sender cells*

1000 sender cells, 5000 receiver cells
Snapshots are taken at the following iteration time steps: 0, 10, 22, 36, 46, 59, 74, 92, 117, 148, 187, 235, 295, and 370
a AHSL
b Senders and receivers
c Aspartate

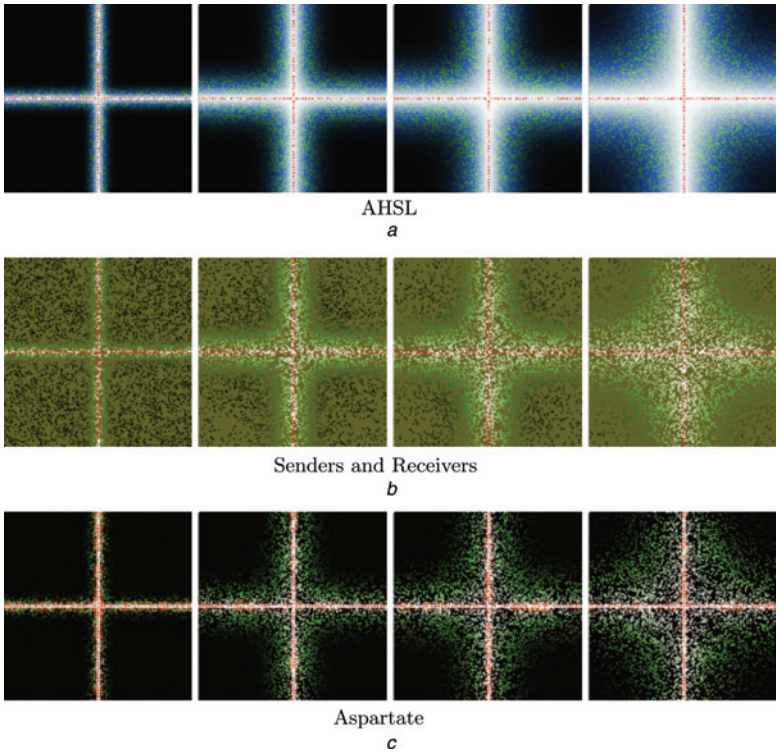

Fig. 7 *Cross arrangement of sender cells*

1000 sender cells, 5000 receiver cells
Snapshots are taken at the following iteration time steps: 0, 10, 22, 36, 46, 59, 74, 92, 117, 148, 187, 235, 295, and 370
a AHSL
b Senders and receivers
c Aspartate

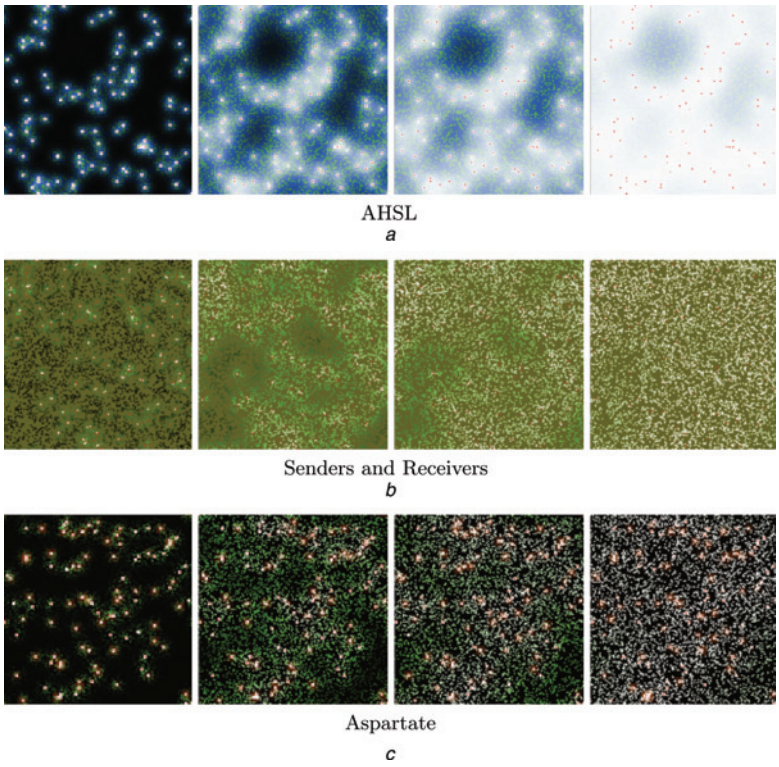

Fig. 8 *Random arrangement of sender cells*

Number of sender cells was reduced to 100
5000 receiver cells
Snapshots are taken at the following iteration time steps: 0, 10, 22, 36, 46, 59, 74, 92, 117, 148, 187, 235, 295, and 370
a AHSL
b Senders and receivers
c Aspartate

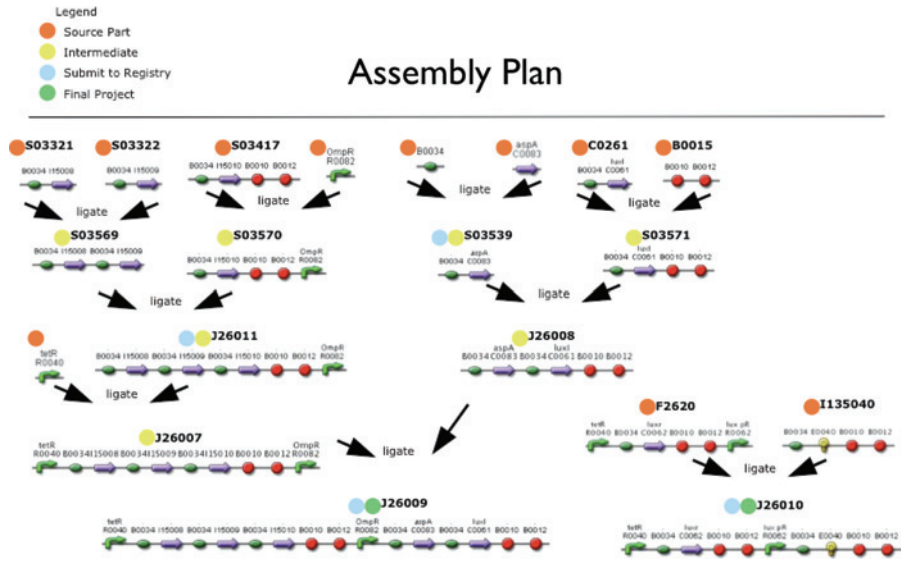

Fig. 9 *Plan for the assembly of parts necessary to implement the senders and receivers*

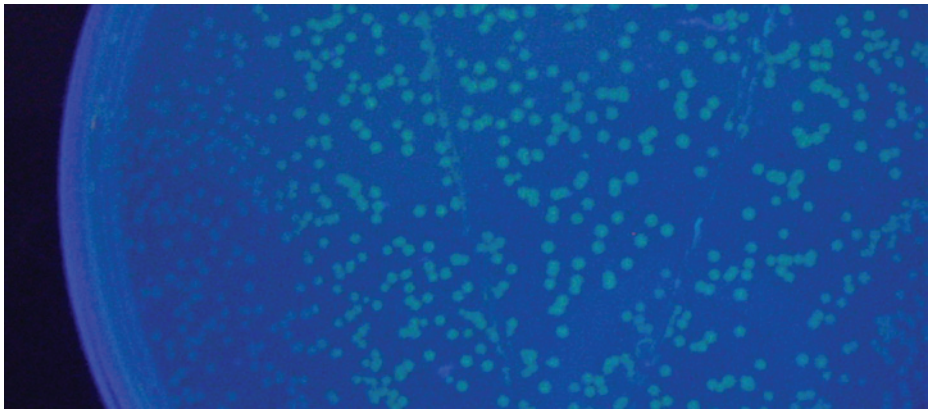

Fig. 10 *AHSL gradient*

of the intended device was constructed. Part F2620, a constitutively expressed luxR gene, and a lux promoter was combined with part I13504, which is a GFP.

Thus, whenever AHL is present, GFP is expressed. In Fig. 10 this is demonstrated by adding a small quantity of AHSL (Sigma) in the middle of the plate. As the AHSL diffuses, GFP expression is induced.

4 Lessons learnt . . .

We lost a bit of our momentum through the summer, especially when it came down to actually applying the creative designs in the wet lab. Three of us, however, kept the project afloat and were dedicated to bring it to a successful end – although this proved to be much harder than anticipated due to the time-consuming and error-prone nature of wet lab experiments. But this is part of the lessons to be learnt. Dealing with reprogrammed bacteria is not precision engineering, nor is it as straightforward as computer programming. We were quite anxious when it came to present our project at the iGEM Jamboree. To our great surprise, our team won the First Place in the Conquest of Adversity category, which recognised our team's effort to bring this project to a successful end. The current stage provides the first step for an expansion of the Dancing Swarm Bacteria for 2007.

5 Conclusion

The work done has set the stage for further work in the field. The use of PCR has so far been successful but there is still concern that many rounds of amplification can introduce mutations. Thus the method will be validated by serial amplification of a large part (2000 bp) for as many cycles as would be required to make the proposed construct. If after 30 cycles there are few or no mutations, it would be safe to continue to use PCR instead of isolating plasmid DNA. In the future we intend to add novel parts as well as devices into the registry, as well as build on our first-year experience to build more sophisticated genetically engineered machines.

6 Acknowledgments

We would like to thank Phillipa Sessini and Boris Shabash for their contributions to our project. We particularly thank Lisa Allen for supporting us in the lab.

7 References

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2 Wilensky, U.: 'NetLogo', available at: http://ccl.northwestern.edu/netlogo/