Molecular and Cellular Computing

Lecture series at Universidad Politécnica de Madrid

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Day 2: From *in vitro* to *in vivo*
2. Laboratory implementations

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Introduction

- We have seen several theoretical models of DNA computation
- Now we will introduce several laboratory implementations
- We begin with a theoretical model that has been successfully realised using molecules

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Tile Assembly Model

- A *constructive* model of DNA computation, based on the principle of *self-assembly*
- Molecular self-assembly gives rise to a vast number of complexes in nature, including crystals and the DNA double helix itself
- The growth of such structures is controlled, at a fundamental level, by *natural* computational processes

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TAM

- Due to Rothemund and Winfree, the TAM is a formal model for the assembly of complexes such as proteins or DNA on a square lattice
- Extends the theory of tiling by Wang tiles to encompass the physics of self-assembly
- Within the model, computations occur by the self-assembly of square tiles, each side of which may be labelled

P.W.K. Rothemund and E. Winfree. The program-size complexity of self-assembled squares. Proc. STOC 1999, pp. 459-468.

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Tile Labels

- Different labels represent ways in which tiles may bind together, the strength (or “stickiness”) of the binding depending on the *binding strength* associated with each *side*
- Rules within the system are therefore encoded by selecting tiles with specific combinations of labels and binding strengths
- We assume the availability of an unlimited number of each tile – a computation begins with a specific seed tile, and proceeds by the addition of single tiles
- Tiles bind together to form a growing complex representing the state of the computation only if their binding interactions are of sufficient strength (that is, the complex is stable)

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Example – binary counting

- We have seven different tiles, four rule tiles (labelled either “1” or “0”, two border tiles (“L”, “R”) and a seed tile (“S”)
- Sides depicted by a *single* line have binding strength=1, those with a *double* line, of 2
- We impose the following important restrictions:
  - A tile may only be added to the assembly if it is held in place by a combined binding strength of 2
  - A labelled tile (rule) may only be added if the labels on its side match those of its proposed neighbour

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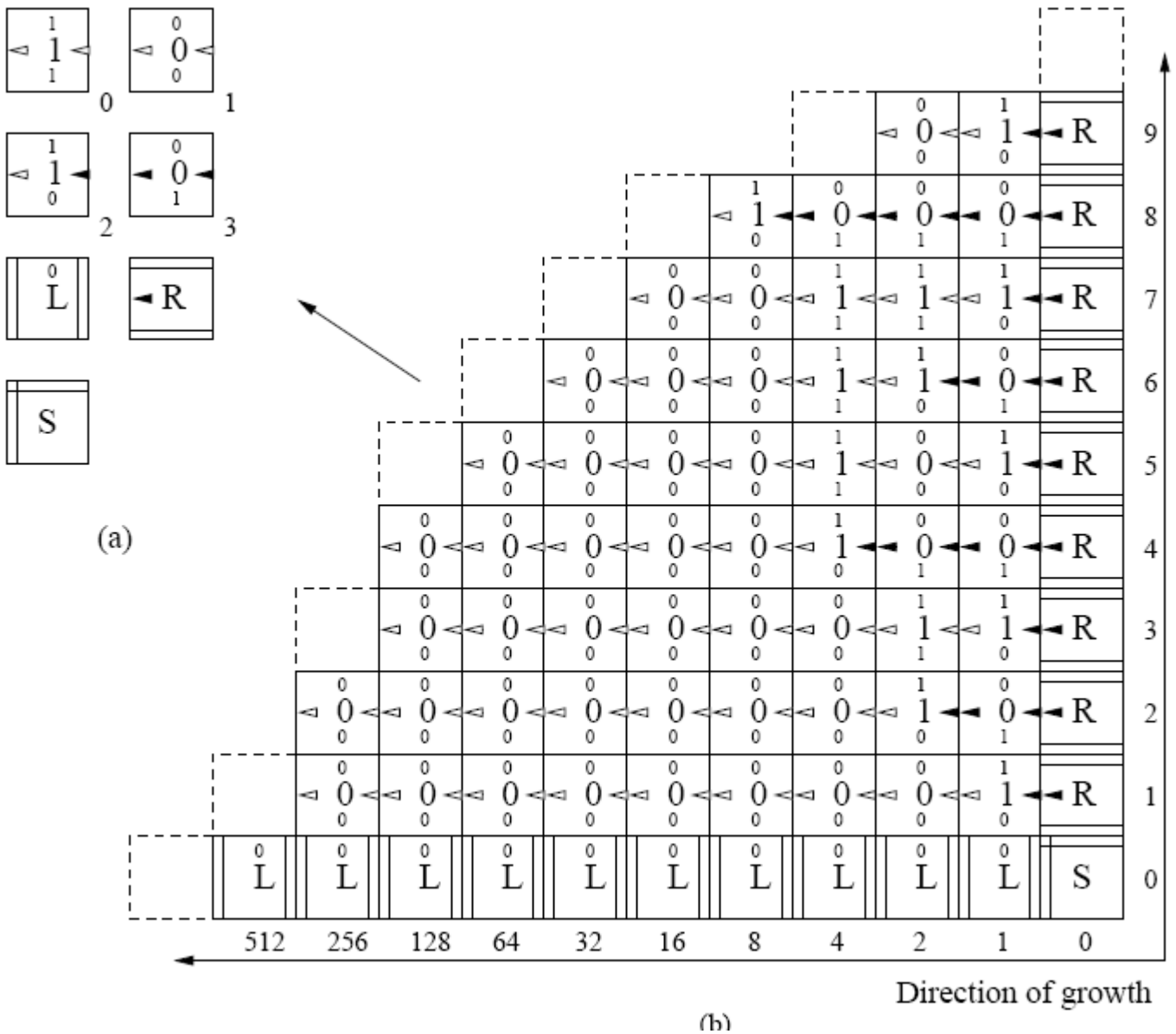

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TAM in action

- Two rule tiles cannot bind, since their combined bond strength is only 1
- Crystallized by the seed tile, a “scaffold” of L and R tiles emerges to support the assembly, as a result of the binding strengths
- At the beginning, with one S, one L and one R, the only tile that matches is rule 2, so it is added to the complex, and so on...

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TAM in the lab

- Excitingly, the model has actually been demonstrated in the lab
- Details beyond the scope of this lecture, but 2-dimensional “pads”, made up of a number of DNA strands, have been shown to self-assemble into a programmed lattice
- By altering the binding interactions between different molecules, arbitrary sets of tiles may be constructed

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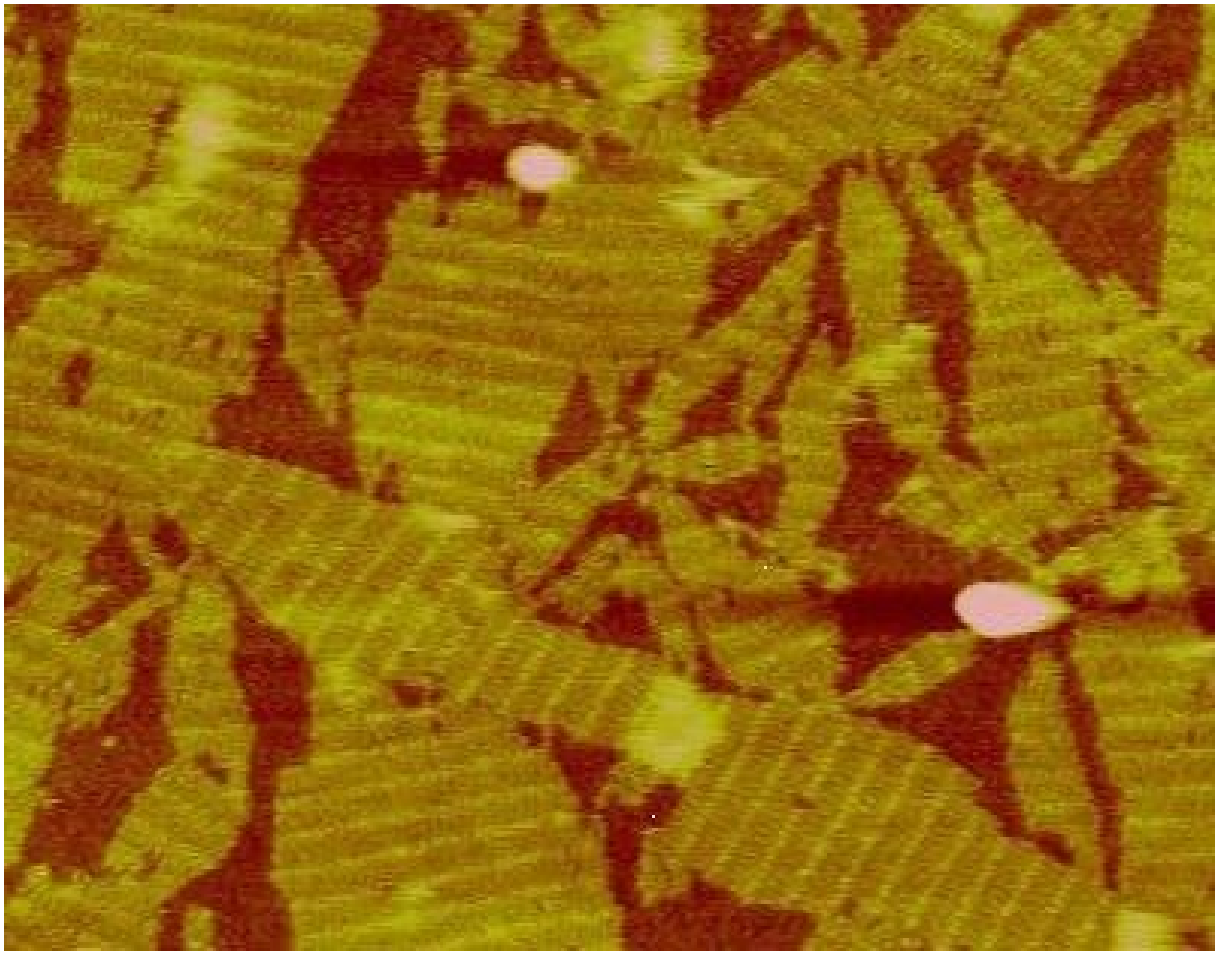

Design and self-assembly of two-dimensional DNA crystals.
Erik Winfree, Furong Liu, Lisa Wenzler, Nadrian C. Seeman.
Nature **394**, 539-544, 1998

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DNA origami

- Takes the entire 7kb single-stranded genome of M13mp18 phage DNA and folds it into a two-dimensional structure
- Uses computer program that takes in a desired shape and calculates position (and therefore sequence) of short “staple” strands that pin the ssDNA in place
- Possible applications as nano-scale “breadboard” for attaching components, or for making nano-wells for reaction vessels

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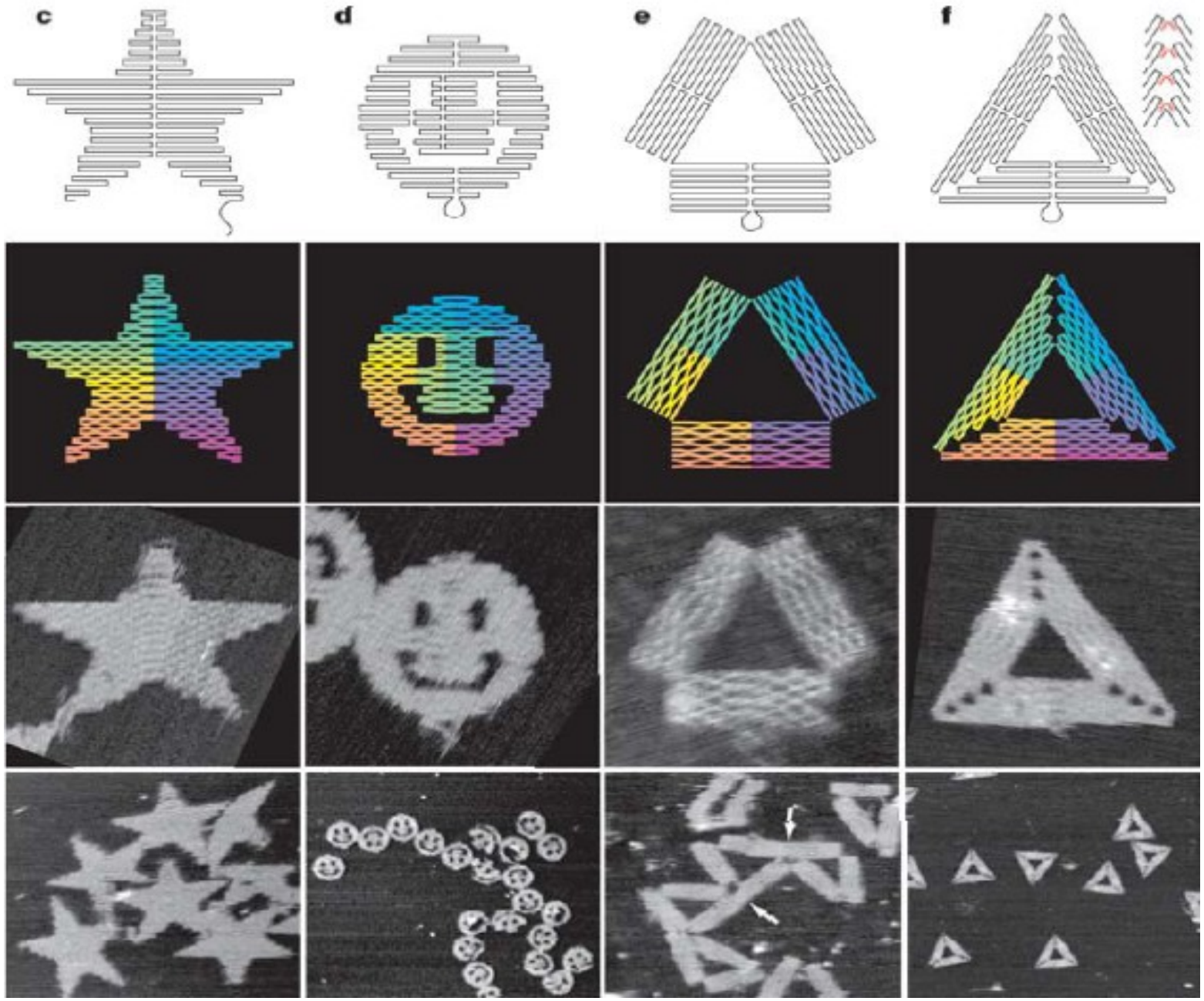

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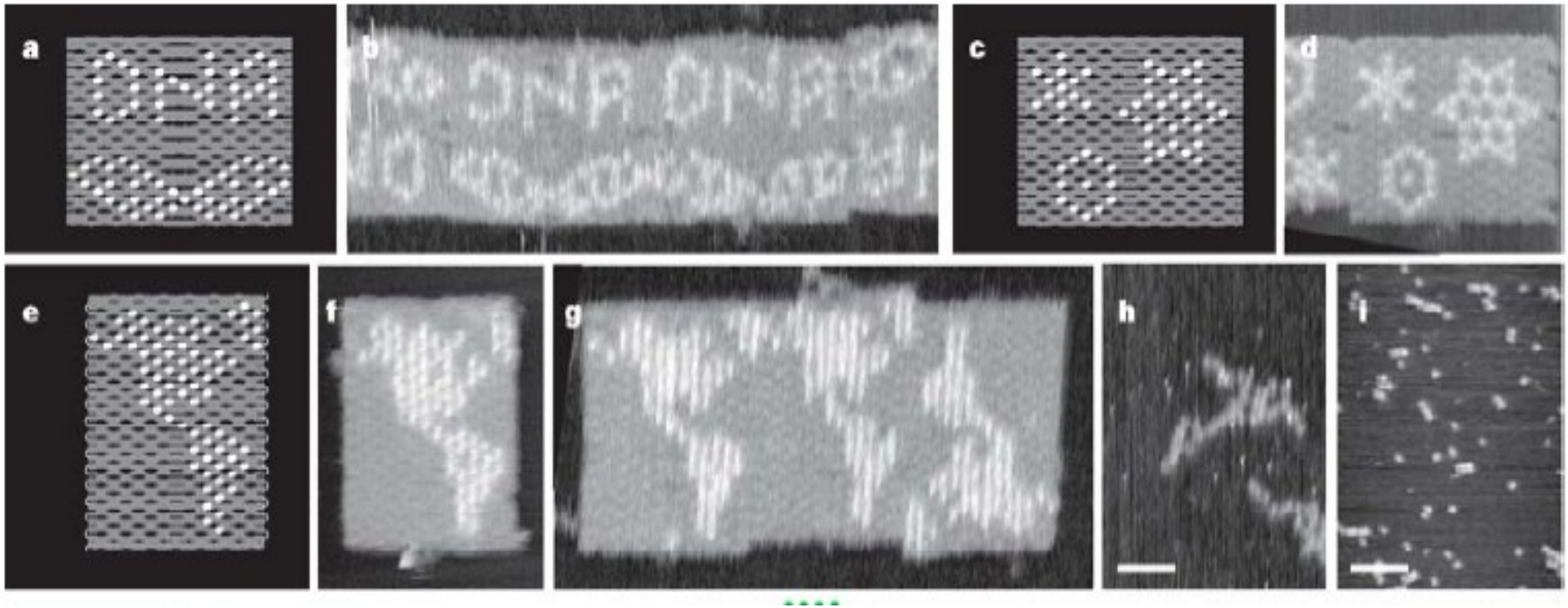

Folding DNA to create nanoscale shapes and patterns. Paul W. K. Rothemund, *Nature* **440**, 297-302, 16 March 2006

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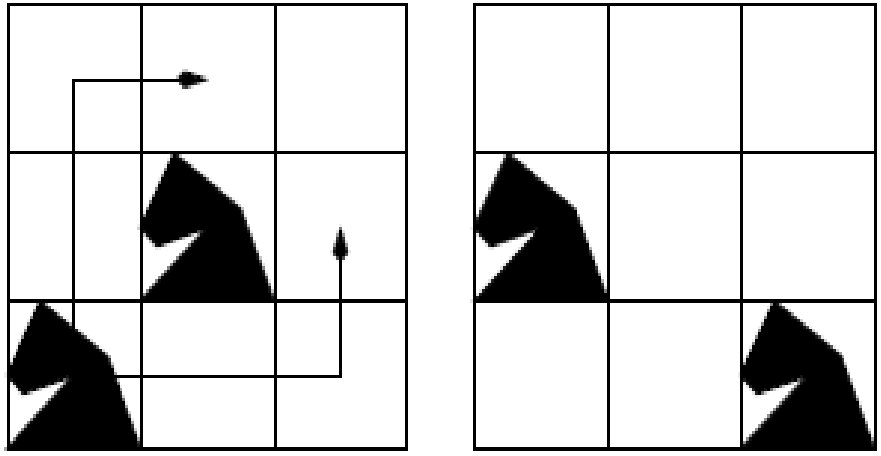
Other implementations

- Faulhammer *et al*. described a solution to a variant of SAT that uses RNA rather than DNA
- The so-called “Knight Problem” seeks configurations of knights on an *n*x*n* chess board such that no knight is attacking another



Chess Games

- Uses the “mark and destroy” paradigm, but utilises RNA (see later lecture) plus Rnase H digestion – acts as a “universal restriction enzyme”
- Used a 3x3 board, with variables *a-i* representing the squares (1=knight present, 0=knight absent)
- Problem can be represented as an instance of SAT

simplified (using the rules of Boolean algebra) to ((NOT h AND NOT f) OR NOT a) AND ((NOT g AND NOT i) OR NOT b) AND ((NOT d AND NOT h) OR NOT c) AND ((NOT c AND NOT i) OR NOT d) AND ((NOT a AND NOT g) OR NOT f)

a	b	c
d	e	f
g	h	i

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Chess Games

- Construct initial library of all possible 9-bit binary strings
- For each square, sequentially, split the library into two tubes, labelled 1 and 2. After destruction, 1 contains strands encoding a knight at that square, and 2 those strands that do not
- In tube 1, destroy any strands that have *no knight* at that square, as well as any strands that have a knight in an attacking position
- In tube 2, destroy any strands that *have* a knight at that square
- Pool tubes 1 and 2, and repeat for next square
- Any remaining strands encode a legal “board”

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Results

- Out of 127 knights placed on 43 boards sampled, only one was placed illegally, giving an overall success rate of 97.7%
- Experiment played a valuable role in getting research over to a general audience

Dirk Faulhammer, Anthony R. Cukras, Richard J. Lipton, and Laura F. Landweber. Molecular computation: RNA solutions to chess problems. *Proceedings of the National Academies of Science*, 97(4):1385–1389, 2000.

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Computing on Surfaces

- Another experiment using the mark and destroy paradigm, but this time with strands tethered to a support, rather than being allowed to float freely in solution
- Idea is to simplify the automation of the (potentially very many) repetitive chemical processes required during the performance of an experiment
- Reported a DNA-based solution to a small instance of SAT: (w OR x OR y) AND (w OR NOT y OR z) AND (NOT x OR y) AND (NOT w or NOT y)

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Computing on Surfaces

- Strands representing each of the 16 unique assignments were synthesized, and each set was then fixed to a specific region of a gold-coated surface using chemical attachment
- The algorithm proceeds in a round of “mark”, “destroy”, “unmark” operations, the idea being to destroy strands that do not satisfy each clause in turn
- Marked strands are *protected* from destruction by using exonuclease to “chew up” unmarked strands

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Results

- Results clearly showed that only legal assignments were retained at the end of the experiment
- There are, however, issues of scalability imposed by the nature of the 2D surface

Liu. Q, et al. DNA computing on surfaces. *Nature* **403**:175-179, 2000

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Gel-Based Computing

- A much larger (20 variable) instance of 3-SAT was successfully solved by Adleman's group in 2002
- The largest problem instance successfully solved by a DNA computer; indeed, “this computational problem may yet be the largest solved by nonelectronic means.”

Braich, R.S et al, Solution of a 20-variable 3-SAT problem on DNA computer, *Science* **296**:499-502, 2002

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Gel-Based Computing

- Architecture related to the Sticker Model, but only using separation (no application of stickers)
- Probes immobilised in gel-filled modules, and strands are pulled through them by electrophoresis
- Strands are removed (retained in the module) by virtue of their “sticking” to the probes, with other strands free to pass through the module for further processing

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Potential Benefits

- Use of electrophoresis *minimizes* the number of (messy, error-prone) laboratory operations (DNA shear, etc.)
- Since strands are not (deliberately) damaged in any way, they, together with the gel modules, are potentially *reusable* for multiple computations
- Whole process is potentially *automatable* – fully integrated DNA computer?

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Instance Solved

- 20 variable, 24 clause SAT instance:

$$\Phi = (\neg x_{13} \vee x_{16} \vee x_{18}) \wedge (x_5 \vee x_{12} \vee \neg x_9) \wedge (\neg x_{13} \vee \neg x_2 \vee x_{20}) \wedge (x_{12} \vee x_9 \vee \neg x_5) \wedge (x_{19} \vee \neg x_4 \vee x_6) \wedge (x_9 \vee x_{12} \vee \neg x_5) \wedge (\neg x_1 \vee x_4 \vee \neg x_{11}) \wedge (x_{13} \vee \neg x_2 \vee \neg x_{19}) \wedge (x_5 \vee x_{17} \vee x_9) \wedge (x_{15} \vee x_9 \vee \neg x_{17}) \wedge (\neg x_5 \vee \neg x_9 \vee \neg x_{12}) \wedge (x_6 \vee x_{11} \vee x_4) \wedge (\neg x_{15} \vee \neg x_{17} \vee x_7) \wedge (\neg x_6 \vee x_{19} \vee x_{13}) \wedge (\neg x_{12} \vee \neg x_9 \vee x_5) \wedge (x_{12} \vee x_1 \vee x_{14}) \wedge (x_{20} \vee x_3 \vee x_2) \wedge (x_{10} \vee \neg x_7 \vee \neg x_8) \wedge (\neg x_5 \vee x_9 \vee \neg x_{12}) \wedge (x_{18} \vee \neg x_{20} \vee x_3) \wedge (\neg x_{10} \vee \neg x_{18} \vee \neg x_{16}) \wedge (x_1 \vee \neg x_{11} \vee \neg x_{14}) \wedge (x_8 \vee \neg x_7 \vee \neg x_{15}) \wedge (\neg x_8 \vee x_{16} \vee \neg x_{10})$$

with a unique satisfying assignment of

$$x_1 = F, x_2 = T, x_3 = F, x_4 = F, x_5 = F, x_6 = F, x_7 = T, x_8 = T, x_9 = F, x_{10} = T, x_{11} = T, x_{12} = T, x_{13} = F, x_{14} = F, x_{15} = T, x_{16} = T, x_{17} = T, x_{18} = F, x_{19} = F, x_{20} = F.$$

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The Solution

- “Mix and split” technique similar to that used for chess game solution used to generate 300-base library sequence for each of the 1,048,576 possible assignments
- For each clause, a gel module is constructed containing probes designed to capture only those strands that satisfy the clause
- So the first module contains probes to capture X_{13}=F, X_{16}=T, X_{18}=T
- Retained strands act as input to the next module
- The final module contains only strands that satisfy the entire formula

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Other Notable Results

- Guarnieri *et al* (1996) described a DNA-based algorithm for binary addition. Used ssDNA to add two non-negative binary numbers. Notable in that it involved DNA for direct numeric representation and addition, as opposed to for massively-parallel random search

All references are in the bibliography supplied with the lectures.

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Other Notable Results

- Tendency of DNA molecules to self-anneal was exploited by Sakamoto et al (2000)
- Solved a small instance of SAT by encoding the formula in “literal strings”
- A formula is satisfiable if there exists a literal string that does not contain any variable together with its negation
- If each variable is encoded as a sequence of DNA, with its negation as the WCC, then any strands containing a variable and its negation will self-anneal, forming a “hairpin” structure
- Such structures can be easily distinguished from other strands
- Only requires temperature cycling, no physical manipulation
- But, requires an exponential number of literal strings

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Other Notable Results

- Algorithmic self-assembly (as previously described) has been used to build tiled structures that execute four steps of a logical XOR operation (Mao et al, 2000)
- Uses triple-crossover molecules – four strands that interact to form three double helices in a roughly planar structure, with sticky regions
- Successful, but scalability questionable, due to requirement for proper hairpin formation in very long single-stranded molecules

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Other Notable Results

- Benenson *et al* (2003) built on their earlier work on the construction of biomolecular machines
- Describe the construction of a molecular automaton that uses the process of DNA backbone hydrolysis and strand hybridization, fuelled by the potential free energy stored in the DNA itself
- “DNA acts as both data and fuel”

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Other Notable Results

- Stojanovic and Stefanovic (2003) describe a molecular automaton that plays tic-tac-toe (noughts and crosses) against a human
- Automaton is a collection of deoxyribozymes implementing a Boolean network, arranged in a 3x3 well formation
- Player signals a move by adding a short strand, and the “computer's” move is signalled by fluorescence in a particular well
- Plays a perfect strategy, can never be beaten
- Also see work of Ehud Shapiro and his group