On Chemical Reaction Network Design by a Nested Evolution Algorithm

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Inria Saclay, Lifeware group, Palaiseau, France

Abstract. One goal of synthetic biology is to implement useful functions with biochemical reactions, either by reprogramming living cells or programming artificial vesicles. In this perspective, we consider Chemical Reaction Networks (CRN) as a programming language, and investigate the CRN program synthesis problem. Recent work has shown that CRN interpreted by differential equations are Turing-complete and can be seen as analog computers where the molecular concentrations play the role of information carriers. Any real function that is computable by a Turing machine in arbitrary precision can thus be computed by a CRN over a finite set of molecular species. The proof of this result gives a numerical method to generate a finite CRN for implementing a real function presented as the solution of a Polynomial Initial Values Problem (PIVP). In this paper, we study an alternative method based on artificial evolution to build a CRN that approximates a real function given on finite sets of input values. We present a nested search algorithm that evolves the structure of the CRN and optimizes the kinetic parameters at each generation. We evaluate this algorithm on the Heaviside and Cosine functions both as functions of time and functions of input molecular species. We then compare the CRN obtained by artificial evolution both to the CRN generated by the numerical method from a PIVP definition of the function, and to the natural CRN found in the BioModels repository for switches and oscillators.

1 Introduction

One goal of Synthetic Biology is to implement useful functions using biochemical reactions, either by reprogramming living cells, or by programming artificial devices. While the former approach is mainstream in synthetic biology [47,41,19,17,12,1], examples of the later approach are given by the whole field of DNA computing [2,38,11,15,8] or analog computing with enzymatic reactions [42], possibly encapsulated in artificial vesicles [13].

Chemical Reaction Networks (CRN) are used to describe systems of chemical reactions. In this article, we consider CRN as a programming language [20,46]. We focus on their continuous semantics defined by Ordinary Differential Equations (ODE) on the molecular concentrations. We study the CRN program synthesis problem, i.e. the problem of designing a CRN for implementing a given function, either a function of time (problem 1) or a function of input molecular species (problem 2):

Problem 1. Given a function $f : [0, a] \to \mathbb{R}$, construct a CRN such that one of the species has a concentration A implementing the function f. That is $A(t) = f(t), t \in [0, a]$.

Problem 2. Given a function $f : [0, a] \to \mathbb{R}$, construct a CRN such that, when initialized for a given input, one of the species converge to the desired result. That is $\forall x \in [0, a], X(t = 0) = P_I(x) \Rightarrow A(t = 1) = f(x)$ where X is the vector of species concentrations and P_I a polynomial of degree one.

A recent result proving the Turing completeness of continuous CRN [20], restricted to mass action law kinetics and with at most two reactants, has given rise to a numerical method for compiling computable real functions and programs in finite CRN. This approach is implemented in Biocham-4[1] [20] and CRN^{++}[2] [46] with some different design choices. The theoretical result states that one can restrict to real functions presented as solutions of polynomial differential equations and polynomial initial values as functions of the inputs (PIVP). For a positive PIVP, the transformation can use a CRN inference algorithm from ODEs such as [29] or [21] initially dedicated to importing MatLab models in SBML [25]. For negative variables, the transformation can use the dual-rail encoding of a real variable by the difference between two positive variables for the positive and negative parts respectively [37]. In the generated CRN the molecular concentration play the role of information carriers, similarly to analog computation performed by protein complexes in cells [17,43].

In this article, we study an alternative CRN design method based on artificial evolution, and compare the results to the numerical method. In [15] a complete search method is described for scanning the space of CRN that may implement a given function with a limited number of species and reactions, and then optimizing the kinetic parameters using a metropolis like algorithm. This enumerative algorithm is limited to small size CRN with only a handful of species and reactions. Another method described in [6] consists in sketching a CRN in broad outline and letting an optimization algorithm complete the holes to perform the desired function. This method thus requires prior knowledge on the CRN. In the framework of gene regulatory networks, [33] gives a method to infer network parameters but with also prior knowledge on the structure of the network. In [18] a method to evolve reaction networks is presented using the DNA toolbox and one single genetic algorithm for both structure and parameters.

The method presented here does not require prior knowledge. It is based on artificial evolution with the hope of finding CRN more akin to comparison to natural CRN present in BioModels. The idea is to let an evolutionary algorithm (EA) evolve a population of CRN to approximate a real function given on a finite set of values. These data are either finite traces for approximating a function of time (problem 1), or dose-response diagrams for approximating a function of input (problem 2). The general idea is to let evolve a population of potential CRN while selecting them according to a fitness that is the distance between

[1] `http://lifeware.inria.fr/biocham4`
[2] `https://github.com/marko-vasic/crnPlusPlus`

their output and the desired target function. We take here as a distance the L2 norm evaluated in a specified set of points.

Such a CRN design method by artificial evolution can also be seen as a machine learning procedure [45] to learn a CRN from traces either observed in the systems biology perspective, or desired in the synthetic biology perspective. This method may be seen as a learning problem akin to those currently solved with neural networks or linear regression. The input is a set of points (x_i, y_i) called *data* and the goal is to find a function f from a certain class that minimizes the difference between the y_i and $f(x_i)$. The output of the approximation is the best function f. However, as the approximation function f is computed through a CRN, we also hope for a network that will gives us some explanatory power in systems biology, as well as some implementability in synthetic biology.

We present a nested evolution algorithm which combines a genetic algorithm for evolving the CRN structures of a population of CRN, with a black-box continuous optimization procedure, namely the covariance matrix adaptation evolutionary strategy (CMA-ES) [28], for optimizing the kinetic parameters of the CRN by evolving a population of parameter settings. This nesting of two levels of evolution has been proposed, see for instance [4] for mixed-variable optimization problems in the framework of non-linear optimization problems, refined in [5] to evolve a cell model with structure optimization and parameter optimization. The authors suggest to use a better genetic algorithm for parameter optimization and to parallelize the genetic algorithms. This is what we show here with the choice of CMA-ES [28] for parameter optimization, and the parallelization of both populations of the genetic algorithm and CMA-ES.

In the next section, we recall basic definitions of continuous CRN, their Turing completeness, and the automatic design method based on this approach. In Sec. 3 we present our two level evolutionary algorithm for learning CRN structure and kinetic parameters, and its parallel implementation. In Sec. 4 we evaluate the results obtained with this algorithm on both functions of time and input/output functions, by focusing in both cases, on the Heaviside function as an ideal sigmoid function, and on the cosine function as an ideal oscillator. On these examples, we compare the CRN obtained by artificial evolution to the CRN obtained by compilation. In Sec. 5, we compare the CRN obtained by our algorithm for the cosine function, to CRN present in BioModels using the subgraph epimorphism method [24,23], and discuss their relationship to circadian clock models.

2 CRN Design by PIVP Compilation

In [20] we have shown that any computable function over the reals can be implemented by a CRN over a finite set of molecular species. This result relies on previous results on analog computation and complexity showing the Turing completeness of polynomial initial value problems (PIVPs), i.e. numerical integration with arbitrary precision of polynomial ODEs from polynomial initial conditions [3]. More precisely, we showed

Theorem 1 ([20]). *A function over the reals is computable (resp. in polynomial time) if and only if it is computable by a CRN with mass action law kinetics using only synthesis reactions with at most two catalysts, of the form*

`- => z` *or* `_ =[x]=> z` *or* `_ =[x+y]=> z`

and annihilation reactions of the form

`x_p + x_m => _`

(resp. with trajectories of polynomial length).

This form of reactions based solely on catalytic synthesis and degradation by annihilation is given by the proof of Turing completeness but is not very appealing from a biochemical point of view. It should be understood as an abstract form of reactions which can be realized with real reactions, for instance by replacing an annihilation reaction by a complexation reaction forming an inactive complex, a synthesis reaction by a transformation reaction from an inactive form, e.g. by a phosphorylation reaction where the catalyst is a kinase, etc. Such a realization of abstract CRN in CRN with real enzymes is described in [14] using the BRENDA database of enzymes[3] and has been used for instance in [13] to implement designed circuits with real enzymes in artificial vesicle.

Example 1. The cosine function of time can be specified by the PIVP

$$df/dt = z \quad dz/dt = -f \quad f(0) = 1 \quad z(0) = 0$$

The CRN generated by the PIVP method introduces variables for the positive and negative part of f and z together with annihilation reactions given with a sufficiently high value rate constant *fast*:

```
biocham: compile_from_expression(cos, time, f).
  _ = [z2_p] => f_p.
  _ = [z2_m] => f_m.
  _ = [f_m] => z2_p.
  _ = [f_p] => z2_m.
  fast*z2_m*z2_p for z2_m+z2_p => _.
  fast*f_m*f_p for f_m+f_p => _.
  present (f_p, 1).
biocham: list_ode.
  d(f_p)/dt = z2_p-fast*f_m*f_p
  d(f_m)/dt = z2_m-fast*f_m*f_p
  d(z2_p)/dt = f_m-fast*z2_m*z2_p
  d(z2_m)/dt = f_p-fast*z2_m*z2_p
```

The inference of general CRN has a low time complexity, linear in the number of variables and monomials for generating general reactions [21]. However, the transformation to reactions with at most two reactants, may take exponential time in the worst case [9].

[3] `https://www.brenda-enzymes.org/`

3 CRN Design by Artificial Evolution

3.1 Nested Evolution Algorithms for Structure and Kinetics

The goal here is to learn a CRN that minimizes the error between the input trace and the simulation trace of the CRN. Thanks to Thm 1 we know that we can restrict ourselves to elementary reactions with at most two reactants of some simple form and with mass action law kinetics, i.e. to PIVPs with monomials of degree at most 2.

While the structure of the CRN/PIVP defines what is possible, the value of the parameters (i.e. kinetic parameters and initial concentrations) is essential to the function and a bad exploration of the parameter space might lead to a wrong rejection of an actually good structure. To solve this difficulty we use two nested evolutionary algorithms where the first optimizes the structure and the second optimizes the parameter values, as summarized in Fig. 1.

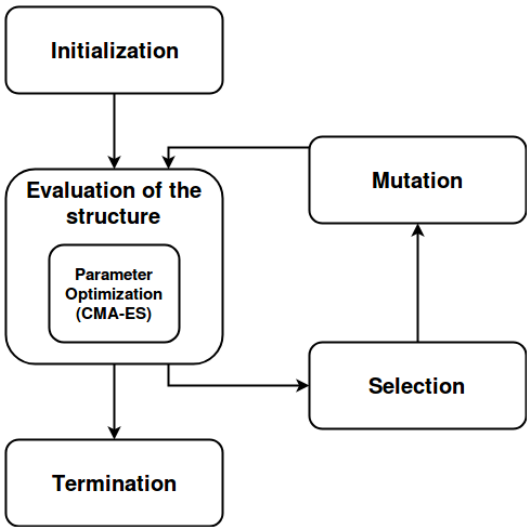


Fig. 1. Schematic representation of the nested evolution algorithm. The initialization corresponds to the creation of different random individuals. The evaluation of a given structure implies a sub-call to the CMA-ES algorithm with a limited time budget, and only the best set of parameters is kept to compute the fitness of the individual in the main algorithm. Elite selection is used where half the population is deleted while the second one is copied, this scheme allows for fast selection (no random number is needed) and keep a constant population size. The mutation scheme incorporates adding or removing variables or monomials, and switching signs. Termination is enforced by fixing the number of generations.

The Covariance Matrix Adaptation Evolution Strategy (CMA-ES) [28] is a black-box derivative-free continuous optimization algorithm used here to optimize the kinetic parameters at each step of the CRN structure evolution. This algorithm enjoys many invariance properties with respect to scales as well as linear, rotational or any order-preserving transformations. It is a state-of-the-art algorithm that shows best performances on the hardest non-convex even discontinuous problems [27]. Here the parameters are positive and are searched according to a logarithmic scale. CMA-ES has been used in Biocham for parameter search and robustness optimization with respect to quantitative temporal logic properties [22,40,39] with notable success in both systems biology [30] and synthetic biology [13] as well as external control of cell processes [44].

Algorithm 1 Structure Optimization

```
function Evolution(f)
    population ← InitializePopulation(f)
    for g in generation do
        for p in population do
            StructureFitness(f,p)
        end for
        pop_new ← Select(population)
        population ← pop_new + Mutate(pop_new)   ▷ Concatenate the two lists
    end for
    return Select_best(population)
end function
```

Algorithm 2 Parameter Optimization

```
procedure StructureFitness(f, pivp)
    u ← Random(0, 1)
    if u == 1 then
        x0 ← RandomInitialState(pivp)
    else
        x0 ← InitialStateFromBest(pivp)
    end if
    fit_value, fit_param ← CmaEs(FitnessFunction(pivp),x0)
    UpdateBestParameters(pivp, fit_value, fit_param)
end procedure
```

The top-level genetic algorithm for learning the structure of the PIVP is given in Alg. 1. Each monomial has a sign, + or -. To handle the constraint of positivity of the concentration variables, a monomial in the ODE of one variable cannot have a negative sign if the variable is not present in the monomial. For example, the derivative of x can depend on $-xy$ but not on $-y$ which is equivalent to

Algorithm 3 Fitness function for functions of time

```
function FitnessFunction(f, pivp, param)
    coeff, y0 ← ParamToCoeff(param)
    sol ← Integrate(pivp, coeff, y0)
    return Loss(sol, f)
end function
```

Algorithm 4 Fitness function for functions of input

```
function FitnessFunction(f, pivp, param)
    coeff, y0 ← ParamToCoeff(param)
    value ← 0
    for x_i in x do
        sol ← Integrate(pivp, coeff, y0(x_i))
        value ← value + Loss(sol, f_i)
    end for
    return value / len(x)
end function
```

say that a degradation reaction must have the degraded species as reactant. This constraint ensures the positivity of the system (Prop. 1 in [21]). With this provision, there exists a canonical (yet not unique) way of associating a CRN to a PIVP, by associating a possibly catalyzed synthesis reaction to each positive monomial, with mass action law kinetics with the monomial coefficient as rate constant, and similarly a degradation reaction to a negative monomial.

The population of possible solutions is initialized with random PIVPs. The number of variables is chosen in a given range with a uniform distribution. They have 1 or 2 monomials per variable with the same probability.

To evaluate the fitness score of a given structure we rely on CMA-ES to find a set of optimized kinetic parameters and initial concentration (Alg. 2). As CMA-ES is a stochastic algorithm and its result may vary from one call to another even for the same structure, only the best parameter set found so far is used to affect the score (that is we do not allow the fitness to decrease). To keep the computation time tractable, CMA-ES is called with a time limit (of the order of the minute depending on the problem at hand). This also mean that, even with a proper initialization of the CMA-ES seed, the result of this optimization may vary according to the current state of the processor. However limiting the number of function evaluations may drastically slow down the algorithm as large PIVP are difficult to integrate.

As for selection, an *elitist* strategy is applied: the 50% best polynomials are kept and then mutated. It is worth noting that this criterion is only based on the ranking of the individual solutions according to their evaluation and is thus robust with respect to the precise fitness definition.

Several types of mutations are used :

Adding a monomial with a coefficient $\exp(v)$ where v is sampled according to the normal law $\mathcal{N}(0, 1)$.

Removing a monomial Note that if a variable has no more monomials, a random monomial is added to this variable (thus avoiding an empty ODE).

Adding a variable to avoid empty variable, two monomials are added with coefficients sampled as just explained, and the initial concentration is sampled with the same law.

Removing a variable all the monomials of this variable are deleted too.

Restarting That is replacing the PIVP by a new random one having the same number of variables and 1 or 2 monomials per variable (also called a restart, this enables the exploration of the search space)

At each generation, one of the mechanisms above can happen. The probability of adding or removing a monomial is 0.35. While the other mutations have a probability of 0.1. Moreover, at each generation, the sign of a monomial is changed (if it satisfies the positivity constraint). This high rate of mutation is tempered by the fact that only one half of the population is mutated while the other half is kept unchanged, thus ensuring that the best structures will never be lost through the mutation operator. Hence the term of elitist selection. During the mutation, the good parameters found during evaluation are kept. Note that no cross-overs are implemented in our mutation operator.

After a fixed number of generations, the best structure of the population is selected as the final result and CMA-ES is called a last time with an extended time limit to fine tuned the parameters.

3.2 Parallel Implementation

The previous algorithm has been implemented in Python 3.6.3 using the CMA-ES package `cma`, the numerical integrator LSODA of the `scipy` library, and the parallelization package `mpi4py` [16].

In our evolutionary algorithm, the most time consuming part is the evaluation part since it requires numerical integration to compare the simulation trace to the objective trace. However, since the evaluations of the different individuals in the population are independent, they can be parallelized. Furthermore, since our algorithm uses two nested evolutionary algorithms, it can benefit from two sources of parallelism. For each of them, we can achieve a linear speedup in the number of processors.

The first layer of parallelism is easy to implement, as there is a function `Scatter` that takes a list from a root process and scatter it to all processes. The function `Gather` can perform the inverse operation. Adding the second layer is more difficult, especially in our case where it is not possible to transform the two layers into one layer. To overcome this challenge, using the function `Split`, new communicators are created. There are as many new communicators as individuals in the population. At each generation, the population is scattered between the different groups of processes. Each group is dedicated to one individual. Each group performs the evaluation of the individual. The population of CMA-ES is set here to 10 individual parameter sets. In a group, during the evaluation part of CMA-ES, each individual of CMA-ES is thus evaluated by a different process, through a scatter/gather mechanism.

4 Evolved CRN for Mathematical Functions

4.1 Functions of Time

In all this section, we use as fitness function the mean square error between our goal and the simulation trace evaluated on a finite set of time points.

Cosine. Interestingly, the algorithm evolves PIVPs that can be reduced to the following CRN of 3 species and 7 reactions[4]:

```
962*A*B for A =[B]=> _.
4.78*C for _ =[C]=> A.
544*A*B for B =[A]=> _.
0.477*B for _ =[B]=> B.
1.5 for _ => B.
0.648*B for _ =[B]=> C.
0.346*C for C => _.
```

```
present(A, 1).
present(B, 0.0028).
present(C, 0.539).
```

On a trace of two periods, the obtained fitness value is $L_2(A, \cos) = 0.042$. This is an excellent fit comparable to the fitness value of $L_2(f_p, \cos) = 0.039$ obtained with the numerical method (Ex. 1) with a `fast` parameter set to 100.

In this CRN, the positive and negative parts of the cosine are the species A and B. It is worth noticing that these two species are subjected to a fast bi-degradation similar to the encoding of signed variables with two variables for the positive and negative parts shown in Ex. 1. However, this CRN uses the two parts in a non symmetric fashion that makes it unreachable for our compiler.

The oscillatory behavior of this CRN is the limit cycle of the ODE and is reached for a large space of initial conditions. We can also gather together the reactions that have similar monomials (reactions 1 and 3 for example) and even if this affect the precise fit of the cosine, we check that the oscillatory behavior is preserved.

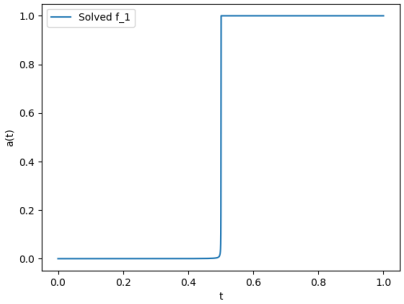
Heaviside. The Heaviside function

$$\Theta(t) = \begin{cases} 0 & \text{if } t < 0.5, \\ 1 & \text{if } t \geq 0.5. \end{cases} \tag{1}$$

is a discontinuous (hence non-computable) function that cannot be defined by a PIVP. Because it is an ideal step function, it is interesting to see what CRN can emerge to best approximate that function. It takes nearly 10 times more generations to evolve a good approximation for Heaviside than it takes to evolve the cosine function. The best result found, with a fitness $\phi = L_2(A, \Theta) = 0.0078$, is of the following form:

[4] The evolved CRN of this section are available at `https://lifeware.inria.fr/wiki/Main/Software#CMSB19b`

```
1.05e-4*C*C for _ =[C+C]=> A.
1.79*C*C for _ =[C+C]=> B.
3.07*C*C for _ =[C+C]=> C.
1*A*B for _ =[A+B]=> C.
1.38*B*C for C =[B]=> _.
```



To understand the dynamics of this CRN, we see that the amplitude of the output species is tuned by the parameter of the first reaction. The fourth reaction (`_ =[A+B]=> C`) is actually unessential to the function even if it can result in a better approximation, its suppression does not significantly modify the trace. The core of the CRN is thus on the competition between species `B` and `C`, both starting at low concentration and increasing abruptly around the desired threshold. There, the last reaction imposes a slow-down that makes `C` disappear and `B` stabilize. The species of interest `A` is then only a linear scaling of `B` to respect the constraints imposed by the fitness function.

It is worth remarking that this solution is surprisingly close to the PIVP method to transform a function of time in a function of input by multiplying each monomial by a variable that decreases exponentially fast, hence halting the computation on the desired state [20]. Here an exploding variable is halted in its course to simulate non-linearity.

Another way our artificial evolutionary algorithm manages to produce Heaviside like functions is through the generation of sigmoidal functions. The CRN below

```
k*A*B for B =[A]=> A.
```

```
present(A, C0).
present(B, 1.0).
```

satisfies the relation $C_0 \simeq \exp(-\frac{k}{2})$ in order to have the jump around one half, and k large makes the jump as sharp as possible. Practically, this make the initial concentration of `A` vanishingly small.

This mechanism may have several variants depending on the evolutionary history followed by the algorithm. A common one is to implement the sigmoid function through two species with large concentration in order to produce a sharp exponential and to report the output on a third one that is a linear rescaling of a previous one as is the case in the previous example.

4.2 Functions of Input Variables

In this section, the performance of a CRN is measured by recording only the final value of its output species and using a mean square error as global loss function. Here, final value mean the value at a predefined time ($t = 1$), to ensure that this correspond to the steady state, the norm of the derivative is used as a

penalty. Note that the choice $t = 1$ has nothing particular as the time may be rescaled.

Cosine. The PIVP structure that commonly emerges by artificial evolution is the following one:

$$\frac{da}{dt} = -2.9 \cdot 10^{2} \cdot a - 1.8 \cdot 10^{-1} \cdot c \tag{2}$$
$$\frac{db}{dt} = -2.2 \cdot 10^{9} \cdot ac - 4.2 \cdot 10^{-10} \cdot c \tag{3}$$
$$\frac{dc}{dt} = 3.8 \cdot 10^{-5} \cdot ab - 1.0 \cdot 10^{0} \cdot c^2 \tag{4}$$

We choose to start from the PIVP to emphasize the importance of neutral transformation in the final result of our EA. Here, both time $2.9 \cdot 10^2 t \to t$ and the last variable $7.7 \cdot 10^6 c \to c$ can be rescaled, giving:

$$\frac{da}{dt} = -a - 8.1 \cdot 10^{-11} \cdot c \tag{5}$$
$$\frac{db}{dt} = -ac - 1.9 \cdot 10^{-19} \cdot c \tag{6}$$
$$\frac{dc}{dt} = ab - 4.5 \cdot 10^{-10} \cdot c^2 \tag{7}$$

where all the variable are of the order of unity. The final term in c may safely be ignored and this let us with the same PIVP as the one feed to our compiler to generate the cosine of input:

$$\frac{da}{dt} = -a \tag{8}$$
$$\frac{db}{dt} = -ac \tag{9}$$
$$\frac{dc}{dt} = ab \tag{10}$$

Here again, a plays the role of a halting species and b and c compute the cosine and sine functions that are halted on the desired value.

Sum of two inputs. The purpose here is to find a CRN to implement the sum function $f(x, y) = x + y$, as a first example of a case with two inputs. The best solution found by evolution is:

```
k1*A*A for A =[A]=> B.
k2*A*A for _ =[A+A]=> C.
k2*B*B for _ =[B+B]=> C.
k2*A*B for _ =[A+B]=> 2C.
k2*C*C for C =[C]=> _.
```

```
present(A, input1).
present(B, input2).
present(C, 1.44).
```

where we have labeled with the same name the rate constants found nearly equal by artificial evolution. On the other hand the values of `k1` and `k2` do not play any role. Actually, `k1` may even be chosen to be null, highlighting a symmetry of the fitness function. The rest of the reaction implements the equation: $\dot{c} = (a+b)^2 - c^2$, where the square enforces a faster dynamics and thus a better convergence.

The general idea is however to balance the positive and negative monomials of variable c to stabilize it on the desired result. A direct consequence of this mechanism is that the initial concentration of `C` has no importance in the final output. While a human engineer would thus fixed it to 0, our evolutionary algorithm proposes a random value. We may however suspect that this is the mean of the output used to train our CRN as such a choice would accelerate the mean convergence time and decrease the final error.

That mechanism may be more clear if written without the squaring:

```
k1*A for _ =[A]=> C.
k2*B for _ =[B]=> C.
k3*C for C => _.
```

```
present(A, input1).
present(B, input2).
```

Interestingly, this also gives a generalization where different values for the rates allow us to compute the function:

$$f[k_1, k_2, k_3](x, y) = \frac{k_1}{k_3}x + \frac{k_2}{k_3}y. \tag{11}$$

Several remarks should be done on this CRN. First, it provides a CRN to compute online since a modification of the concentration of the input will be transmitted to the output. This online CRN algorithm will have the form of an exponential decay with a time rate $\frac{1}{k_3}$. A fast dynamics (high k_3) means that either the output is small, or the energetic cost will be high. Finally, it is known from the study of such process that on the stochastic regime, the distribution of species C will follow a Poisson distribution with a variance equal to its mean. In comparison, the solution found by our EA converges faster and present a higher signal to noise ratio in stochastic semantics.

Heaviside. When considered as a function of input, the non-linearity of the Heaviside function is no longer an issue as it can be computed with continuous function such as a bi-stable switch. For example, starting from the simplest bistable PIVP $\frac{da}{dt} = a(1-a)(2a-1)$, our compiler generates the following PIVP:

```
3*A*A for _ =[A+A]=> A.
A for A => _.
2*A*B for A =[B]=> _.
6*A*B for _ =[A+B]=> B.
4*B*B for B =[B]=> _.
2*B for B => _.
```

```
present(A,input).
present(B,input2).
```

where `input2` have to be the square of `input`. Interestingly, this switch CRN is an instance of the Approximate Majority distributed algorithm extensively studied and related to cell division cycle progression models in [7].

Another solution would be to activate a production and degradation cycle based on the remainder of the input after a fast comparison to a predefined threshold:

```
fast*A*C for A+C => _.
 A  for _ =[A]=> B.
A*B for B =[A]=> _.
```

```
present(A,input).
present(C,0.5).
```

The best CRN found by evolution in our experiments is however very different from these two solutions, and plays upon the dynamics of the network and the precise point of evaluation defined by the input trace to fit, here at time $t = 1$. The CRN found displays a transient state of variable length τ_t where the output is near zero, and a single steady state where the output is near unity. The value of the input impacts the time to reach the steady state so that if the input is greater than one half then $\tau_t < 1$ and the output is near 1 at evaluation time, while in the other case, $\tau_t > 1$ and at the evaluation time $t = 1$ the output is null but just temporarily. This illustrates the typical problem of generalization in machine learning for an input/output function specified by a finite trace.

5 Comparison to Natural CRNs in BioModels

One first remark illustrated by the previous examples, is that when we usually end up with parameters value taking involved values it is often possible, by clever variable transformation, to recover more natural values thus highlighting several symmetries of our fitness function. This is typically the case when evolving the cosine function, the scale of the sine function is undefined and creates a whole variety of solutions that are strictly equivalent up to a rescaling of two parameters. This kind of symmetry of the fitness function is one of the plague of biology as it is often difficult to identify them and they may obfuscate a simpler design [26].

A second discussion may be raised by the two CRN for the cosine function of time obtained respectively by the numerical method and the artificial evolution algorithm. Fig. 2 shows that those CRN can be compared to the circadian clocks present in eukaryote and prokaryote albeit with strong differences in their implementations. In prokaryotes, a circadian clock has been shown to be implemented through a cycle of phosphorylation and dephosphorylation, such as of the two sites S and T of a KaiC protein polymer found in cyanobacteria[35]. This mechanism is usually presented through a four step process:

$$ST \rightarrow SpT \rightarrow pSpT \rightarrow pST \rightarrow ST$$

where a p preceding a letter indicates that the corresponding site is phosphorylated. This mechanism is similar to the compiled version of our cosine function where 4 species activates one another in a circular fashion :

$$\cos^+ \rightarrow \sin^+ \rightarrow \cos^- \rightarrow \sin^- \rightarrow \cos^+$$

where the upper signs denote the positive and negative part of the function.

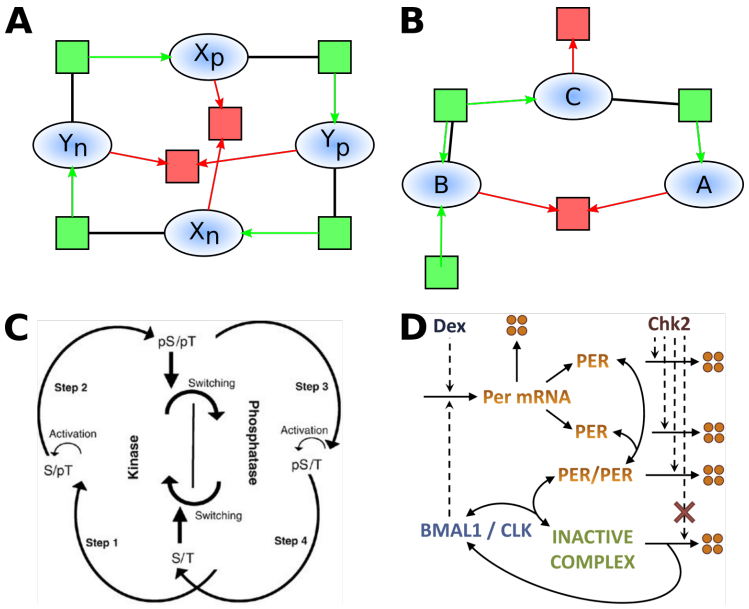


Fig. 2. Representation of our compiled (panel A) and evolved (panel B) CRN as reaction graphs for the cosine function of time. Ellipses represent species, squares indicate reactions in green for productions and red for degradations. Catalysts are linked to their reactions with a bold black line. Panel C shows the molecular oscillator of cyanobacteria as described in[35]. Panel D shows the genetic oscillator of mammal (similar in most eukaryotes) as proposed in [32].

The eukaryotes use a transcriptional mechanism where a CLOCK protein activates the transcription of its own inhibitor. The action of the inhibitor is however delayed through several steps that may vary from one organism to another. Usually this involves translation, dimerization and transportation to and from the nucleus. By using the graph matching method based on subgraph epimorphism (SEPI) [24] to compare our evolved CRN to models of the BioModels repository, we found a mapping from the mammalian circadian clock models such as [32] toward the simple oscillator in panel B of Fig. 2 presented above. The SEPI mapping makes `B` play the role of the transcription factor that activates the transcription of the messenger RNA `C` that then became the protein `A` that will bind to the transcription factor to form an inactive complex, thus inhibiting the transcription.

Table 1 shows general SEPI comparison results with models in BioModels [10]. We see that models presenting oscillations (circadian clock and cell cycle) are far more likely to exhibit a SEPI toward one of our evolved network than the MAPK models (the SEPIs found come from model 026 [36] and 010 [34]). The

Model type	Nb. of models	Nb. SEPI matchings (min/mean/max)	% SEPI
Circadian clock	13	3/7.8/12	60%
Cell cycle	22	6/10.1/17	45%
Mapk	9	0/0.7/2	08%

Table 1. SEPI matchings from biological models in BioModels having between 5 and 15 species to the 10 best evolved CRNs (over 60 runs) for the cosine time function.

evolved model that harbors nearly all SEPI from both cell cycle and circadian clock models is the one with the best fit presented in section 4.1.

These connections between the result of our evolutionary algorithm and the actual mechanisms found in biology suggest a form of evolutionary convergence. It is well known in biology that organs in species that have evolved totally independently may in fact be similar. There might be actually few ways to implement a clock that are easily found by evolution so much that every solution that appears is actually a close repetition from the existing one. This kind of evolution convergence has already been emphasized in the case of the evolution of a biological logarithm were every successful run displays the same core mechanism [31]. This provides a tool to decipher the function and constraints of a biochemical network by comparing it to an idealized mathematical framework while still being able to make relevant connection with the biological case.

6 Conclusion

We have described a nested evolution algorithm to learn both the structure and the kinetic parameters of a CRN for approximating a function given by a finite trace of either time points for a function of time, or input values.

On a Heaviside function of time, the results obtained by artificial evolution lead to a remarkably simple CRN of 3 molecular species and 5 reactions with double catalysts which provide a very stiff transition although using mass action law kinetics. This solution is more economical than the CRN generated by the PIVP method for sigmoid functions [20]. On a Heaviside function of input, the CRN found by evolution are slightly more complicated than the bistable switch found in cell cycle CRN for instance, but much less complex than the MAPK signaling network that plays a similar role [34].

On the cosine function of time, the best CRN found by evolution contains an annihilation reaction similar to the CRN generated by the numerical method for positive and negative variables, but one less reaction thanks to an intriguing non symmetric use of the two variables which preserves the limit cycle. Interestingly, the evolved and the PIVP generated structures could be compared to prokaryote and eukaryote models of the circadian clock found in BioModels.

On the cosine function of input, a CRN surprisingly emerges with the structure of the CRN for cosine function of time, using the same trick as in [20] to stop time at the desired input value.

These results are encouraging to further develop artificial evolution methods for CRN design in both perspectives of systems biology with the study of evolution convergence, and synthetic biology in addition to rational design.

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