Objectives

• The promises of Molecular Programming:
  • In Science & Medicine
  • In Engineering
  • In Computing

• The current practice of Molecular Programming
  • DNA technology
  • Molecular languages and tools
  • Example of a molecular algorithm
The Hardware Argument

Smaller and smaller things can be built
Smaller and Smaller

**First working transistor**
John Bardeen and Walter Brattain, Dec. 23, 1947

**First integrated circuit**

50 years later

**25nm NAND flash**
Intel&Micron, Jan. 2010. ~50 atoms

**Single molecule transistor**
Observation of molecular orbital gating
*Nature*, 2009; 462 (7276): 1039

**Molecules on a chip**

~10 Moore’s Law cycles left!

Building the *Smallest* Things

- How do we build structures that are by definition smaller than your tools?
- Basic answer: you can’t. Structures (and tools) should build themselves!
- By *programmed self-assembly*

www.youtube.com/watch?v=Ey7Emmddf7Y
Molecular IKEA

- Nature can self-assemble. Can we?

- “Dear IKEA, please send me a chest of drawers that assembles itself.”

- We need a magical material where the pieces are pre-programmed to fit into each other.

- At the molecular scale many such materials exist...

Programmed Self-Assembly

Proteins

Membranes

DNA/RNA
The Software Argument

Smaller and smaller things can be programmed
We can program...

- Computers.
  - Completely!
We can program...

- Physical systems.
  - Completely!
    (Modulo sensors/actuators)
We can program...

- **Matter**
  - Completely and directly!
  - Currently: only DNA/RNA.

*It's like a 3D printer without the printer!*  
[Andrew Hellington]
What can we do with “just” DNA?

- Organize ANY matter [caveats apply]
- Execute ANY kinetics [caveats: up to time scaling]
- Build Nano-Control Devices
- Interface to Biology
Organizing Any Matter

- Use one kind of programmable matter (e.g. DNA).
- To organize (almost) ANY matter through it.

"What we are really making are tiny DNA circuit boards that will be used to assemble other components."

Greg Wallraff, IBM

6 nm grid of individually addressable DNA pixels

Executing Any Kinetics

- The kinetics of any finite network of chemical reactions, can be implemented (physically) with especially programmed DNA molecules.

- Chemical reactions as an executable programming language for dynamical systems!

DNA as a universal substrate for chemical kinetics

PNAS

David Soloveichik, Georg Seelig, and Erik Winfree
Building Nano-Control Devices

- All the components of nanocontrollers can already be built entirely and solely with DNA, and interfaced to the environment.
Interfacing to Biology

- A doctor in each cell

Fig. 1 Medicine in 2050: “Doctor in a Cell”
The Biological Argument

Biological systems are already ‘molecularly programmed’
Abstract Machines of Biology

- **Gene Machine**
  - Nucleotides
  - Regulation
  - Direct genome construction
  - Send signals
  - Hold receptors, host reactions

- **Protein Machine**
  - Amino acids
  - Make proteins
  - Enact fusion, fission
  - Metabolism, Propulsion
  - Signaling, Transport

- **Membran Machine**
  - Phospholipids
  - Enact fusion, fission
  - Confinement, Storage
  - Bulk Transport

- **Glycan Machine**
  - Surface and Extracellular Features

Biological Languages

Gene Machine

Protein Machine

Membrane Machine

Molecular Interaction Maps

Gene Networks

Transport Networks

A B C x y P Q

Biological Languages
But ...

- Biology is programmable, but (mostly) not by us!

- Still work in progress:
  - Gene networks are being programmed in synthetic biology, but using existing ‘parts’
  - Protein networks are a good candidate, but we cannot yet effectively design proteins
  - Transport networks are being looked at for programming microfluidic devices manipulating vesicles
Molecular Languages

... that we can execute
Action Plan

- Building a full software/hardware pipeline for a new fundamental technology
  - Mathematical Foundations [~ concurrency theory in the 80's]
  - Programming Languages [~ software engineering in the 70's]
  - Analytical Methods and Tools [~ formal methods in the 90's]
  - Device Architecture and Manufacturing [~ electronics in the 60's]

- To realize the potential of Molecular Programming

- “With no alien technology” [David Soloveichik]

- This is largely a ‘software problem’ even when working on device design
Our Assembly Language: Chemistry

- A Lingua Franca between Biology, Dynamical Systems, and Concurrent Languages

- Chemical Reaction Networks
  - A + B →, C + D (the program)

- Ordinary Differential Equations
  - d[A]/dt = -r[A][B] ... (the behavior)

- Rich analytical techniques based on Calculus

- But prone to combinatorial explosion
  - E.g., due to the peculiarities of protein interactions
Chemistry as a Concurrent Language

- A connection with the theory of concurrency
  - Via Process Algebra and Petri Nets

![Diagram showing connections between continuous and discrete chemistry, process algebra, and stochastic semantics.](image)
How do we “run” Chemistry?

• Chemistry is not easily executable
  • “Please Mr Chemist, execute me this bunch of reactions that I just made up”

• Most molecular languages are not executable
  • They are descriptive (modeling) languages

• How can we execute molecular languages?
  • With real molecules?
  • That we can design ourselves?
  • And that we can buy on the web?
Molecular Programming with DNA

Building the cores of programmable molecular controllers
The role of DNA Computing

• Non-goals
  • Not to solve NP-complete problems with large vats of DNA
  • Not to replace silicon

• Bootstrapping a carbon-based technology
  • To precisely control the organization and dynamics of matter and information at the molecular level
  • DNA is our engineering material
    • Its biological origin is “accidental” (but convenient)
    • It is an information-bearing programmable material
    • Other such materials will be (are being) developed
Domains

- Subsequences on a DNA strand are called **domains**
  - *provided* they are “independent” of each other

- Differently named domains must not **hybridize**
  - With each other, with each other’s complement, with subsequences of each other, with concatenations of other domains (or their complements), etc.
Short Domains

Reversible Hybridization

DNA double strand
Long Domains

Irreversible Hybridization
Strand Displacement

“Toehold Mediated”
Strand Displacement

Toehold Binding
Strand Displacement

Branch Migration
Strand Displacement

Displacement
Strand Displacement

Irreversible release
Bad Match
Bad Match
Bad Match
Bad Match

Cannot proceed
Hence will undo
Two-Domain Architecture

- Signals: 1 toehold + 1 recognition region

- Gates: “top-nicked double strands” with open toeholds

Garbage collection “built into” the gate operation
Plasmidic Gate Technology

• Synthetic DNA is length-limited
  • Finite error probability at each nucleotide addition, hence ~ 200nt max
• Bacteria can replicate plasmids for us
  • Loops of DNA 1000’s nt, with extremely high fidelity
  • Practically no structural limitations on gate fan-in/fan-out

Only possible with two-domain architecture
Transducer
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$

**Built by self-assembly!**

**ta** is a *private* signal (a different ‘a’ for each xy pair)
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$

Active waste
Transducer $x \rightarrow y$
So far, a **tx signal** has produced an **at cosignal**. But we want signals as output, not cosignals.
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$
Here is our output \textbf{ty signal}. But we are not done yet:
1) We need to make the output irreversible.
2) We need to remove the garbage.
We can use (2) to achieve (1).
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$
Transducer $x \rightarrow y$

Done.

N.B. the gate is consumed: it is the energy source
(no proteins, no enzymes, no heat-cycling, etc.; just DNA in salty water)
Transducer $x \rightarrow y$
Join $x + y \rightarrow z$
Tools and Techniques

A software pipeline for Molecular Programming
High(er)-Level Languages

- Gene Networks
  - Synchronous Boolean networks
    - Stewart Kauffman, etc.
  - Asynchronous Boolean networks
    - René Thomas, etc.

- Protein Networks
  - Process Algebra (stochastic $\pi$-calculus etc.)
    - Priami, Regev-Shapiro, etc.
  - Graph Rewriting (kappa, BioNetGen etc.)
    - Danos-Laneve, Fontana & al., etc.

- Membrane Networks
  - Membrane Computing
    - Gheorghe Păun, etc.
  - Brane Calculi
    - Luca Cardelli, etc.

- Waiting for an architecture to run on...
Molecular Compilation

Programs

“High-Level” Language

Intermediate Language

Boolean Networks

Petri Nets

Chemical Reaction Networks

Gates

4-domain Signals

3-domain Signals

2-domain Signals

Sequences

Architecture

Devices

Molecules
Development Tools
MSRC Bio Computation Group

Visual DSD
A Development Environment for DNA Strand Displacement

A programming language for composable DNA circuits
Andrew Phillips and Luca Cardelli
A Language for DNA Structures

- Describe the initial structures

```python
def T(N,x,y) -
    new a
    ( N; t^a 
    | N; y t^a 
    | N; t^a:[x t^a]:[a t^a]:[a] ("Input gate") 
    | N; [x]:[t^a y]:[t^a a]:t^a ("Output gate")
    )

( t^a x | T(1,x,y) )
```
Compute Species and Reactions

- Recursively computed from the initial structures
Reaction Graph and Export
Simulation

- Stochastic
- Deterministic
- "JIT"
State Space Analysis

INITIAL STATE:
- \( (1) \)
- \( (1) \)
- \( (1) \)
- \( (1) \)

TERMINAL STATE:
- \( (1) \)
- \( (1) \)
- \( (1) \)
- \( (1) \)
Modelchecking

- Export to PRISM probabilistic modelchecker
Verification

• Quantitative theories of system equivalence and approximation.
Related Work Supporter by our Tools

**Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades**

Lulu Qian and Erik Winfree

- Square root of a 4-bit number

**Neural network computation with DNA strand displacement cascades**

Lulu Qian, Erik Winfree, and John E. Bruck

- Associative memory
Execution

A software/wetware pipeline for Molecular Programming
Output of Design Process

- Domain structures
  - (DNA sequences to be determined)

“Ok, how do I run this for real”
From Structures to Sequences

NUPACK BETA nucleic acid package

DSD Structure → “Dot-Paren” representation

Output Sequences

Thermodynamic Synthesis

“Ok, where do I buy these?”

www.nupack.org
"DNA Synthesis"
From Sequences to Molecules

- Copy&Paste from nupack
“Ok, how do I run these?”
Add Water
Execute (finally!)

- Fluorescence is your one-bit ‘print’ statement
Output
Debugging

- A core dump
Delivery!

Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA
David Yu Zhang, et al.
Science 318, 1121 (2007);
DOI: 10.1126/science.1148532
A Molecular Algorithm

Running something interesting with DNA
Approximate Majority Algorithm

- Given two populations of agents (or molecules)
  - Randomly communicating by radio (or by collisions)
  - Reach an agreement about which population is in majority
  - By converting all the minority to the majority
    [Angluin et al., Distributed Computing, 2007]

- 3 rules of agent (or molecule) interaction
  - $X + Y \rightarrow B + B$
  - $B + X \rightarrow X + X$
  - $B + Y \rightarrow Y + Y$

“our program”
Surprisingly good (in fact, optimal)

- Fast: reaches agreement in $O(\log n)$ time w.h.p.
  - $O(n \log n)$ communications/collisions
  - Even when initially $#X = #Y!$ (stochastic symmetry breaking)

- Robust: true majority wins w.h.p.
  - If initial majority exceeds minority by $\omega(\sqrt{n \log n})$
  - Hence the agreement state is stable

Stochastic simulation of worst-case scenario with initially $#X = #Y$
DNA Implementation, at U.W.

- Programmable chemical controllers made from DNA
  [Yuan-Jyue Chen, Neil Dalchau, Niranjan Srinivas, Andrew Phillips, Luca Cardelli, David Soloveichik and Georg Seelig]

i. $X + Y \xrightarrow{k} 2B + PB$

\[
\begin{align*}
&x \rightarrow t, 
y \rightarrow y, 
&x'y \rightarrow xy, 
y' \rightarrow u1' \\
&X' \rightarrow t', 
y' \rightarrow t', 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&ig \rightarrow t, 
&b \rightarrow u3, 
&pb \rightarrow t, 
&b' \rightarrow u2 \\
&PB \rightarrow B, 
&PB' \rightarrow u2, 
&RQ \rightarrow ROX, 
&pb' \rightarrow u3' \\
&x \rightarrow t, 
y \rightarrow y, 
&x'y \rightarrow xy, 
y' \rightarrow u1' \\
&X' \rightarrow t', 
y' \rightarrow t', 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&ig' \rightarrow t', 
&b' \rightarrow u3', 
&pb' \rightarrow t', 
&b'' \rightarrow u2' \\
&PB' \rightarrow B', 
&PB'' \rightarrow u2', 
&RQ' \rightarrow ROX, 
&pb'' \rightarrow u3' \\
&x' \rightarrow t, 
y' \rightarrow y, 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&X'' \rightarrow t'', 
y'' \rightarrow t'', 
&x'y'' \rightarrow xy'', 
y'' \rightarrow u1'' \\
&ig'' \rightarrow t'', 
&b'' \rightarrow u3'', 
&pb'' \rightarrow t'', 
&b''' \rightarrow u2'' \\
&PB'' \rightarrow B'', 
&PB''' \rightarrow u2'', 
&RQ'' \rightarrow ROX, 
&pb''' \rightarrow u3'' \\
\end{align*}
\]

ii. $B + X \xrightarrow{k} 2X + PX$

\[
\begin{align*}
&x \rightarrow t, 
y \rightarrow y, 
&x'y \rightarrow xy, 
y' \rightarrow u1' \\
&B' \rightarrow b', 
&PX \rightarrow px, 
&px \rightarrow px' \\
&ig \rightarrow t, 
&x \rightarrow u3, 
&px \rightarrow px', 
&px' \rightarrow px'' \\
&B'' \rightarrow B', 
&PX' \rightarrow px, 
&px'' \rightarrow px''' \\
&x' \rightarrow t, 
y' \rightarrow y, 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&X' \rightarrow t', 
y' \rightarrow t', 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&ig' \rightarrow t', 
&x' \rightarrow u3', 
&px' \rightarrow px'', 
&px'' \rightarrow px''' \\
&B' \rightarrow B', 
&PX'' \rightarrow px, 
&px''' \rightarrow px''' \\
&x' \rightarrow t, 
y' \rightarrow y, 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&X'' \rightarrow t'', 
y'' \rightarrow t'', 
&x'y'' \rightarrow xy'', 
y'' \rightarrow u1'' \\
&ig'' \rightarrow t'', 
&x'' \rightarrow u3'', 
&px'' \rightarrow px''' \\
&B'' \rightarrow B'', 
&PX''' \rightarrow px, 
&px''' \rightarrow px''' \\
\end{align*}
\]

iii. $B + Y \xrightarrow{k} 2Y + PY$

\[
\begin{align*}
&x \rightarrow t, 
y \rightarrow y, 
&x'y \rightarrow xy, 
y' \rightarrow u1' \\
&Y' \rightarrow py, 
&Y' \rightarrow py', 
&PY \rightarrow py, 
&py \rightarrow py' \\
&ig \rightarrow t, 
&y \rightarrow u3, 
&py \rightarrow py', 
&py' \rightarrow py'' \\
&Y'' \rightarrow py, 
&PY' \rightarrow py, 
&py'' \rightarrow py''' \\
&x' \rightarrow t, 
y' \rightarrow y, 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&X' \rightarrow t', 
y' \rightarrow t', 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&ig' \rightarrow t', 
&y' \rightarrow u3', 
&py' \rightarrow py'', 
&py'' \rightarrow py''' \\
&Y' \rightarrow py, 
&PY'' \rightarrow py, 
&py''' \rightarrow py''' \\
&x' \rightarrow t, 
y' \rightarrow y, 
&x'y' \rightarrow xy', 
y' \rightarrow u1' \\
&X'' \rightarrow t'', 
y'' \rightarrow t'', 
&x'y'' \rightarrow xy'', 
y'' \rightarrow u1'' \\
&ig'' \rightarrow t'', 
&y'' \rightarrow u3'', 
&py'' \rightarrow py''' \\
&Y'' \rightarrow py, 
&PY''' \rightarrow py, 
&py''' \rightarrow py''' \\
\end{align*}
\]

\[X_0 = 0.7, \quad Y_0 = 0.3\]
Final Remarks
A Brief History of DNA

Turing Machine, 1936

DNA, -3,800,000,000

Transistor, 1947

Digital Computers

Computer programming

20th century

Software

systematic manipulation of information

<??>

every systematic manipulation of matter

DNA Algorithm, 1994

DNA Computers

Molecular programming

21st century

Structural DNA Nonotech, 1982
Acknowledgments

- Microsoft Research
  - Andrew Phillips, Biological Computation Group
- Caltech
  - Winfree Lab
- U.Washington
  - Seelig Lab
Questions?
Resources

• Visual DSD at MSR

• Molecular Programming Project at Caltech
  http://molecular-programming.org/

• Georg Seelig’s DNA Nanotech Lab at U.W. CS&E
  http://homes.cs.washington.edu/~seelig/