Automatic design of digital synthetic gene circuits

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Summary

- Automatic gene circuit design: the problem.
- The Karnaugh map method in biology.
- Comparison with a different design.
- Circuit complexity and performance.
- Conclusion and future work.

Automatic gene circuit design: previous approaches.

- Given the output, how to derive the corresponding circuit (structure and parameter values?)
- Brute force optimization via evolutionary algorithm (François and Hakim, *PNAS* **101**, 580, 2004)
- Similar implementations: OptCircuit (Dasika and Maranas, *BMC Syst. Bio.* **2**, 24, 2008); Genetdes (Rodrigo *et al*., *Bioinformatics* **23**, 1857, 2007).

Problems:

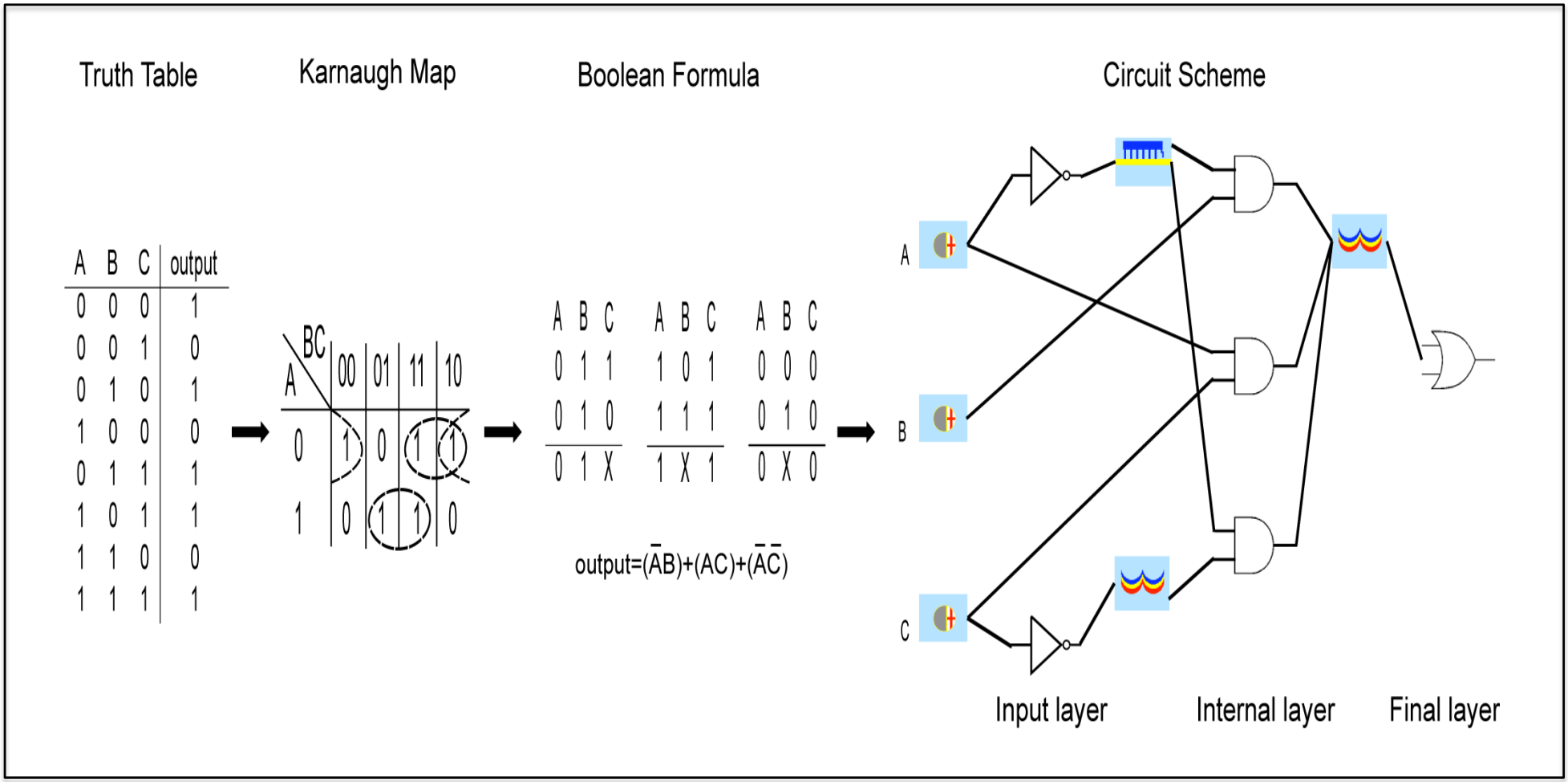
- Transcription units as bio-bricks (instead of parts).
- Limited model (translation as single-step event).
- Double optimization procedure: long computational time.

Looking for a different strategy.

Digital gene circuits

- Input/Output relation fully described by a truth table.
- The Karnaugh map method converts a truth table into a circuit scheme – no optimization required.
- Boolean gates due to promoter and RBS regulation mechanisms.
- Important application as biosensors.

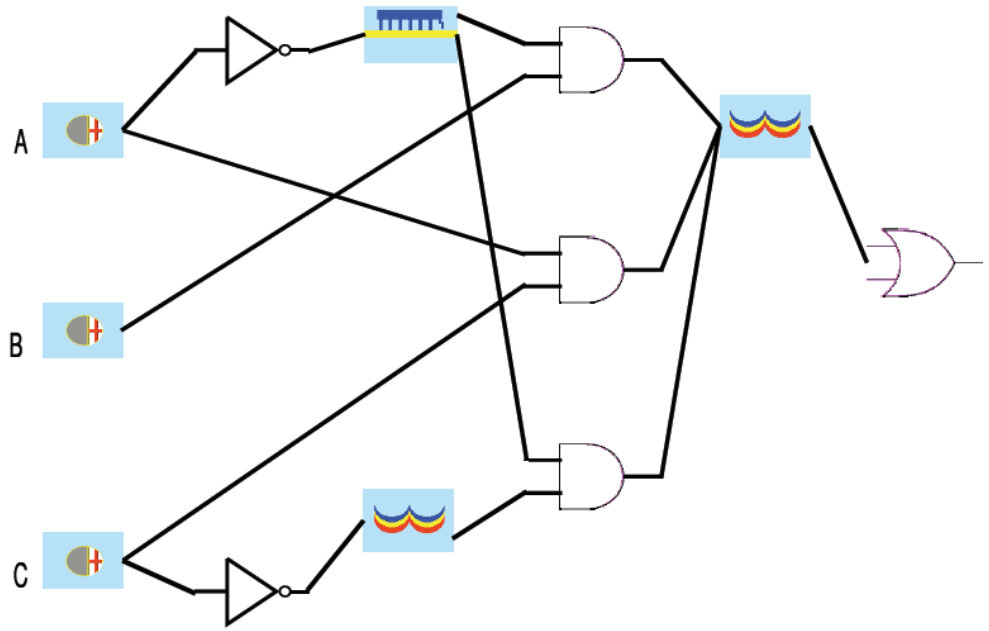
The Karnaugh map method



Circuit **structure** in three layers – **No optimization** required

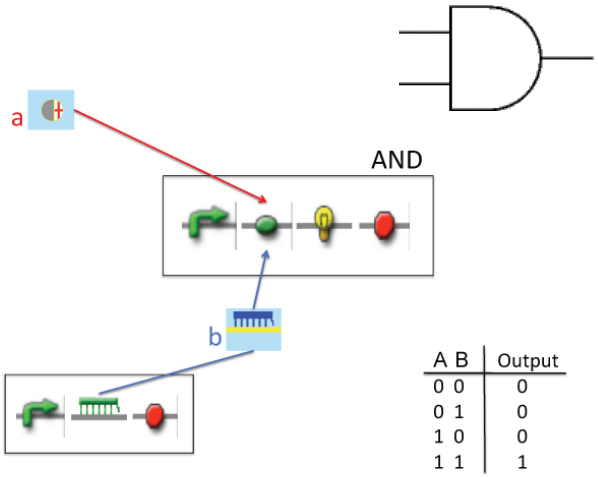
Circuit characteristics

- Activated/Repressed Promoters and RBSs (Bintu *et al.*, *Curr. Opin. Genet. Dev.* **15**, 125, 2005; Isaacs et al., *Nat. Biotech.* **22**, 841, 2004).
- **Pools** of transcription factors, sRNAs, and chemicals (M.A. Marchisio and J. Stelling, "Computational design of synthetic gene circuits with composable parts." *Bioinformatics*, **24**, 1903, 2008).
- A circuit takes up to four inputs (chemicals) and produces a single output (fluorescent protein).

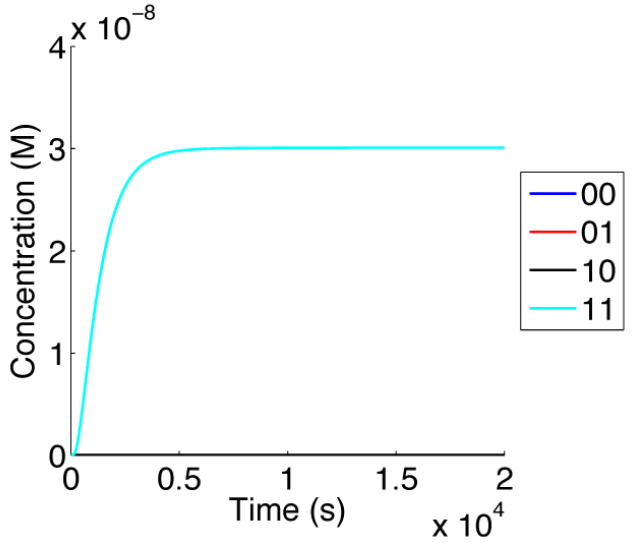


Gate structure and new designs.

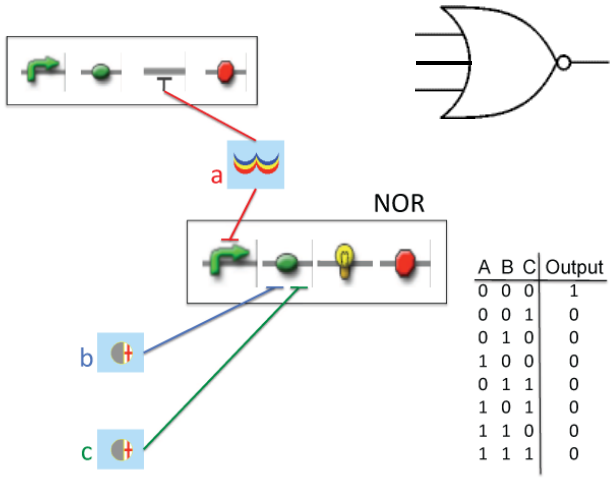
- Riboswitches + sRNA on the RBS.



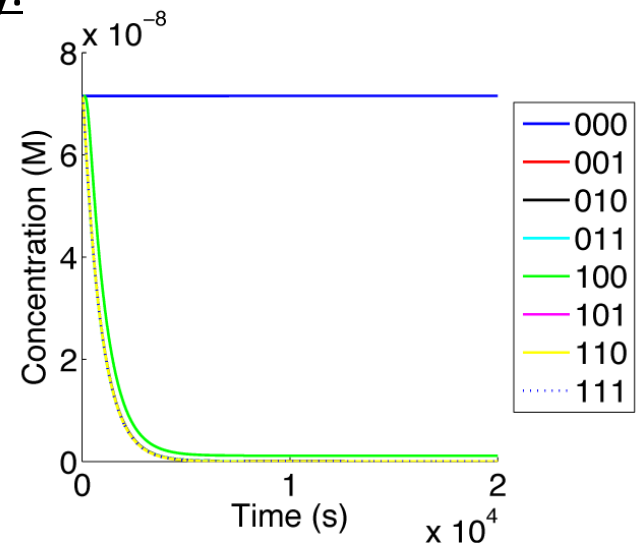
A	B	Output
0	0	0
0	1	0
1	0	0
1	1	1



- Promoters and RBS are controlled simultaneously.



A	B	C	Output
0	0	0	1
0	0	1	0
0	1	0	0
1	0	0	0
0	1	1	0
1	0	1	0
1	1	0	0
1	1	1	0



Comparison with electronics

- Every truth table corresponds to two Boolean formulas: CNF (POS) and DNF (SOP).
- In electronics the minimal circuit is given by the formula with the lowest number of clauses of NOT operations.
- In biology several circuit schemes arise from the same Boolean formula.
- How to define a **minimal** circuit in biology?

The complexity score

- **Regulatory factors** matter more than gene number.
- Only a handful of repressors and activators is currently used.
- Engineering new proteins is more difficult than synthesizing antisense small RNAs.
- Riboswitches simplify the structure of a gate.
- We define as minimal the circuit with the lowest complexity score defined as

$$S = 2^{R-1} + 2^{A-1} + n$$

where: R, repressor number (>= 1); A, activator number (>=1),n antisense sRNA number

- A circuit should avoid to re-use the same kind of transcription factors and prefer RBS controls to the promoter ones.
- Riboswitches do not increase the circuit complexity.

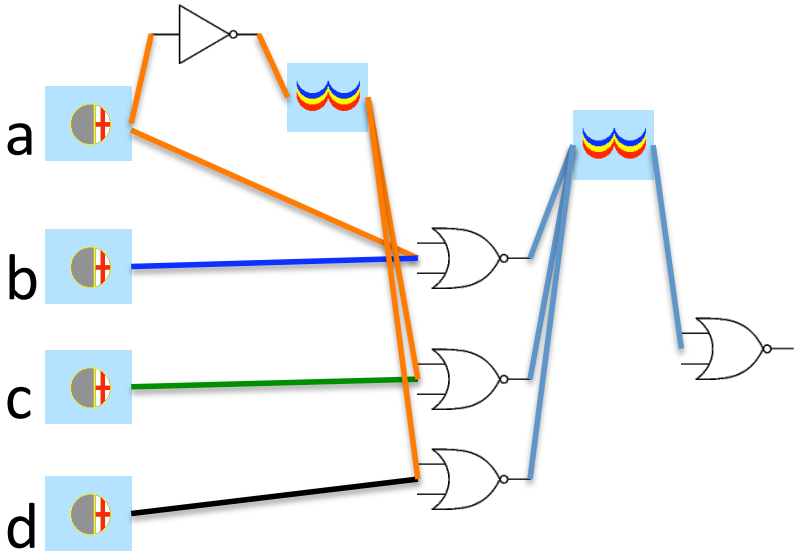
Our tool

- The truth table is the only input.
- All the schemes compatible with POS and SOP formulas are computed (less than 1s up to 8s).
- They are ranked according to their complexity score.
- The user can choose a solution: this is built by parts, pools, and device composition and encoded in MDL (Model Definition Language) to be visualized in ProMoT (http://www.mpimagdeburg.mpg.de/projects/promot/).

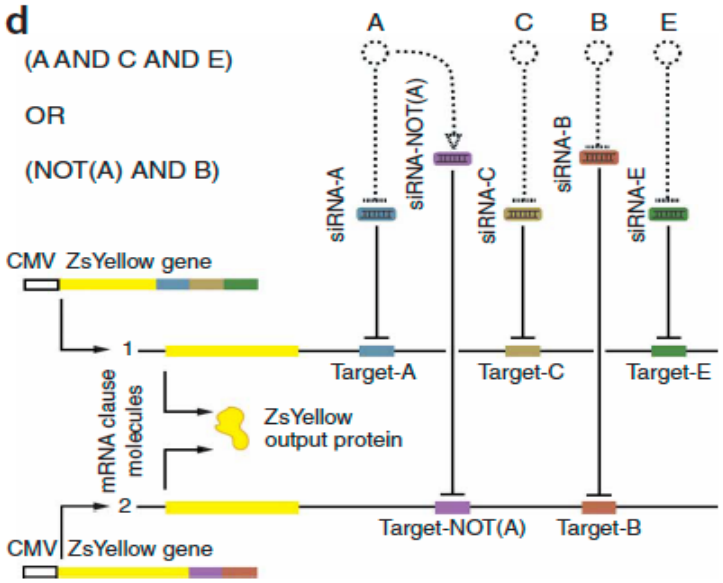
Comparison with RNAi-based design

(Rinaudo *et al.*, *Nat. Biotech.*, **25**, 795, 2007)

Our best solution
(a+b)(A+c)(A+d)



Rinaudo's solution
(acd)+(Ab)

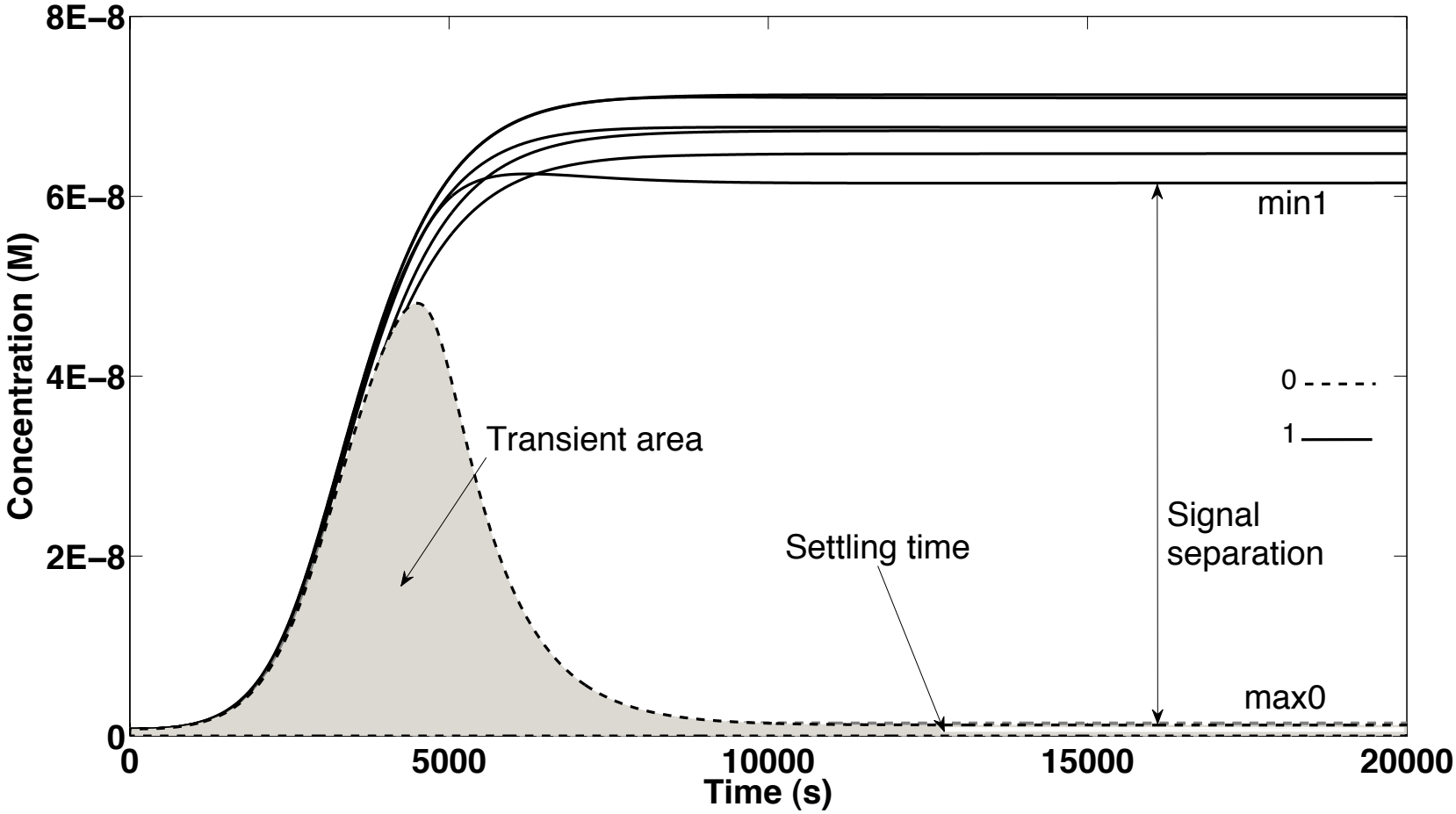


Circuit	Score	A	R	RNA
Our best POS	2	0	2	0
Our best SOP	4	2	1	1
Rinaudo	5	0	0	5

Our tool found 15 designs with complexity lower than 5.

How do these circuits work?

- Circuit **performance** is estimated through **signal separation** and **transient** calculation and depends both on structure and parameter values.

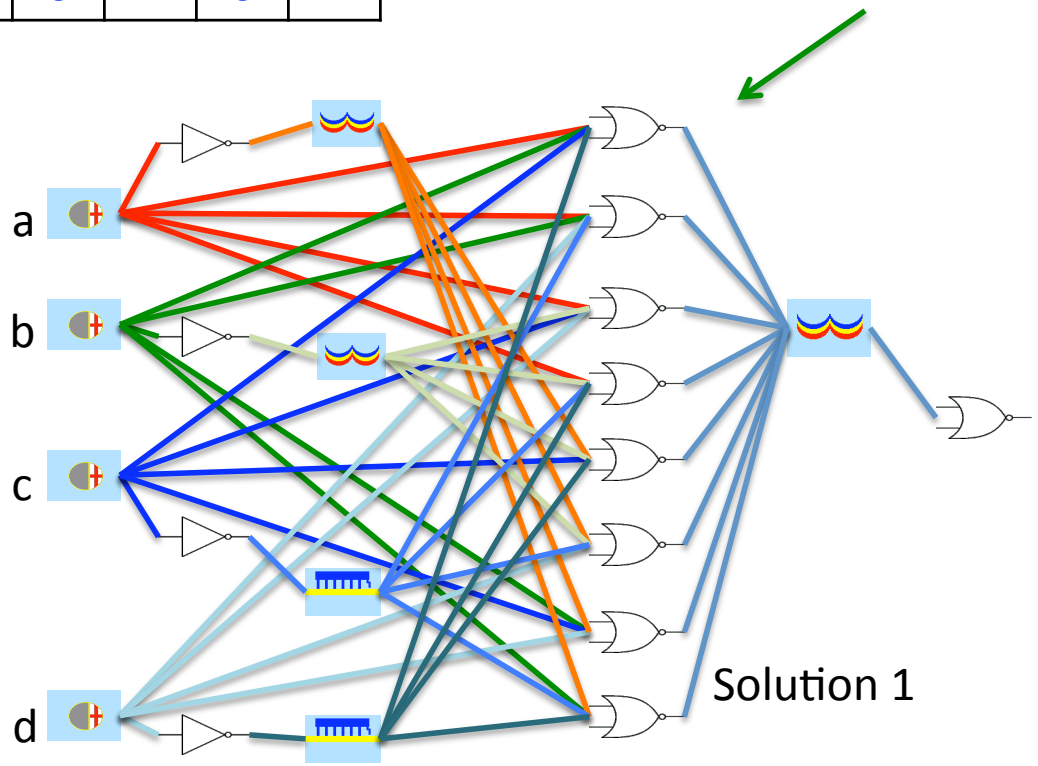


A benchmark

a	b	c	d	O
0	0	0	0	1
0	0	0	1	0
0	0	1	0	0
0	1	0	0	0
1	0	0	0	0
0	0	1	1	1
0	1	0	1	1
1	0	0	1	1
0	1	1	0	1
1	0	1	0	1
1	1	0	0	1
0	1	1	1	0
1	0	1	1	0
1	1	0	1	0
1	1	1	0	0
1	1	1	1	1

ab \ cd	00	01	11	10
00	1	0	1	0
01	0	1	0	1
11	1	0	1	0
10	0	1	0	1

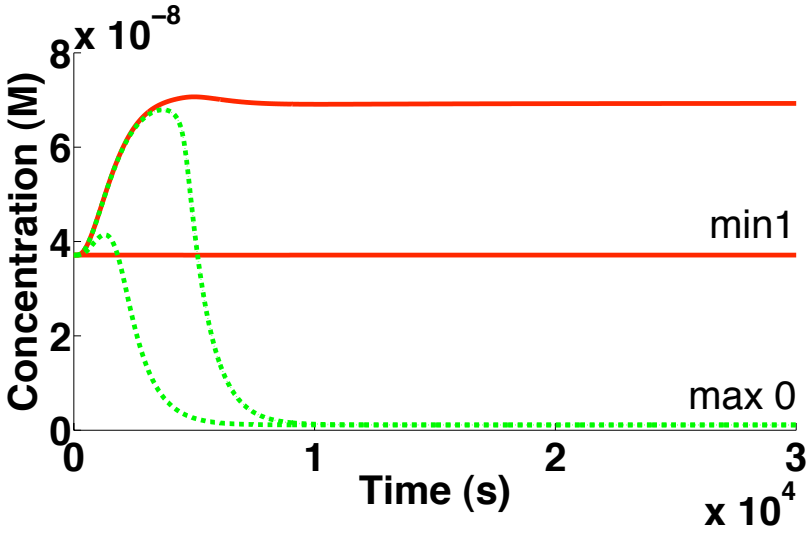
- POS and SOP formulas have 8 clauses each of which contains 4 inputs.
- 48 possible schemes with S varying from 20 to 2062.



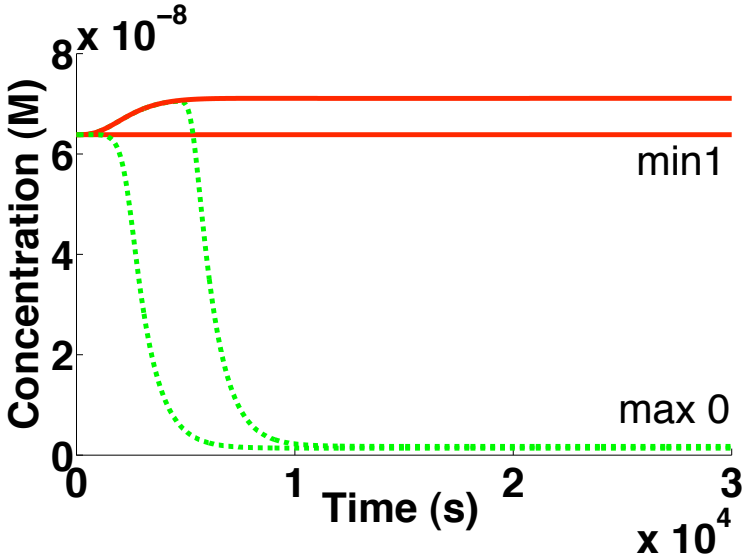
Solution 1

Comparison of two possible solutions

Solution 1



Solution 4

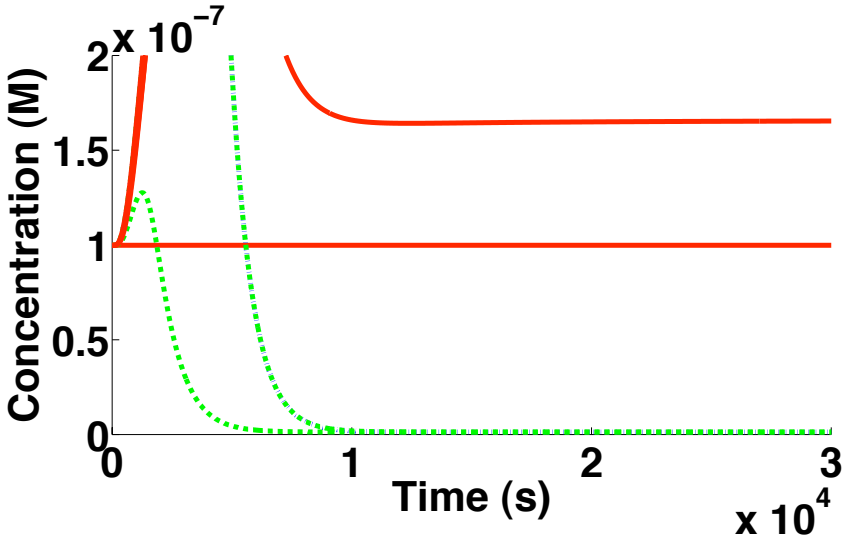


Solution	Rank	Score	A	R	RNA	Gene	Separation	Transient
1	1	20	2	5	2	17	36 nM	3709
4	25	548	10	6	4	21	62.1 nM	5112

Higher complexity seems to guarantee better performance.

Improving the performance

- The signal separation is mostly influenced by parameters belonging to the **final gate**.
- Tuning only **one** parameter (the strength of the promoter in the final gate) the signal separation can be drastically amplified.



- Stochastic algorithms can be avoided but a good set of default parameter values is required.

Conclusion and future work

- Automatic design of digital synthetic gene circuits via the Karnaugh map method.
- Circuit structure calculation does not require any optimization procedure.
- Theoretical new design of Boolean gates where promoter and RBS are simultaneously.
- Computer simulations show an unequivocal signal separation between 0/1 outputs with our choice of default parameter values.

- Insertion of other translation regulation mechanisms.
- Extension to eukaryotic cells.
- **MAIN GOAL**: Wet-lab implementation of single gates and more complex circuits.