Two Different Template Replicators Coexisting in the Same Protocell: Stochastic Simulation of an Extended Chemoton Model

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Abstract

The simulation of complex biochemical systems, consisting of intertwined subsystems, is a challenging task in computational biology. The complex biochemical organization of the cell is effectively modeled by the minimal cell model called chemoton, proposed by Gánti. Since the chemoton is a system consisting of a large but fixed number of interacting molecular species, it can effectively be implemented in a process algebra-based language such as the BlenX programming language. The stochastic model behaves comparably to previous continuous deterministic models of the chemoton. Additionally to the well-known chemoton, we also implemented an extended version with two competing template cycles. The new insight from our study is that the coupling of reactions in the chemoton ensures that these templates coexist providing an alternative solution to Eigen’s paradox. Our technical innovation involves the introduction of a two-state switch to control cell growth and division, thus providing an example for hybrid methods in BlenX. Further developments to the BlenX language are suggested in the Appendix.

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Introduction

The simulation of complex biochemical systems, consisting of intertwined subsystems, is a challenging task in computational biology. The complex biochemical organization of the cell is effectively modeled by the minimal cell model called chemoton, put forward by Gánti [1,2,3,4]. In this paper we show an application of the process-algebra based programming language BlenX [5,6,7] for implementing the chemoton as a stochastic model of a complex chemical system. BlenX was developed for modeling systems whose step of computation is a monovalent event, or multivalent interaction between subcomponents [6]. As such it is well suited for modeling elementary or complex chemical reactions [6], Lotka-Volterra type predator-prey dynamics [5] and community dynamics [8].

The language is based on the concept of boxes equipped with binders with π-like processes inside. In the chemical reactions example, boxes represent different molecules, whereas binders express their interaction capabilities and processes handle the manipulation of the binders and drive the internal behavior of the boxes. In classical process calculi, boxes can interact provided that they have identical channel names. BlenX, being a specific type of process calculus itself, has its interactions guided by the compatibility of the binders, which is expressed through an affinity function applied to the type of the binder types [7].
Although information is limited, it is still information. The system carries is the distribution of polymers of different lengths. The actual growth rate of each component is defined by their kinetic constant \( k \), times the amount of the reactants (with appropriate exponents) producing it. The calculated growth rate function is then plugged into the specific event representing the reaction, as the rate of the event. The kinetic factors of a reversible reaction:

\[
A_1 + X \xrightleftharpoons[k_1]{k_1^\prime} A_2
\]

are defined as follows:

\[
\text{let } k1 : \text{const} = 2.0; \\
\text{let } k1r : \text{const} = 0.1; \\
\text{let } rate1 : \text{function} = k1 \ast |A1| \ast |X|; \\
\text{let } rate1r : \text{function} = k1r \ast |A2|;
\]

where \(|s|\) indicates the cardinality (amount) of component \( s \) in the system and \( k1r \) in the code equals to \( k1^\prime \) in the equation. The reactions are called for as events of the following form:

\[
\text{when}(A1, X :: \text{rate1}) \text{ join}(A2); \\
\text{when}(A2 :: \text{rate1r}) \text{ split}(A1, X);
\]

Since division is a global event that cannot be modeled from molecule to molecule, but only concerning the cell as a whole, we decided to implement it as a non-stochastic process. Division is controlled by a global switch, which starts the growing or the dividing phase of the cell. The standard chemoton reactions are suspended during cell division. Although switching cell states is deterministic and happens instantaneously, the division of amounts is still a stochastic process as will be explained below.

In Gánti’s models cell division is triggered automatically when the surface area of the cell doubles. Even if more realistic scenarios exist (for example, in a stochastic model [15] the trigger is the changing osmotic pressure) we chose to use the original assumption for the sake of simplicity. Thus, in our model, cell division always initiates when the surface of the membrane (actually, the amount of \( T_m \) molecules incorporated into the membrane) reaches a certain, predefined size. At this point cell division starts deterministically by switching the cell from the phase of cell growth to the phase of cell division. (For a smoother splitting mechanism, see [16], where the precise volume is calculated depending on the shape of the cell during division.)

The two-state switch (acting as a global signal) is implemented by boxes called Growth and Division:

\[
\text{when}(\text{Growth} : |Tm| = 1000 : \text{inf}) \text{ split}(\text{Division}, \text{Nil}); \\
\text{when}(\text{Division} : |Tm| = 500 : \text{inf}) \text{ split}(\text{Growth}, \text{Nil});
\]

The two-state switch is a previously undocumented addition to the BlenX armament of tools. It is a deterministic operator that can be extended to handle more than two states as well. By referring to the state of the switch, multiple events can be triggered immediately. While the referred component (\( T_m \) here) is properly handled, no infinite loops should occur.

All the stochastic events are conditional on the presence of these signals: the basic chemical reactions of the chemoton are only active when the Growth signal is present; when the Division signal is present all these reactions freeze until cell division is finished.

\[
\text{when}(A1, X :: |\text{Growth}| > 0 : \text{rate1}) \text{ join}(A2); \\
\]

Conversely, division has been implemented as a set of eliminating reactions which are only active when the Division signal is present. Cell division is modeled by halving all molecular amounts in the system. Only the halving of the amount of \( T_m \) membrane molecules is deterministic and precise, all other molecules are deleted with their specific deletion-rate until the membrane reaches its post-division size, that is, until \( T_m \) reaches its lower bound.

\[
\text{when}(\text{Growth} : |Tm| = 1000 : \text{inf}) \text{ split}(\text{Division}, \text{Nil}); \\
\text{when}(\text{Division} > 0 : \text{delrate}(Tm)) \text{ delete}(1);
\]
when (Division > 0 ; deleteA1) delete(1);
when (Division > 0 ; deleteA2) delete(1);
// delete every other component
when (Division : |Tm| = 500 : inf) split (Growth, Nil);

where deletion rates are defined as:
let factor : const = 10.0;
let delrateA1 : function = factor * |A1|;
let delrateA2 : function = factor * |A2|;
...

The food molecule X either has a constant concentration, or is constantly added to the system with a specific (fast) rate, which sets the pace for the chemoton, for example:
let influx : const = 10.0;
...

when (X : |X| = 0 ; influx) new(1);

Template polymerization follows the method of [14]. Initially a double-stranded homopolymer of length \( n/2 \) is present (it consists of two \( n/2 \)-length polymer strands; \( n \) monomers in sum). This state of the polymer is called \( pV(0) \), indicating that zero extra monomer was added to it so far above the basic \( n \). During polymerization the double-stranded polymer is growing by successively adding extra V molecules (e.g. \( pV(0) + V \rightarrow pV(1) + R \)) until the total number of V molecules in the polymer reaches \( 2n-1 \). This state is denoted \( pV(n-1) \). Adding a further V to the polymer, it splits into two \( pV(0) \) molecules, initiating thus further autocatalytic template cycles. In each of the models discussed here \( n = 6 \). We entirely ignored the polycondensation threshold value (usually present in chemoton models, e.g. [3,14,17]), because it is not necessary in our model for maintaining the growth and division cycles of the chemoton. The mechanism of replication is deliberately kept as a black box: we take a worst-case approach by assuming that replication can result in exponential growth. We are aware of the complication that non-enzymatic replication of nucleic acid templates in general is an unsolved problem [18], but here we address a different issue.

In the double-template model (Figure 1), the template monomers V and W have a common precursor, U. Whether U will be converted to V or W in reversible reactions depends entirely on the availability of food molecules Z1 and Z2. Since Z1 and Z2 are represented as entities of constant concentration...
(assuming that the outside environment is stable and abundant in food), the proportion of V and W (and thus $p_V$ and $p_W$) will be regulated by the kinetic factors of the forward ($k_V$ and $k_W$) and backward reactions ($\ell_V$ and $\ell_W$), and the rates of polymerization ($k_{V9}$ and $k_{W9}$), and the rates of polymerization ($k_{V6}$, $k_{V7}$, $k_{W6}$ and $k_{W7}$).

In the simple model, there is only one template polymerization cycle which is directly connected to the metabolic cycle. There is no precursor (U), food molecules Z1 or Z2; V is directly produced by metabolism.

Note that the time scale of simulations is dimensionless; with the proper setting of rates and initial amounts, the behavior scales appropriately with time.

**Results and Discussion**

**Basic model**

Figure 2 shows the behavior of the basic chemoton in BlenX, with similar kinetic constants as used by [14,17,19]. These deterministic models agree that the chemoton maintains stable cell cycles in various conditions. However, in Munteanu and Solé’s model [20], small changes in the concentration levels may lead to drastic changes in the replication period. The cause of these differences is still unclear. In the only stochastic model of the chemoton [21] stable cell cycles were found. Our model also confirmed these results.

The run was initialized with 200 $A_1$, 20 $p_V(0)$, 10 $T_m$ molecules and 1 Growth signal (and either 200 X and an influx rate, or a constant amount of X). The critical amount of $T_m$ was 200: division initiates when the number of $T_m$ molecules grows above 200. Right after division, the cell membrane contains 100 $T_m$ molecules. The initial concentrations need not be close to the adapted concentrations of the chemoton, as the system self-regulates: each component, independently of their initial amount, reaches its typical value, which is maintained throughout the oscillations. The chemoton can exist stably, with clockwork-like oscillations. Its internal processes are synchronized to the division process, which solely relies on the amount of membrane molecules.

**Double template model**

Figure 3 shows now a second template subsystem can coexist with the first one. We investigated the effect of different polymerization rates on this model. In Figure 3 top row the two templates have identical polymerization rates, and both V and W are created in reactions with identical kinetic constants. As expected, the two templates stably coexist.

In the second experiment, one of the polymers has a higher polymerization rate. Usually, if an autocatalytic entity has a higher growth rate than another one, its amount increases (due to the autocatalytic nature of the template) to a point where the other template is practically diluted to extinction. On the contrary, in the chemoton two homopolymers can stably coexist, even if they have different individual growth rates. Figure 3 middle and bottom rows show that templates stably coexist when $p_W$ has a polymerization rate 10 or even 100 times higher than that of $p_V$, respectively. In the chemoton all subsystems are stoichiometrically coupled, meaning that the growth of each component is synchronized with the overall growth of the chemoton.

We also tested what happens if the external source of X is not constant, but has a low influx rate (Figure 4). This introduces both
Figure 3. Dynamics of the chemoton with two different templates, $pV$ and $pW$, when the concentration of food molecules is constant. $k_6 = k_7 = k_{87} = 1$, critical $T_m = 1000$. Top row: the polymerization rate of $V$ ($k_7$) and $W$ ($k_{87}$) are identical (1). Middle row: $k_{87} = 10$. Bottom row: $k_{87} = 100$. In the last two cases, $k_7 = 1$. Volume and surface variables are omitted from the figure. Initial amounts: 100 $A_1$, 100 $pV(0)$, 100 $pW(0)$, 100 $T_m$ and 1 Growth. $X$ has constant amount at 20, $Z_1$ and $Z_2$ at 10. Note that since division is set to a 100 times slower than in Figures 2 and 4, removal of molecules is actually slower than growth. This has no effect on the outcome of the simulation, as the removal process is deterministic. $\Sigma A_i$ stands for the total amount of all metabolites, $\Sigma pV_i$ and $\Sigma pW_j$ for the total amount of all $pV$ and $pW$ polymer stages, respectively.

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Figure 4. Dynamics of the chemoton with two different templates, $pV$ and $pW$, when the main food molecule ($X$) has a low influx rate. $k_6 = k_7 = k_{87} = 1$, critical $T_m = 1000$. Top row: the polymerization rate of $V$ ($k_7$) and $W$ ($k_{87}$) are identical. Middle row: $k_{87} = 10$. Bottom row: $k_{87} = 100$. In the last two cases, $k_7 = 1$. Volume and surface variables are omitted from the figure. Initial amounts: 100 $A_1$, 100 $X$, 100 $pV(0)$, 100 $pW(0)$, 100 $T_m$ and 1 Growth. Influx rate of $X$ is 10; $Z_1$ and $Z_2$ are still constant.

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extra stochasticity, and a reduced pace for the oscillatory cycles (note time scale), but still the experiments yield similar results.

Although the whole template subsystem is still stoichiometrically coupled with metabolism and membrane growth the two templates have internal dynamics. These are independent of the dynamics of the chemoton as a whole: the total amount of $P_V(0)$ and $P_W(0)$ polymers is the same as the amount of $p_N(0)$ in case of the basic model. The internal template-competition is the consequence of the OR type branching of the reactions of $U$ ($U+Z_1\rightarrow V$ or $U+Z_2\rightarrow W$). The reversible nature of the reactions producing $V$ and $W$ and the periodic fission of the chemoton ensures that the faster template cannot wipe out the slower one.

To test the robustness of the chemoton, we have investigated different metabolic subsystems as well. Figure 5 compares the behavior of the model with $m=12$ metabolites with $m=5$ metabolites: the difference is almost undetectable, indicating that the model is capable of handling larger autocatalytic cycles as well. Smaller metabolic subsystems can be crafted, though somewhat forcibly: Gánti has defined the metabolic subsystem of five members as the minimal cycle consisting of elementary reaction steps. Fewer metabolites means that some of the elementary reaction steps should be combined, yielding thus non-elementary reactions. This, having no effect on the simulation at all, blurs the clarity of the original chemoton model, thus we mention it only briefly that results for $m=3$ are almost identical to those for $m=5$.

Conclusions

We have shown in this paper how to implement a stochastic version of the chemoton with the help of the BlenX programming language. BlenX was developed for implementing systems that can be built up by the basic interactions of components; thus, chemical reactions are quite straightforward to implement in this language. Since the chemoton is a theoretically important and well-studied system, this study could be a milestone for BlenX applications.

A specific, somewhat contradictory solution was the introduction of a deterministic two-state switch in our BlenX code. It might seem strange to introduce a deterministic process in a stochastic model; however, it was very important for controlling the timing of the different stages of the cell cycle, namely, the growth and the division of the chemoton, being thus a prime example of hybrid methods available in BlenX.

The new theoretical result of our model is that two competing templates can coexist in the chemoton thanks to the topology of the coupling of its chemical reactions. This finding suggests that the constraint on existence of different templates, as assumed in the formulation of Eigen’s paradox, may generally be more relaxed than previously thought. Results are robust for both polymer-size and the size of the metabolic subsystem.

Notes and suggestions for BlenX

The most important next step would be to allow the chemoton to host a large set of possibly interacting templates, yielding heteropolymers as well. This would open the scene for novel combinations and also for mutations (i.e. hereditary variations) to appear. By this way the evolution of templates could be introduced into the model. However, evolution cannot be directly modeled in BlenX. Evolution requires the random generation of sufficient variability but at present, there is no method or function in BlenX that could generate random variation (we are aware of the fact that this is work in progress). At present, the only way to work around is to manipulate the results of successive BlenX simulations by external scripts [22]. A random number generator therefore should be integrated with box/process/interface creation, to allow the generation of new, programmable boxes on the fly, or the mutation of existing boxes, processes, binder types, and binder affinities during runtime.

This also requires the referencing of boxes or processes that were not declared prior to running. An alternate naming system could rely on parental relations rather than structural congruence: each entity could be tracked, even if their internal structure is unknown, by their parent entities. We conclude that the BlenX language is a very good medium to simulate closed systems, with predefined actors, such as predator-prey dynamics, or chemical reactions. However, as evolutionary biologists, we would like to see a more convenient way to model evolutionary systems as well. The addition of the random number generator, its integration with process-generation, and the possible referencing of undeclared entities would open the door for evolutionary modeling in BlenX, and possibly would give a boost for the productivity of the language by making it a very useful tool in a number of fields.

Provided that these improvements are present in a future release of BlenX, real evolutionary simulations will become feasible with the existing powerful capabilities of the language. Simulating more advanced chemoton models in a stochastic way in BlenX would yield important insights about the coexistence of replicators in compartments, something which has been a holy grail for researchers of prebiotic evolution for decades.

Figure 5. Comparison of different metabolic subsystems. The chemoton on the left consists of a 5-member metabolic cycle ($A_1$ to $A_5$), while the chemoton on the right harbors a 12-strong metabolism ($A_1$ to $A_{12}$). The extra metabolites feed on $X$ and the previous metabolite, and produce $V$ and $W$ polymers in case of the OR type branching of the reactions of $U$ ($U+Z_1\rightarrow V$ or $U+Z_2\rightarrow W$). The reversible nature of the reactions producing $V$ and $W$ and the periodic fission of the chemoton ensures that the faster template cannot wipe out the slower one.
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Author Contributions
Conceived and designed the experiments: AF IZ ES. Performed the experiments: AF IZ. Analyzed the data: AF IZ ES. Wrote the paper: AF IZ ES.

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