Digitally Programmed Cells

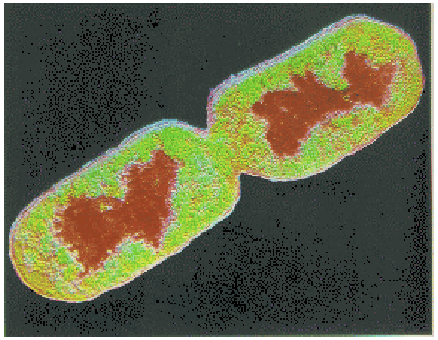
Ron Weiss

PI: Tom Knight

MIT Artificial Intelligence Laboratory

Goal

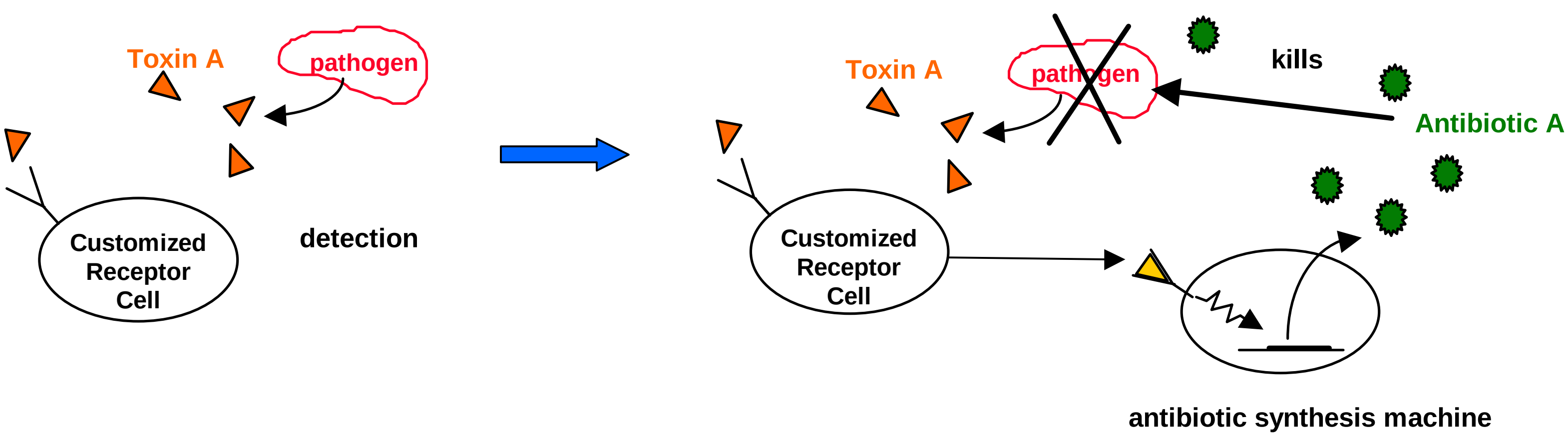
- Process-Control Cellular Computers -- Microbial Robotics



- 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
  - selective binding to membrane sites
  - collective behavior
    - autoinducers
    - slime molds
    - pattern formation
- Example: timed drug-delivery in response to toxins



A New Engineering Discipline

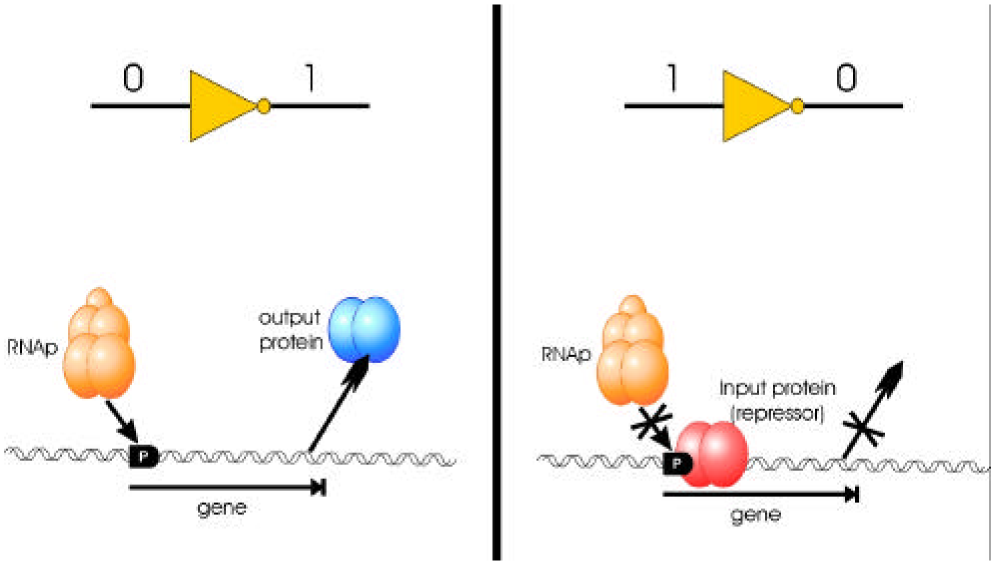
- System design:
  - interfaces to sensors
  - *in-vivo* logic circuits
  - interfaces to actuators
- Strategy: reuse and modify existing mechanisms
  - characterize, then combine control elements
  - modify elements to generate large component libraries
  - implement transgenic signalling pathways for I/O

Outline

- Implementing in-vivo computation
- Experimental effort
- System design methodology
- Programming Cooperative behavior
- Challenges

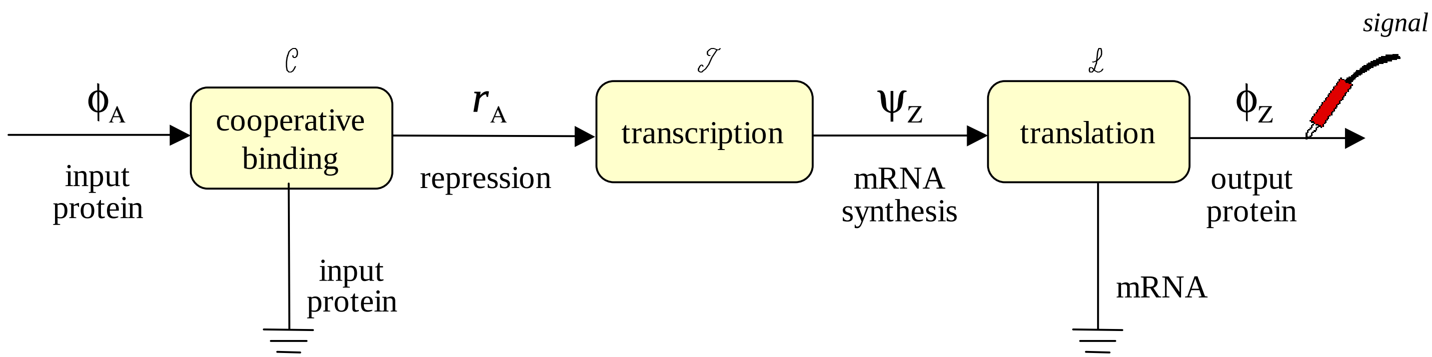
Implementing the Digital Abstraction

- *In-vivo* digital circuits:
  - signal = concentration of specific protein
  - computation = regulated protein synthesis + decay
- The basic computational element is an **inverter**



☞Allows building any (complex) digital circuit in individual cells!

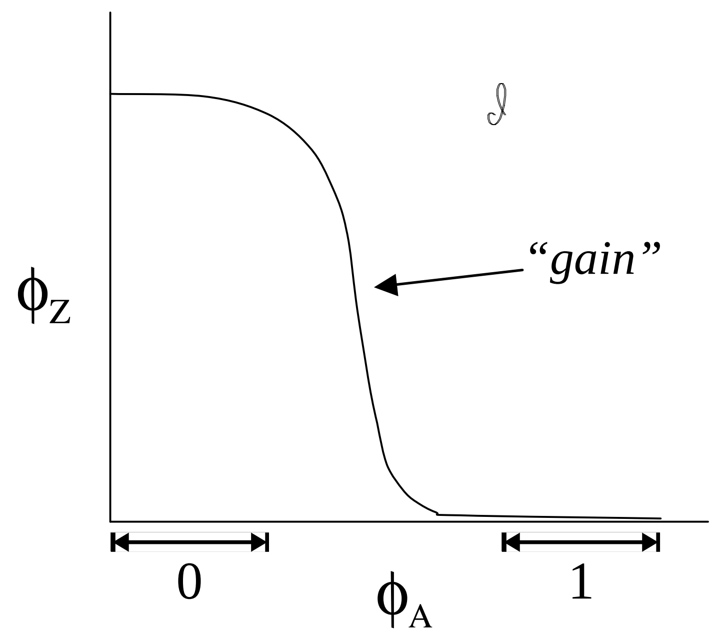
Inverter Characteristics



- inversion relation $\mathcal{I}$:

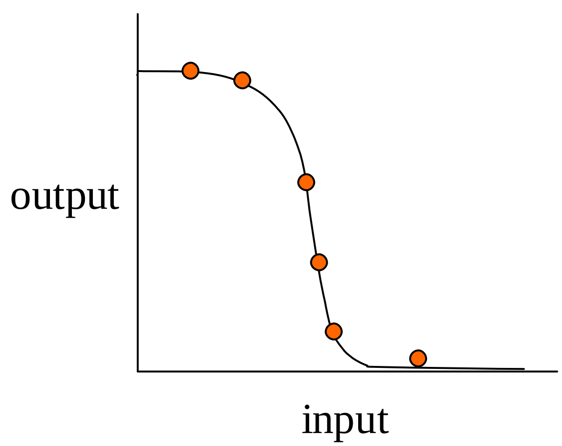
$$\boxed{\phi_Z = \mathcal{I}(\phi_A) = \mathcal{L} \circ \mathcal{T} \circ \mathcal{C}(\phi_A)}$$

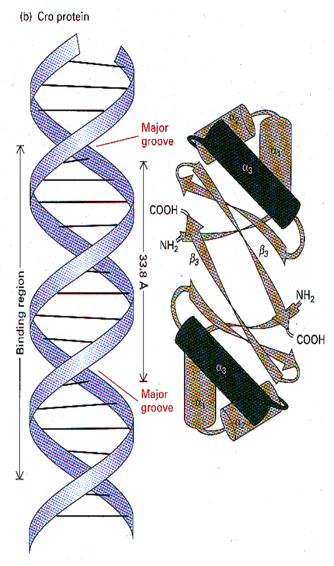
- "ideal" transfer curve:
  - gain (flat,steep,flat)
  - adequate noise margins



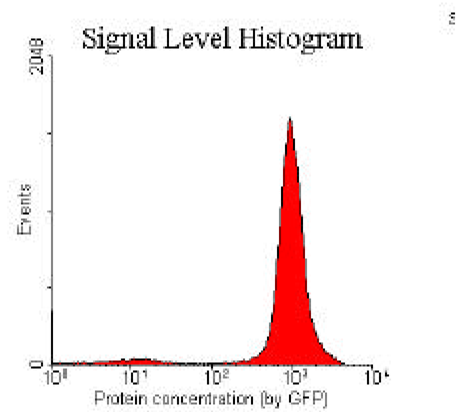
Experimental Effort

- First, characterize several inverters
  - genes from Lambdoid phages (cI, P_R)
  - measure points on the transfer function



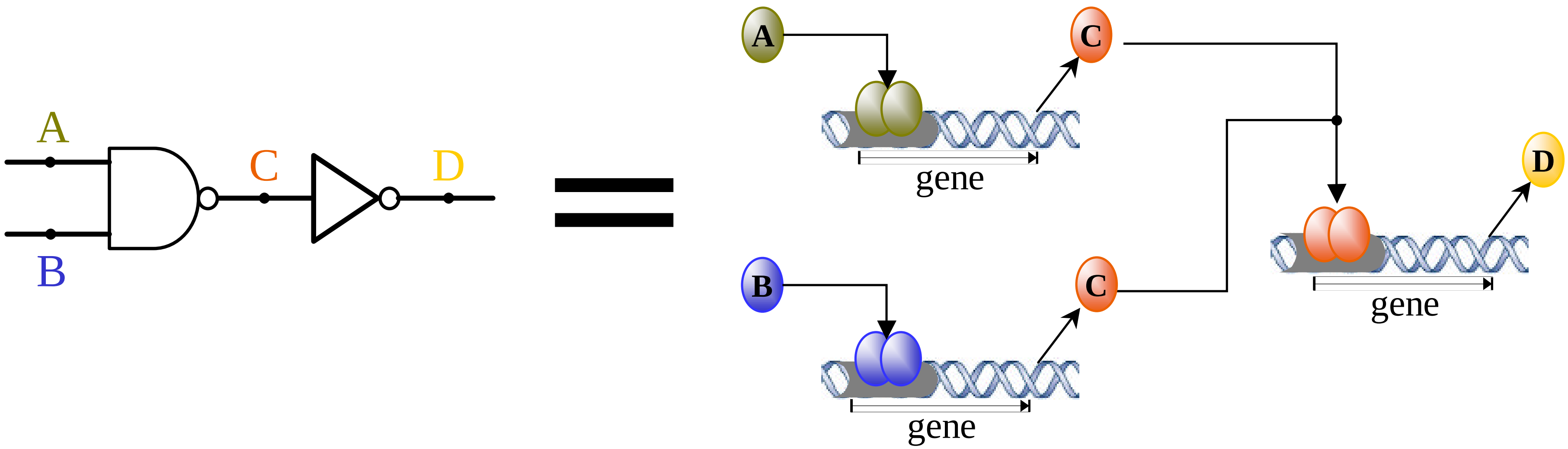


- Typical fluctuations in signal levels:
  - constitutive expression of GFP with a synthetic promoter



Digital Circuits

- With properly designed inverters, any (finite) digital circuit can be built

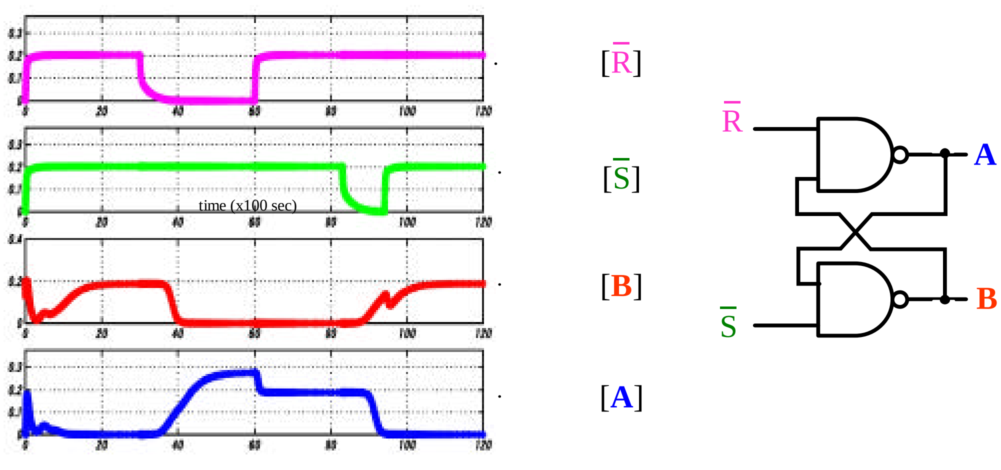


- proteins are the wires, genes are the gates
- NAND gate = “wire-OR” of two genes

“Proof of Concept” Circuits

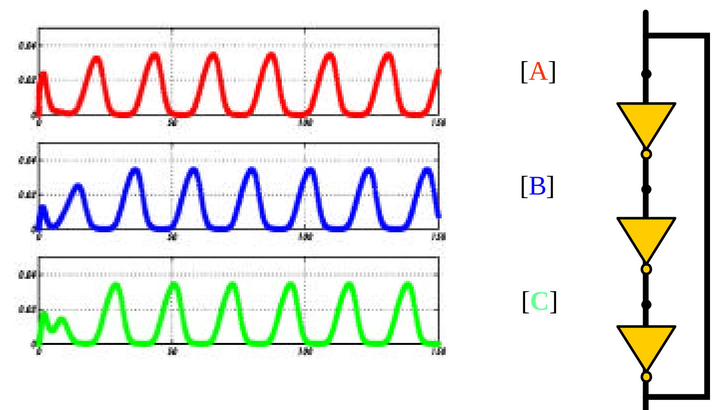
- Building several simple circuits
- Simulation results are promising:

RS-Latch (“flip-flop”)



time (x100 sec)

Ring oscillator



time (x100 sec)

BioCircuit Design (“TTL Data Book”)

- Data sheets for components
  - imitate existing silicon logic gates
  - new primitives from cellular regulatory elements
    - e.g. an inverter that can be “induced”
- Assembling a large library of components
  - modifications that yield desired behaviors
- Constructing complex circuits
  - matching gates is hard
  - need standard interfaces for parts

☞ *from black magic to “you can do it too”*

Naturally Occurring Sensor and Actuator Parts Catalog

Sensors

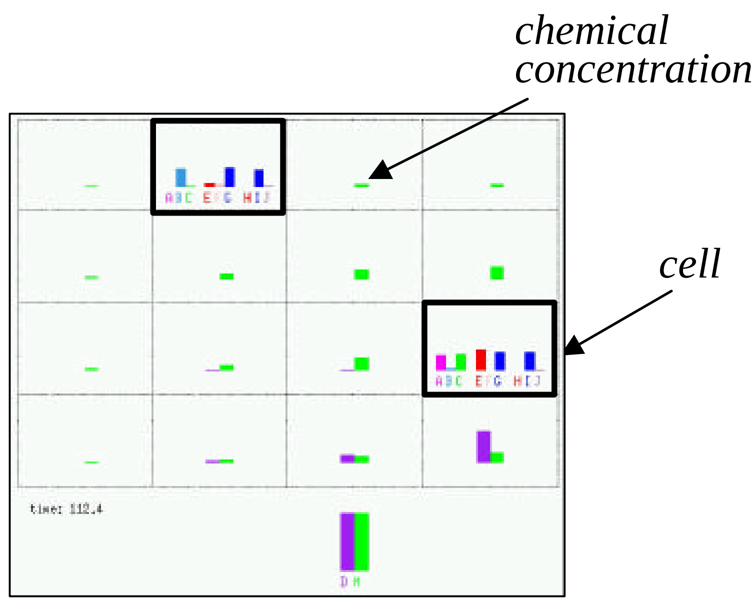
- Light (various wavelengths)
- Magnetic and electric fields
- pH
- Molecules
  - Ammonia
  - H2S
  - maltose
  - serine
  - ribose
  - cAMP
  - NO
- Internal State
  - Cell Cycle
  - Heat Shock
- Chemical and ionic membrane potentials

Actuators

- Motors
  - Flagellar
  - Gliding motion
- Light (various wavelengths)
- Fluorescence
- Autoinducers (intercellular communications)
- Sporulation
- Cell Cycle control
- Membrane transport
- Exported protein product (enzymes)
- Exported small molecules
- Cell pressure / osmolarity
- Cell death

Tools

- BioSpice – a prototype simulation & verification tool
  - simulates protein and chemical concentrations
  - intracellular circuits, intercellular communication



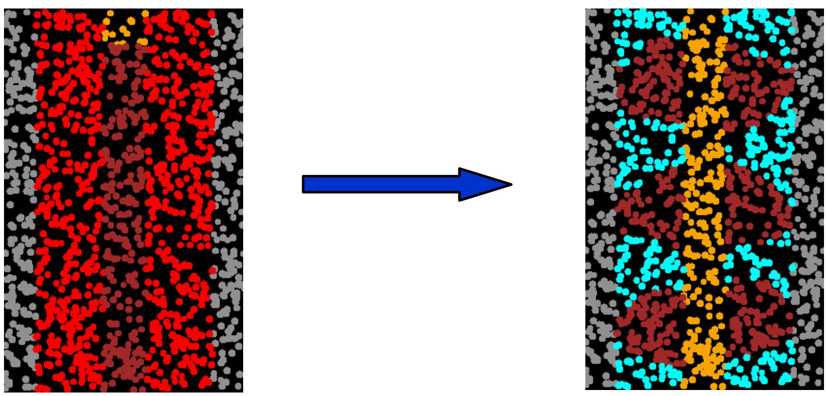
Simulation snapshot

Programming Cooperative Behavior

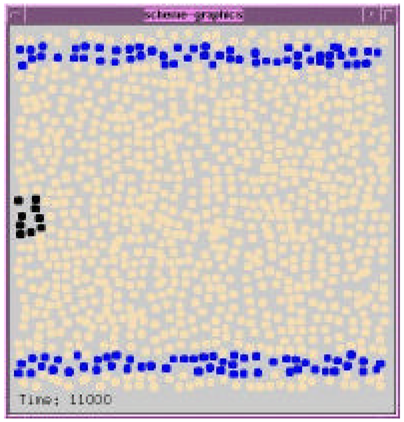
- Engineer loosely-coupled multicellular systems that display coordinated behavior
- Use localized cell-to-cell communications
- Robust programming despite:
  - faulty parts
  - unreliable communications
  - no global synchronization
- Control results in
  - Patterned biological behavior
  - Patterned material fabrication
  - Massively parallel computation with local communication
  - Suitable for problems such as physical simulation

High Level Programming

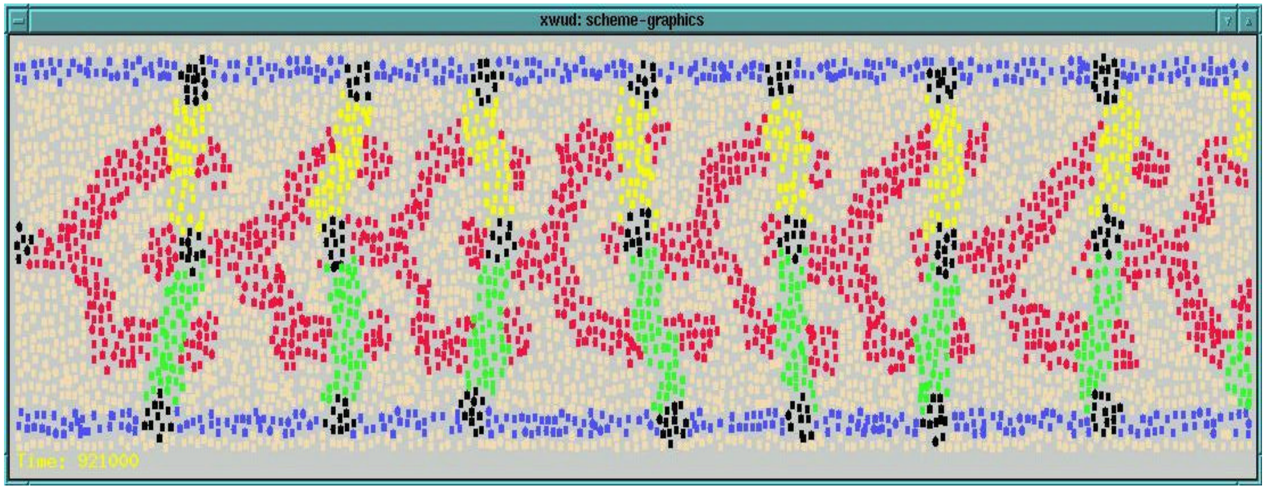
- Requires a new paradigm
  - colonies are amorphous
  - cells multiply & die often
  - expose mechanisms cells can perform reliably
- Microbial programming language
  - example: pattern generation using aggregated behavior



Pattern Formation in Amorphous Substrates



Example: forming a chain of "inverters" using only local communications



Limitations

- DNA Binding Protein Logic is Slow
  - milli Hertz (even with 10^{12} cells, still too slow)
- Limited number of intra- and inter-cellular signals
- Amount of extracellular DNA that can be inserted into cells
- Reduction in cell viability due to extra metabolic requirements
- We need a writeable long term storage

Challenges

- Engineer the system support for experimental cellular engineering into living cells
- Engineer component interfaces
- Develop instrumentation and modeling tools
  - Obtain missing data in spec sheet fields
  - Discover unknown fields in the spec sheet
- Create computational organizing principles
  - Invent languages to describe phenomena
  - Builds models for organizing cooperative behavior
- Create a new discipline crossing existing boundaries
  - Educate a new set of engineering oriented students