Molecular and Cellular Computing

Lecture series at Universidad Politécnica de Madrid

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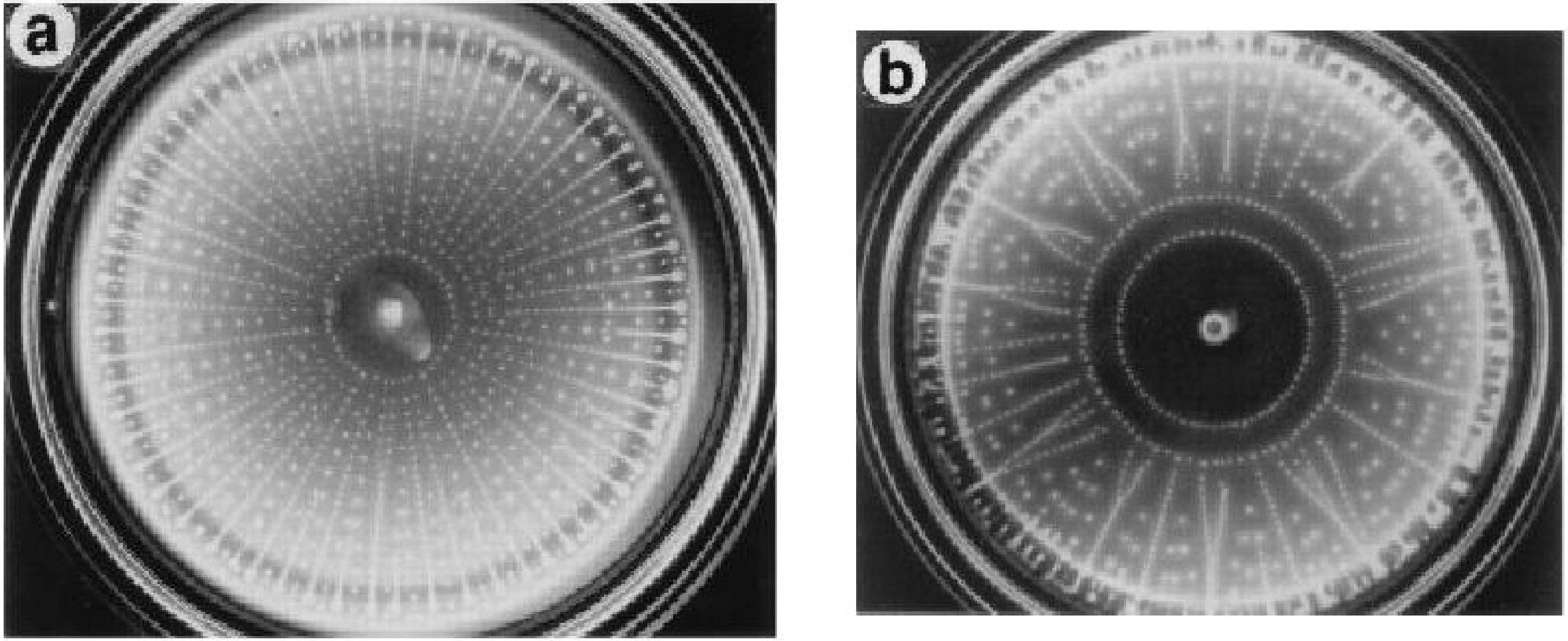
Day 3: Biological Engineering
3. Synthetic Biology II

Engineered Communication

- Towards the end of his life, Alan Turing did some foundational work on *pattern formation* in nature, in an attempt to explain how zebras get their striped coats or leopards their spots
- The study of *morphogenesis* is concerned with how cells split to assume new roles and communicate with another to form very precise shapes, such as tissues and organs
- Turing postulated that the diffusion of chemical signals both *within* and *between* cells is the main driving force behind such complex pattern formation

A.M. Turing. The chemical basis of morphogenesis. *Phil. Trans. Roy. Soc* B **237**:37-72, 1952

Pattern Formation in Bacteria



Howard Berg

Signalling

- Although Turing's work was mainly concerned with the processes occurring amongst cells inside a developing embryo, it is clear that chemical signalling also goes on between *bacteria*
- Ron Weiss (Princeton) particularly interested in Vibrio fischeri, a bacterium that has a symbiotic relationship with a variety of acquatic creatures, including the Hawaiian squid
- This relationship is due mainly to the fact that the bacteria exhibit *bioluminescence* – they generate Luciferase (coded by the *Lux* gene), a version of which is also found in fireflies, and which causes them to glow when gathered together in numbers

Quorum Sensing

- Cells within the primitive light organs of the squid draw in bacteria from the seawater and encourage them to grow
- Once enough bacteria are present in the light organ they produce a signal to tell the squid cells to stop attracting, and only then do they begin to glow
- The mechanism by which the *Vibrio* “know” when to start glowing is known as quorum sensing, since there have to be sufficient “members” present for luminiscence to occur

Quorum Sensing

- The bacteria secrete an autoinducer molecule, known as VAI (Vibrio Auto Inducer), which diffuses through the cell wall
- The *Lux* gene (which generates the glowing chemical) needs to be activated (turned on) by a particular protein which attracts the attention of the polymerase – but the protein can only do this with help from the VAI
- The concentration of VAI is absolutely crucial; once a critical threshold has been passed, the bacteria “know” that there are enough of them present, and they begin to glow

Harnessing Quorum Sensing

- Weiss realised that this quorum-based cell-to-cell communication mechanism could provide a powerful framework for the construction of bacterial devices
- Possible application: a tube of solution containing engineered bacteria that can be added to a sample of seawater, causing it to glow only if the concentration of a particular pollutant exceeds a certain threshold

Harnessing Quorum Sensing

- Weiss set up two colonies of E. coli, one containing “sender”, and the other “receivers”
- The idea was that the senders would generate a chemical signal made up of VAI, which could diffuse across a gap and then be picked up by the receivers
- Once a strong enough signal was being communicated, the receivers would glow using GFP to say that it had been picked up
- Weiss cloned the appropriate gene sequences into his bacteria, placed colonies of receiver cells on a plate, and the receivers started to glow in acknowledgment

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Circuit Evolution

- In late 2002, Weiss and colleagues examined how rigourous engineering principles may be brought to bear on the problem of designing and building entirely new genetic circuitry
- “Biological circuit engineers will have to confront their inability to predict the precise behavior of even the most simple synthetic networks, a serious shortcoming and challenge for the design and construction of more sophisticated genetic circuitry in the future.”

Yohei Yokobayashi, Ron Weiss, and Frances H. Arnold. Directed evolution of a genetic circuit. *Proc. Natl. Acad. Sci. USA* **99**(26):16587-16591, 2002

Circuit Evolution

- Two stage stategy: first, design a circuit from the bottom up, a la Elowitz, and clone it into bacteria
- Such circuits are highly unlikely to work first time, “because the behavior of biological components inside living cells is highly context-dependent, the actual circuit performance will likely differ from the design predictions, often resulting in a poorly performing or nonfunctional circuit.”
- Rather than simply abandoning their design, decided to then tune the circuit *inside* the cell itself, by applying the principles of *evolution*

A “Real” Genetic Algorithm?

- By inducing mutations in the DNA that they had just introduced, they were able to slightly modify the behaviour of the circuit that it represented
- Many of these changes would be catastrophic, but, occasionally, they observed a minor improvement
- In that case, they kept the “winning” bacteria, and subjected them to another round of mutation, in a repeated cycle
- In a microcosmic version of Darwinian evolution, mutation followed by selection of the fittest took an initially unpromising pool of broken circuits and transformed them into working constructs

Commentary

- "Ron is utilizing the power of evolution to design networks in ways so that they perform exactly the way you want them to" Jim Collins
- In a commentary article in the same issue of the journal, Jeff Hasty called this approach "design then mutate"
- "The approach we have outlined should serve as a robust and widely applicable route to obtaining circuits, as well as new genetic devices, that function inside living cells."

Jeff Hasty. Design then mutate. *Proc. Natl. Acad. Sci. USA* (PNAS), **99**(26):16516-16518, 2002.

Space

- Problem of *space* – specifically, how to get a population of bacteria to cover a surface with a specific density
- Could be useful when designing bacterial bio-sensors – devices that detect chemicals in the environment and produce a response
- By controlling the density of the microbial components, it might be possible to tune the sensitivity of the overall device
- More importantly, the ability for cells to control their own density would provide a useful “self-destruct” mechanism were they ever to be released into the environment for “real world” applications

Space

- Weiss *et al* built on their previous results to demonstrate the ability to keep the density of an E. coli population artificially low – below the “natural” density that could be supported by the available nutrients
- Designed a genetic circuit that caused the bacteria to generate a *different Vibrio* signalling molecule; a sufficient concentration would flip a switch inside the cell, turning on a killer gene, encoding a protein that was toxic in sufficient quantities

Lingchong You, Robert Sidney Cox III, Ron Weiss, and Frances H. Arnold. Programmed population control by cell-cell communication and regulated killing. *Nature*, **428**:868-871, 2004

Space

- The culture grew at an exponential rate for seven hours, before hitting the defined density threshold
- At that point population dropped sharply, as most cells were killed
- Population settled at a steady density significantly (ten times) lower than an unmodified "control" colony.
- "The population-control circuit lays the foundations for using cell-cell communication to programme interactions among bacterial colonies, allowing the concept of communication -regulated growth and death to be extended to engineering synthetic ecosystems."

Pattern Formation

- Programme cells to form *specific* spatial patterns in the dish
- Generally achieved using some form of chemical signalling, combined with a differential response
- Different cells, although genetically identical, may “read” the environmental signals and react in different ways, depending on their internal state

Pattern Formation

- Used a variant of the sender-receiver model, only this time adding a "distance detection" component to the receiver circuit
- Senders were placed in the centre of the dish, and the receivers distributed uniformly across the surface
- Receivers constructed so that they could measure the strength of the signal from the senders, which decays over distance
- Cells were engineered so that only those that were either "near" to the senders or "far" from the senders would generate a response (those in the middle region were instructed to do nothing)

Subhayu Basu, Yoram Gerchman, Cynthia H. Collins, Frances H. Arnold, and Ron Weiss. A synthetic multicellular system for programmed pattern formation. *Nature*, **434**:1130-1134, 2005

Pattern Formation

- Cells are genetically identical, and uniformly distributed over the surface – the differential response comes in the way that they assess the strength of the signal, and make a decision on whether or not to respond
- Power of the system was increased further by making the “near” cells glow green, and those “far away” glow red (using a different fluorescent protein)

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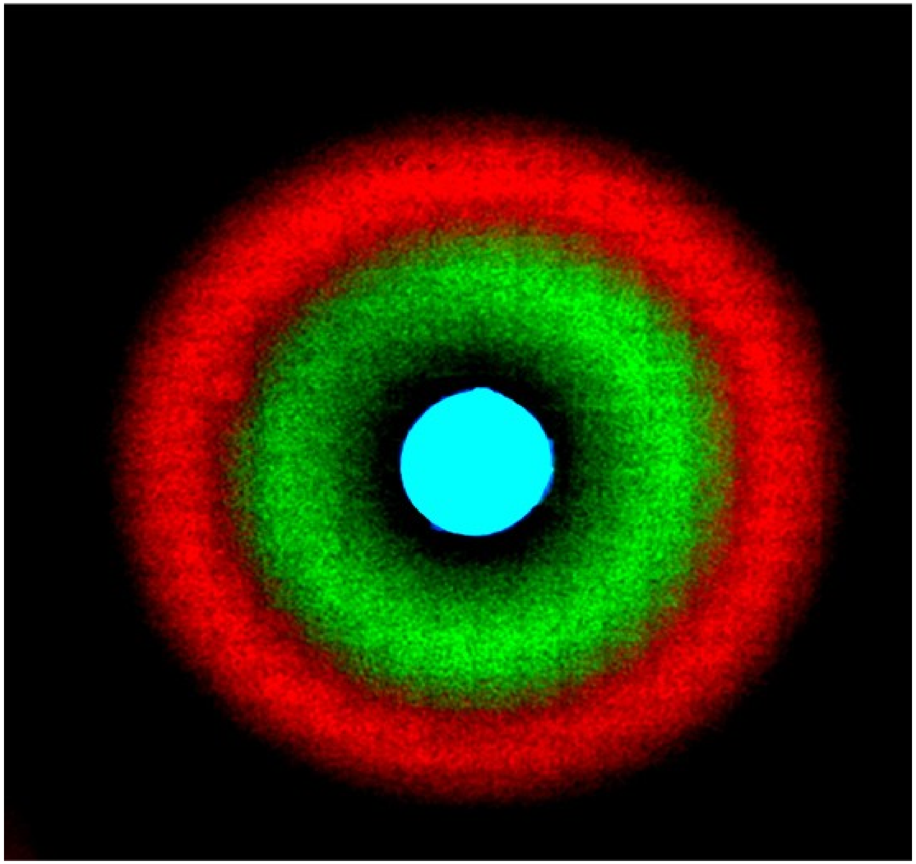

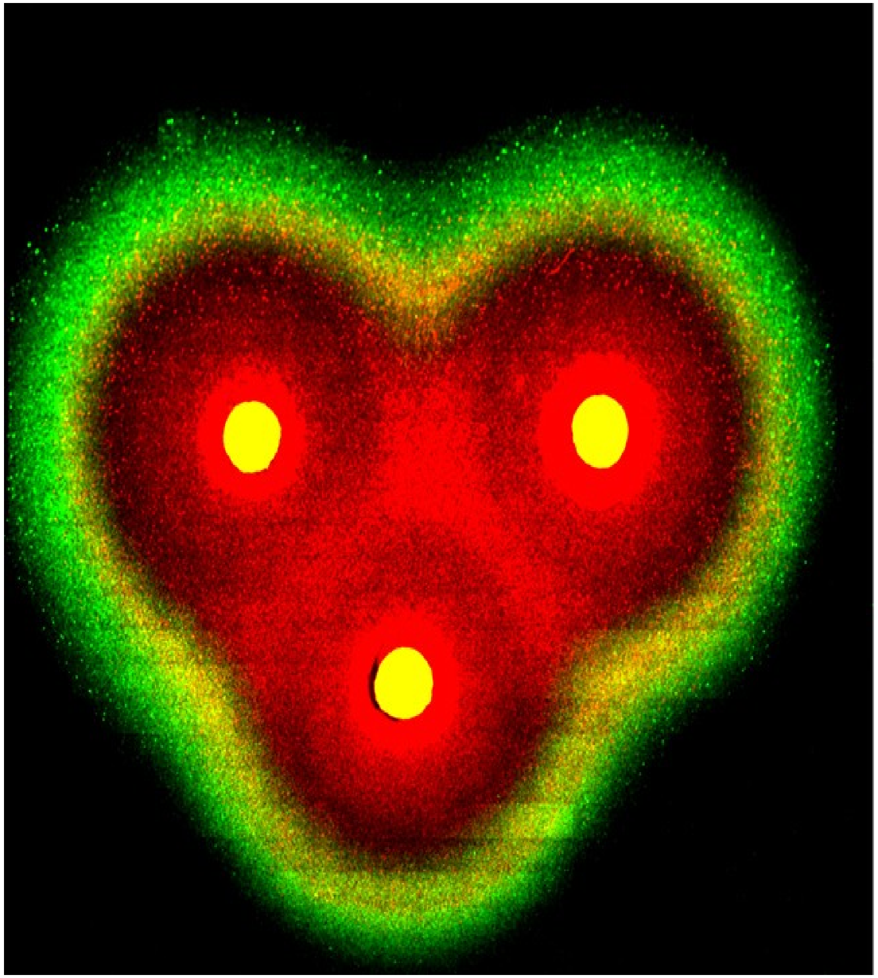

Ron Weiss

Bacterial Camera

- Rather than *generating* light, a different team decided to use bacteria to *detect* light
- The world's first microbial camera
- By engineering a dense bed of E. coli, a team of students led by Chris Voight at Berkeley developed light-sensitive "film" capable of storing images at a resolution of 100 megapixels per square inch
- E. coli are not normally sensitive to light, so they took genes coding for photoreceptors from blue-green algae, and spliced them in
- When light was shone on the cells, it turned on a genetic switch that cause a chemical inside them to permanently darken, thus generating a black "pixel"
- By projecting an image onto a plate of bacteria, were able to obtain several monochrome images

Anselm Levskaya et al. Engineering *Escherichia coli* to see light. *Nature*, **438**:441-442, 2005

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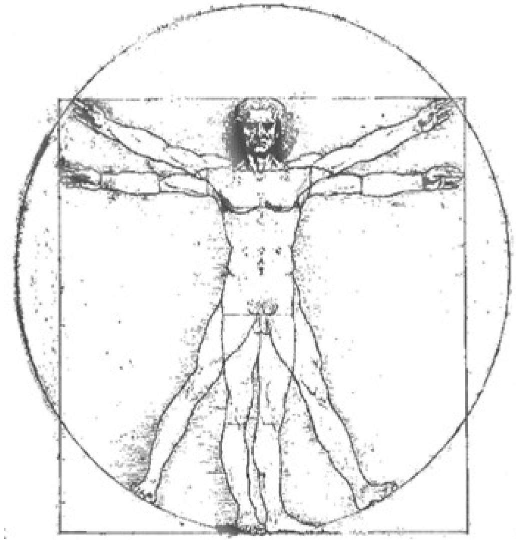
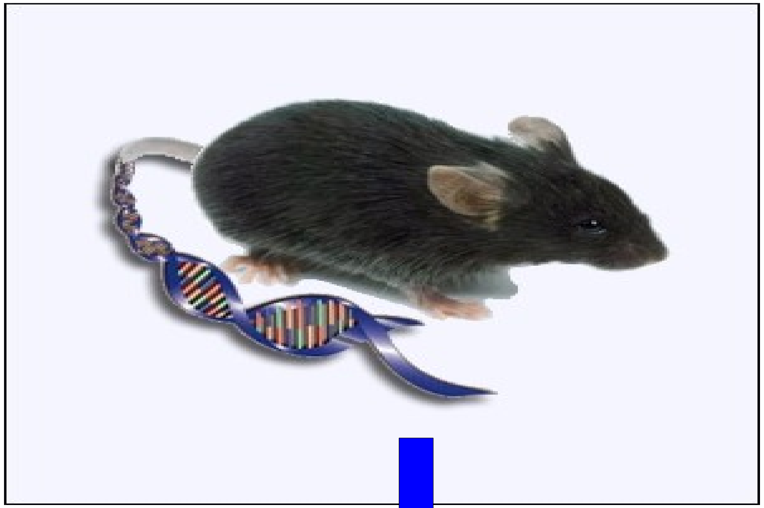
Living photograph: Scientists at UCSF and U. of Texas have been creating living photographs by genetically engineered microbes to become "biological film." Bacteria exposed to light produce dark pigment, while those in the dark do not.

Credit: Aaaron Chevalier (UT-Austin)





Bio-bricks

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“Our team have designed and modelled an bio-sensor that can detect detect several different concentrations of arsenic and emit a pH signal in response. The device can detect the WHO guideline level of 10 ppb and the Bangladeshi standard of 50 ppb for arsenic in drinking water. A proof of concept Bio-brick construct has shown a pH response to a concentration of arsenic of 5 ppb”

THE UNIVERSITY OF EDINBURGH 2006 iGEM

Future Directions

- “The integration of such systems into higher-level organisms and with different cell functions will have practical applications in three-dimensional tissue engineering, biosensing, and biomaterial fabrication.” Ron Weiss
- In addition to building structures, others are engineering cells to act as miniature drug delivery systems – fighting disease or infection from the inside
- Adam Arkin and Chris Voight currently investigating the use of modified it *E. coli* to fight cancer tumours
- Jay Keasling and co-workers at Berkeley are looking at engineering circuits into the same bacteria to persuade them to generate a potent antimalarial drug that is normally found in small amounts in wormwood plants

Postscript

"I think that synthetic biology...will be as important to the 21st century as [the] ability to manipulate bits was to the 20th." Roger Brent

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