BIOSIGNAL-BASED COMPUTING BY AHL INDUCED SYNTHETIC GENE REGULATORY NETWORKS

From an in vivo Flip-Flop Implementation to Programmable Computing Agents

T. Hinze, T. Lenser, N. Matsumaru, P. Dittrich
Bio Systems Analysis Group, Friedrich-Schiller-Universität Jena, Ernst-Abbe-Platz 1–4, D-07743 Jena, Germany
{*hinze,thlenser,naoki,dittrich*}*minet.uni-jena.de*

S. Hayat
Computational Biology Group, Universität des Saarlandes, Im Stadtwald, PF 15 11 50, D-66041 Saarbrücken, Germany
s.hayat@bioinformatik.uni-saarland.de

Keywords: biologically inspired computing, gene regulatory networks, bistable toggle switch, NP complete problem

Abstract: Gene regulatory networks (GRNs) form naturally predefined and optimised computational units envisioned to act as biohardware able to solve hard computational problems efficiently. This interplay of GRNs via signalling pathways allows the consideration as well as implementation of interconnection-free and fault tolerant programmable computing agents. It has been quantitatively shown in an *in vivo* study that a reporter gene encoding the green fluorescent protein (*gfp*) can be switched between high and low expression states, thus mimicking a NAND gate and a RS flip-flop. This was accomplished by incorporating the N-acyl homoserine lactone (AHL) sensing lux operon from *Vibrio fischeri* along with a toggle switch in *Escherichia coli*. *gfp* expression was quantified using flow cytometry. The computational capacity of this approach is extendable by coupling several logic gates and flip-flops. We demonstrate its feasibility by designing a finite automaton capable of solving a knapsack problem instance.

1 INTRODUCTION

Molecular biosignals of GRNs are suited for computing purposes. Beyond error-prone molecular computers *in vitro*, the idea of computing *in vivo* becomes more and more attractive (Kobayashi et al., 2004; Weiss et al., 1999). Motivated by the presence of naturally predefined functional units found in microorganisms, their exploration towards computing agents seems promising. GRNs can be viewed as computational devices of pro- and eukaryotic cells, triggering and directing responses to external inputs influenced by genetically stored information. Emerged from an evolutionary optimisation, they form reliable modularised systems with obvious advantages in carrying out massively parallel calculations.

Recently, more than $6,000$ transcription factors acting as signal inducers or transmitters in GRNs have been listed in the public Transpath and Transfac database. Selection of GRN candidates in concert with their ability for composition via specific receptor-controlled interfaces is of particular interest for creating computing agents. Following the pioneering implementation of a bistable toggle switch *in vivo* (Gardner et al., 2000), we could confirm its practicability in a previous study (Hayat et al., 2006). Two extensions in the experimental setup were investigated: Firstly, the effects of isopropyl-β-D-thiogalactopyranoside (IPTG) and AHL as appropriate intercellular inducers for flip-flop setting were shown. Secondly, flow cytometry was used to quantitatively measure protein concentrations within the flip-flop implementation. Section 3 briefly explains underlying methods and materials, while section 4 describes the experiment. Based on these experimental results, we identify GRNs able to function as NAND gates and RS flip-flops (section 5). For these computational units, we present a homogeneous dynamical model using Hill kinetics (Mestl et al., 1995). Unit composition is exemplified by design of a finite automaton for a knapsack problem instance (section 6). We transfer this automaton into a minimised logic circuit consisting of interacting NAND gates. A subsequent simulation of the corresponding computing agent on the GRN level demonstrates a constructive approach towards programmable *in vivo* computers encouraged by the Turing completeness of (bio)chemical reaction networks (Magnasco, 1997).

2 RELATED WORK

First ideas about principles of interconnection-free biomolecular computation were introduced in (Aoki et al., 1992; Thomas, 1991). Along with the growing knowledge in genetics and proteomics, the imagination of computing *in vivo* came into the focus of scientific research (Weiss et al., 1999). Several approaches address engineering of GRNs using predefined network motifs (Guido et al., 2006; Kaern et al., 2003; Kobayashi et al., 2004). Inspired by advantageous properties of specific GRNs for computational tasks, construction and implementation of genetic circuits has been successfully explored (Gardner et al., 2000; Sprinzak and Elowitz, 2005; Yokobayashi et al., 2004). Recently, these circuits have been used to form logic gates, clocks (oscillators), switches, or sensors. As a next step, their combination to achieve higher integrated problem-specific designs will be investigated. A DNA-based solution to the knapsack problem *in vitro* was introduced in (Hinze et al., 2002). An alternative approach *in vivo* generates a variety of encoding plasmids that are translated into proteins (Henkel et al., 2007).

3 BIOLOGICAL PRINCIPLES AND PREREQUISITES

3.1 Quorum Sensing and Autoinduction via AHL

In quorum sensing, bacterial species regulate gene expression based on cell-population density (Miller and Bassler, 2001). An alteration in gene expression occurs when an intercellular signalling molecule termed autoinducer, produced and released by the bacterial cells reaches a critical concentration. Termed as quorum sensing or autoinduction, this fluctuation in autoinducer concentration is a function of bacterial cell-population density. *Vibrio fischeri*, a well studied bacterium, colonises the light organs of a variety of marine fishes and squids, where it occurs at very high densities ($10^{10}\frac{\text{cells}}{\text{ml}}$) and produces light. The two genes essential for cell density regulation of luminescence are: luxI, which codes for an autoinducer synthase (Schaefer et al., 1996); and luxR, which codes for an autoinducer-dependent activator of the luminescence genes. The luxR and luxI genes are adjacent and divergently transcribed, and luxI is the first of seven genes in the luminescence or lux operon. LuxI-type proteins direct AHL synthesis while LuxR-type proteins function as transcriptional regulators that are capable of binding AHL signal molecules. Once formed, LuxR-AHL complexes bind to target promoters of quorum-regulated genes. Quorum sensing is now known to be widespread among both Gram-positive and Gram-negative bacteria.

3.2 Bioluminescence in *Vibrio fischeri*

Bioluminescence in general is defined as an enzyme catalysed chemical reaction in which the energy released is used to produce an intermediate or product in an electronically excited state, which then emits a photon. It differs from fluorescence or phosphorescence as it is not depended on light absorbed. The mechanism for gene expression and the structure of the polycistronic message of the lux structural genes in *Vibrio fischeri* have been thoroughly characterised (Hastings and Nealson, 1977). Briefly, there are two substrates, luciferin, which is a reduced flavin mononucleotide ($FMNH_2$), and a long chain ($7-16$ carbons) fatty aldehyde (RCHO). An external reductant acts via flavin mono-oxygenase oxidoreductase to catalyse the reduction of FMN to $FMNH_2$, which binds to the enzyme and reacts with O_2 to form a 4a-peroxy-flavin intermediate. This complex oxidises the aldehyde to form the corresponding acid (RCOOH) and a highly stable luciferase-hydroxyflavin intermediate in its excited state, which decays slowly to its ground state emitting blue-green light $h\nu$ with a maximum intensity at about 490nm:

$$\begin{array}{l} \mathrm{FMNH_2} + \mathrm{RCHO} + \mathrm{O_2} \xrightarrow{\text{lucif.}} \\ \mathrm{FMN} + \mathrm{H_2O} + \mathrm{RCOOH} + h\nu \end{array} \qquad (1)$$

3.3 Transcription Control by LacR and λCI Repressor Proteins

Escherichia coli cells repress the expression of the lac operon when glucose is abundant in the growth medium. Only when the glucose level is low and the lactose level is high, the operon is fully expressed. The Lac repressor LacR is a 360 residue long protein that associates into a homotetramer. It contains a helix-turn-helix (HTH) motif through which it interacts with DNA. This interaction represses transcription by hindering association with RNA polymerase and represents an example of “combinatorial control” widely seen in prokaryotes and eukaryotes (Buchler et al., 2003). The CI repressor of bacteriophage lambda is the key regulator in lambda’s genetic switch, a bistable switch that underlies the phage’s ability to efficiently use its two modes of development (Ptashne, 1992).

3.4 Flow Cytometry

Flow cytometry refers to the technique where microscopic particles are counted and examined as they pass in a hydro-dynamically focused fluid stream through a measuring point surrounded by an array of detectors. Previously, flow cytometry analyses were performed by us using a BD LSRII flow cytometer equipped with 405nm, 488nm and 633nm lasers. 488nm laser was used for *gfp* and yellow fluorescent protein (*yfp*) quantification.

4 IMPLEMENTATION OF A COMPUTATIONAL UNIT *IN VIVO*

4.1 Experimental Setup

We have shown that an *in vivo* system (Hayat et al., 2006) can potentially be used to mimic a RS flip-flop (Huffman, 1954; Morris Mano, 1991) and have quantified its performance using flow cytometry. The presence or absence of the inducers IPTG or AHL in combination with temperature shift acts as an input signal. The toggle switch comprising of structural genes for reporter/output proteins fused to promoter regions that are regulated by input signals is visualised as a RS flip-flop, see figure 1. The functional modularity of the input and output circuits is maintained so that the artificial GRN used can be easily extended for future studies.

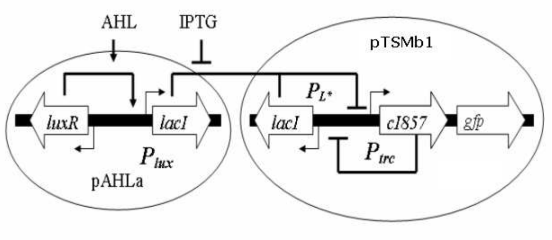


Figure 1: A schematic diagram of an AHL biosensor module interfaced with the genetic toggle switch adapted from (Hayat et al., 2006). The transgenic artificial GRN consists of a bistable genetic toggle switch (Gardner et al., 2000) which is interfaced with genes from the lux operon (Engebrecht and Silverman, 1984) of the quorum sensing signalling pathway of *Vibrio fischeri* (Schaefer et al., 1996).

This design endows cells with two distinct phenotypic states: where the λCI activity is high and the expression of lacI is low (referred to as high or 1 state), or where the activity of LacR is high and the expression of λCI is low (referred to as low or 0 state). *gfp* is expressed only in the high λCI/low LacR state. Figure 2 shows the experimental results obtained by flow cytometry.

4.2 Results and Discussion

For co-relational purposes, all experiments were conducted with both BL21 and Top10 strains of *Escherichia coli*. The concentration of IPTG used in all the experiments was 2mM and that of AHL was 1μM. Experiments conducted without the use of inducers, lead to an unreliable shifting of the states, signifying the use if inducers in a tightly, mutually regulated circuit. Further experiments conducted to understand the switching dynamics of the circuit revealed that in the current scenario, it was easier to switch from a high to a low state than vice versa. This discrepancy in switching behaviour is attributed to the differing modes of elimination of LacR and λCI repressor proteins. While switching from low to high state, the repression due to IPTG-bound Lac repressor needs to be overcome by cell growth. Switching from high to low state is effected by immediate thermal degradation of the temperature-sensitive λCI. Experiments were also conducted to test the sustainability of states. The plug and play property of the circuit was examined by employing *yfp* as the reporter gene instead of *gfp*. As shown in figure 2, the circuit could reliably mimic a RS flip-flop. The massive parallelism permissible by the use of large quantities of cells can compensate for the slow speed of switching. Further tests are to be performed to confirm this hypothesis.

5 DEFINITION OF COMPUTATIONAL UNITS

Artificial GRNs have been instrumental in elucidating basic principles that govern the dynamics and consequences of stochasticity in the gene expression of naturally occurring GRNs. The realisation as computational circuits infers inherent evolutionary fault tolerance and robustness to these modular units.

In a more abstract view, generalised building blocks adopted from previous *in vivo* studies can be identified. In terms of predefined GRN modules, they form computational units. We introduce two artificial GRNs for logic gates and describe their dynamical behaviour quantitatively by an ordinary differential equation model using Hill kinetics (Mestl et al., 1995). A variety of specific signalling proteins (transcription factors) given by their concentration over the time course enables communication between as well as coupling of computational units. Thus, circuit

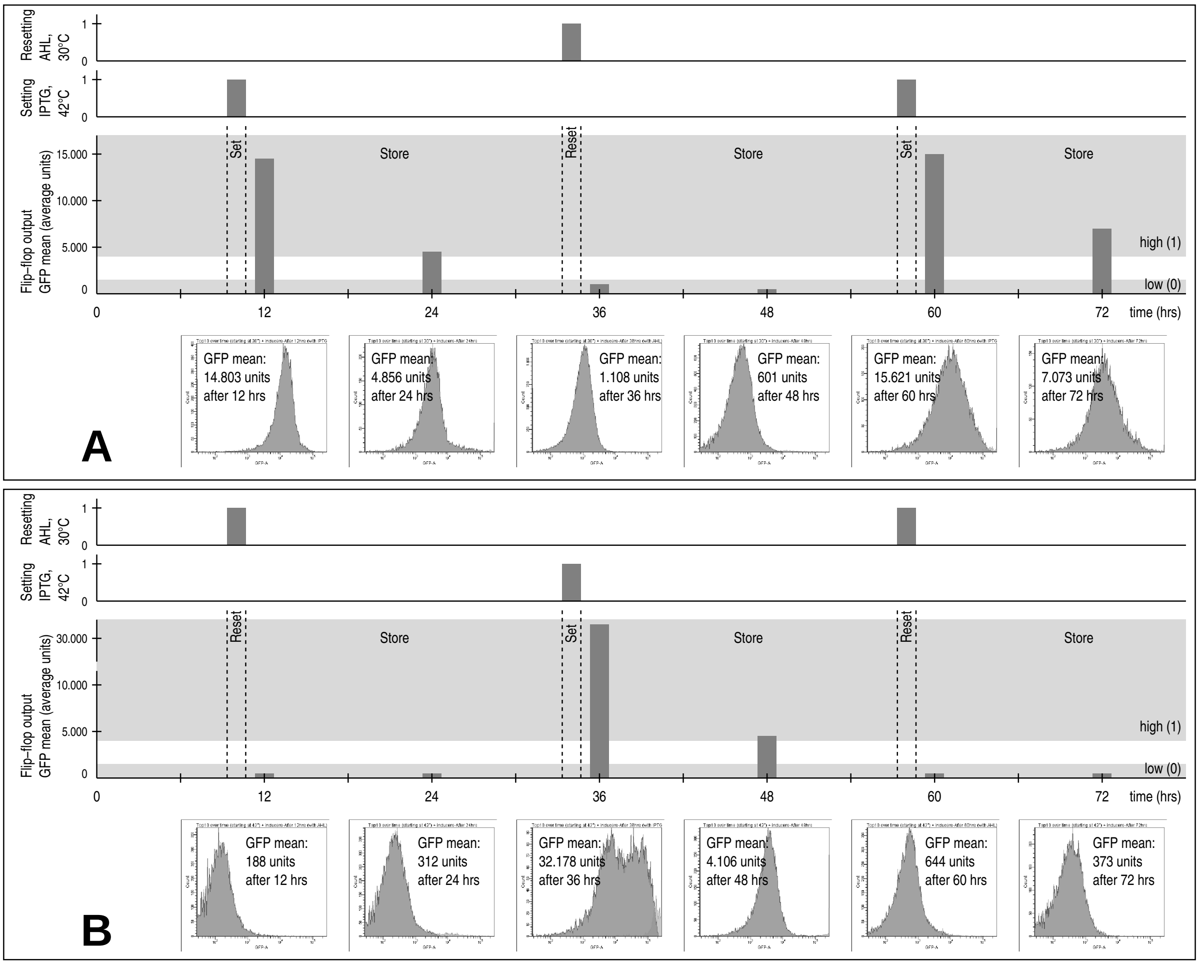


Figure 2: Inducer-dependent switching. Repeated activation and deactivation of the toggle switch based on inducers and temperature. Temperature was switched every 24 hours. Cells were incubated with inducers for 12 hours, followed by growth for 12 hours without inducers, initially kept at 30°C (**A**) and 42°C (**B**). The cells successfully switched states thrice.

engineering becomes feasible. Resulting computing agents can serve as templates for experimental setups. We distinguish computational units within a circuit by index *i*.

Hill kinetics represents a homogeneous approach to model cooperative and competitive aspects of interacting biochemical reaction networks. It formulates the relative intensity of gene regulations by sigmoid-shaped threshold functions h of degree *m* and threshold $\Theta > 0$ such that $x \geq 0$ specifies the concentration level of a transcription factor that activates resp. inhibits gene expression. Function value h then returns the normalised change in concentration level of the corresponding gene product. It reaches a concentration level of 50% iff $x = \Theta$.

Activation (upregulation) $\rightarrow$:

$$\mathrm{h}^{+}(x,\Theta,m) = \frac{x^m}{x^m + \Theta^m} \tag{2}$$

Inhibition (downregulation) $\perp$:

$$\mathrm{h}^{-}(x,\Theta,m) = 1 - \mathrm{h}^{+}(x,\Theta,m) \tag{3}$$

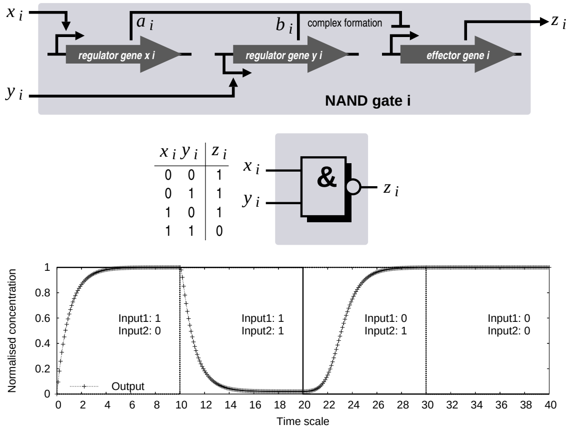
5.1 NAND Gate

Input: concentration levels of transcription factors x_i, y_i

Output: concentration level of gene product z_i

The dynamical system behaviour is defined by ordinary differential equations 4, 5, and 6.

$$\begin{aligned}
\dot{a}_i &= \mathrm{h}^{+}(x_i,\Theta_{i1},m_{i1}) - a_i && (4)\\
\dot{b}_i &= \mathrm{h}^{+}(y_i,\Theta_{i2},m_{i2}) - b_i && (5)\\
\dot{z}_i &= 1 - \mathrm{h}^{+}(a_i,\Theta_{i3},m_{i3}) \cdot \mathrm{h}^{+}(b_i,\Theta_{i4},m_{i4}) \\
&\quad -z_i && (6)
\end{aligned}$$



x_i	y_i	z_i
0	0	1
0	1	1
1	0	1
1	1	0

Figure 3: GRN mimicking a NAND gate. Dynamical behaviour shown for $m_{ij} = 2$, $\Theta_{ij} = 0.1$, $j = 1, \ldots, 4$, $a(0) = 0$, $b(0) = 0$, $z(0) = 0$ (Copasi ODE solver simulation)

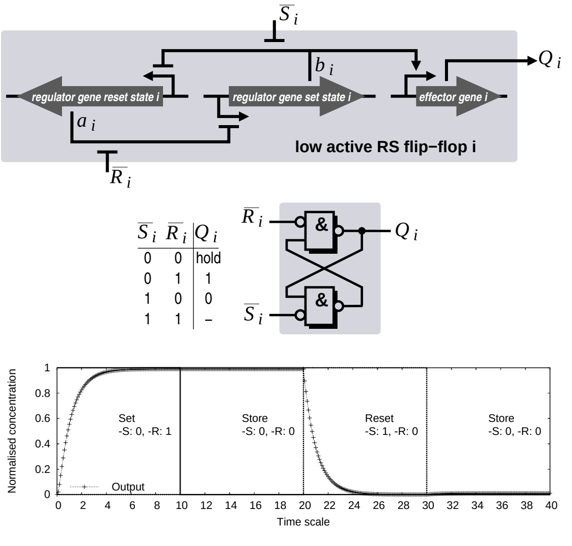
5.2 RS Flip-Flop

Input: concentration levels of transcription factors $\overline{S_i}, \overline{R_i}$

Output: concentration level of gene product Q_i

The dynamical system behaviour is defined by ordinary differential equations 7, 8, and 9.

$$\begin{aligned}
\dot{a}_i &= 1 - \mathrm{h}^+(b_i, \Theta_{i1}, m_{i1}) \cdot \mathrm{h}^-(\overline{S_i}, \Theta_{i2}, m_{i2}) \\
&\quad - a_i && (7)\\
\dot{b}_i &= 1 - \mathrm{h}^+(a_i, \Theta_{i3}, m_{i3}) \cdot \mathrm{h}^-(\overline{R_i}, \Theta_{i4}, m_{i4}) \\
&\quad - b_i && (8)\\
\dot{Q}_i &= \mathrm{h}^+(b_i, \Theta_{i1}, m_{i1}) \cdot \mathrm{h}^-(\overline{S_i}, \Theta_{i2}, m_{i2}) \\
&\quad - Q_i && (9)
\end{aligned}$$



$\overline{S}_i$	$\overline{R}_i$	Q_i
0	0	hold
0	1	1
1	0	0
1	1	–

Figure 4: GRN mimicking a low active RS flip-flop. Dynamical behaviour shown for $m_{ij} = 2$, $\Theta_{ij} = 0.1$, $j = 1, \ldots, 4$, $a(0) = 0$, $b(0) = 0$, $Q(0) = 0$ (Copasi ODE solver simulation)

A homologous model of a bistable toggle switch was introduced in (Gardner et al., 2000). In case of the forbidden input signalling $\overline{S_i} = 1$, $\overline{R_i} = 1$, the normalised concentrations of both proteins a_i and b_i converge to 0.5. By setting or resetting input signalling, the flip-flop restores.

6 AN ARTIFICIAL GRN FOR KNAPSACK PROBLEM SOLUTION

We demonstrate the feasibility of unit composition for solution of more complex problems, exemplified by the integer knapsack problem. It is known to be NP complete, defined by n natural numbers $a_1, \ldots, a_n$ representing weights of corresponding objects $1, \ldots, n$ and a reference weight b given by a natural number. Is there a subset $I \subseteq \{1, \ldots, n\}$ such that $\sum_{i \in I} a_i = b$? Brute force approaches enumerating the whole search space consider up to 2^n solution candidates.

Regarding a reaction network-based algorithm, the dynamic programming approach introduced in (Baum and Boneh, 1999) provides an appropriate framework to be adapted for our purpose. Here, the problem parameters are encoded into a directed graph $\mathcal{G} = (V, E)$ with a $(b+1) \times (n+1)$ grid of nodes $V = \{v_{(i,k)} \mid \forall i = 0, \ldots, b\ \forall k = 0, \ldots, n\}$ and edges $E \subset V \times V$ as follows: $E = \{(v_{(i,k)}, v_{(i,k+1)}) \mid \forall i = 0, \ldots, b\ \forall k = 0, \ldots, n-1\} \cup \{(v_{(i,k)}, v_{(i+a_i,k+1)}) \mid \forall i = 0, \ldots, b : i + a_i \leq b\ \forall k = 0, \ldots, n-1\}$. The answer to the knapsack problem is yes iff there exists a path through $\mathcal{G}$ from $v_{(0,0)}$ to $v_{(b,n)}$.

As an example for GRN network composition, we choose the problem instance $n = 3, a_1 = 3, a_2 = 1, a_3 = 2, b = 3$. Upper part of figure 5 illustrates graph $\mathcal{G}$ in this case. Having in mind the presence of a separate signal generator module, we can interpret $\mathcal{G}$ as a finite automaton. Assume that the generator module continually disseminates transmitter substances representing binary strings. Computing agents are able to receive these substances in parallel. So, one agent randomly obtains one binary string for subsequent processing in terms of input data. Acting as a (finite) automaton, the agent can reach a final state which is coupled to the expression of *gfp*.

In this example, the agent checks whether or not the binary string as a candidate solution meets the answer “yes”. Therefore, the first three input bits are interpreted as presence (1) or absence (0) of an object corresponding to the bit position. Accordingly, binary strings 011 and 100 lead to the positive answer. In order to construct the finite automaton from grid $\mathcal{G}$, we

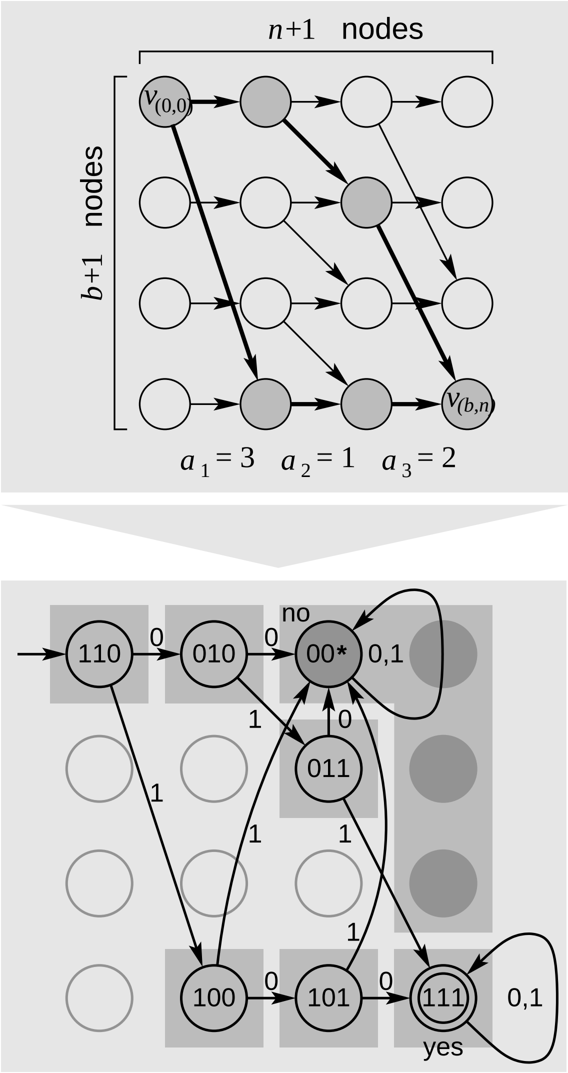


Figure 5: Construction of a deterministic finite automaton (lower part) from the dynamic programming approach (upper part)

transform the nodes into states whereas $v_{(0,0)}$ is the initial and $v_{(b,n)}$ the final state. Unreachable states are removed, and all four states that yield the answer "no" are subsumed into one, see lower part of figure 5. Finally, we obtain seven states. For preparation of the NAND-based network as template for GRN composition, we term each state by a three bit binary number $b_1b_2b_3$. With regard to a minimal number of NAND gates in the final circuit, the Gray code schema is used for state numbering. So, each transition flips at most one bit. Additionally, one state may carry a one-bit don't-care term marked by $*$ that arbitrarily embodies 1 or 0. Using Karnaugh optimisation, the binary number $b'_1b'_2b'_3$ for the new state based on the previous state $b_1b_2b_3$ and the input bit x is defined by:

$$\begin{aligned}
b'_1 &= (b_1 \wedge b_2 \wedge b_3) \vee (b_1 \wedge \overline{b_2} \wedge \overline{x}) \\
&\quad \vee (b_2 \wedge b_3 \wedge x) \vee (b_1 \wedge b_2 \wedge x) \qquad (10) \\
b'_2 &= (b_1 \wedge b_2 \wedge b_3) \vee (b_1 \wedge b_2 \wedge \overline{x}) \\
&\quad \vee (b_1 \wedge b_3 \wedge \overline{x}) \vee (\overline{b_1} \wedge b_2 \wedge x) \qquad (11) \\
b'_3 &= b_3 \vee (\overline{b_2} \wedge \overline{x}) \vee (\overline{b_1} \wedge x) \qquad (12)
\end{aligned}$$

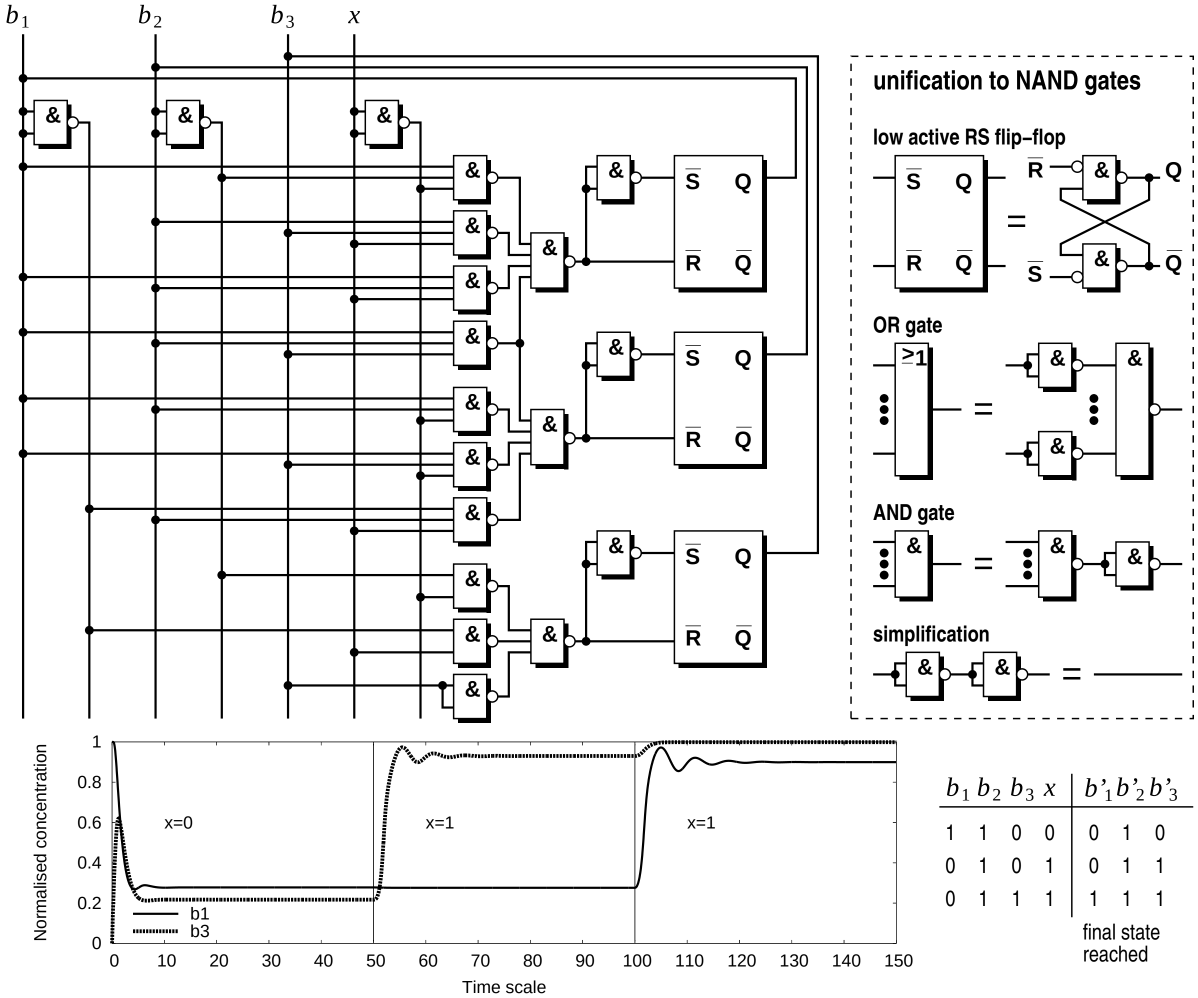
The resulting NAND-based network shown in the upper part of figure 6 was directly derived from these transition rules. After backtransformation of this network into the description level of coupled GRNs, we obtain an artificial biosignalling system consisting of 115 interacting activation resp. repression pathways. The lower part of figure 6 depicts the normalised concentrations of the signalling substances encoding state bits b_1 and b_3 over the time course for three state transitions. Effects of signal weakening, also observed in laboratory studies *in vivo* (see figure 2), are reflected by the model based on Hill kinetics.

7 CONCLUSIONS

This paper addresses three strongly interconnected aspects of biomolecular computing based on biosignals processed by GRNs: wetlab implementation of computational units (NAND gate, low active RS flip-flop) *in vivo*, homogeneous dynamical modelling of these units (Hill kinetics), and their composition to computing agents able to solve real world problems, initially shown in a conceptual study *in silico*. We incorporated AHL as an additional intercellular transcription factor suitable to switch logic gates. Despite being rather slow, they convince by their reliability as a requirement for scalability. Flow cytometry provides a promising method to visualise amounts of output proteins resulting from computing processes *in vivo*. Further studies will consider parallel interactions of GRNs.

ACKNOWLEDGEMENTS

This work is part of the ESIGNET project (Evolving Cell Signalling Networks *in silico*), which has received research funding from the European Community's Sixth Framework Programme (project no. 12789). Further funding from the Federal Ministry of Education and Research (BMBF, grant 0312704A) is acknowledged. We are very grateful to J.J. Collins for providing us with the plasmids and their sequences; to W. Pompe, G. Rödel, K. Ostermann, and L. Brusch



b_1	b_2	b_3	x	b'_1	b'_2	b'_3
1	1	0	0	0	1	0
0	1	0	1	0	1	1
0	1	1	1	1	1	1

final state reached

Figure 6: Minimal NAND-based logic circuit derived from the finite automaton and dynamical simulation of the system resulting from coupled GRNs (Copasi ODE solver). Up to 115 regulatory processes interact via signalling molecules (input/output substances of underlying 22 computational units). The diagram depicts the path $110 \xrightarrow{0} 010 \xrightarrow{1} 011 \xrightarrow{1} 111$ through the finite automaton states $b_1 b_2 b_3$. While state bit b_2 remains at the high level (not shown), b_1 and b_3 switch twice resp. once. Hill kinetic parameters were set as follows: $m_{ij} = 2$, $\Theta_{ij} \in [0.1, 0.3]$ in accordance with achieving stable system states corresponding to binary numbers. Normalised concentration levels between 0 and 0.3 are treated as binary 0, values between 0.7 and 1 represent binary 1.

from Dresden University of Technology for their scientific support and V. Helms from Saarland University for administrative support.

REFERENCES

Aoki, T., Kameyama, M., and Higuchi, T. (1992). Interconnection-free biomolecular computing. *IEEE Computer*, 25:41–50.

Baum, E. and Boneh, D. (1999). Running dynamic programming algorithms on a DNA computer. *DIMACS*, 44:77–86. Proceedings Second Meeting on DNA-Based Computers.

Buchler, N., Gerland, U., and Hwa, T. (2003). On schemes of combinatorial transcription logic. *Proc. Natl. Acad. Sci. USA*, 100(9):5136–5141.

Engebrecht, J. and Silverman, M. (1984). Identification of genes and gene products necessary for bacterial bioluminescence. *Proc. Natl. Acad. Sci. USA*, 81:4154–4158.

Gardner, T., Cantor, C., and Collins, J. (2000). Construction of a genetic toggle switch in escherichia coli. *Nature*, 403:339–342.

Guido, N., Wang, X., Adalsteinsson, D., McMillen, D., Hasty, J., Cantor, C., Elston, T., and Collins, J. (2006). A bottom-up approach to gene regulation. *Nature*, 439:856–860.

Hastings, J. and Nealson, K. (1977). Bacterial bioluminescence. *Annu. Rev. Microbiol.*, 31:549–595.

Hayat, S., Ostermann, K., Brusch, L., Pompe, W., and Roedel, G. (2006). Towards in vivo computing: Quantitative analysis of an artificial gene regulatory network behaving as a RS flip-flop and simulating the system in silico. In *Proc. Bionetics Cavalese*. IEEE. in print.

Henkel, C., Baeck, T., Kok, J., Rozenberg, G., and Spaink, H. (2007). DNA computing of solutions to knapsack problems. *Biosystems*. in print.

Hinze, T., Hatnik, U., and Sturm, M. (2002). An object oriented simulation of real occurring molecular biological processes for DNA computing and its experimental verification. In *DNA Computing*, volume 2340 of *Lecture Notes in Computer Science*, pages 1–13.

Huffman, D. (1954). The synthesis of sequential switching circuits. *Journal of the Franklin Institute*, 257(3):161–190.

Kaern, M., Blake, W., and Collins, J. (2003). Engineering of gene regulatory networks. *Annu. Rev. Biomed. Eng.*, 5:179–206.

Kobayashi, H., Kaern, M., Araki, M., Chung, K., Gardner, T., Cantor, C., and Collins, J. (2004). Programmable cells. *Proc. Natl. Acad. Sci. USA*, 101:8414–8419.

Magnasco, M. (1997). Chemical kinetics is Turing universal. *Physical Review Letters*, 78(6):1190–1193.

Mestl, T., Plahte, E., and Omholt, S. (1995). A mathematical framework for describing and analysing gene regulatory networks. *J. Theor. Biol.*, 176:291–300.

Miller, M. and Bassler, B. (2001). Quorum sensing in bacteria. *Annu. Rev. Microbiol.*, 55:165–199.

Morris Mano, M. (1991). *Digital Design*. Prentice-Hall, second edition.

Ptashne, M. (1992). *Genetic Switch: Phage Lambda and Higher Organisms*. Blackwell.

Schaefer, A., Val, D., Hanzelka, B., Cronan jr., J., and Greenberg, E. (1996). Generation of cell-to-cell signals in quorum sensing: Acyl homoserine lactone synthase activity of a purified vibrio fischeri LuxI protein. *Proc. Natl. Acad. Sci. USA*, 93:9505–9509.

Sprinzak, D. and Elowitz, M. (2005). Reconstruction of genetic circuits. *Nature*, 438:443–448.

Thomas, R. (1991). Regulatory networks seen as asynchronous automata: a logical description. *J. Theor. Biol.*, 53:1–23.

Weiss, R., Homsy, G., and Knight jr., T. (1999). Toward in-vivo digital circuits. *DIMACS*. Workshop on Evolution as Computation.

Yokobayashi, Y., Weiss, R., and Arnold, F. (2004). Directed evolution of a genetic circuit. *Proc. Natl. Acad. Sci. USA*, 99:16587–16591.