

Computational Models of Signalling Networks for Non-linear Control

Luis A. Fuente^{a,*}, Michael A. Lones^a, Alexander P. Turner^a, Susan Stepney^b, Leo S. Caves^c, Andy M. Tyrrell^a

^aDepartment of Electronics, University of York

^bDepartment of Computer Science, University of York

^cDepartment of Biology, University of York,

York Centre for Complex Systems Analysis (YCCSA), York, YO10 5DD, UK

Abstract

Artificial Signalling Networks (ASNs) are a computational approach inspired by the signalling processes inside cells that decode outside environmental information. Using evolutionary algorithms to induce complex behaviors, we show how chaotic dynamics in a conservative dynamical system can be controlled. Such dynamics are of particular interest as they mimic the inherent complexity of non-linear physical systems in the real world. Considering the main biological interpretations of cellular signalling, in which complex behaviours and robust cellular responses emerge from the interaction of multiple pathways, we introduce two ASN representations: a stand-alone ASN and a coupled ASN. In particular we note how sophisticated cellular communication mechanisms can lead to effective controllers, where complicated problems can be divided into smaller and independent tasks.

Keywords: cellular signalling, biochemical networks, crosstalk, evolutionary algorithms, chaos control.

1. Introduction

Cellular signalling needs to engage in many forms of communication to enable cells to sense and respond to the outside world. This capability is vital for cells to survive and adapt to constantly fluctuating environments. In multicellular organisms, the role of cellular signaling is especially significant as it is responsible for the coordination of complex multicellular interactions and the production of collective responses.

Broadly speaking, cellular signaling is a sequence of events triggered by a biochemical signal that requires a cellular response. Signalling pathways are the simplest cellular structures connecting the outside environment with the genes they regulate. A closer inspection reveals that cellular signalling starts when a surface receptor binds an extracellular messenger, which diffuses an intracellular signal to an effector protein inside the cell. This then produces secondary messengers, which transmit the information further into the cell along signalling pathways. Spatially or temporally variable catalytic reactions or cascades of protein kinases lead to changes in gene expression, bringing about a change in cellular activity. Cells also show a complex internal organization, which regulates the number of cellular components activated by secondary messen-

gers and guides the interactions between cellular regions. Crosstalk (Schwartz and Baron, 1999) captures the interaction between signaling pathways that lead to the formation of complex networks that produce a coordinated response.

In this paper, we extend our previous work on Artificial Signalling Networks (ASNs) (Fuente et al., 2012) and suggest the use of crosstalk as a mechanism to model the structural and temporal topologies of cellular signalling, capturing its intrinsic dynamics. In order to test our model, we apply it to the control of a numerical dynamical system, whose properties mirror the complexity of the cellular environment.

This paper is organised as follows: Section 2 presents a brief overview of dynamical systems, Section 3 reviews the modeling of ASNs, highlighting the challenges this involves, Section 4 presents the new model and proposes the evolutionary algorithm used to induce model instances, Section 5 presents results and analysis and Section 6 concludes the paper.

2. Dynamical Systems

A dynamical system is a mathematical model consisting of a state space and a function, or *evolution rule*, that specifies its current state within the space state based on an initial condition (Stepney, 2011). The evolution rule defines the motion and behaviour of the system across the state space. Dynamical systems can be *autonomous* or *non-autonomous*. The former is a closed system whose dynamics are not perturbed by the outside word. The

*Corresponding author

Email addresses: laf509@york.ac.uk (Luis A. Fuente), michael.lones@york.ac.uk (Michael A. Lones), apt503@york.ac.uk (Alexander P. Turner), susan.stepney@cs.york.ac.uk (Susan Stepney), leo.caves@york.ac.uk (Leo S. Caves), andy.tyrrell@york.ac.uk (Andy M. Tyrrell)

latter defines an open system changing over time, as inputs are received from an external environment. Likewise, dynamical systems can be *discrete* or *continuous* in time, depending on the type of evolution rule: difference equations in the former and differential equations in the latter.

Given a set of initial points within a discrete state space, the evolution rule defines their *trajectories* as a sequence of states over a period of time. A dynamical system where trajectories do not contract to a limited region of the state space is known as a *conservative* system.

Dynamical systems can display a wide range of behaviours. The most interesting are those involving holistic irregular and unpredictable properties; this atypical dynamism is known as *chaos*. Despite being deterministic, chaotic systems display aperiodic behaviours characterised by an exponential sensitivity to initial conditions and the existence of strange attractors. Whereas the former suggests that small changes in the initial conditions convey highly different trajectories throughout the state space, the latter defines fractal and non-linear regions where trajectories may converge.

2.1. Chirikov's Standard Map

Chirikov's standard map (Chirikov, 1962) is a conservative and discrete two-dimensional dynamical system representing iteratively the interactions of two canonical variables:

$$\begin{aligned} x_{n+1} &= (x_n + y_{n+1}) \bmod 1 \\ y_{n+1} &= y_n - \frac{k}{2\pi} \sin(2\pi x_n) \end{aligned} \quad (1)$$

One of the map's main properties is its capacity to represent different dynamics as its nonlinearity increases. Thus, low values of k preserve an ordered state where trajectories lead to periodic and quasi-periodic trajectories bounded on the y -axis (see Fig. 1a). As k increases, chaotic dynamics arise in the form of chaotic islands along the y -axis (see Fig. 1c). The type of trajectories depend on the map's initial conditions. The map shows a behavioural inflection point, k_c , at $k \approx 0.972$ (see Fig. 1b). Initial impermeability progressively disappears as $k > k_c$ (see Fig. 1d), enabling trajectories to vertically travel across the map. The example in Fig. 1 shows the permeability of the map increasing as k increases, characterised by the gradual encroachment of the chaotic regions.

Chirikov's map is a model of many types of complex physical systems that occur in the real world (Izraelev, 1980). Being able to control Chirikov's map could have benefits for a range of real world problems, such as controlling spacecraft trajectories in n-body gravitational systems or autonomous robot manipulators.

3. Artificial Signalling Networks

Activities and functions of biological organisms emerge from the interactions amongst the biochemical networks

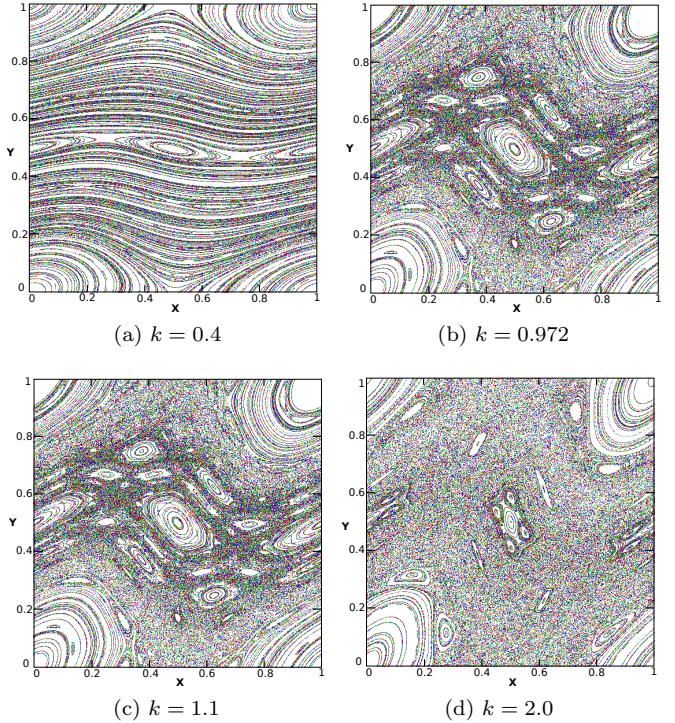


Figure 1: Sampled trajectories of Chirikov's standard map using different values of k , showing the transition from the ordered to the chaotic state. Each map is plotted using 400 randomly chosen initial points across the unit interval over 800 iterations.

operating within cells. These networks are categorised in three domains: genetic networks, which derive new behaviour via genetic regulation (Banzhaf, 2004); metabolic networks, preserving the physiological equilibrium inside cells (Fontana, 1992); and signalling networks, translating externals inputs into meaningful biological signals (Bray, 1995). Computational models of these biochemical structures are collectively termed Artificial Biochemical Networks (Lones et al., 2010; Turner et al., 2012). The ability of biochemical networks to encapsulate cellular interactions has led to a growing interest in the integration of biological knowledge into computational models, which represent and modulate the dynamics of these networks.

An ASN is an abstraction of interacting molecules within cellular signalling pathways. It closely models the attributes that enable cells to take chemical signals as inputs and generate some adaptive output. From a computational perspective, the importance of ASNs lies in their capacity to coordinate the set of events within cells that trigger robust, efficient and specific responses, their ability to work as independent working units, and their capacity to adapt to environmental perturbations.

The understanding of complex biological processes inside cells has led to a large diversity of ASN representations that attempt to mimic their dynamics. In particular, ASN modelling is highly dependent on the type of exper-

imental data, the amount of prior information and the modeling context. This means that a concise classification of ASN models is hard to produce. Therefore, the amount of available information related to a signalling process is the primary form of classification (Kestler et al., 2008).

One possibility to model ASNs relies on the quantitative description of specific pathways. Experimental and mathematical approaches facilitate the identification of the functional elements as well as their interactions in concrete pathways, thereby simplifying their modelling. Initial steps in such models conceive signal pathways as linear stimuli chains, where cellular responses are quantified as fluctuations in protein activity (Kholodenko et al., 1997). The inclusion of feedback loops has helped to integrate dynamical behaviors leading to a new generation of computational models (Nevers and Iyengar, 2002). For instance, the fuzzy model suggested in (Huang and Hahm, 2008) computes the dynamics of the IL-6 pathways based on the state of the components, the initial inputs and a set of fuzzy rules. Likewise, the validity of logic-based modelling has been widely demonstrated in (Morris et al., 2010). As an alternative to the physical basis of the previous models, (Said et al., 2003) takes a more abstract approach, modelling the interaction between two participant elements, applying this to simulate MAP kinase cascades as Markov chains. However, the reconstruction of these pathways is insufficiently accurate to capture the complexity of signalling components. Additional abstractions include Boolean models, which are considered in (Decraene et al., 2009; Réka, 2004).

Qualitative approaches offer an alternative approach to signaling pathway modeling. ASNs are commonly described as protein-protein interaction networks, where nodes are molecules and edges are undirected reactions (Bray, 1995). Such models constitute graph-based approaches aiming to encapsulate the spatial properties of signaling pathways. However, many of them are unable to fulfill this objective, or it is only partially achievable, as node connectivity is strictly constrained. An example of this limitation can be found in the acyclic nature of Bayesian network-like models (Tulupyyev and Nikolenko, 2005; Sachs et al., 2002). Extensions to these representations are, for example, probabilistic networks (Friedman et al., 2000), reaction-diffusion networks (Dale, 2006) and network motifs (Milo et al., 2002).

Another way to design ASNs is the use of evolutionary algorithms. They can induce complex behaviours in a concise and evolvable way (Lones et al., 2010) and some specific functionalities are achievable only through evolutionary processes (Decraene et al., 2009). Evolved ASNs have been successfully used to capture simple forms of biological signal processing (Bray and Lay, 1994; Deckard and Sauro, 2004). In this paper we propose an alternative approach: we use generic evolved interaction graphs for the modelling of artificial signalling networks, where no specific information of either the participating elements or their interactions is needed. By doing this, we avoid

connectivity-based constraints and encourage the creation of a topology resulting uniquely from the interaction of the ASN with its environment. This increases its adaptability when facing different types of environments and facilitates overcoming the limitations found in enzymatic connectivity (Klamt et al., 2006).

4. State Space Targeting with ASNs

The signal transduction processes inside cells depend on complex interaction between enzymes. Although these interactions vary in number of participants, they are essential in the generation of a cellular response. In fact, enzymes are not functional unless they are assembled together into a biological structure. Likewise, some of the main cellular functions are only achievable under certain configurations.

Despite the diversity of models aiming to capture the properties of intracellular signaling networks, in this paper we concentrate on directed interaction graphs to capture the topological and temporal relationships intrinsic to intracellular signaling pathways. A directed interaction graph is a mathematical abstraction representing the dynamical behaviours and interactions of multi-component systems interacting over time. Therefore, an ASN constitutes an indexed set of enzyme-based nodes and a set of directed connections representing their biochemical reactions. Each reaction relates the substrates of a set of indexed enzymes in order to calculate the real-valued product concentration. This approach extends the Artificial Metabolic Network model in (Lones et al., 2010) by using a layered internal representation to deal with the spatial properties of signalling pathways. Formally: $\text{ASN} = \langle E, R, I_E, O_E \rangle$, where:

E set of enzymes $\{e_0, e_1, \dots, e_n : e_i = \langle S_i, P_i, m_i \rangle\}$.

S_i enzyme's substrates.

P_i enzyme's products.

$m_i : \mathbb{R}^n \rightarrow \mathbb{R}^n$ substrate-product mapping functions.

R set of enzymatic reactions $\{r_0, r_1, \dots, r_n : r_i = \{+, -\}\}$.

$I_E \subset E$ set of enzymes used as inputs.

$O_E \subset E$ set of enzymes used as outputs.

The execution of a stand-alone ASN starts with the random initialisation of its enzymatic concentrations (S_i and P_i), if not previously executed. Concentrations of external inputs are explicitly set according to the indices specified in I_E . At each time step, each enzyme e_i applies its mapping function m_i to the current concentrations of its substrates S_i to determine the new concentration of its product P_i . This new concentration is the mean output of all different contributing enzymes. After the network has iterated a certain number of times, outputs are explicitly gathered from those enzymes whose indices belong to O_E . A The execution of a stand-alone ASN is also illustrated in Algorithm 1.

Algorithm 1 Execution of a stand-alone ASN

Require: network size $> |I_E| + |O_E|$

```
if first execution then
    initialize  $e_i \in E$ 
end if

Set  $I_E$  concentrations from external inputs
for  $i = 0$  to ASN time steps do
    for  $j = 0$  to network size do
         $S_j = \text{mean}(e_j \in r_j)$ 
         $P_j = m_j(S_j)$ 
    end for
end for
External outputs =  $P(e \in O_E)$ 
```

4.1. Coupling Artificial Signalling Networks

The ASN model just described represents a single signalling pathway. In biological cells, responses are often a result of the interaction between multiple pathways. An initial interaction takes place when an external chemical binds to several membrane receptors of different pathways. A secondary level happens internally and links multiple pathways together in a manner in which their cooperation produces of a joint response. This phenomenon is usually termed crosstalk and can be seen as non-linear signal transduction, which encourages robustness in cellular responses.

There has been a growing interest in the study of crosstalk in order to make possible the development of more complex networks (Hucka et al., 2003). Additionally, this combination of several models into an single network more closely reflects biological systems. Since information exchange in biological networks is not yet fully understood, pathway integration is still a challenge. In this paper, we propose an indexed set of ASNs, each of which represents a cascade of protein kinases and are composed of a set of indexed enzymes and a set of biochemical reactions. Pathway crosstalk is simulated by a set of directed edges connecting to neighbouring ASNs. Formally: coupled-ASN = $\langle \text{ASN}, C_P, I_E, O_E \rangle$:

ASN set of indexed artificial signaling networks

$$\{\text{asn}_0, \text{asn}_1, \dots, \text{asn}_n : \text{asn}_i = \langle E, R, I_E, O_E \rangle\}.$$

$C_P \in [0, 1]$ enzyme's crosstalk probability.

$I_E \subset E$ set of enzymes used as inputs, $|\text{ASN}| = |I_E|$.

$O_E \subset E$ set of enzymes used as outputs.

Enzymes involved in crosstalk have their product concentration asymptotically reduced to half of their maximum value. This limitation attempts to imitate low production rates associated with high energy consumption in crosstalk reactions.

We also look at extended models which take into consider multiple-bound enzymes. In particular, we look

at the effect of having two types of enzymes depending on the number of times they are phosphorylated: single or double. This more accurately represents the different phosphorylation levels in cascades of protein kinase.

The execution of a coupled-ASN is shown in Algorithm 2.

Algorithm 2 Execution of a coupled-ASN

Require: network size $> |I_E| + |O_E| \wedge |\text{ASN}| = \text{number of external inputs}$

```
if first execution then
    Initialize ASN  $\rightarrow e \in E$ 
    for each  $e_i \in \text{coupled-ASN}$  do
        if random number  $< C_P(e_i)$  then
            Add crosstalk R
        end if
    end for
end if

Set  $I_E$  concentrations from external inputs

for  $i = 0$  to  $|\text{ASN}|$  do
    for  $j = 0$  to ASN time steps do
        for  $k = 0$  to network size do
             $S_k = \text{mean}(e_{ik} \in r_k)$ 
             $P_k = m_k(S_k)$ 
        end for
    end for
end for
External outputs = mean( $O_E$ )
```

4.2. Biochemical Mappings

Four types of parameterisable functions are chosen as enzymatic mappings. These are the most common models of molecular regulatory functions within biological systems (see Table 1).

- *The Hill equation* quantifies the substrate-enzyme cooperativity by measuring the number of substrates that must bind to an enzyme to cause an effect. It is a quasi-sigmoidal function in which $v \in [0, 1]$ is its asymptotic threshold, $k \in [0, 1]$ determines its gradient and $h \in \mathbb{R}$ is the Hill coefficient indicating the degree of cooperativeness. This equation is extended by adding the binding probability $\beta \in [0, 1]$ to stimulate the reaction speed at high substrate concentrations. For multiple inputs $x = \sum_{j=0}^n i_j w_j / n$. Negative concentrations indicate inhibition, with the concentration determined by $m(x)^- = 1 - m(x)$.

- *The Michaelis-Menten equation* characterises the enzyme kinematic reactions, such as gene regulation or enzyme catalytic reactions. It is a hyperbolic function where $v \in [0, 1]$ is the asymptotic threshold and $k \in [0, 1]$ determines its gradient. For multiple inputs, $x = \sum_{j=0}^n i_j w_j / n$, where $i_0 \dots i_n$ are inputs

and $w_0 \dots w_n \in [-1, 1]$ are the corresponding input weights. Negative values indicate inhibition, with the concentration determined by $m(x)^- = 1 - m(x)$.

- *The Multi-Dimensional Michaelis-Menten equation* parameterises the enzymes' kinematics when substrates are produced by multiple enzymes based on a probability of binding $\beta \in [0, 1]$, where $v \in [0, 1]$ is the asymptotic threshold and $k \in [0, 1]$ determines its gradient and $m, n \in \mathbb{R}$ are coefficients indicating activation, when $m = n$, and repression, when $n = 0$ and $m > 0$ (Alon, 2007).
- *The first-order kinetics equation* is the simplest kinetics mapping and describes the enzymatic rate of phosphorylation with respect to the concentrations of its active site and unphosphorylated substrates, where $v \in [0, 1]$ is the asymptotic threshold. For multiple inputs, $x = \sum_{j=0}^n i_j w_j / n$, where $i_0 \dots i_n$ are inputs and $w_0 \dots w_n \in [-1, 1]$ are the corresponding input weights. Negative values indicate inhibition, with the concentration determined by $m(x)^- = 1 - m(x)$.

Table 1: Mathematical functions used in ASNs.

Hill equation:

$$m(x) = vx^h(k^h x^h)^{-1}$$

Michaelis-Menten equation:

$$m(x) = vx(k - x)^{-1}$$

Multi-Dimension Michaelis Menten equation:

$$m(x) = \sum_{i=0}^k \beta_i (x_i/k_i)^{n_i} (1 + \sum_{i=0}^n (x_i/k_i)^{m_i})^{-1}$$

First-order kinematic equation:

$$m(x) = vx^2(1 + x + x^2)^{-1}$$

4.3. Evolved Artificial Signalling Networks

A standard generational evolutionary algorithm with elitism (rate = 0.2), tournament selection (size = 4), uniform crossover (rate = 0.48), and point mutation (rate = 0.16) has been used to evolve both ASN models. This configuration guarantees the preservation of the fittest individuals and mutation of 13% of the solutions. A multi-chromosomal encoding is used to evolve coupled-ASNs (see Fig. 2). The chromosomal representation is beneficial to counteract problems with growing complexity, as it permits division of a complex problem into small tasks, which can be independently modelled using different representations (Mayer and Spitzlinger, 2003) and improves the evolution of complex structures (Cavill et al., 2005). In an attempt to develop larger cellular networks, multiple recombined chromosomes offer a natural possibility to encode separate cellular regions; therefore, we believe that crosstalk becomes an evolutionary parameter controlling

the degree of complexity and the robustness of the global response.

A coupled-ASN is encoded as an indexed array of chromosomes followed by timing information. Each chromosome represents a stand-alone ASN, encoded as an indexed array of enzymes. Crossover points lie between the enzymes' boundaries and chromosome shuffling is not permitted. In an attempt to reduce the complexity of the network, the number of enzymes of each stand-alone ASN has been fixed at 10.

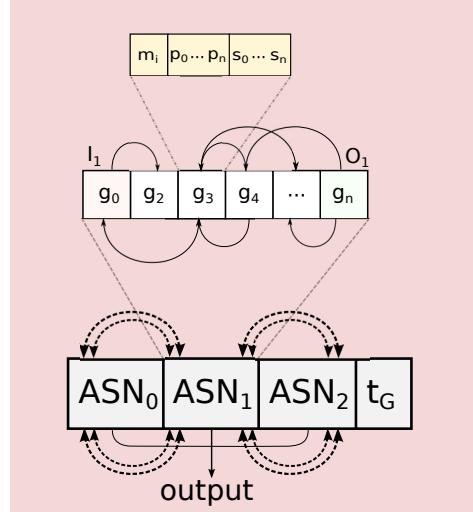


Figure 2: Genetic encoding of a coupled artificial signalling network comprising three chromosomes. Unbroken lines indicate the enhancing and inhibitory catalytic reactions between enzymes. Broken lines indicate crosstalk connections between ASNs.

For both representations, input and output enzymes are determined according to their relative position in the graph (i.e. low- and high-numbered genes). Chemical values and mapping parameters are represented using floating-point and mutated using a Gaussian function with its center at the current value. Continuous values introduces two main benefits. First, they ease the coupling with external environments, such that inputs and outputs do not require a binary encoding. Second, they do no limit the size of the space state. In Boolean Networks, the set of possible states is constrained to 2^N , where N is the number of nodes. Since the state space is finite, the network's dynamics become deterministic and, in the case of small networks, it will eventually converge to a static orbit. The use of continuous values brings about an infinite space state (within the limits of representation), which can lead to more complexity in both the network's dynamics and expression capacity. Nevertheless, mutation is restricted to one of the following operations to meet biochemical plausibility in the graphs (Ziegler and Banzhaf, 2001):

1. Increasing/Decreasing chemical values.

2. Altering mapping function parameters.
3. Changing reactions rates by changing weights.
4. Adding/removing reaction's participants.
5. Varying execution time.

The evolution process of both ASN models starts with the generation of a random initial population. The external inputs are the initial state position, whilst the output is the control parameter and is determined by the chemical value of the highest-numbered gene at the end of execution. Both representations are evolved using a population of 500 individuals. Initial experiments showed this was sufficient to find correct solutions. All runs terminate after 100 generations.

4.3.1. Traversing Chirikov's standard map

A progressive increase of the k value in the standard map entails a reduction of the permeability of its middle region. Therefore, the map becomes navigable from the bottom to the top and, consequently, it is possible to find a controller able to transverse it. In principle, the disappearance of the horizontal chaotic islands is noticeable as long as $k > k_c$ and becomes more visible when $k \approx 1.1$. The goal is to evolve an ASN-based controller able to modulate the parameter k inside the range [1.0, 1.1] and find a path starting in a predefined region at the bottom of the map and ending in a specific region at the top. This range still ensures the presence of chaotic islands in the middle region of Chirikov's standard map, making the task challenging, but also allowing trajectories to traverse it. The inputs of the ASN are the position in the map, (x, y) , and the Euclidean distance from the current position to the top-centre of the map, d ; the output is the value of k . The evolved network is evaluated on 20 trajectories from different points inside the bottom region. The fitness function is the mean number of steps these trajectories take to traverse the map. Controllers are limited to a maximum number of 1000 steps. Controllers exceeding this threshold are penalised with a fitness of 2000 steps.

5. Results

Figure 3 shows the distribution of the number of steps needed to traverse Chirikov's standard map for the evolved controllers at the end of the 100 evolutionary runs. The results indicate that both representations, the stand-alone ASN and the coupled-ASN, led to valid controllers, with scores approaching 100 and 300 steps respectively (see Fig. 4a – 4b for an example of map traversing using both models). The best performance arises from the Michaelis-Menten regulatory function in both scenarios. Although stand-alone ASNs produce better solutions, coupled-ASN are still of interest because their cross-linked ASN individuals also bring about predictable and ordered behaviours. From a dynamical perspective, it is also noticeable that the complex dynamics intrinsic to coupled-ASNs can also be

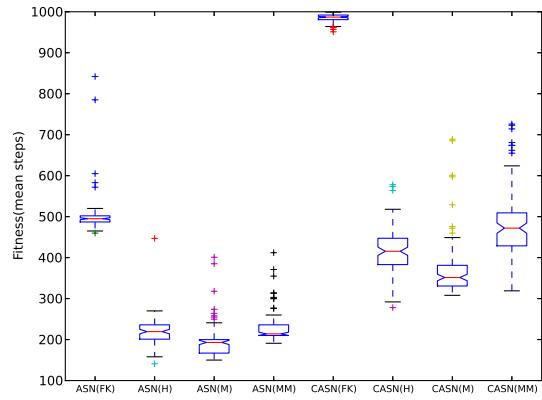


Figure 3: Space state targeting results using evolved ASNs with (H)ill, (M)ichaelis-Menten, (M)ulti-Dimensional (M)ichaelis-Menten ($\beta = 0.5$) and (F)irst-order (K)inematics as regulatory mappings. Summary statistics of the 100 runs are shown as box plots. Low values are better.

modulated by the interactions amongst their functionally independent components.

The choice of regulatory function also has significant consequences upon the plausibility of the solutions. It is apparent that first-order kinematic-based controllers have the worst performance in both models. From a biological perspective, zero- and first-order kinematics model the phosphorylation process when reaction rates are either independent to the concentration of the participating enzymes or dependent on only one enzyme. This suggests that more-complex regulatory functions, which integrate multiple-enzymatic interactions, are beneficial to complex control processes.

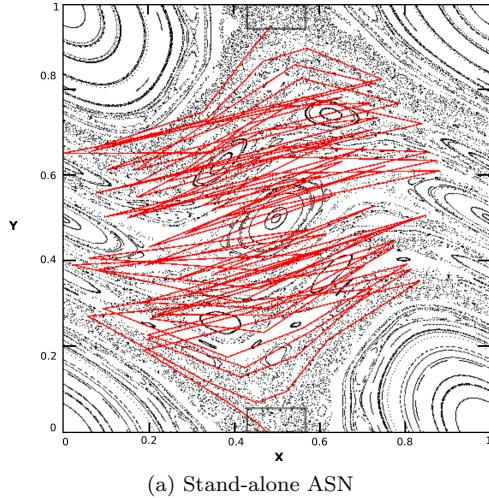
Table 2: Average of ASN-controllers able to traverse Chirikov's map when $k \in [1.0, 1.1]$

Regulatory Function	ASN	C-ASN
Michaelis-Menten equation	81.4%	62.9%
Hill equation	87%	80.1%
Multi-Dim. Michaelis-Menten	97.6%	89.4%
First-order Kinematics	71.1%	8.7%

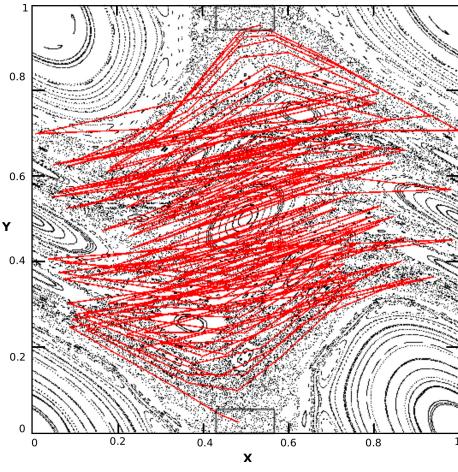
Table 2 analyses the effectiveness of the regulatory functions over 1000 different trajectories. The Hill and Multi-dimensional Michaelis-Menten equations generate the most successful controllers at the end of the evolution process. The fact that both equations estimate multi-binding reaction rates facilitates the expression of enzymatic affinities, which are essential in biological signalling as control mechanisms (Somogyi et al., 1997). While the Hill coefficient (h) measures such binding affinity in the Hill equation, the binding probability (β) does it in the

Multi-dimensional Michaelis-Menten equation.

To gain some insight into the behaviour of the evolved ASNs, we have looked at how the output response (k) varies over the number of steps needed to transverse Chirikov's standard map. Fig. 5a–5b shows examples for different regulatory functions.



(a) Stand-alone ASN

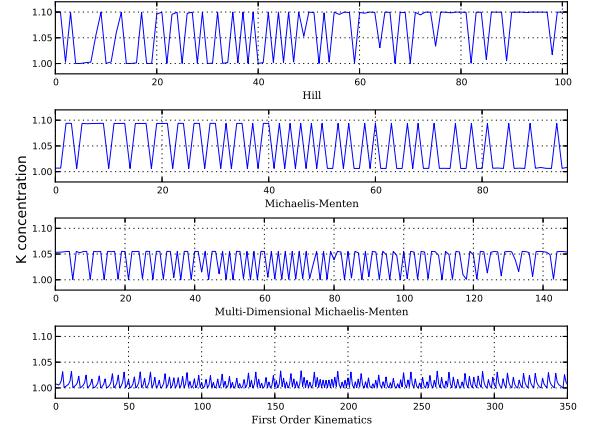


(b) Coupled-ASN

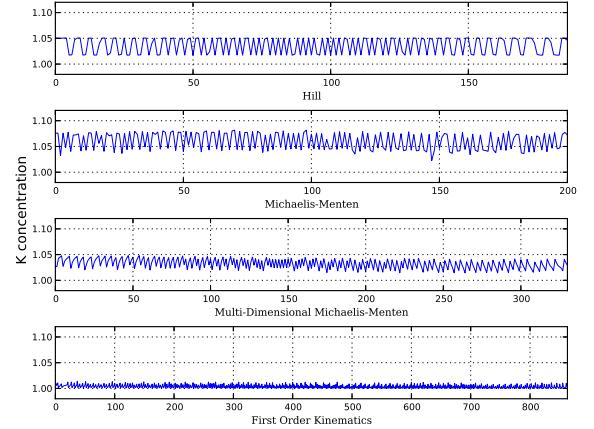
Figure 4: Example of state space targeting from a region at the bottom, $(0.45, 0) \rightarrow (0.55, 0.05)$, to a region at the top, $(0.45, 0.95) \rightarrow (0.55, 1)$, in Chirikov's standard map using both ASN approaches. The stand-alone ASN requires 92 steps to complete the task. The coupled ASN does it in 186. Chirikov's standard map is plotted with $k \approx 1.01$.

Notably, evolved ASN controllers display a symmetrical behaviour in the sequence of movements that the controller needs to move from the lower region to the upper region in the standard map. This phenomenon is clearly seen in the second subgraph in the top of Fig. 5a where the Michaelis-Menten regulatory function is used; however, it is also noticeable in the other representations. As the controller traverses the initial chaotic area, the k value follows an attractor, disappearing when the controllers reach the chaotic islands in the middle region of the map (steps

$0 - 40$ in the stand-alone ASN(M)). Its crossing induces a behavioral change in the form of a stable oscillator (steps $40 - 52$). Finally, the presence of a second attractor leads the controller through the upper chaotic area (steps $52 - 92$). This illustrates the capacity of both ASN models to generate different behaviors upon changes in the external environment.



(a) Stand-alone ASN outputs



(b) Coupled-ASN outputs

Figure 5: k values used to traverse Chirikov's standard map at each step for both ASN models using the regulatory functions in Table 1. k is modulated in the range $[1.0, 1.1]$. Three different behaviours can be seen corresponding to the lower chaotic region, chaotic islands in the middle region and the upper chaotic region.

5.1. Crosstalk as a linking tool

Perhaps the most interesting result is the capacity of the coupled-ASNs to lead to valid solutions when every pathway computes its dynamics independently or quasi-independently. As can be seen in Fig. 6, the degree of crosstalk has a significant effect upon the capacity of the solution to transverse the map. Whilst low crosstalk levels

seem to be slightly beneficial, high values induce uncorrelated noise, which reduces the overall performance. This is clearly illustrated in Table 3, which compares the effectiveness of coupled-ASNs with different crosstalk configurations, averaged over 100 runs and 1000 random trajectories. Similar conclusion on the effect of crosstalk were noted in (Arias and Hayward, 2006). Furthermore, high crosstalk rates produce a decrement in the capacity to infer generic network architectures through evolution, however good solutions can still be found. This is particularly important when modelling large cellular areas, where both pathway differentiation and their ability to respond to perturbation are essential in order to sense different stimuli and produce a coordinated answer.

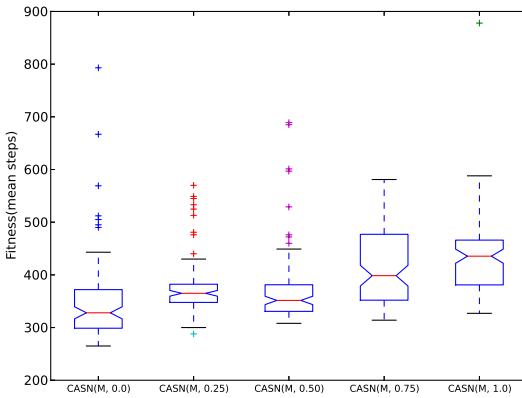


Figure 6: Space state targeting using an evolved coupled-ASN with (M)ichaelis-Menten regulatory map and different crosstalk probabilities, averaged over 100 runs.

It is also important to highlight the ability of coupled-ASNs to traverse the map even when there is no crosstalk (see Table 3). This shows how complex problems can be subdivided into smaller and specialised tasks, which individually produce valid solutions. An analogous organisation can be found inside cells, where the interaction between cellular regions is constrained. This highlights the important role that crosstalk plays in the formation of more complex and realistic nonlinear signalling models. Therefore, we believe that it is possible to develop biologically plausible models imitating the motivating structure of the signalling pathways.

Given a network topology that has entirely arisen from the model's interactions with its external environment – Chirikov's standard map – we believe that coupled-ASNs are able to cope with incomplete or corrupt environmental information. This would considerably increase the model's adaptability when faced with real environments. Initial experimentation upon the modulation of the network output certainly support our previous hypothesis. Fig 7 shows the concentration of both partial and global coupled-ASN outputs at each step during the map traversing process. It becomes apparent that there is an uneven distribution re-

Table 3: Ability to control trajectories of coupled-ASN controllers when $k \in [1.0, 1.1]$ at different crosstalk rates.

Crosstalk Rate	Effectiveness	Average	Best
0.0	73.4%	502	197
0.25	82.6%	433	192
0.5	62.9%	680	198
0.75	60.1%	660	214
1.0	60.3%	616	200

garding the importance that every partial output has upon the global coupled-ASN output.

As can be seen in Fig. 7 the global output follows the pattern generated by the first partial output, but on a different scale. This is a consequence of the modulation effect caused by the computation of the global output as the mean of all contributing partial outputs, which highlights that some inputs may not be needed to traverse the map. Particularly, the output of the second ASN has a relatively low concentration, which appears to have a minimal effect on the output. A similar behaviour is noticed with the use of other regulatory functions.

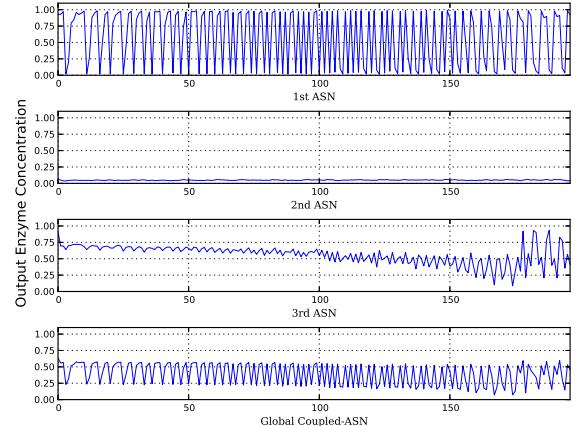


Figure 7: Example of the partial and global outputs of an evolved coupled-ASN. The three upper graphs represent the partial outputs gathered from three different stand-alone ASNs when the current x-position, the current y-position and the Euclidean distance (d) are input respectively into each one of the three networks. The lower graph is the global output of the coupled-ASN.

6. Conclusions

In this paper, we have presented an interaction graph-based approach to the modelling of signalling pathways using evolutionary algorithms. The evolved ASN attains promising results in chaos targeting within Chirikov's standard map. Notably, our results illustrate that effective

controllers can be found when signalling networks are interpreted as an individual pathway or a set of pathways, thereby demonstrating how the topology and adaptability of signalling networks can be evolved. Likewise, an accurate representation of cellular spatial properties was achieved without knowledge of the surrounding environment and limitations in the enzymatic connectivity.

Additionally, this paper has highlighted the network sensitivity to different crosstalk levels when attempting to induce complex dynamics. This shows that crosstalk has a large impact upon the validity of the controllers and can be considered as a powerful mechanism in order to build sophisticated and realistic representations of cellular regions. Similar conclusions have been suggested in (Bhalla and Iyengar, 1999). We aim to extend our understanding of crosstalk's properties in order to produce an effective and dynamical communication protocol amongst signalling pathway-based models.

Our future work will focus on analyzing the complex dynamics and spatial distribution of the coupled-ASN model in an attempt to solve more complex-real word problems. In particular, we intend to develop an ASN-based robot controller. Of special interest is the work presented in (Lones et al., 2011) where the combination of an AGN and an AMN is used to evolve quadrupedal gaits for legged robots. Since robot kinematics can be considered as a set of non-linear equations, a coupled-ASN controller becomes plausible, where every stand-alone ASN is responsible for one equation and crosstalk acts as a reinforcement mechanism. Likewise, we are also looking at different evolutionary strategies in order to promote the importance that crosstalk has upon the performance of the controllers.

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