The Beacon Calculus: A formal method for the flexible and concise modelling of biological systems

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Abstract

Biological systems are made up of components that change their actions (and interactions) over time and coordinate with other components nearby. Together with a large state space, the complexity of this behaviour can make it difficult to create concise mathematical models that can be easily extended or modified. This paper introduces the beacon calculus, a process algebra designed to simplify the task of modelling interacting biological components. Its breadth is demonstrated by creating models of DNA replication dynamics, the gene expression dynamics in response to DNA methylation damage, and a multisite phosphorylation switch. The flexibility of these models is shown by adapting the DNA replication model to further include two topics of interest from the literature: cooperative origin firing and replication fork barriers. The beacon calculus is supported with the open-source bcs simulator to allow users to develop and simulate their own models.

1 Introduction

The ability to quickly create flexible and concise models of biological systems makes mathematical modelling more practical, enables rapid hypothesis testing, and increases the likelihood that modelling will be used to ensure that conclusions drawn from experiments are consistent with data. Process calculi (or process algebras) are valuable tools for assessing the performance, reliability, and behaviour of a system. Each component in a system is abstracted as a process that can perform actions. Communication actions allow processes to interact with one another to perform coordinated behaviours. The semantics of a process calculus sets rigorous rules that govern which actions that processes can perform, enabling formal reasoning about whether a system is ever capable of performing (or

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not performing) a certain sequence of actions. While process calculi have been historically developed to formally reason about programs and algorithms, they are applicable to any concurrent system (such as biological systems).

There have been many process calculi developed in recent decades: The calculus of communicating systems (CCS) [27] and communicating sequential processes (CSP) [23] are early and foundational examples of process calculi where "reachability", or whether a system can ever perform a certain set of actions, can be determined using the language's structural operational semantics. Performance Evaluation Process Algebra (PEPA) assigned a rate to each action so that the system could be mapped onto a continuous time Markov chain (CTMC) [20, 21]. Once expressed as a CTMC, the system can be simulated by generating random paths through the CTMC's states. It also becomes possible to determine the probability that a behaviour occurs within a specified amount of time, and the system's asymptotic behaviour can be determined using the CTMC's stationary distribution [25]. Tools have been developed to map the PEPA language onto a CTMC and perform this analysis, including the PEPA workbench [17], the PEPA eclipse plug-in [41], and a PEPA-to-PRISM compiler [16].

PEPA has been expanded in a number of ways: Bio-PEPA [6] is an extension for the simulation and verification of biochemical networks and can be analysed via the accompanying Eclipse plugin or the Bio-PEPA workbench [1]. PEPAk is an extension of PEPA that includes process parameters and gated actions [7, 8]. PEPA has also been used as an inscription language for stochastic Petri nets, providing a natural framework for modelling mobile systems [18].

The π-calculus encodes models of concurrent processes using a notion of naming, whereby processes can use channels to communicate channel names to dynamically change which processes can communicate with one another [28]. The stochastic π-calculus is an extension of the π-calculus that has been used for performance modelling in a number of biological applications [33]. SPiM is a stochastic π-calculus simulator for large numbers of interacting biological molecules [32]. In addition, several studies have use the stochastic π-calculus to model regulatory networks in biology, for example [4, 24, 34, 35].

This paper introduces the beacon calculus, which makes it simple and concise to encode models of complex biological systems. It is a tool that builds upon the intuitive syntax of PEPA and mobility in the π-calculus to produce models that are shorter, simpler, and more flexible than they would be if they were encoded in either of these languages (Section S2). Section 2 gives a description of the language by way of examples (a formal description of the language is given in Section S1). To demonstrate breadth, Section 3 presents beacon calculus models of three different biological systems from the literature, each of which highlights one of the language's main features: a model of DNA replication dynamics that fits replication timing data (Section 3.1), a model of the gene expression response to DNA methylation damage in which the model qualitatively matches single-cell tracking experiments (Section 3.2), and a stochastic version of an established deterministic multisite phosphorylation model from the literature (Section 3.3).

2 Materials and methods

This section gives an informal introduction to the beacon calculus by way of examples. Usage is demonstrated by gradually building upon a model of kinesin stepping down a microtubule, ultimately leading to a model that can be applied to molecular crowding [26]. In addition to the beacon calculus itself, a contribution of this paper is bcs, an open-source beacon calculus simulator (`https://github.com/MBoemo/bcs.git`). To make it clear how to translate theory to practice, all examples are given in bcs source code so that they can be simulated and experimented with. A more formal and precise specification of the language and its semantics is given in Section S1.

2.1 Language Overview

Models are written in the beacon calculus by representing components in a system as processes that can perform actions. Processes can make an exclusive choice between multiple actions, execute multiple actions in parallel, and perform actions in a sequence. These three simple but powerful combinators are common amongst many process algebras and are used in CCS, PEPA, the π-calculus, and others [2]. The beacon calculus is a stochastic process calculus where each action is specified as an ordered pair together with the rate at which it is performed. The ordered pairs `{a,ra}` and `{b,rb}` specify rates for actions `a` and `b`, respectively. The following three examples of process definitions show how each combinator is used:

- `P = {a,ra}.{b,rb}` uses the unary prefix operator "`{a,ra}.___`" to denote a sequence of actions whereby action `a` is performed at rate `ra` and, once it has finished, action `b` is performed at rate `rb`.
- `P = {a,ra} + {b,rb}` uses the choice operator "+" to denote the exclusive choice between performing action `a` and rate `ra` and performing action `b` at rate `rb`.
- `P = {a,ra} || {b,rb}` uses the parallel operator "`||`" to denote that actions `a` and `b` are performed in parallel at their respective rates.

Choice binds stronger than prefix, and prefix binds stronger than parallel execution. This means that in the process definition,

```
P = {a,ra} || {b,rb}.{c,rc} + {d,rd}.{e,re}
```

process `P` makes an exclusive choice between performing action `b` at rate `rb` and performing action `d` at rate `rd`. If `b` is chosen, `P` then performs action `c` at rate `rc` while if action `d` is chosen, `P` performs action `e` at rate `re`. All the while, `P` can perform action `a` at rate `ra` in parallel.

A process can have a finite sequence of parameters which, in practice, is often used to encode the process's location, a quantity, or a state (though there are many other uses as well). A process `P` with parameters `i1,i2,...,in` is denoted using the notation `P[i1,i2,...,in]`. Processes can change their parameters through recursion. This is often used when a process moves (if the parameter models a location), modifies how much of something it has (if the parameter models a quantity), or otherwise changes state in some way that should influence the process's later behaviour. For example, the following model describes the stepping of the motor protein kinesin down a microtubule:

```
r = 1; //define the rate for walking

//process definition
K[i] = {walk,r}.K[i+1];

//give the initial state of the system
K[1];
```

In this model, a kinesin process `K` has a position given by its parameter `i`. It starts at position `i=1` (Line 7) and then walks at rate `r`. Every time it takes a step, it recurses with the value of its parameter increased by one.

If this model were run in bcs, the kinesin would continue stepping until it hit the maximum number of transitions allowed by the software. To create an effective model, it is necessary to specify a position where the kinesin should stop walking (at the end of a microtubule, for instance). A process can change its behaviour according to its parameter values by using gates. A gate is a condition that must be satisfied for a process to perform a gated action. Gated actions are written using the notation

```
[<condition>] -> {a,r}
```

where action `a` can only be performed if the condition is true. For example, suppose kinesin should accelerate forward until location `i=5` and then stop. The parameter `i` can be used to scale the static constant `r` so that the rate of walking increases as the motor gets closer to `i=5`. This can be expressed as follows:

```
r = 1; //define the rate for walking

//process definition
K[i] = [i<5] -> {walk,r*i}.K[i+1];

//give the initial state of the system
K[1];
```

Once `i=5`, the condition specified in the gate no longer holds and kinesin cannot perform the action `walk`. When a process can no longer perform any actions, it is said to be deadlocked and is removed from the system. If all processes in the system are deadlocked, the simulation stops. In addition to the "less than" comparison operator used in this example, the beacon calculus simulator also supports the following operators in the expression for the gate condition:

- `<=`, less than or equal to,
- `==`, equal to,
- `!=`, not equal to,
- `>`, greater than,
- `>=`, greater than or equal to,
- `&`, logical and,
- `|`, logical or.

In order for the beacon calculus to be useful for biological applications, a process must be able to react in some way to the actions of other processes; they must be able to communicate with one another via special actions. Handshakes are a common type of synchronous communication in many process algebras whereby two separate processes each perform an action at the same time. In the beacon calculus, two processes handshake when the following two actions are performed together:

- A handshake send is written {`@c![i],rs`}; it denotes a handshake that is offered on channel `c` that transmits parameter `i`.
- A handshake receive is written {`@c?[Ω](x),rr`}; it denotes a handshake that can be received on channel `c` so long as the parameter from the sending handshake is a member of the set Ω. The particular parameter received is bound to the variable `x` and can be used subsequently by the process.

A handshake always occurs between exactly two processes at a rate equal to the product of the handshake receive rate and the handshake send rate. A handshake send and a handshake receive must always be performed together. If a process is ready to send a handshake but there is no other process that can receive the handshake, then the first process must wait until another process is ready to perform the handshake receive. There is no crosstalk between channels, meaning two processes cannot handshake by performing actions {`@c![i],rs`} and {`@d?[Ω](x),rr`} because the channel names do not match. The following example includes two kinesin motors stepping on the same microtubule.

```
1  r = 1; //define the rate for walking
2  rr = 2; //handshake receive rate
3  rs = 3.5; //handshake send rate
4  L = 100; //microtubule length
5
6  //process definition
7  K[i] = {@bump?[i+1],rr}.K[i]
8       + {@bump![i],rs}.K[i]
9       + [i<L] -> {walk,r}.K[i+1];
10
11 //initial state of the system
12 K[5] || K[8];
```

This model has two kinesin motors: one starts at `i=5` and the other starts at `i=8`. They can communicate over channel `bump` (Lines 7-8). If they step such that one kinesin is directly ahead of the other (if they were respectively at positions `i=10` and `i=11`, for example) then they can handshake over channel `bump` at rate `rs*rr`. If they do, both kinesin motors remain where they are. This handshake, when it is possible, competes with each motor's ability to step independently (Line 9). Therefore, the handshake has the effect of the motors impeding each other's progress when they are nearby.

While the handshake receive in the previous example could only receive a single value, handshake receives can accept a set of possible values. A set is specified in the beacon calculus simulator using the following operations. Examples are written for each to show the set (right) encoded by each beacon calculus expression (left). Note that set subtraction binds more strongly than set intersection, which in turn binds more strongly than set union.

- `..`, range,
  - `0..3` $\equiv \{0, 1, 2, 3\}$
  - `-1..2` $\equiv \{-1, 0, 1, 2\}$
- `U`, set union,
  - `0..3 U 6..7` $\equiv \{0, 1, 2, 3, 6, 7\}$
  - `-1 U 0..3` $\equiv \{-1, 0, 1, 2, 3\}$
- `I`, set intersection,
  - `0..10 I 8..15` $\equiv \{8, 9, 10\}$

- `0..2 U 8..15 I 4..9` $\equiv \{0, 1, 2, 8, 9\}$

- \, set subtraction.
  - `0..5\3` $\equiv \{0, 1, 2, 4, 5\}$
  - `0..5\8` $\equiv \{0, 1, 2, 3, 4, 5\}$

If a handshake receive can accept multiple values, the receiving process can bind the value it receives to a variable for later use. The process may, for instance, use this value in a rate expression or as a parameter. The binding variable can be used in the rate expression to indicate how different values can be received at different rates; it can bias which value in the set is received. For example, suppose it is more likely that two kinesin motors impede each other as they get closer to one another. The two definitions for kinesin below, `K1` and `K2`, are equivalent.

```
r = 1; //define the rate for walking
rs = 3.5; //handshake send rate
L = 100; //microtubule length

//process definition
K1[i] = {@bump?[i+1..i+5](x),1/abs(i-x)}.K1[i]
      + {@bump![i],rs}.K1[i]
      + [i<L] -> {walk,r}.K1[i+1];
K2[i] = {@bump?[i+1],1}.K2[i]
      + {@bump?[i+2],1/2}.K2[i]
      + {@bump?[i+3],1/3}.K2[i]
      + {@bump?[i+4],1/4}.K2[i]
      + {@bump?[i+5],1/5}.K2[i]
      + {@bump![i],rs}.K2[i]
      + [i<L] -> {walk,r}.K2[i+1];

//initial state of the system
K1[5] || K2[8];
```

While handshakes allow two processes to perform a coordinated action simultaneously, beacons provide the means for asynchronous communication. In practice, beacons can be used to communicate the state change of a process globally to all other processes in the system. Using beacons, a process can cry for help, efficiently indicate to a number of other processes that a task has been accomplished, or keep track of tasks that have been done over time. A process can launch a beacon on a channel that transmits a parameter; the beacon stays active until it is explicitly killed by a process. While active, the beacon can be received any number of times by any process. Processes can also check whether a particular beacon is active, and only carry on if there is no active beacon with a given channel and parameter.

- A beacon launch is written {`c![i], rs`}; it denotes a beacon that is launched on channel `c` that transmits parameter `i`.
- A beacon kill is written {`c#[i], rs`}; it denotes an action that kills a beacon on channel `c` transmitting parameter `i` if one exists. Once a beacon is killed, it can no longer be received unless it is re-launched by a process.
- A beacon receive is written {`c?[`Ω`](x), rs`}; it denotes an action that can only be performed if there is an active beacon on channel `c` transmitting a parameter `i` in Ω. The parameter received is bound to `x` and can be used subsequently in the process.
- A beacon check is written {`~c?[`Ω`], rs`}; it denotes an action that can only be performed if there is no active beacon on channel `c` transmitting a parameter in Ω.

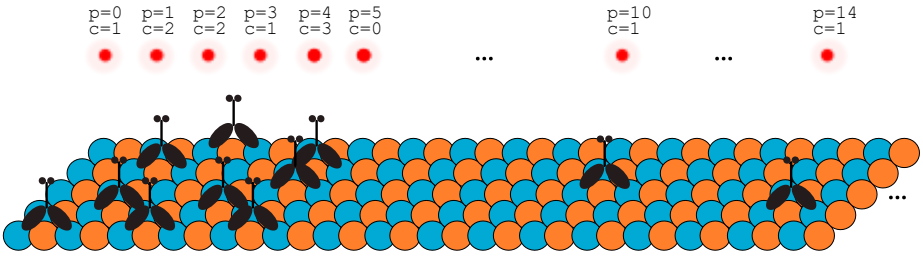
Tau is a neuronal microtubule associated protein that diffuses up and down microtubules [22] and increases the rate of kinesin unbinding [5, 30]. In the following example, a tau protein diffuses on the microtubule and keeps a beacon active at its current position. Kinesin has a chance to unbind from the microtubule when it is at the same position as tau.

```
1  f = 10000; //fast rate
2  r = 1; //kinesin stepping rate
3  rr = 2; //handshake receive rate
4  rs = 3.5; //handshake send rate
5  d = 5; //tau diffusion rate
6  L = 100; //microtubule length
7
8  //process definition
9  tau[i] = [i>0] -> {left,d}.{block#[i],f}.{block![i-1],f}.tau[i-1]
10        + [i<L] -> {right,d}.{block#[i],f}.{block![i+1],f}.tau[i+1];
11
12 K[i] = {@bump?[i+1],rr}.K[i]
13      + {@bump![i],rs}.K[i]
14      + [i<L] -> {walk,r}.K[i+1]
15      + {block?[i],r};
16
17 //initial state of the system
18 K[5] || K[8] || tau[10];
```

In this model, tau diffuses left (Line 9) or right (Line 10) along the microtubule. Both the `left` and `right` actions have the same rate `d`, so the diffusion is unbiased. Tau keeps a beacon active on channel `block` that transmits its position. When it moves left or right, it kills the beacon transmitting its current location and launches a beacon transmitting its new location. These beacon actions should happen immediately so that their rates do not impact the rate of left-right diffusion; they are done at a fast rate (Line 1) relative to other action rates in the system. If there is a beacon active on channel `block` transmitting the same value as kinesin's location, there is a chance for kinesin to receive the beacon (Line 15) and deadlock.

Before moving to applications from the biological literature, the final example of this section is a more advanced use of beacons. Channel names can be strings, as in the previous examples, but the beacon calculus also supports integer-valued channel names. The example below models a microtubule of length `L` as a process `T` that keeps track of the number of kinesin motors at each position using beacons. Each position from `0` through `L` on the microtubule is a beacon channel. A single value is transmitted on each of these integer-valued channels which is the number of kinesin motors at that position. The microtubule process `T` updates each beacon accordingly as kinesin motors step down the microtubule. As this model is intended for demonstration, the parameter values in this example are not biologically representative. However, if the model were parametrised further, it can be applicable to studies of molecular crowding.

(a)



(b)

```
1  f = 10000; //fast rate
2  r = 1; //stepping rate
3  N = 20; //max number of kinesin in the system
4  L = 100; //microtubule length
5
6  //process definitions
7  K[i] = {i?[1..N](c),r*c}.K[i]
8       + {@step![i+1],r}.{@leave![i],f}.K[i+1];
9
10 T[] = {@step?[0..L](p),1}.({p?[0..N](c),f}.{p#[c],f}.{p![c+1],f}.T[]
11                            + {~p?[0..N],f}.{p![1],f}.T[])
12     + {@leave?[0..L](p),1}.({p?[1..N](c),f}.{p#[c],f}.{p![c-1],f}.T[]
13                            + {~p?[1..N],f}.{p![0],f}.T[]);
14
15 //initial state of the system, 5 kinesin at each of positions 10,20,30,40
16 5*K[10] || 5*K[20] || 5*K[30] || 5*K[40] || T[];
```

Figure 1: **Molecular crowding of kinesin stepping down a microtubule. (a)** Kinesin (black) stepping down dimers of tubulin (blue, orange) in a microtubule. A microtubule process `T` maintains a series of beacons (red) where each position `p` is a channel transmitting the current count `c` of kinesin processes `K` at that position. **(b)** Beacon calculus model for molecular crowding.

In the above model, kinesin `K` can receive a beacon on channel `i`, an integer-valued channel name corresponding with kinesin's current position (Line 7). This beacon receive action represents a stall. The values it can receive on channel `i` range from 1 to `N`, the maximum number of kinesin motors in the system. If there is such an active beacon, it can perform the beacon receive on Line 7 and bind the value it receives to `c`, the count of kinesin also at that position. It then makes a choice between stalling at rate `r*c` or stepping forward (Line 8) at rate `r`; the higher the count of kinesin at position `i`, the more likely the stall. If there is no such active beacon, it cannot perform the beacon receive and steps as normal. When kinesin steps forward, it performs a handshake on channel `step` with microtubule process `T` (Line 8). When the microtubule receives the handshake on channel `step`, it binds the position of the kinesin that sent the handshake to `p` (Line 10). It then gets the count of kinesin at that position with a beacon receive and binds that value to `c`. It kills the count of kinesin at position `p` and launches a new beacon that increments the count by one. Kinesin also informs the microtubule of the position that it has left via a handshake on channel `leave`. The microtubule then performs a similar function (Line 12) where it decreases the count of kinesin at that position.

2.2 Simulation

An open-source beacon calculus simulator (bcs) is provided to simulate models written in the beacon calculus (`https://github.com/MBoemo/bcs.git`). The software uses a modified Gillespie algorithm to simulate paths through the model [15]. For each simulation, the software outputs a table of actions sorted in order of ascending time. Each row specifies a time, the action performed at that time, and the process that performed the action (as well as its parameter values at the time when the action was performed). Rather than including plotting capability in the bcs software, the output was designed to be easy to parse so that it can be reformatted into plots that are appropriate for the biological system being modelled. In Section 3, the beacon calculus output has been reformatted into plots that are common in the examples' respective fields. To make it clear how to use the bcs software to simulate biological models, all of the examples in Section 3 are written in bcs source code. Benchmarks for the run time of all models in Section 3 are specified in Section S4.

3 Results

This section applies the beacon calculus to three different areas of biological research: DNA replication (Section 3.1), DNA damage response (Section 3.2), and multisite phosphorylation ultrasensitivity (Section 3.3). These diverse examples demonstrate the breadth of applications for the beacon calculus, and each example showcases a key feature. In the DNA replication model, replication forks use beacons to efficiently coordinate which parts of the chromosome have and have not been replicated. The DNA damage model uses parameters to keep count of damage and repair proteins, showing how to model a population of cells that grows and changes over time. The multisite phosphorylation model shows how receiving a set of possible values in a handshake receive can reduce the number of process definitions required in a model.

3.1 DNA Replication

The mechanisms underlying DNA replication are detailed in a recent review [13] and are briefly summarised here to provide the necessary background for the model. In budding yeast (*S. cerevisiae*), DNA replication initiates during S-phase of the cell cycle from discrete sites on the chromosome known as origins of replication. To maintain genomic integrity, the genome must be fully replicated exactly once per cell cycle. The regulatory mechanism responsible for maintaining this integrity uses an origin recognition complex that binds to the origin and recruits additional proteins to form a pre-replicative complex (pre-RC) in late M-phase and G1-phase when cyclin-dependent kinase (CDK) levels in the cell are low. By the the end of G1, CDK levels have risen (and remain high for the remainder of the cell cycle) so that no new origins can assemble a pre-RC. Those origins that have assembled a pre-RC by S-phase are said to be licensed. The chromosome is replicated when these licensed origins "fire" during S-phase to create bidirectional replication forks that travel along the chromosome in opposite directions from the origin. Forks terminate when they meet a fork travelling in the opposite direction or reach the end of a chromosome.

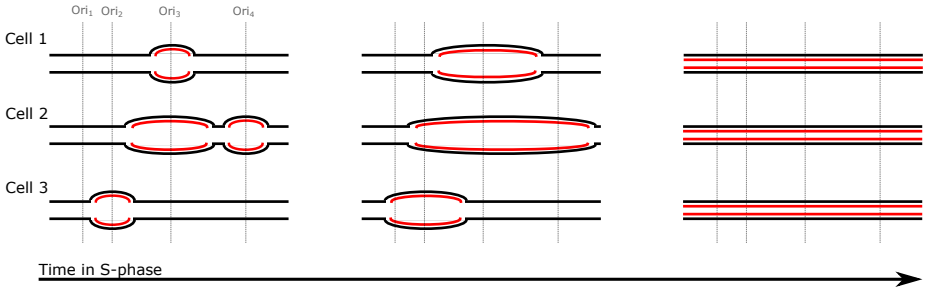
A random subset (but typically not all) of a chromosome's origins initiate replication in S-phase and multiple forks can be active at the same time (Figure 2a). The probability with which origins are licensed is not uniform; some origins are more likely to assemble a pre-RC than others. In addition, of those origins that are licensed, some fire characteristically early in S-phase while others tend to fire late. Therefore, DNA replication is a stochastic process: the set of active origins and the origins responsible for replicating each position on the chromosome will differ from cell-to-cell. Despite this heterogeneity, DNA replication is a remarkably reliable process where errors such as replication fork collapse are rare.

The stochastic nature of DNA replication makes it well-suited to modelling with the beacon calculus: the difference in behaviour between simulations mirrors the heterogeneity between replicating cells, and communication via beacons enables origins and forks to keep track of which chromosomal positions have been replicated. DNA replication is simulated using the beacon calculus model in Figure 2b. The model is comprised of three process definitions: rightward-moving forks `FR`, leftward-moving forks `FL`, and origins of replication `ORI`. The chromosome is of length `L`, and each of these three processes have a single parameter `i` which is taken to be a position on the chromosome between 1 and `L`. Origins have two additional parameters: a licensing probability `q` and a firing rate `fire`. The processes keep track of which chromosomal positions have already been replicated by using beacons: when a fork replicates position `i`, it launches a beacon on channel `chr` with parameter `i`.

The behaviour of an `ORI` process is encoded on Line 6 of Figure 2b. An origin is licensed or not licensed, which is modelled by the choice between the actions `licensed` and `nlicensed`. If the origin is not licensed, the origin can perform no further actions; it is said to be deadlocked. If the origin is licensed, it fires by performing a beacon check action on channel `chr` at its position `i` to ensure that it only fires if that chromosomal position has not yet been replicated by another fork. Once the origin fires, the `ORI` process continues on as two parallel processes: a rightward-moving fork (`FR`, Line 8) and a leftward-moving fork (`FL`, Line 9). The forks first launch a beacon on channel `chr` with their position to indicate to all other forks and origins that the position has been replicated. After launching the beacon, forks use a gate to ensure they have not yet reached the end of the chromosome. If they have not, the forks verify that the position ahead has not yet been replicated by performing a beacon check on that position. If there is no active beacon at that location, the position has not yet been replicated and the fork moves forward by increasing (for `FR`) or decreasing (for `FL`) the position parameter `i`. Like all processes in the beacon calculus, fork movement is stochastic, but forks will tend to the same average velocity over long timescales. Replication has finished when all processes have deadlocked. The initial processes in the system (Line 12) are all `ORI` processes with positions corresponding to known origin locations on *S. cerevisiae* chromosome II [39]. As shown in Figure 2c, when the initial processes in the system are set to be origins with the positions, licensing probabilities, and firing rates from the literature [9], simulations of the beacon calculus model in Figure 2b give good agreement ($R^2 = 0.76$) with established replication timing profiles [29].

The simplicity of the DNA replication model in the beacon calculus makes it quick and simple to test biological hypotheses. For example, the licensing probability, affinity for firing factors, and the spatial distribution of origins across the chromosome will all have an effect on the replication

(a)



(b)

```
L = 813; //length of S. cerevisiae chrII in kilobases
fast = 100000; //fast rate
v = 1.4; //fork velocity in kilobases per minute

//process definitions
ORI[i,fire,q] = {licensed,q*fast}.{~chr?[i],fire}.(FL[i]||FR[i])
              + {nlicensed,fast*(1-q)};
FR[i] = {chr![i],fast}.[i < L] -> {~chr?[i+1],v}.FR[i+1];
FL[i] = {chr![i],fast}.[i > 0] -> {~chr?[i-1],v}.FL[i-1];

//initial processes in the system
ORI[1,0.025,0.522] || ORI[6,0.201,0.097] || ... ;
```

(c)

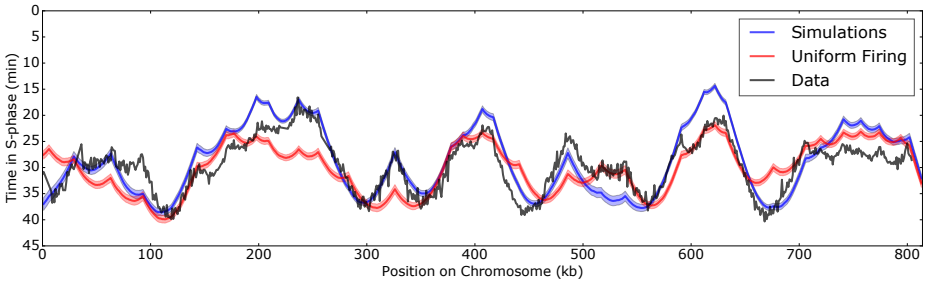


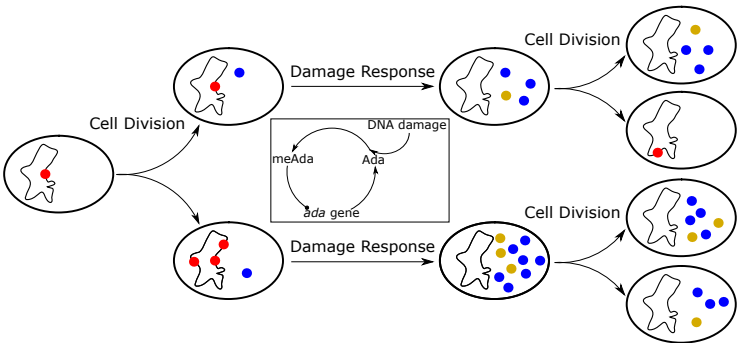
Figure 2: **Replication timing from beacon calculus simulations. (a)** Diagram of ongoing DNA replication in the same chromosome segment of three different cells. Replication can begin from four discrete locations (origins of replication). Each cell successfully replicates its DNA despite having different patterns of origin activation. **(b)** DNA replication model written in the beacon calculus. **(c)** Curves showing the mean time at which each position on *S. cerevisiae* chromosome II was replicated, taken from the beacon calculus model where each origin has a licensing probability and firing time taken from [9] (blue), the beacon calculus model where all origins are licensed and have the same firing rate of 0.015 (red), and experimental data from [29] (grey). The beacon calculus results are averaged over 500 simulations and shaded regions show the standard error of the mean. The system line has been truncated for clarity (see Section S3).

timing profile; the model can be easily modified to investigate the effect of the spatial distribution of origins alone. The red curve in Figure 2c shows the timing profile for a modified version of the model where all origins are licensed and the firing rate of all origins is set to the same value. While the timing profile does not match the data as well ($R^2 = 0.49$), the main features of the replication profile are still captured. This suggests that the primary factor influencing the replication timing profile is the spatial distribution of origins, and that an origin's affinity for licensing and firing factors play a more minor role. As shown in Section 3.4, making other minor modifications to the the beacon calculus model in Figure 2b allows for modelling cooperative origin firing and replication fork progression through fork barriers. More broadly, this modelling strategy is applicable to coordinated movement by biological components within a reference frame.

3.2 Cellular Response to DNA Damage

To show how the beacon calculus can be used to model systems at the population level, this section models the *E. coli* DNA methylation damage system studied in [43]. The effective identification and repair of DNA damage is essential to genome integrity. Unrepaired methylation damage is particularly cytotoxic and mutagenic [3]. In *E. coli*, DNA methylation damage is repaired by the Ada methyltransferase protein: Ada repairs the damage by transferring a methyl group from O^6-Methylguanine or O^4-Methylthymidine to itself [38]. The resulting methylated Ada (meAda) significantly upregulates transcription of the *ada* gene, creating a positive feedback loop that increases Ada levels. This leads to a spike in Ada level following DNA repair which is reduced back to basal levels over generations by successive cell divisions (Figure 3a).

(a)



(b)

```
k_basal = 0.03125; //1.25 molecules per generation, 1.25*(1/40min)
k_me = 0.0375; //1.5 molecules per generation, 1.5*(1/40min)
k_dmg = 0.1;//0.0001;// //aquire DNA damage
k_division = 0.025;//E.coli replication time about 40 min
rho = 0.01625; // 0.65 per generation, 0.65*(1/40min)
kMax = 10; //max rate to generate Ada
fast = 10000;

//process definitions
CELL[A,mA,d] = {generate_Ada, k_basal + kMax*(mA/(mA+10))}.CELL[A+1,mA,d]
             + [d>0 & A>0] -> {generate_meAda,k_me*d*A}.CELL[A-1,mA+1,d-1]
             + {dna_dmg, k_dmg}.CELL[A,mA,d+1]
             + {startRep,k_division}.DIV[A,mA,d,0,0,0,0];

DIV[A,mA,d,A1,M1,A2,M2] = [A>0] -> ({seg, fast}.DIV[A-1,mA,d,A1+1,M1,A2,M2]
                                  + {seg, fast}.DIV[A-1,mA,d,A1,M1,A2+1,M2])
                        + [mA>0] -> ({seg, fast}.DIV[A,mA-1,d,A1,M1+1,A2,M2]
                                   + {seg, fast}.DIV[A,mA-1,d,A1,M1,A2,M2+1])
                        + [A==0 & mA==0] -> {finish,fast}.(CELL[A1,M1,d]
                                                          || CELL[A2,M2,d]);

//initial processes in the system
CELL[0,0,0];
```

(c) (d)

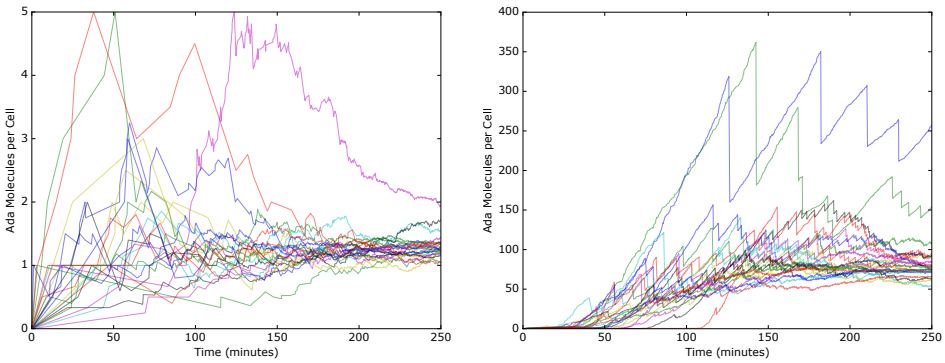


Figure 3: **DNA damage from beacon calculus simulations.** **(a)** Cells undergo DNA damage (red) and may carry it forward for generations before an Ada molecule (blue) is generated to repair it. Ada is methylated (gold) as it repairs DNA damage creating a positive feedback loop whereby methylated Ada upregulates transcription of the *ada* gene. Ada levels are reduced through successive cell divisions. **(b)** DNA damage model written in the beacon calculus. **(c-d)** Average total Ada and meAda per cell over time. Each trace corresponds to a simulation of a growing population of cells for **(c)** low DNA damage ($k_{dmg} = 0.0001$) and **(d)** high DNA damage ($k_{dmg} = 0.01$). In each of panels **(c-d)**, 25 simulations are shown.

A cell keeps Ada levels low in order to perform a delicate balancing act: Excessive Ada levels are thought to be cytotoxic [14], but the cell must still produce enough Ada to repair DNA methylation damage in a timely fashion. This is accomplished by expressing the *ada* gene at very low levels such that on average only one Ada protein is produced per generation [43]. Such a low rate of production means that due to stochasticity, DNA damage may go unrepaired for one or more generations before an Ada protein is produced to repair it (Figure 3a).

A stochastic model can provide insight into this repair system by showing the Ada response in rare but important simulations where DNA methylation damage has gone unrepaired for several generations (see, for example, the complementary model in [42]). By varying the DNA damage rate, a model can also predict how the repair system responds to both high and low rates of DNA methylation damage. The beacon calculus makes modelling this system straightforward by representing an *E. coli* cell as a process that can repair DNA damage and divide into two daughter cells (Figure 3b). The cell process keeps track of DNA damage and Ada levels using parameters and the value of these parameters can scale the rate at which the process performs certain actions.

The process `CELL` is defined on Line 10 of the beacon calculus model in Figure 3b. `CELL` has parameters that keep track of three quantities: the number of Ada molecules (`A`), the number of methylated Ada molecules (`mA`), and the number of sites where DNA has been damaged (`d`). The cell can generate an Ada molecule with action `generate_Ada` (Line 10). The parameter `mA` is used in the rate calculation of this action so that Ada is generated at a basal rate if `mA=0`, but the rate scales to saturation with the value of `mA` to reflect the upgregulation of the *ada* gene by meAda. If the cell has DNA damage and at least one Ada molecule to repair it, `CELL` can fix the damage by first performing action `generate_meAda` and then converting Ada to meAda (Line 11). Damage repair requires interaction between Ada and a methylated base, so the rate of this action scales with the value of `d` and `A`. The cell's DNA is damaged at a static rate (Line 12) which increments parameter `d`.

The cell can divide at the mean rate of replication for *E. coli* cells (Line 13). When cell division begins, the `CELL` process carries on as a new process `DIV` for a dividing cell. The `DIV` process (Line 15) encodes how Ada, methylated Ada, and damage are segregated between two daughter cells. In addition to the original three parameters of the dividing cell, this process has four additional parameters: the amount of Ada and meAda that segregates to one daughter cell (`A1` and `M1`, respectively) and the amount of Ada and meAda that segregates to the second daughter cell (`A2` and `M2`). These new parameters each start at zero (Line 13). For each Ada and methylated Ada molecule in the parent cell (Lines 15 and 17, respectively) a random choice is made as to which daughter cell inherits the protein. When a choice has been made for each molecule of Ada and meAda, the `DIV` process starts two new daughter `CELL` processes (Lines 19-20).

The initial condition for each simulation is a single cell with no Ada and no DNA methylation damage (Line 23). As the cell divides, the system is comprised of an exponentially growing population of the initial cell's descendents. Computing the average Ada per cell for this exponentially growing colony shows that the amount of Ada stays near the basal average amount of 1.25 molecules per cell when the rate of DNA damage is low (Figure 3c, highest spike at 5 Ada molecules per cell). Some

colonies exhibited sharp spikes in Ada levels caused by DNA damage that had gone unrepaired for several generations. However, this happened infrequently and the elevated Ada levels tended back towards zero as the Ada was diluted by successive cell divisions. When the rate of DNA damage was high, the spikes in Ada level were higher in magnitude (Figure 3d, highest spike at 350 Ada molecules per cell). In addition, Ada levels stayed elevated over time and did not tend back towards zero. These observations are qualitatively consistent with the results from [43] of Ada levels in individual *E. coli* cells under both high and low DNA damage conditions.

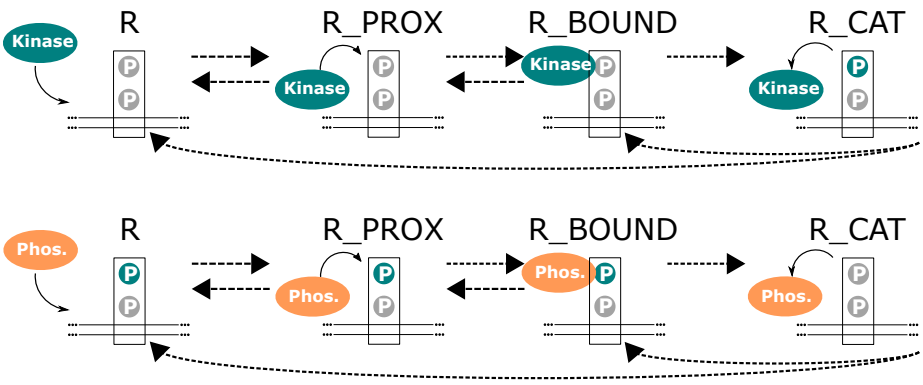
Communication between processes in the beacon calculus means that the model can be easily extended to incorporate cell-to-cell interactions or cell-to-environment interactions using handshakes and beacons. More generally, the beacon calculus makes it simple to model a growing and changing population. While this example focused on how a population of cells responds to DNA damage, a similar approach can be taken to model more diverse applications such as the spread of disease through a population.

3.3 Multisite Phosphorylation

Cellular signalling relies on post-translational modifiacations and, in many instances, substrates are modified on multiple sites. This is thought to confer specific information processing functions such as switch-like responses [12, 19, 40] (see [37] for a review). One example is the reversible phosphorylation of membrane-anchored receptors or adaptors by extrinsic kinases and phosphatases, which applies to a large class of receptors known as non-catalytic tyrosine-phosphorylated receptors (NTRs) of which the T-cell antigen receptor (TCR) is a member [10]. NTRs are known to have multiple phosphorylation sites (20 in the case of TCRs) and are phosphorylated and dephosphorylated by kinases and phosphatases that are also confined to the plasma membrane. Given that these receptors often control cellular responses, their phosphorylation is tightly regulated and consequently, will be highly sensitive to the relative concentration or activity of their regulating kinases and phosphatases.

The beacon calculus model in Figure 4 is similar to, and inspired by, that of Dushek *et al.* who modelled the phosphorylation of TCRs when they were phosphorylated by kinase Lck and dephosphorylated by phosphatase CD45 [11]. The model shown here corroborates the authors' findings: In order to achieve ultrasensitivity, an enzyme must dwell for a short period after modifying the phosphorylation of a receptor.

(a)



(b)

```
intlPhosphatase = 100;
intlKinase = 100; //varied in Fig. 3c
intlReceptors = 10;
pSites = 20;
kPlus = 0.1;
kOn = 100000;
kMin = 1000;
kOff = 1;
kr = 0.1;
mu = 1; //turned on or off in Fig. 3c

//process definitions
ENZYME[e] = {@proximalEnzyme![e], 1};
R[p] = {@proximalEnzyme?[0..1](e),kPlus}.R_PROX[p,e];
R_PROX[p,e] = {enzLeave, kMin}.(R[p] || ENZYME[e])
            + [e==0 & p<pSites]->{bindK, kOn*(pSites-p)}.R_BOUND[p,e]
            + [e==1 & p>0]->{bindP,kOn*p}.R_BOUND[p,e];
R_CAT[p,e] = {enzymeLeave, kMin}.(R[p] || ENZYME[e])
           + {dwell,mu}.([e==0 & p<pSites]->{bindK,kOn*(pSites-p)}.R_BOUND[p,e]
                       + [e==1 & p>0]->{bindP,kOn*p}.R_BOUND[p,e]);
R_BOUND[p,e] = {unbindEnzyme, kOff}.R_PROX[p,e]
             + [e==0]->{phosphorylate,kr}.R_CAT[p+1,e]
             + [e==1]->{dephosphorylate,kr}.R_CAT[p-1,e];

//initial processes in the system
intlKinase*ENZYME[0] || intlPhosphatase*ENZYME[1] || intlReceptors*R[0];
```

(c)

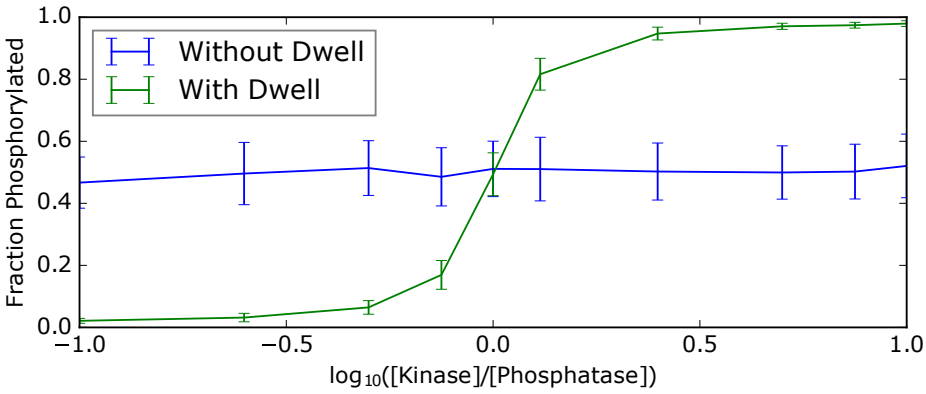


Figure 4: **T cell receptor ultrasensitivity from beacon calculus simulations. (a)** An enzyme enters the proximity of a receptor, binds to the receptor, and phosphorylates the receptor if the enzyme is a kinase or dephosphorylates the receptor if the enzyme is a phosphatase. **(b)** T cell receptor model written in the beacon calculus. **(c)** The fraction of phosphorylated receptors is ultrasensitive to the relative concentration of kinase and phosphatase when the enzyme dwells after modifying a receptor (green) but loses ultrasensitivity if the dwell is removed (blue). Points (shown with standard errors) are the average of 50 simulations taken after the system reaches a steady state.

Each TCR can be phosphorylated 20 times. When an enzyme enters the proximity of a receptor, it can either bind to the receptor or leave (Figure 4a). The enzyme can phosphorylate the receptor if it is a kinase or dephosphorylate the receptor if it is a phosphatase. Once the enzyme phorphorylates or dephosphorylates the receptor, there is a period of inactivity (or a dwell) before the enzyme can bind to the receptor again. The number of phosphorylation sites, together with the action of two types of enzyme, leads to a high number of distinct species in the system; this can make a differential equation model cumbersome to write down and integrate. The beacon calculus makes modelling this system straightforward by representing enzymes and receptors as processes, whereby receptor processes keep track of their phosphorylation and the type of enzyme bound to them using parameters.

A model in the beacon calculus for TCR phosphorylation is shown in Figure 4b. Each `ENZYME` process (Line 13) has parameter `e`, whereby the enzyme is a phosphatase if `e=1` or else it is a kinase if `e=0`. A receptor process `R` (Line 14) has parameter `p` which keeps track of the number of times the receptor has been phosphorylated. An enzyme enters the proximity of a receptor via a handshake on channel `proximalEnzyme`, whereby the enzyme transmits its parameter `e` to the receptor to indicate whether it is a kinase or a phosphatase. After the handshake, the enzyme deadlocks while the receptor carries on as a new process `R_PROX` (Line 15) that encodes the behaviour of a receptor with an enzyme in close proximity. The reverse reaction can occur if `R_PROX` performs action `enzLeave` (Line 15) where `R_PROX` then carries on as `R` and `ENZYME` in parallel. If the enzyme is a kinase and the receptor is not fully phosphorylated, the enzyme can bind at a rate proportional to how many sites on the receptor are unphosphorylated (Line 16). If the enzyme is a phosphatase, the enzyme binds at a rate proportional to how many sites on the receptor are phosphorylated (Line 17). When `R_PROX` binds an enzyme, it carries on as process `R_BOUND`. In this new process, the enzyme can either unbind (Line 21), phosphorylate the receptor if the bound enzyme is a kinase (Line 22), or dephosphorylate the receptor if the bound enzyme is a phosphatase (Line 23). If the enzyme phosphorylates or dephosphorylates the receptor, the bound receptor `R_BOUND` carries on as process `R_CAT` in which the enzyme is proximal to the receptor but briefly inert. The enzyme can either leave (Line 18) or rebind once the inert period is over (Line 19-20).

The above model is similar to that of [11], and the results agree with the authors' findings (Figure 4c). When the enzyme dwells after modifying the phosphorylation of a receptor, the fraction of receptors that are phosphorylated is ultrasensitive with respect to the relative concentration of kinase and phosphatase; it displays switch-like behaviour. If the dwell is removed and all other parameter values are kept constant, the ultrasensitivity is lost. While the beacon calculus is able to reproduce an established model in only a few lines of code, the language also makes it simple to expand upon the model. For example, the model in Figure 4b can be extended to model groups of receptors on different areas of the membrane. A group of receptors can use beacon to signal a state change in that group which can cause other groups located elsewhere to respond.

3.4 Extensions to the DNA Replication Model

To demonstrate the flexibility of models written in the beacon calculus, the DNA replication model from Section 3.1 is extended to include two

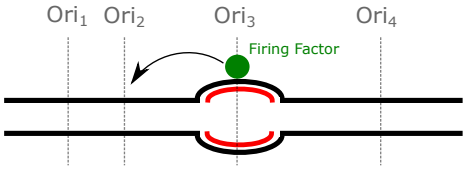
topics of interest from the field: cooperative origin firing and the effect of a replication fork barrier.

It has been hypothesised that the probability of a replication origin firing increases if a nearby origin fires [31]. This may be due to stoichiometrically limiting firing factors which are more likely to interact with an origin if they have already interacted with another origin nearby. The beacon calculus model in Figure 5a extends the DNA replication model so that when an origin fires, it launches a beacon on channel `coop` transmitting its location to induce firing of nearby origins. If an origin has not yet fired, it can fire at rate `fire` which is taken to be the origin's base affinity for firing factors (Line 7). The modified model includes an additional pathway to firing where an origin can receive a beacon on channel `coop` that is transmitting a parameter within 50 kb of the origin's location (Line 8). The rate at which this beacon is received is inversely proportional to the distance between the origin and the transmitted parameter. If the beacon is received, the origin fires, launches its own beacon on channel `coop` transmitting its position to other origins, and starts two replication forks from its position. Therefore, origin firing is either due to the origin's natural affinity for firing factors or due to another origin firing nearby (if there is one).

When proteins bind tightly to DNA, they may act as a replication fork barrier (RFB) that can stall replication forks moving in a particular direction. One role of RFBs is preventing collisions between replication and transcription machinery [36]. This is incorporated into the beacon calculus model as shown in Figure 5b. First, the location of the fork barrier is specified on the chromosome (Line 5) along with the rate of the stall (Line 6). If a rightward moving fork makes it to this position, it stalls (Line 12) before it ultimately recovers and continues stepping.

Simulations of the models shown in Figure 5a-5b are shown in Figure 5c. The replication fork barrier causes a sharp change to the timing profile near the location of the replication fork barrier at chromosomal coordinate `i=200` while the cooperative firing behaviour makes the whole chromosome replicate slightly earlier. However, the additional parameters added in these two models were not fit to data; these two extensions are only intended to demonstrate the ease with which beacon calculus models can be extended. With further parametrisation, however, these extensions can be useful in making meaningful biological predictions about DNA replication systems.

(a)

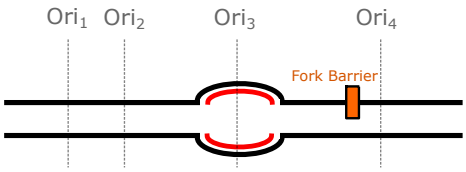



```
//rates and constants
L = 813; //chromosome length in kb
fast = 100000; //fast rate
v = 1.4; //fork velocity in kilobases per minute

//process definitions
ORI[i,q,fire] = {license,fast*q}.({~chr?[i],fire}.{coop![i],fast}.(FL[i]||FR[i])
        +{coop?[i-50..i+50](x),1/abs(i-x)}.{ ~chr?[i],fast}.{coop![i],fast}.(FL[i]||FR[i]))
              + {nlicensed,fast*(1-q)};
FR[i] = {chr![i],fast}.[i < L] -> {~chr?[i+1],v}.FR[i+1];
FL[i] = {chr![i],fast}.[i > 0] -> {~chr?[i-1],v}.FL[i-1];

//initial processes in the system
ORI[1,0.025,0.522] || ORI[6,0.201,0.097] || ... ;
```

(b)




```
//rates and constants
L = 813; //chromosome length in kb
fast = 100000; //fast rate
v = 1.4; //fork velocity in kilobases per minute
rfb = 200; //location of replication fork barrier
sr = 0.14; //stall rate

//process definitions
ORI[i,q,fire] = {license,q}.{~chr?[i],fire}.(FL[i]||FR[i])
              + {nlicensed,1-q};
FR[i] = {chr![i],fast}.([i<L & i!=rfb] -> {~chr?[i+1],v}.FR[i+1]
                      + [i==rfb] -> {stall,sr}.FR[i+1]);
FL[i] = {chr![i],fast}.[i > 0] -> {~chr?[i-1],v}.FL[i-1];

//initial processes in the system
ORI[1,0.025,0.522] || ORI[6,0.201,0.097] || ... ;
```

(c)

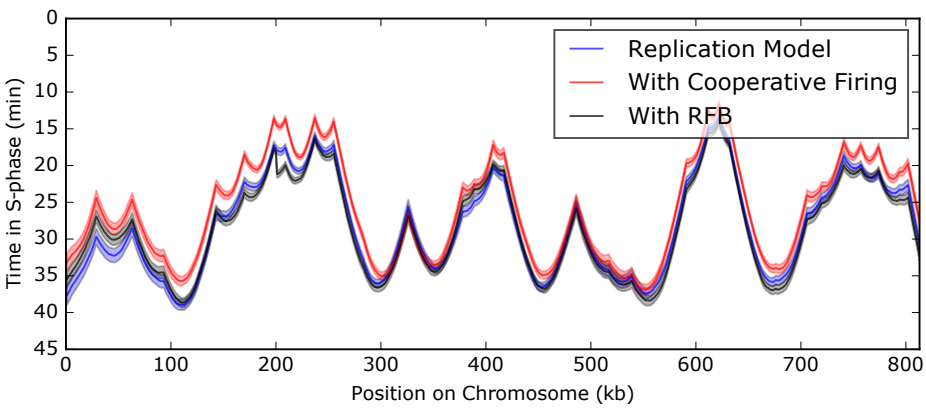


Figure 5: **Extensions to the DNA Replication Model.** The DNA replication in Figure 2b is modified to include either **(a)** cooperative origin firing or **(b)** fork progression through a replication fork barrier. All changes to the model in Figure 2b are highlighted in blue. **(c)** Results from Figure 2b compared with the simulated models from **(a)** and **(b)** where each curve is the average over 500 simulations. Shading shows the standard error of the mean. The system line has been truncated for clarity (see Section S3).

4 Discussion

Process calculi are a natural framework in which to model biological systems, but they are an underutilised tool within systems biology; to the authors' knowledge, process calculi have never before been applied to DNA replication, DNA methylation damage, or receptor ultrasensitivity. The beacon calculus makes it quick and easy to create models of systems where processes can change both their actions and interactions over time. Beacons make it simple for a process to influence the actions of all other processes in the system. This paper has shown how this paradigm is used to model both the complex behaviour of cells and macromolecular structures in only a few lines of code.

A language that makes it simple and concise to encode biological models has advantages beyond saving time: It changes the way the tool is used. Simplicity increases confidence that the user has actually encoded what they think they have encoded and have not introduced bugs into the model. It also leads to models that are easy to change, modify, and extend. This flexibility encourages experimentation where hypotheses can be rapidly tested, and any conclusions drawn from laboratory experiments investigated, to ensure that they are consistent with the biological data.

The features in the beacon calculus are all geared toward models that are quick to encode and easy to modify. As shown in Section 3.4, the DNA replication model in Figure 2b can be modified to include features of interest from the literature such as cooperative origin firing and a replication fork barrier that stalls replication forks at a particular chromosomal coordinate. The flexibility of the beacon calculus means that these changes are straightforward to incorporate from the model in Figure 2b and come at the expense of only one or two lines of code.

While this paper has shown that the beacon calculus can easily produce flexible and concise models of biological systems from the current literature, it is not appropriate for every task. When the beacon calculus is compared with the stochastic π-calculus (Section S2.1), the Kappa language (Section S2.2), and Bio-PEPA (Section S2.3), there are situations where these tools can be more appropriate. In particular, Kappa is better suited to modelling protein-protein interaction networks and large chemical reaction networks while Bio-PEPA is better suited to modelling chemical regulatory networks under general kinetic laws (Michaelis-Menten kinetics and Hill kinetics). For these applications, the features of the beacon calculus add little benefit and other tools may deal with them more naturally. The beacon calculus finds its niche in applications where system components must be able to easily coordinate with each other or with a global reference frame (such as in the DNA replication model) or adapt behaviour in response to complex and changing environmental conditions (such as a cell responding to DNA damage or multisite phosphorylation).

The beacon calculus is well-suited to these applications, but there are many more applications throughout biology where the beacon calculus can be an ideal tool for modelling and simulation. Section 3 illustrated three examples from cell biology and molecular biology, but modelling at the population level is possible as well. A stochastic version of the SIR model for a population's response to an infectious disease would be straightforward: Each individual is a process, whether they are susceptible, infected, or recovered from an infection is kept track of with a parameter, a response to nearby individuals could be modelled using the ability of handshake receives to accept a range of parameters, and beacons could be used to signal

some state change within a city or area as the disease evolves. There are a wide range of applications within biology, and while the beacon calculus was developed for biological applications, there is nothing biology-specific in the language; it can be used for applications in engineering and other fields as well.

One of the biggest challenges in creating a simulation tool is ensuring the user is simulating what they think they are simulating; if the user has made an error encoding the model, this can lead to incorrect conclusions being drawn about the underlying biology. An advantage of process algebras is that the language's semantics, together with automated theorem proving techniques, can be used to prove whether a certain combination of actions is ever possible in the model. In the DNA replication model, for example, a user may wish to verify that replication forks cannot step through each other in the model that they have encoded. If this action is possible, then there is an error in the model and the simulation results will not accurately reflect the biological reality. A planned extension of the bcs tool is allowing the user to specify certain actions or properties that should not be allowed in the model. The tool will check these properties before beginning the simulations to ensure that they are not possible, giving the user greater confidence in the validity of the result.

5 Conclusion

The beacon calculus is a language that makes it fast and easy to encode concise, flexible models of biological systems. It is particularly well-suited for systems where interactions between components change over time, where components need to change the state of many other components, or where components need to respond to events happening within a certain region. Its breadth is demonstrated by creating models of DNA replication and DNA damage repair from the literature, as well as creating a stochastic version of an established deterministic multisite phosphorylation model. To support the language, a contribution of this work is an open-source simulator called bcs which, together with the provided examples, makes it easy for users to create and simulate their own models.

Acknowledgements

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Author Contributions

MAB created and simulated the models, wrote the software, and designed the study; MAB and CAN created the DNA replication models; MAB and LC designed the language; CAN obtained the funding; MAB wrote the paper with input from all authors.

Conflict of Interests

The authors declare no conflict of interests.

Software and Data Availability

The beacon calculus simulator is available at `https://github.com/MBoemo/bcs.git`.

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