Molecular and Cellular Computing

Lecture series at Universidad Politécnica de Madrid

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Day 3: Biological Engineering
2. Synthetic Biology

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Synthetic Biology

- “Where top down meets bottom up”
- “The work on restriction nucleases not only permits us easily to construct recombinant DNA molecules and to analyze individual genes, but also has led us into the new era of *synthetic biology* where not only existing genes are described and analyzed but also new gene arrangements can be constructed and evaluated.” Waclaw Szybalski, Nobel prizes and restriction enzymes (1978). *Gene* **4**: 181-2

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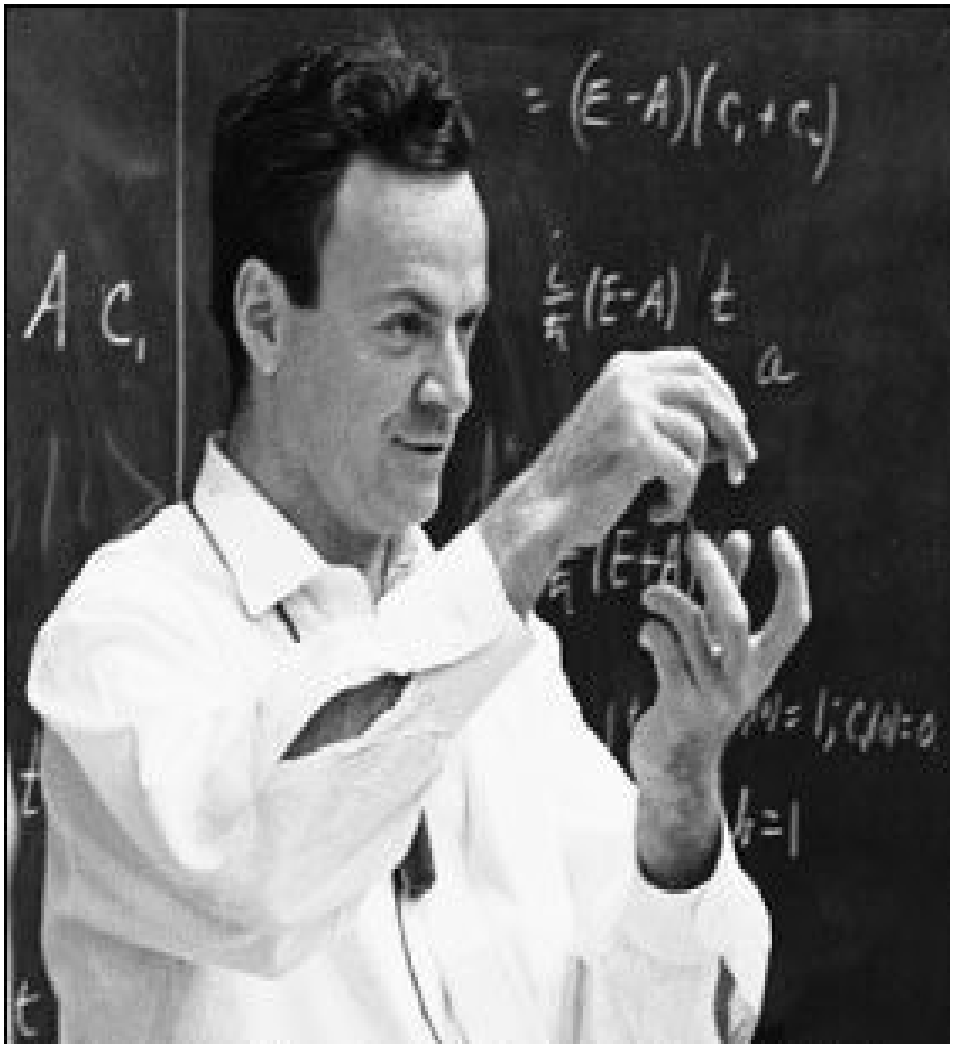
Synthetic Biology



"What I cannot create,
I cannot understand"

– Richard P. Feynman

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Synthetic Biology

"*Biology* is the nanotechnology that *works*."

- Tom Knight, MIT

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build, only smaller

smaller

smaller

smaller

smaller

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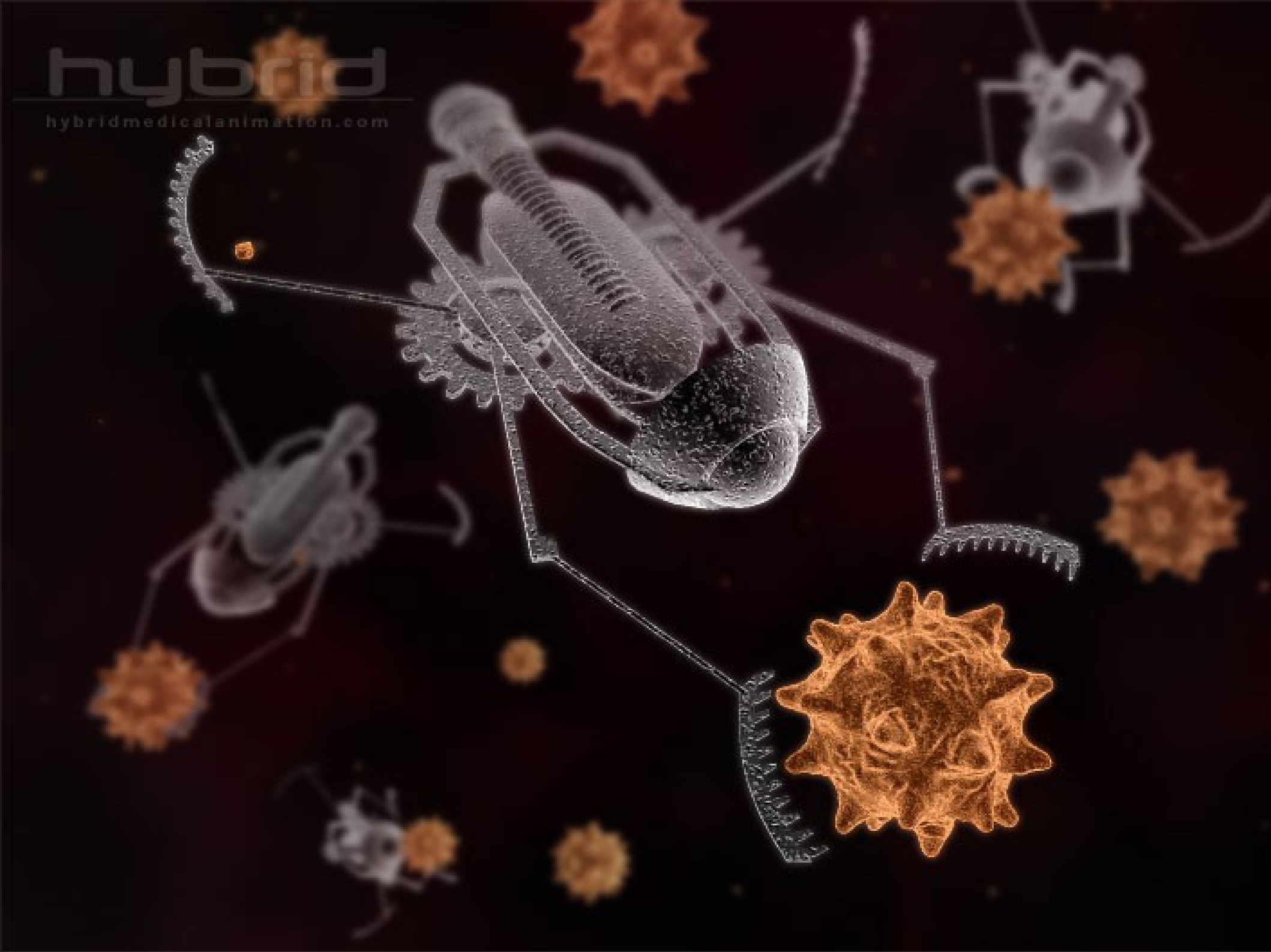
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hybridmedicalanimation.com


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copy existing designs

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US Centennial of
Flight Commission

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Definitions

- An emerging field at the intersection of biology, computer science, chemistry, physics, mathematics, ... that aims to design and build novel biological systems (and therefore understand them better)
- Eventually, we would like to do this in the same way as engineers design and build mechanical or electronic systems
- Hierarchical and modular features allow biological systems to be understood, and make SB possible

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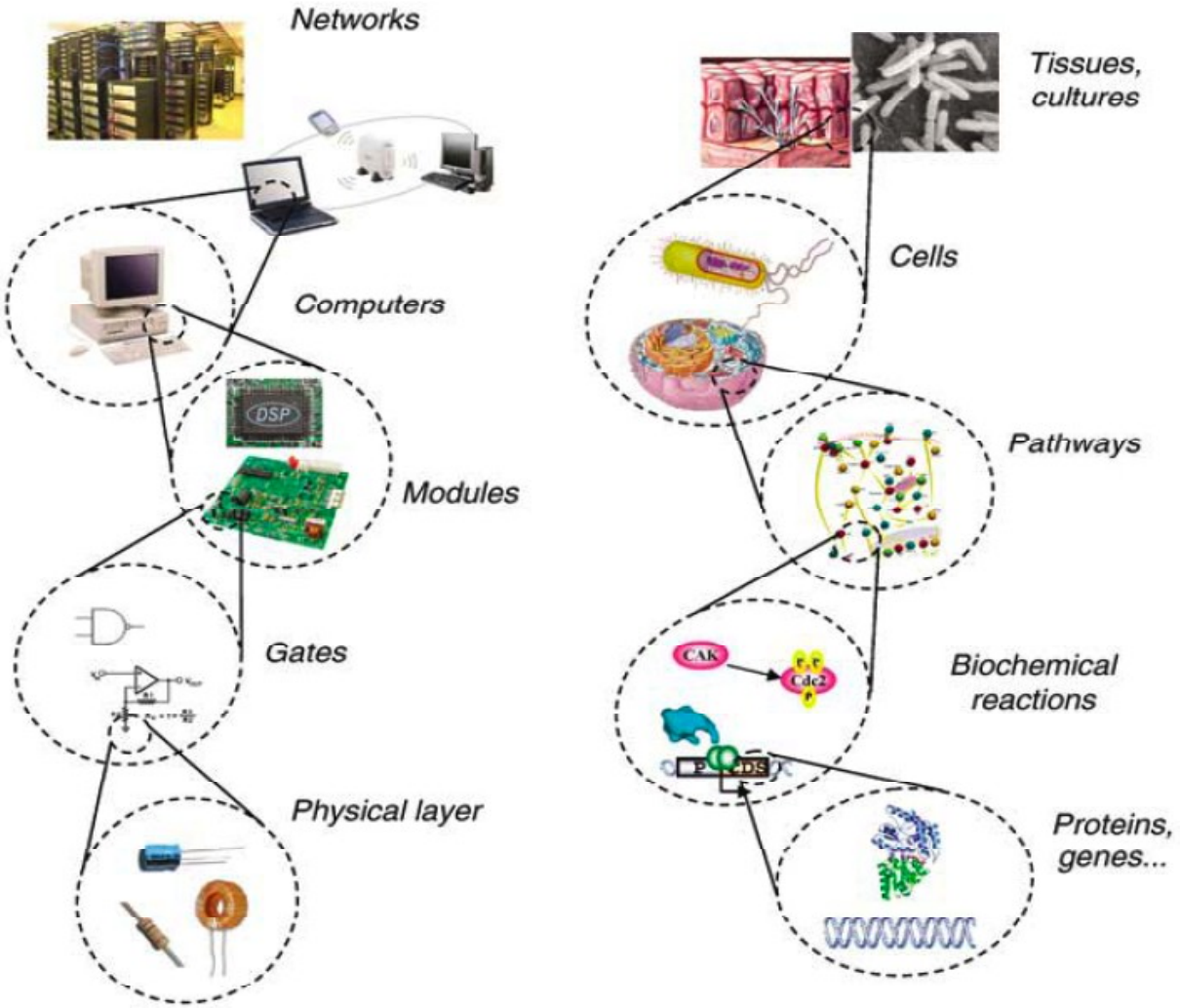
Possible Metaphor



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Why use Cells?

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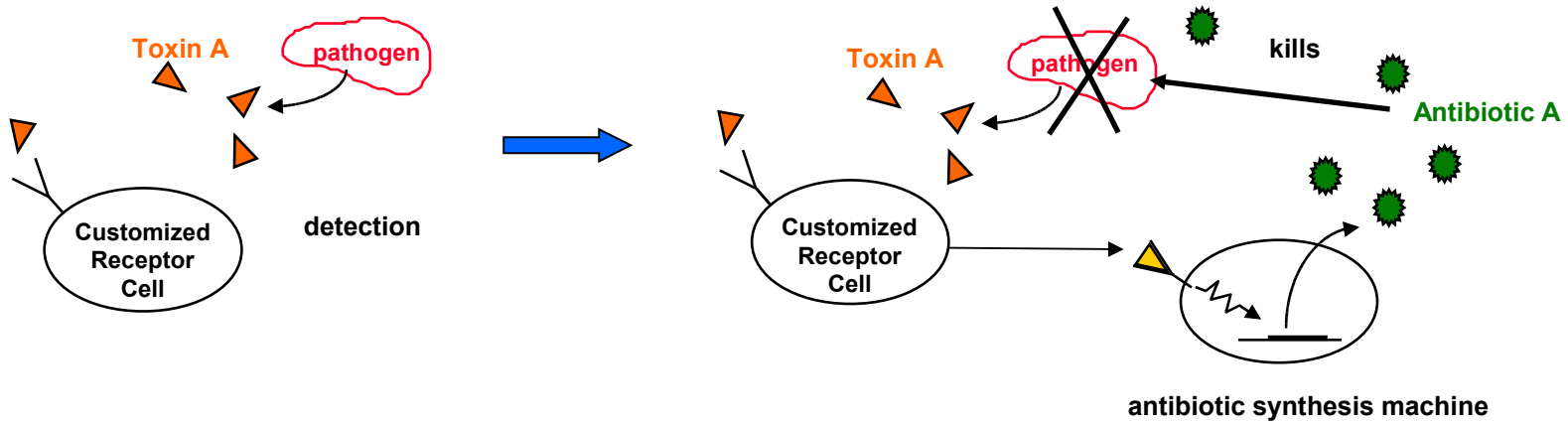
Why use Cells?

- Unique features:
  - small, self-replicating, energy-efficient
- Purposes:
  - Biomedical applications
  - Environmental applications (sensors & effectors)
  - Embedded systems
  - Interface to chemical world
  - Molecular scale engineering

Microbial Robotics

- Potential to engineer behavior into bacterial cells:
  - phototropic or magnetotropic response
  - control of flagellar motors
  - chemical sensing and engineered enzymatic release
  - selective protein expression
  - molecular scale fabrication
  - collective behavior
    - autoinducers
    - slime molds
    - pattern formation
- Example: timed drug-delivery in response to toxins



Ron Weiss

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Genetic Process Engineering - Dry

- Methodology for modifying the DNA encoding of existing genetic elements to achieve desired input/output behaviour for constructing reliable circuits of significant complexity
- Construct library of well-characterised (understood) genes, with their inputs and outputs defined – circuit components
- Take a circuit for a given task (eg. if-then-else clause) and map it onto the component library

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Genetic Process Engineering - Wet

- Then clone the required genes into your target organism
- May take many months, but then have limitless supply
- Choose one or more output genes to yield detectable signal
- Cell development and metabolism simulates the circuit

Laboratory Implementations

- Elowitz and Leibler described the construction of an oscillator network that causes colony of *E. coli* to periodically flash – oscillation cycle slower than reproduction cycle, showing that oscillation state was transmitted from one generation to the next

M.B. Elowitz and S. Leibler, A synthetic oscillatory network of transcriptional regulators, *Nature* **403**:335—338, 2000

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Elowitz and Leibler

- Elowitz and Leibler succeeded in constructing an artificial genetic oscillator in cells, using a synthetic network of repressors called the *repressilator*)
- Rather than investigating existing oscillator networks, decided to build one entirely from first principles
- Chose three repressor-promoter pairs that had already been sequenced and characterised, and first built a mathematical model in software
- Identified from their simulation results certain molecular characteristics of the components that gave rise to so-called limit cycle oscillations; those that are robust to perturbations

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Elowitz and Leibler

- This information from the model's results lead them to select strong promoter molecules and repressor molecules that would rapidly decay
- In order to implement the oscillation, they chose three genes, each of which affected one of the others by repressing it, or turning it off
- For the sake of illustration, we call the genes A, B and C.
- The product of gene A turns off (represses) gene B. The absence of B (which represses C) allows C to turn on. C is chosen such that it turns gene A off again
- The three genes loop continuously in a "daisy chain" effect, turning on and off in a repetitive cycle

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Elowitz and Leibler

- However, some form of reporting is necessary in order to confirm that the oscillation is occurring as planned
- Green fluorescent protein (GFP) is a molecule found occurring naturally in the jellyfish Aequorea victoria
- When placed under ultraviolet light, it glows
- Biologists quickly sequenced the gene responsible for producing this protein, as they realised that it could have many applications as a reporter

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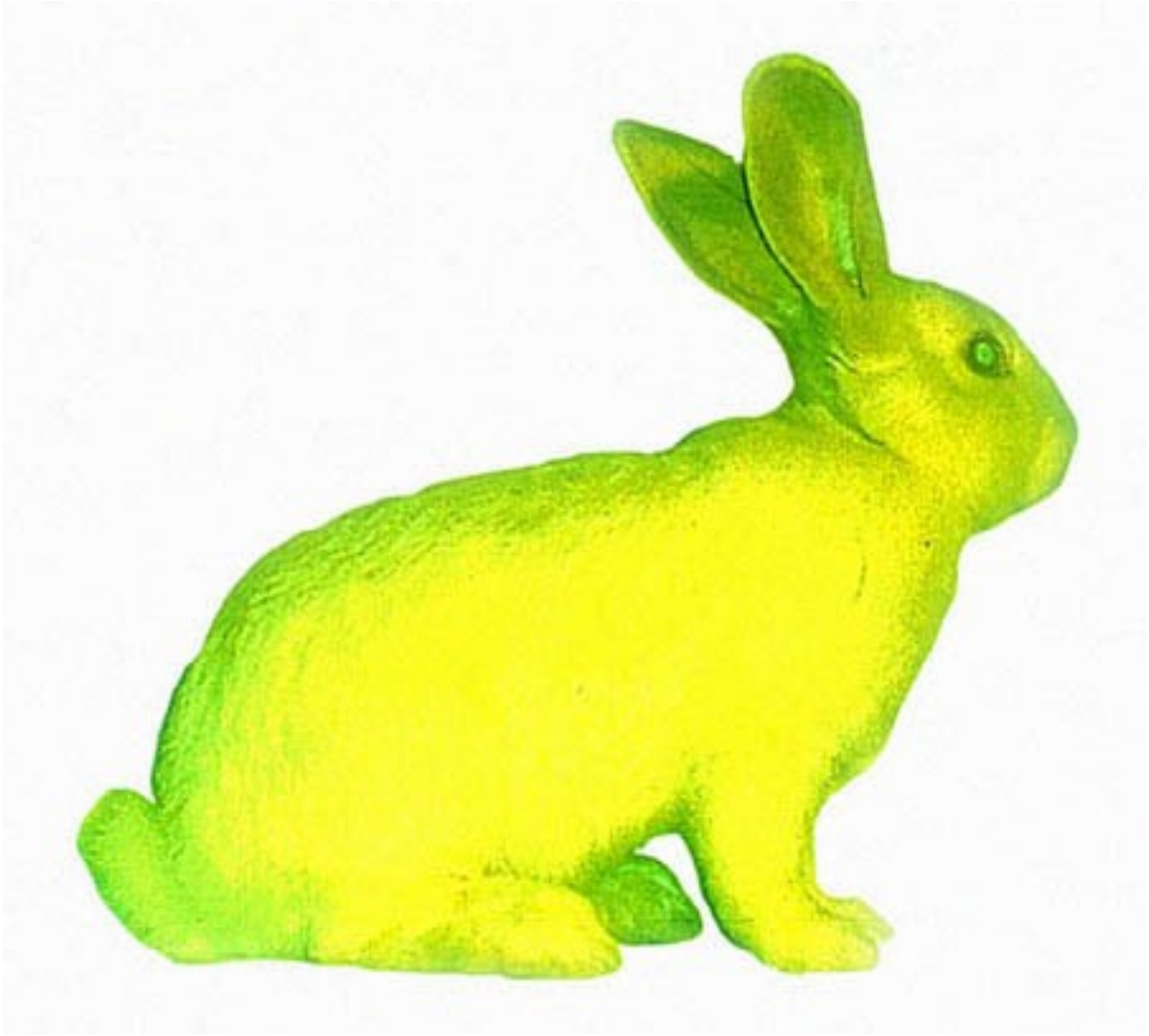

Henry Art Gallery

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Elowitz and Leibler

- Elowitz and Leibler set up their gene network so that the GFP gene would be expressed whenever gene C was turned off
- When it was turned on, the GFP would gradually decay and fade away
- They synthesised the appropriate DNA sequences and inserted them into a plasmid, eventually yielding a population of
- bacteria that blinked on and off in a repetitive cycle
- Moreover, and perhaps most significantly, the period between flashes was longer than the time taken for the cells to divide, showing that the state of the system had been passed on during reproduction

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Toggle Switch

- In the same issue, Gardner *et al.* describe the construction of a genetic toggle switch that is flipped from one state to another by either chemical or heat induction – molecular memory

T.S. Gardner, C.R. Cantor and J.J. Collins, Construction of a genetic toggle switch in *Escherichia coli*, *Nature* **403**:339—342, 2000

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Toggle Switch

- Rather than model an existing circuit and then altering it, Elowitz and Leibler had taken a “bottom up” approach to learning about how gene circuits operate
- In 2000, Gardner, Collins and Cantor observed that genetic switching had not yet been “demonstrated in networks of non-specialised regulatory components”
- That is to say, at that point nobody had been able to construct a switch out of genes that hadn’t already been “designed” by evolution to perform that specific task

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Toggle Switch

- The team had a similar philosophy to that of Elowitz and Leibler, in that their main motivation was being able to test theories about the fundamental behaviour of gene regulatory networks.
- “Owing to the difficulty of testing their predictions, these theories have not, in general, been verified experimentally.
- Here we have integrated theory and experiment by constructing and testing a synthetic, bistable [two-state] gene circuit based on the predictions of a simple mathematical model.”

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Toggle Switch

- Chose two genes that were mutually inhibitory – that is, each produced a molecule that would turn the other off
- One important thing to note is that the system didn't have a *single* input
- Although they acknowledged that bistability *might* be possible using only a single promoter that regulated itself, they anticipated possible problems with robustness and experimental tunability

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Toggle Switch

- Instead, used a system whereby each “side” of the switch could be “pressed” by a different stimulus – the addition of a chemical on one, and a change in temperature on the other
- If the system was in the state induced by the chemical, it would stay in that state until the temperature was changed, and would only change back again if the chemical was reintroduced
- Importantly, these stimuli did not have to be applied continuously – a “short sharp” burst was enough to cause the switch to flip over
- Also used GFP as the system state reporter, so that the cells glowed in one state, and looked “normal” in the other

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Toggle Switch

- “As a practical device, the toggle switch...may find applications in gene therapy and biotechnology.”
- “Finally, as a cellular memory unit, the toggle forms the basis for ‘genetic applets’ – self-contained, programmable, synthetic gene circuits for the control of cell function.”