Crosstalk and the Cooperation of Collectively Autocatalytic Reaction Networks

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***Abstract*— We examine a potential role of signalling crosstalk in Artificial Cell Signalling Networks (ACSNs). In this research, we regard these ACSNs or Artificial Biochemical Networks (ABNs) as collectively autocatalytic (i.e., closed) reaction networks being able to both self-maintain and to carry out a distinct signal processing function. These signalling crosstalk phenomena occur naturally when different biochemical networks become mixed together where a given molecular species may contribute simultaneously to multiple ACSNs. It has been reported in the biological literature, that crosstalk may have effects that are both constructive (e.g., coordinating cellular activities, multi-tasking) and destructive (e.g., premature programmed cell death). In this paper we demonstrate how crosstalk may enable distinct closed ACSNs to cooperate with other. From a theoretical point of view, this work may give new insights for the understanding of crosstalk in natural biochemical networks. From a practical point view, this investigation may provide novel applications of crosstalk in engineered ABNs.**

I. Introduction

Cell Signalling Networks (CSNs) are biochemical networks occurring in cells which are capable of signal processing or cognitive abilities. These abilities coordinate the cellular activities in response to internal and external stimuli. CSNs are responsible for the intricate functioning and ultimately survival of a cell in its dynamic environment. By taking the *in silico* counterparts of real CSNs - Artificial CSNs (ACSNs) we use an evolutionary simulation platform to identify new computational paradigms which are directly inspired by nature [1]. This evolutionary system is built upon Artificial Chemistries (AC) which have been shown to provide a suitable framework to model, simulate and analyse ABNs [2].

As CSNs are contained in cells and are randomly distributed to offspring cells during cellular division, a mechanism is necessary to ensure the replication of CSNs prior to the cellular division. This assertion applies to systems where a genetic subsystem is present, as the latter still requires a minimal CSNs to coordinate the translation of the genetic code (which may produce further CSN's components). Closure is one candidate mechanism which would enable the CSN's self-replication or maintenance: A collectively autocatalytic reaction network (i.e., a closed system) is able to produce all the catalysts and substrates for its reactions, thus achieving the self-maintenance of the system.

Based on above assumption, we conjecture that ACSNs are subsets of collectively autocatalytic reaction networks, see Fig. I. Closure in ACSNs is also of interest from a practical point of view, e.g., engineering ABNs which are autonomous self-maintaining/repairing cognitive systems.

We may identify ABNs as networks which are made up of more than one specific ACSNs, each responsible for a distinct signal processing function (involving an input/output relationship) see Fig. I. Interactions between different ACSNs may occur and this phenomenon is called signalling crosstalk. This arises very naturally in real CSNs due to the fact that the molecules from all pathways may share the same physical reaction space (the cell). Depending on the relative specificities of the reactions there is then an automatic potential for any given molecular species to contribute to signal levels in multiple pathways.

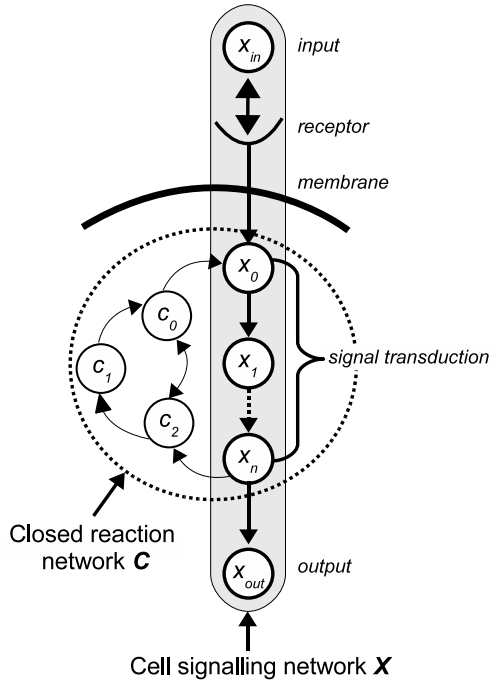

Fig. 1. Cell Signalling Network X being a subset of the closed reaction network C

In traditional communication and signal processing engineering, crosstalk is regarded as a defect, an *unintended* or *undesigned* interaction between signals, that therefore has the potential to cause system malfunction. This can also clearly be the case of crosstalk in CSNs.

However, in the specific case of CSNs, crosstalk also has additional potential functionalities, which may actually be

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constructive:

- Even where an interfering signal is, in effect, adding uncorrelated noise to a functional signal, this may sometimes improve overall system behaviour. This is well known in conventional control systems engineering in the form of so-called dither. Compare also, [3], [4] on constructive biological roles of noise.
- The crosstalk mechanism provides a very generic way of creating a large space of possible modifications or interactions between signalling pathways. Thus, although many cases of crosstalk may be immediately negative in their impact, crosstalk may still be a key mechanism in enabling incremental evolutionary search for more elaborate or complex cell signalling networks.

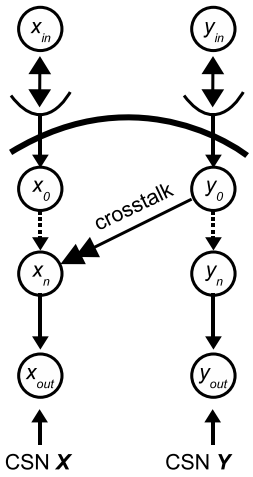

Fig. 2. Crosstalk between Cell Signalling Networks X and Y

In this paper we present another potential constructive role of crosstalk in ABNs: Signalling crosstalk is a key feature allowing distinct collectively autocatalytic reaction networks to cooperate when occurring in the same reaction space.

Our seminal inspirations to this work originate from specific experiments carried out by Fontana with the Alchemy system [5]: When mixing two collectively autocatalytic reaction networks (which were obtained from previous independent experiments), two outcomes could be observed according to the level of interaction between the two reaction networks:

1) If no molecular interactions (i.e., no crosstalk) exist between the two networks then one would displace the other network.
2) If, to the contrary, some molecular interactions occur between the two crosstalking networks then a "meta" closed reaction network emerges which contains and maintains both seed closed reaction networks.

We extend this seminal investigation on crosstalk in ABNs using an Artificial Chemistry (AC) called MCS.bl based on the Molecular Classifier Systems (MCS) and Holland's broadcast language (BL) [6]. A number of key differences exist between Alchemy and the MCS.bl:

- Alchemy is based on the $\lambda-$calculus formalism, whereas the MCS.bl employs the broadcast language (a term-rewriting system which was the precursor to Holland's Learning Classifier Systems).
- Similarly to Alchemy, molecules may interact and compete with each other. In addition to this first level of selection we introduced a higher level of selection: Molecules are contained in multiple reactors (i.e., cells) which are capable of competing with each other through a cellular division process.
- We defined mutation operators at both the molecular and cellular level. No evolutionary operators were specified in Alchemy.
- We evolved the seed closed reaction networks to carry-out pre-specified tasks. The meta reaction network having to therefore functionally carry out both pre-specified tasks. In Alchemy, the reaction networks were self-organized without any target functions.

This paper is organized as follows: We first introduce the MCS.bl, we then present a first series of experiments involving non-crosstalking reaction networks. Following this, we examine a second series of experiments using crosstalking reaction networks where only cell level mutation applies. Finally, a third series of experiments is described where we employ crosstalking reaction networks where both cellular *and* molecular mutations occur. We finally outline potential future work and conclude this paper.

II. The Artificial Chemistry

We first present the MCS metaphor and outline the Holland broadcast language which is employed to specify the molecular reactions. We then describe the reactor algorithm which was implemented on a concurrent system (using a cluster of computers).

A. The Molecular Classifier Systems

Molecular Classifier Systems are a class of string-rewriting based AC inspired by Learning Classifier Systems (LCS). As opposed to traditional string-rewriting systems, operations are stochastic and reflexive (no distinction made between operands and operators). The behaviour of the condition (binding) properties and action events (enzymatic functions) is defined by a language specified within the MCS. This "chemical" language defines and constrains the complexity of the chemical reactions that may be modelled and simulated. In this AC, all reactants are catalytic in the sense that they are not consumed during reactions. These reactions result from successful molecular interactions which occur at random. When a reaction occurs, a product molecule is inserted into the reactor.

We proposed a simplification of the Holland broadcast language [1] which is used as the MCS chemical language resulting in the MCS.bl system. The MCS.bl differs from the original MCS [7] by introducing more complex chemical reactions (only replications may occur in the MCS). A molecule may contain several condition/action rules which define the binding and enzymatic properties. A reaction

between molecules occurs if at least one conditional part from any rules in a molecule A matches a target molecule B. A is regarded as an enzyme whereas B is regarded as a substrate molecule. When a reaction occurs, the action part from the satisfied rule in A is utilized to perform the enzymatic operations upon the bound substrate molecule B. This operation results in the production of another offspring (product). If several rules in A are satisfied by B, then one of these rules is picked at random and employed to carry out the enzymatic function.

A number of differences exist between our simplified broadcast language (BL) and the LCS, e.g., the LCS's alphabet is $\lambda = \{ 1, 0, \#\}$ whereas the BL includes additional symbols $\Lambda = \{1, 0, *, :, \Diamond, \triangle, ', \nabla\}$. The basic elements of the BL are strings made from Λ called *broadcast devices*. A broadcast device is parsed into zero, one or more *broadcast units*, where each unit represents a single condition/action rule. The symbol $*$ separates broadcast units within a broadcast device. The symbol : separates a condition from an action within a single broadcast unit. 0s and 1s are basic informational symbols. $\{\Diamond, \nabla, \triangle\}$ are single/multiple character(s) wildcards that may also transpose matched strings into output strings. Quoted symbols (preceded by $'$) are prevented from interpretation. Fig. 3 depicts an example broadcast device which may bind and react with a copy of itself, this reaction is presented in Fig. 4 .

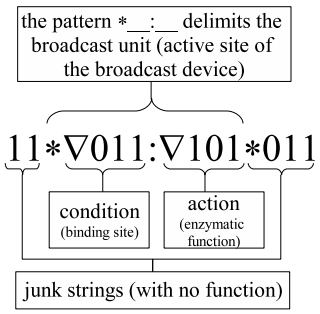

Fig. 3. An example broadcast device

Enzyme	substrate	product	operation
$*\nabla 1:\ \nabla 0$	$1:0$	$\emptyset$	no reaction
$*\nabla 1:\ '*\nabla$	$0:1$	$*0:1$	activation
$*\ '*0\nabla:0\nabla$	$*0:1$	$0:1$	inhibition
$*\nabla:\nabla$	$*00:11$	$*00:11$	universal replication
$*\nabla 0:\nabla 0$	$*\nabla 0:\nabla 0$	$*\nabla 0:\nabla 0$	self-replication
$*\nabla 1:\nabla 10$	$*0:1$	$*0:10$	concatenation
$*\nabla 1:\nabla$	$*0:1$	$*0:$	cleavage

TABLE I

EXAMPLE OPERATIONS REALIZED WITH THE MCS.BL

A detailed description is omitted in this paper, see [8] for full specification of our BL implementation. Table I presents a number of example operations that can be realized with the MCS.bl.

B. Multi-level selectional and concurrent model

We implemented the MCS.bl as a multi-level selectional model, we introduced multiple reactors where each of them

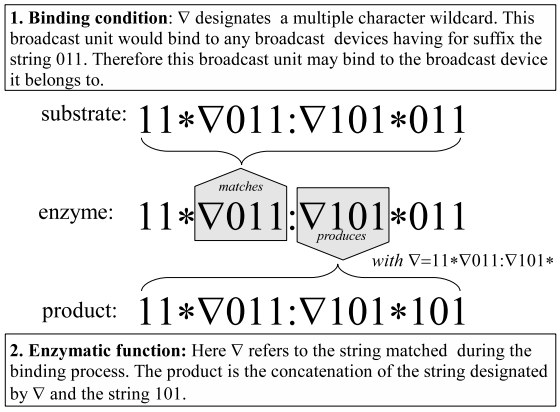

Fig. 4. Example reaction

contains a population of molecules. These reactors or *cells* may be subjected to cellular division, which results in the replacement of the parent cell and creation of two offspring cells. However, the number of cells in the universe is fixed. As a result such a cellular division also triggers the removal of another cell selected at random. In a similar manner to molecules, cells are competing with each other which is regarded as the second level of selection.

Successful reactions do not lead to the removal or degradation of molecules in the reaction space. Thus the number m of molecules contained in a cell may increase until the cell is full (i.e., when m is equal to the cell maximum capacity c). When a cell is full, a division occurs as follows: Half of the molecules contained in the cell are selected at random, then these molecules are removed from this cell and are inserted into the offspring cell. This newly created cell is then inserted into the cellular population. Finally, a cell is picked at random (other than the offspring and parent cell) and removed from the cell population, see Fig. 5.

```
1) Initialize molecular species, go to 3.
2) If simulation termination criterion is
   satisfied go to 8 else go to 3.
3) Collide two molecules selected at random, go
   to 4.
4) If the two selected molecules can react with
   each other go to 5 else go to 2.
5) Create and insert product molecule into the
   cell, go to 6.
6) If the cell contains c molecules then go to 7
   else go to 2.
7) Divide and displace another cell selected at
   random, go to 2.
8) End of simulation.
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Fig. 5. Multi-level reactor algorithm, each single cell/CPU runs this algorithm simultaneously.

Furthermore this multi-level model was implemented as a concurrent system where each cell is run on a single CPU. In this concurrent model, the fittest cells would not only be the cells that exhibit a high rate of successful reactions (when compared to the total number of molecular collisions), but also cells that contain molecules that are fast

to compute. For example let us consider two cells containing complete reaction networks (i.e., all molecular collisions lead to the successful production of molecules). Those cells would moreover contain molecules having different computational complexities. In here the cell which possesses a smaller overall molecular computational complexity will have the selective advantage. This computational complexity introduced in our model a notion of chemical kinetics and may alter the cellular growth rate (i.e., the cells fitness).

III. Experiments

We present three series of experiments, in which we explore the effects of signalling crosstalk in systems where closed reaction networks are employed. We first define the different reaction networks X, Y and Z which are utilized throughout these series of experiments, see Table II.

X	Y	Z
$A = *\nabla 00 : \nabla 01$	$E = *\nabla 10 : \nabla 11$	$I = *\nabla 10 : \nabla 00$
$B = *\nabla 00 : \nabla 00$	$F = *\nabla 10 : \nabla 10$	$J = \nabla 1 * \nabla 00 : \nabla 10$
$C = *\nabla 0\diamond : \nabla 00$	$G = *\nabla 1\diamond : \nabla 10$	$K = *\nabla 10 : \nabla 10$
$D = *\nabla 0\diamond : \nabla 01$	$H = *\nabla 1\diamond : \nabla 11$	$L = \nabla 1 * \nabla 00 : \nabla 00$

TABLE II
Molecular species contained in ACSNs X, Y and Z

No molecular species from X may interact with any molecular species from Y and vice versa. X and Y are non-crosstalking reaction networks. The species A, B, C and D from X may interact with species I, J, K and L from Z, whereas species J and L may interact with B and C from X. X and Z are crosstalking reaction networks. X, Y and Z were obtained from previous experiments in which they were evolved to maximize the production of molecular species A, E and I respectively. Fig. 6 depicts the bipartite reaction network graphs of ACSNs X, Y and Z, note that X and Y possess the same network topology. A cell dominated by a molecular species A is denoted as C_A. The number of molecules of a given species A contained in a cell i is denoted as n_A^i. All experiments are run for a pre-defined amount of time $t_{max} = 3600$ (seconds). Finally, no self-replication reactions are allowed in these experiments (as was the case in analogous Alchemy experiences).

A. Non-crosstalking networks

In this first series of experiments, we investigate the dynamics of a system in which the non-crosstalking closed reaction networks X and Y are used. 30 concurrent cells are employed and initialized with 10 molecules from each species from both X and Y. A cell i divides if $n_A^i \geq 200 \wedge n_E^i \geq 200$. As previously presented in Section II-B, during cellular division half of the molecules in the parent cell are selected at random and transferred into the offspring cell. During this stochastic process some molecular species may not be transmitted to the offspring cells, resulting in a "mutant" cell containing a different reaction network (which may not be closed). We refer to this error prone transmission

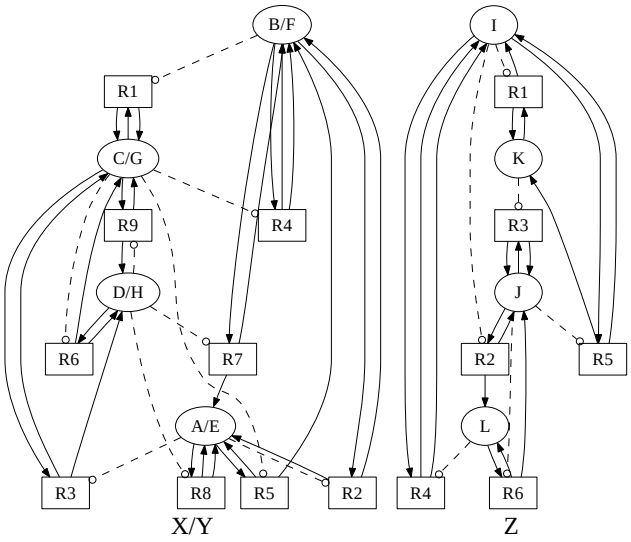

Fig. 6. Bipartite reaction network graphs of ACSNs X/Y and Z. The topology of molecular interactions of X and Y are equivalent, e.g., the reaction $R4$ would involve the molecular species B and C in X, whereas $R4$ would involve the molecular species F and G in Y.

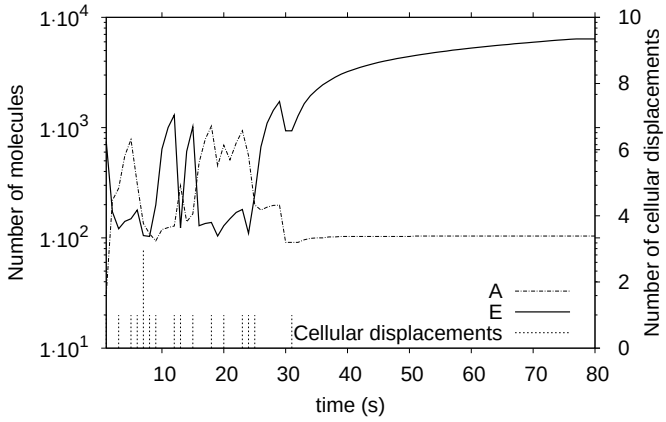

Fig. 7. Growth of molecular species A and E in a sample cell $c1$. The impulses represent the number of cellular displacements (i.e., the sum of all events where $c1$ displaced another cell *and* was itself displaced by another cell).

as mutation at the cell level. No other mutations (e.g., at the molecular level) occur in the system at present.

In Fig. 7, we observe an early phase where molecular species A and E dominate each other in an alternating fashion. In each of these alternated domination periods, n_A^{c1} or n_E^{c1} is increasing rapidly, typically 7 to 10 times higher than the molecular amount of the other species. Moreover this phase is associated with recurrent displacement events which punctuate each domination phase (showing a level of activity at the cell population level). At $t \approx 32$ a displacement event occurs, following this we note that n_E^{c1} is now rapidly increasing, reaching up to 8.10^3 whereas n_A^{c1} stagnates at 2.10^2. This cell is now saturated with molecular species E and will not grow and divide any further. In addition we do not observe any further displacements that may be due to other cells, this suggests that the growth of

the other cells is also null (which could be due to a similar behaviour where a molecular species saturates the cell).

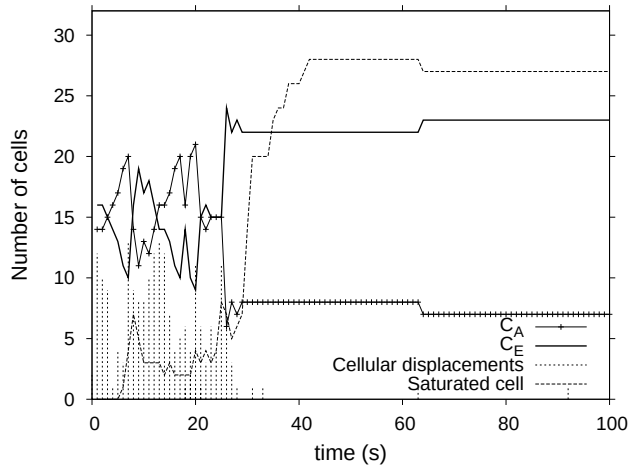

Fig. 8. Domination of molecular species A and E at the cell level. A cell i is dominated by A if $n_A^i > n_E^i$ and vice versa. The impulses represent the total number of cellular displacements occurring in the cellular population.

Fig. 8 provides a cell population view of the simulation in which we may observe the domination of A and E at the cell level. We first note that the early phase previously shown in a given cell can be generalized at the cell population level, i.e., the domination of cell C_A and C_E switches rapidly and is associated with a high overall cellular activity (i.e, the cellular growth rate which is best captured by the number of cellular displacements). We also distinguish that the number of saturated cells increases rapidly when $t \approx 30$ which correlates with previous observations conducted in Fig. 7. However we note that the number of saturated cells does not exceed 28 throughout the simulation. A complementary investigation (not documented here) revealed that the non-saturated cells contained reaction networks in which no successful reactions could occur. These reaction networks resulted from cell level mutation.

Additional experiments were conducted to explore any potential differing dynamics. The above phenomenon was found to be exhibited in all of these experiments.

Although based on a different AC, these experiments shared a key property with experiments conducted in Alchemy: When two non-crosstalking closed reaction networks are mixed together, one displaces the other one.

B. Crosstalking networks

In the remaining sections, cellular species are discriminated by the specific reaction network contained in a given cell (and not by the dominant molecular species as in previous section). We now investigate the effects of crosstalking closed reaction networks upon the system's dynamics. In this experiment, the cells are seeded with molecular species from the crosstalking reaction networks X and Z. A cell i divides if $n_A^i \geq 200 \wedge n_I^i \geq 200$. Any other experimental conditions are identical to those described in the previous section.

Our results showed that the interactions between molecular species from X and Y led to the production of new molecular species M, N, O and P (which may engage in novel reactions with existing molecular species). This new cellular species, denoted as C_1, contains both networks X and Y, and presents an increased level of complexity (the reaction network now contains 12 molecular species and 55 reactions, see Fig. 9). Moreover these C_1 cells were able to self-maintain for a sustained period of time. This first observation also applied in analogous experiments conducted in Achemy, in which a meta-reaction network emerged and had the ability to maintain both seed closed reaction networks throughout the simulation.

However, an additional phenomenon occurred which was not observed in Fontana's AC. We distinguished a selective displacement event between C_1 and a new cellular species. In this series of experiments, a level of diversity was maintained due to cell level mutation, as depicted in Fig. 10. At $t \approx 380$ we note the emergence of a new cellular species, denoted as C_2 and shown in Fig. 9, which later displaced C_1. During this displacement phase, we note that the cell diversity also increased suggesting that other cellular species may also have contributed to the displacement of C_1 cells.

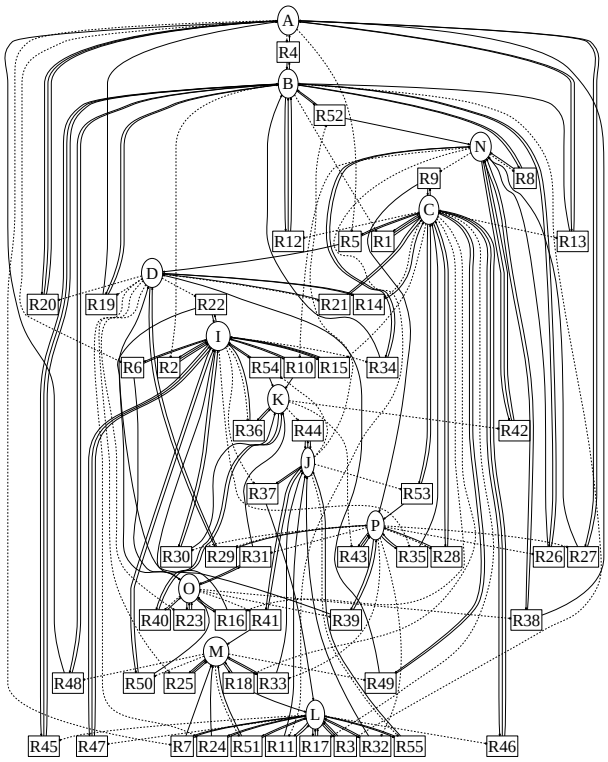

Fig. 9. Reaction network of cellular species C_1 which contains all molecular species from ACSNs X and Z in addition to new molecular species M, N, O and P.

In Fig. 11 we compare the fitness of reaction networks C_1 and C_2. The fitness of a given cell i is defined as the necessary (real) time t_i to satisfy the condition $n_A^i \geq 200 \wedge n_I^i \geq 200$. With the present concurrent system, if a cell is faster to satisfy the condition, then by definition it is a fitter cell.

We note in Fig. 11 that C_2 cells produce molecular species

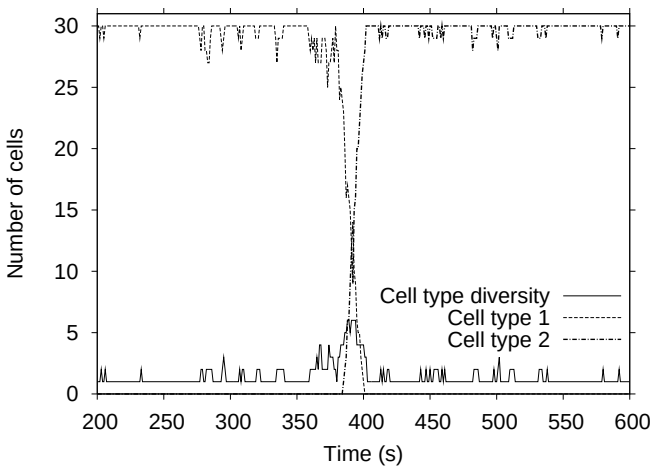

Fig. 10. Cellular species displacement between C_1 and C_2. The cellular species diversity refers to the number of different (from a qualitative point of view) reaction networks present at a given time.

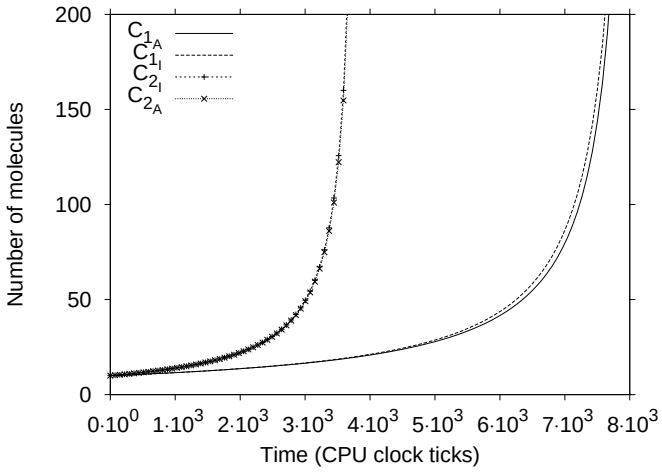

Fig. 11. Comparison of molecular growth of species A and I in C_1 and C_2. The growth of compared molecular species were obtained through solving the Ordinary Differential Equation systems extracted from SBML models of C_1 and C_2. We conducted additional experiments to measure t_{C_1} and t_{C_2} in CPU clock ticks. These measurements were then employed to rescale the time course of the different molecular species' growth.

A and I at a faster pace than C_1 cells (i.e., $t_{C_2} > t_{C_1}$). According to our definition of fitness, C_2 cells are therefore fitter than C_1 cells. We observed a selective displacement in which C_2 evolved its qualitative properties and exploited crosstalk to maximize the production of molecular species A and I. We may also identify this increase in fitness in Fig. 12, in which we distinguish a net increase in the overall cellular growth rate following the displacement event. The "multi-tasking" C_2 cells were able to self-maintain throughout the entire simulation while cell level mutation continued to occur.

Moreover the modifications due to cell level mutations resulted in the removal of specific molecular species from X and Z in C_2. The evolved cellular species C_2 was no longer maintaining the seed reaction networks X and Z. As we cannot identify X and Z in C_2, a natural open-question follows: does C_2 still contain crosstalking ACSNs? Such

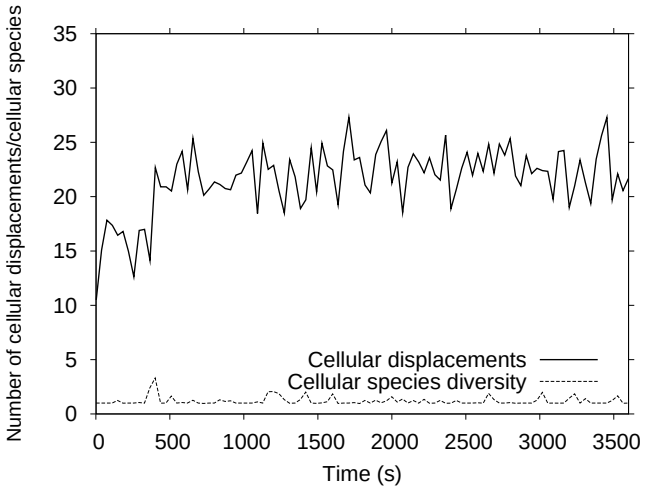

Fig. 12. Dynamics of cellular growth rate and diversity. The cellular growth rate is represented by the total number of displacements (where cells displaced other cells). A spline function was employed to approximate the cellular displacement and cellular species diversity curves.

a question could be addressed if we employ an adequate formalism and identify distinct ACSNs as subsystems in C_2. This issue is nevertheless beyond the scope of this paper but the reader may find further details in [9], where an abstract cell model is proposed to investigate such issues.

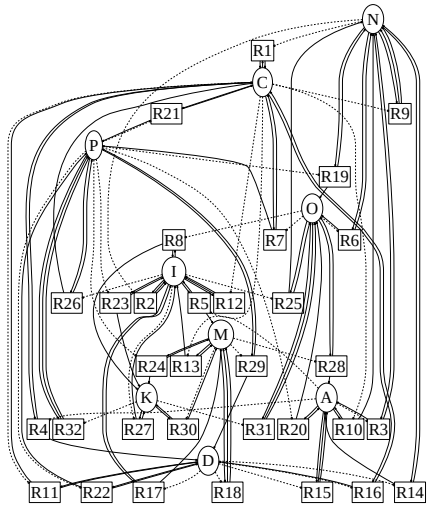

Fig. 13. Reaction network present in cellular species C_2 in which molecular species B, J and L from ACSNs C_1 are absent.

C. Crosstalking networks and molecular mutation

We finally examine the effects of molecular mutation in systems where the crosstalking reaction networks X and Z are used. Molecular mutations are defined and occur as follows:

- When a new molecule is produced, a mutation with probability $p_{sym} = 0.00005$ may be applied to each of its symbols. Therefore, the longer the molecule, the higher the probability of mutation occurring.
- Three types of mutation are distinguished and are applied with equal probabilities:

– *Symbol flipping:* The current symbol is replaced with a symbol picked uniformly at random from Λ.
– *Symbol insertion:* A symbol is picked uniformly at random from Λ and inserted after the current symbol.
– *Symbol deletion:* The current symbol is removed.

Complementary experimental parameters are identical to those presented in Section III-B. Using above conditions, we conducted an experiment in which we identified the following distinctive behaviour.

We first note in Fig. 14 that the variance of cell level activity shares some similarities with the cellular growth rate exhibited in the previous experiment (depicted in Fig. 12). Indeed we observe a common early phase where the cellular activity is approximately equal to 16 cellular displacements per second, then at $t \approx 250$ the cellular growth rate starts to increase. This common behaviour is due to the presence of C_1 (i.e., the meta-reaction network containing both seed reaction networks X and Z) which was also able to self-maintain for a period of time. Then this phase was followed by the emergence of mutant cells which increased the level of cellular growth rate. However due to molecular mutations occurring, a significant difference exists in the cellular species diversity. Here a high level of diversity is exhibited (i.e., averaging at 20.3061 different cellular species compared to 1.1991 in the previous experiment) and is maintained throughout the evolutionary simulation.

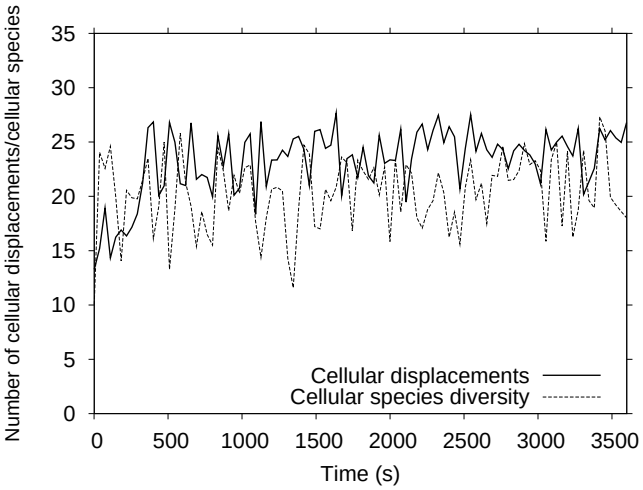

Fig. 14. Dynamics of cellular growth rate and diversity when molecular mutations occur. A spline function was employed to approximate the cellular displacement and cellular species diversity curves

A cellular species displacement is defined as follows: Such a displacement occurs when cells from a specific cellular species continuously dominate the cellular population for at least 50 seconds. In Fig. 15, we note that 52 cellular species displacements occurred (compared to the unique displacement observed in Fig. 10). In addition, it can be observed that the cells' domination rarely exceeded half of the population.

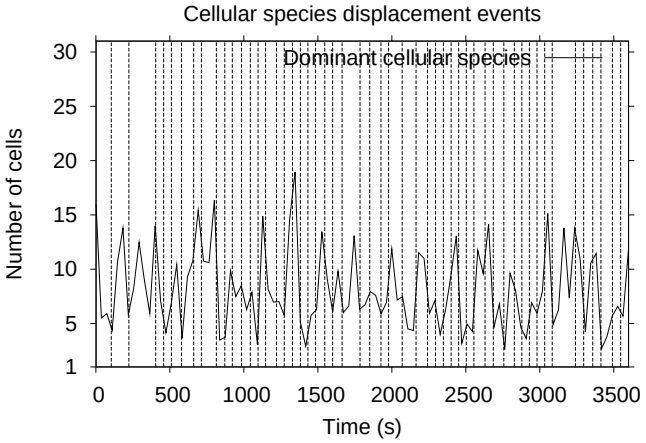

Fig. 15. Dynamics of dominant cellular species. A spline function was employed to approximate the cellular species curve. The vertical lines identify the cellular species displacement events. Only displacements which led to the domination of a given cellular species for at least 50 seconds are shown.

We compare the fitness of the final dominant cellular species, denoted as C_3 see Fig. 16, resulting from this evolutionary simulation. The cellular species C_3 shared an equivalent level of complexity (containing 13 molecular species and 66 reactions) with C_1 cells.

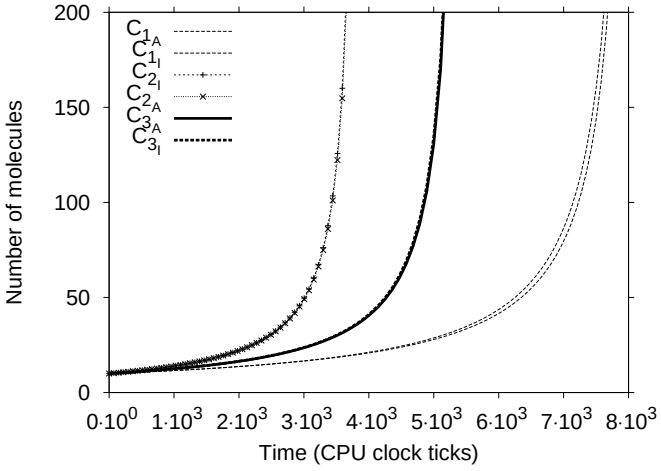

Fig. 16. Comparison of molecular growth of species A and I in C_1, C_2 and C_3.

Since $t_2 < t_3 < t_1$ and the above definition of fitness, it may be argued that the cellular species C_3 is fitter than C_1 and less fit than C_2. Our definition of fitness may thus not be applicable here (otherwise we would expect the cellular species C_2 to be the dominant species and not C_3). In typical evolutionary simulations it is usually expected to observe an incremental improvement in the species' fitness. However an additional investigation of the different successive dominant cellular species revealed that this incremental evolutionary improvement (according to our definition of fitness) did not occur.

When comparing the overall cellular growth rate depicted in Fig. 12 and Fig. 16, we identify a roughly equivalent level of cellular growth rate (≈22 cellular displacements per second). This would thus indicate that although C_2 are fitter (producing molecular species A and I more rapidly) than C_3, the latter (or potentially the cell population as a whole) may have developed other features which maintained a similar cellular growth rate.

At present we have not fully understood the details of this particular evolutionary dynamic, nevertheless we formulate a number of potential explanations:

- Our simplistic view of fitness may not be appropriate in the current experiment. As molecular mutation is now occurring, the cellular species *or* the cellular population as a whole may have developed new features to cope with negative mutation effects. These features may have enabled the cellular population to maintain a competitive overall cellular growth rate while mutations occur. Such features improving the cellular growth rate should then be accounted for in the cellular species' fitness.
- Our classification of cellular species may not expose the dominant cellular species properly. A different classification scheme may be defined which would be based on some key properties of the cell's reaction network (and not only on the species being present in the cell).
- The chaotic nature of the dominant cellular species dynamics (see Fig. 15) may also suggest that the observed displacements might not only be due to selection. This chaotic behaviour may have resulted from the relatively small cellular population size employed here. This parameter may have increased the sensibility of the cellular population to statistical fluctuations.

This experiment presented a range of interesting and unexpected issues which resulted directly from the key differences existing between Alchemy and the MCS.bl system. Further analytical work using adequate tools such as Organization Theory [10] may clarify these complex issues.

IV. Conclusion

We introduced our work which hypothesises that CSNs are subsest of closed reaction networks being able to both self-maintain and to carry out a distinct signal processing function. The nature and potential roles of signalling crosstalk were presented in real CSNs and engineered communication systems. Inspired by specific experiments related to crosstalk conducted with Alchemy by Fontana, we investigated a potential constructive role of crosstalk: To allow distinct closed reaction networks to cooperate with each other when occurring in the same reaction space. We indicated the similarities and key differences between the Alchemy system and our MCS.bl, which we briefly introduced. Three series of experiments were then detailed:

1) Two non-crosstalking closed reaction networks were employed. Although significant differences exist between the MCS.bl and Alchemy, we essentially identified a similar behaviour: one reaction network would displace the other.
2) Two crosstalking closed reaction networks were utilized. We first noted a phenomenon (which also occurred in the corresponding Alchemy experiments), in which a meta-reaction network emerged and contained both seed closed-reaction networks. This new cellular species was able to self-maintain for a sustained period of time. However a second phenomenon occurred (which was not observed in Alchemy), in which a selective displacement took place. A mutant cell emerged and displaced the meta-reaction network. This mutant cellular species was no longer maintaining the seed reaction networks but was in fact fitter at performing the pre-specified tasks.
3) Two crosstalking closed reaction networks were used and molecular mutations were applied. Multiple successive cellular species displacements were observed and presented evolutionary dynamics which are not fully understood yet. The role of crosstalk in this particular evolutionary process remains unclear.

These experiments demonstrated the constructive role of signalling crosstalk in enabling cooperation to occur between closed reaction networks. The evolutionary process was also able to optimize the reaction networks (which exhibited a higher complexity) and their crosstalk properties to carry out the pre-defined multi-task function. However future analytical work remains necessary as the final series of experiments presented intriguing evolutionary dynamics.

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