IMSV: bacteria simulator design
Version 0.0

M. Dinh and S. Fischer

June 29, 2016

Contents

1 Introduction

The aim of this document is not to go into technical details of the implementation (the code is documented using Doxygen, technical details are therefore best found in the Doxygen-generated manual). Rather we wish to walk through the choices in design that have been made. The technical manual hopefully contains necessary information for understanding the concept behind each class and how to use it. However it does not tell you what classes are central in the architecture and it is difficult to see at a glimpse how classes interact.

Describing global design should be more agreeable to read than the purely technical document. It helps understand how we tackled a certain number of efficiency issues (should it be speed or maintainability). Most efficiency issues in programming are related to architecture design rather than class implementation. Efficiency of an architecture can be related to information flowing between classes. Restricting information access through classes and dependencies between classes is generally considered good style, as it limits data corruption and enhances maintainability. Achieving this increases the probabilities that the simulator behaves the way it should and facilitates further expansions.

Once data protection and maintainability are ensured, speed issues are addressed only if they can be identified. Most parts of the simulator are not critical in that regard and do not need a particularly refined design or implementation. If speed issues arise, two level of solutions can be worked on. At the lowest level, class implementations can be changed to perform some routines more quickly (generally leading to at most a couple-fold speed increase). At the highest level, communication between classes can be tuned to ensure that only the necessary computations are done (generally leading to a drastic speed increase and a complexification of the architecture with new classes that "filter" communications).

To sum up, description at a global level gives critical insight into how the simulator works and where speed/design issues have been identified during development. It should facilitate discussions even with non-programmers (or at least non-C++-programmers).

2 Global presentation of the components of the simulator

2.1 Components of the simulator

The simulator can be decomposed into several large modules that handle specific tasks during simulation (Fig. 1). First of all, there is an **input/output** module that creates everything that is needed for the simulation from an input file (Fig. 1 - Initialization). **Reactants** and **reactions** are user-specified and need to be created on demand, as well as **events** happening throughout the simulations and more technical aspects about which algorithm to use to perform the integration. Once everything is set up, the **solver** follows a simple loop that can be decomposed in three steps (Fig. 1 - Loop). Integration occurs reaction by reaction, at each loop, we go forward one reaction, update the simulation

time, concentrations and reaction rates.

1. At the beginning of the loop, the **input/output** process checks whether **events** should occur at the current simulation time and whether it needs to write some concentrations to an output file.

2. It then hands control over to the **solver**, which is based on Gillespie's approach to integrate a network of chemical reactions. The Gillespie algorithm needs the current reaction rates of all **reactions** and draws a random reaction with a probability proportional to its rate. This task is delegated to a **rate manager**, which uses state-of-the-art methods to maintain the rate list updated and perform the drawing efficiently.

3. Once a **reaction** is drawn, it is performed *i.e.* the concentrations (and the state, see below) of its **reactants** is modified.

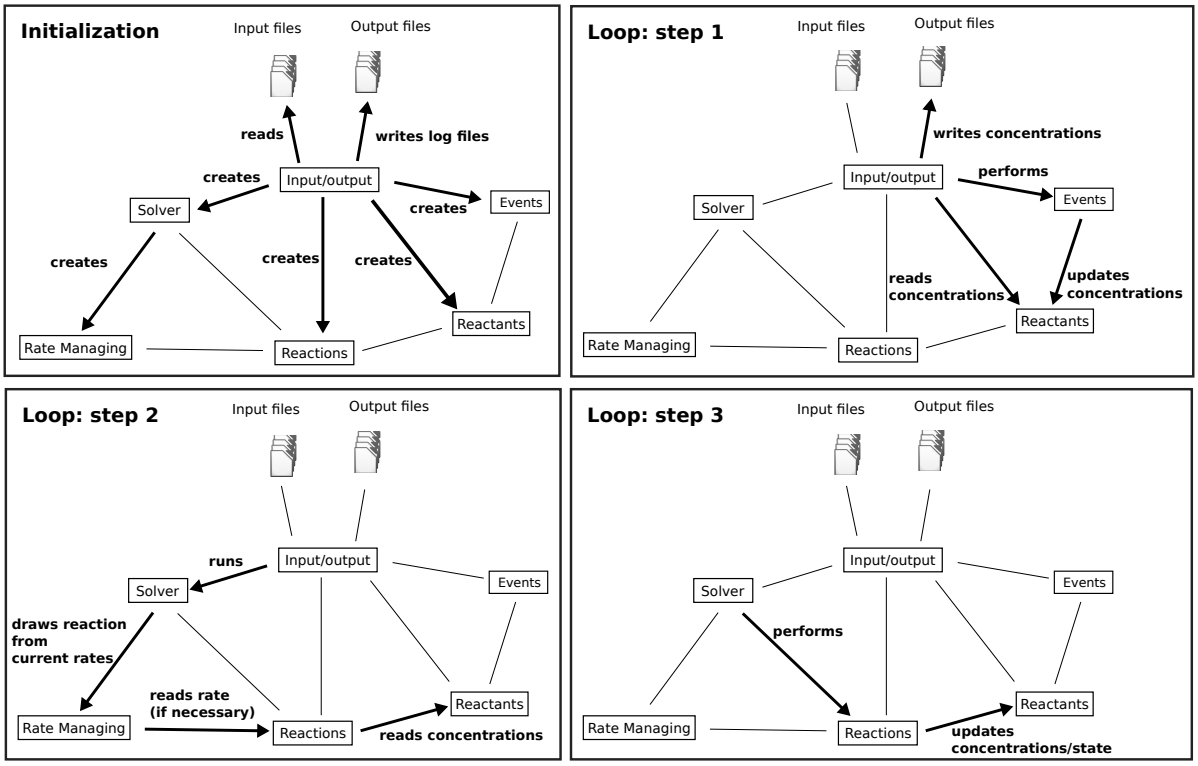
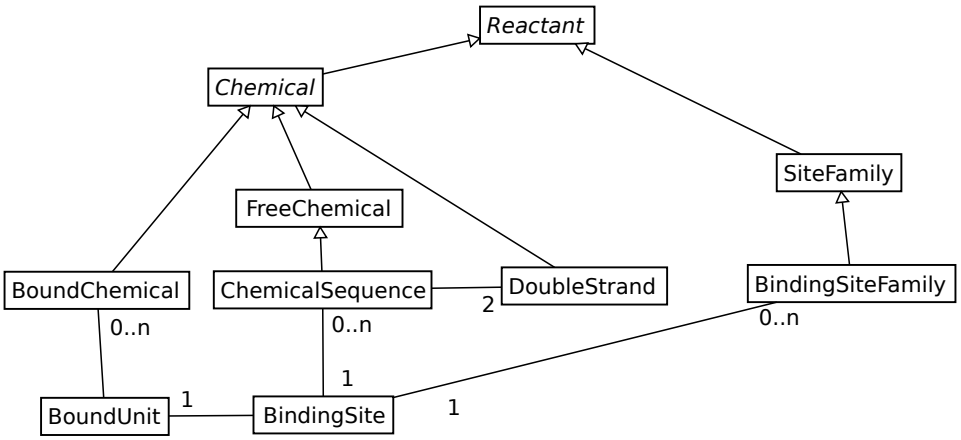


Figure 1: Schematical view of the simulator.

2.2 Reactant hierarchy

This section gives a quick overview of the contents of the **reactant** module. More details about how reactants are implemented can be found later.

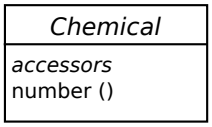
2.2.1 UML class diagram



2.2.2 Reactant

`Reactant` is a global abstract interface. All entities that can participate in a reaction *must* inherit from it.

2.2.3 Chemical

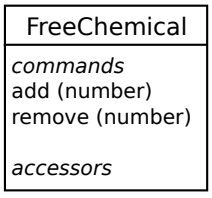


`Chemical` is an abstract class. It defines all standard chemical entities. `Chemical` represents a *pool* of a given chemical species, meaning that one may access its current number at any time.

2.2.4 FreeChemical

Input format

```
FreeChemical <name> [<initial quantity>]
```

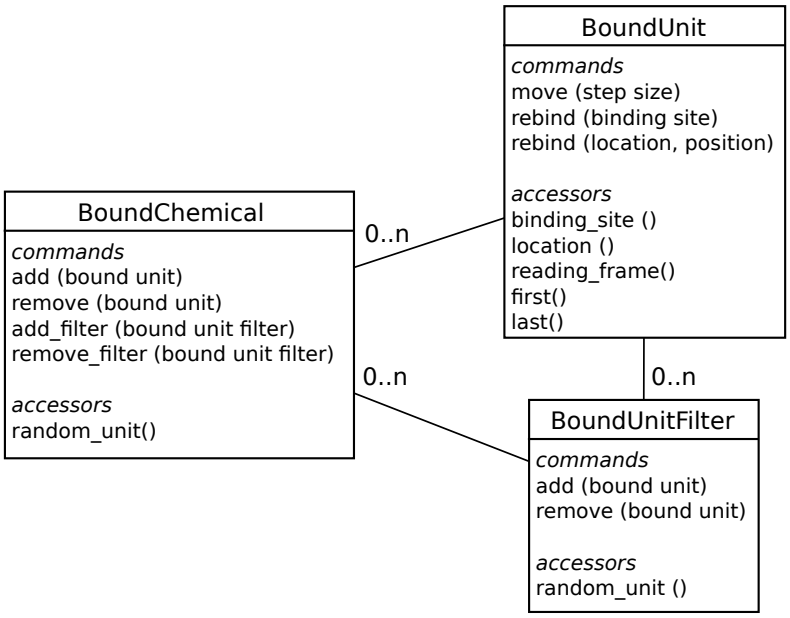


`FreeChemical` is a subclass of `Chemical` that represents free chemical (*e.g.* molecules diffusing in the cytosol or extracellular medium).

2.2.5 BoundChemical

Input format

```
BoundChemical <name>
```



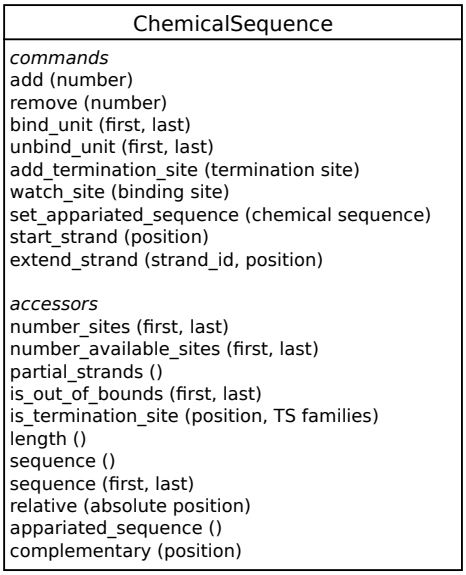
`BoundChemical` is a subclass of `Chemical` that represents chemicals that are bound to a sequence. It is important to note it only represents molecules bound to the sequence, *not* the complex formed by the chemical and the sequence. Even though `BoundChemical` represents a pool of molecules, single elements are not interchangeable, they are defined by their position on a sequence. `BoundChemical` uses class `BoundUnit` to represent molecules individually. It uses `BoundUnitFilter` to organize bound units according to outside criteria needed for reactions (classify according to binding sites, motifs read, etc.).

2.2.6 ChemicalSequence

Input format

```
ChemicalSequence <name> sequence <sequence> [<initial quantity>]
TransformationTable <name> [<parent_letter> <product_letter>,]^{1..n}
ProductTable <name> <transformation table>
ChemicalSequence <name> product_of <parent sequence> \
  <starting position> <ending position> <product table> [<initial quantity>]
```

`ChemicalSequence` is a subclass of `FreeChemical`. It is defined by a sequence and the ability to bind elements. However, instances of a sequence are *not* treated individually, it is impossible to tell to which instance a given chemical bound. An object called

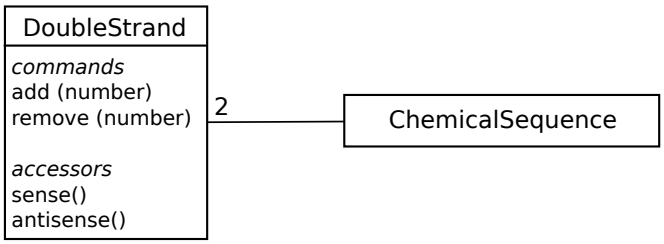


`SequenceOccupation` maintains occupation levels at sites of interest. For example, suppose the sequence is an mRNA carrying a ribosome binding site for the protein DnaA. The number of available sites is obtained by removing the number of bound chemicals occupying the site from the number of instances of the mRNA currently in the cell. A `ChemicalSequence` can be appariated to another `ChemicalSequence`. A `ChemicalSequence` can be created from a sequence or as a product of another sequence, in which case a `TransformationTable` is needed to generate the product's sequence from the parent's, and a `ProductTable` stores the parent/product relationship.

2.2.7 DoubleStrand

Input format

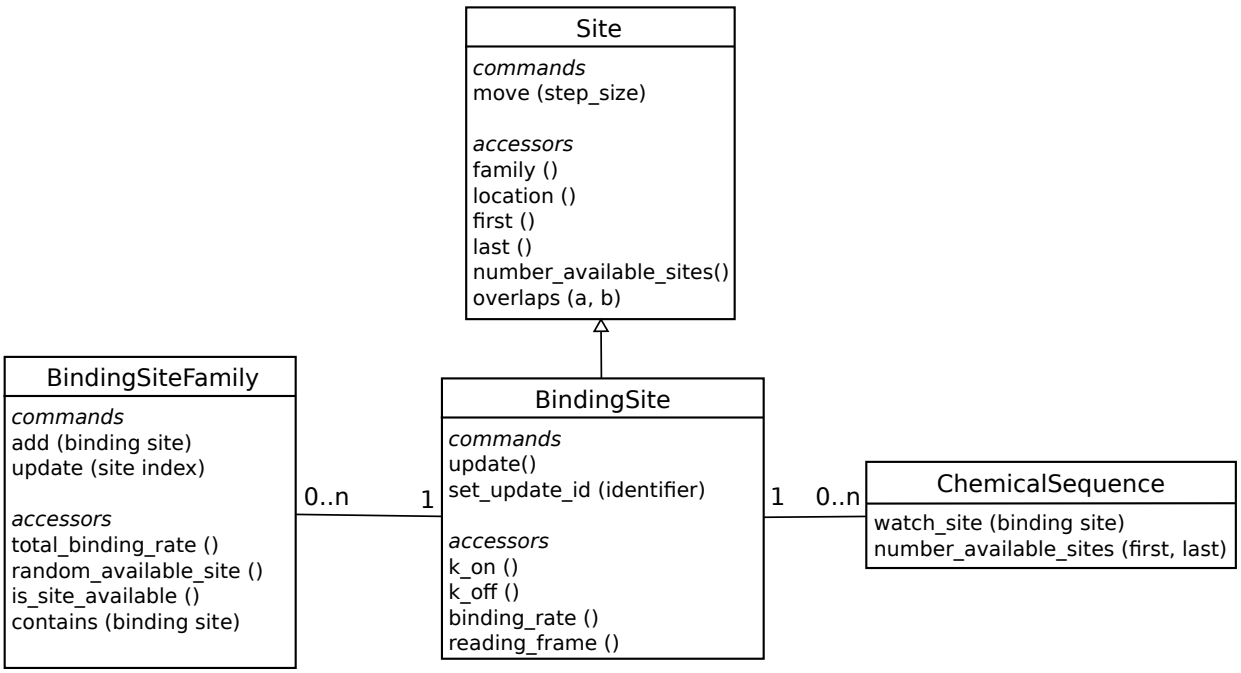
```
TransformationTable <name> [<letter> <complementary_letter>,]^{1..n}
DoubleStrandSequence <name> <name_sense_sequence> <sense_sequence> \
  <name_antisense_sequence> <transformation_table> [<initial quantity>]
```



`DoubleStrand` links two `ChemicalSequence` together that are biochemically linked (*e.g.* DNA), one sequence being complementary to the other. It enables segment extension on the appariated strand and free end binding (see interface of `ChemicalSequence`). A `DoubleStrand` is created from a sense sequence that is specified similarly to a `ChemicalSequence`.

However, the complementary sequence is created from a `TransformationTable` that specifies how to transform the sense sequence into antisense sequence (*e.g.* for DNA, $A \to T$, $T \to A$, $C \to G$, $G \to C$).

2.2.8 BindingSiteFamily



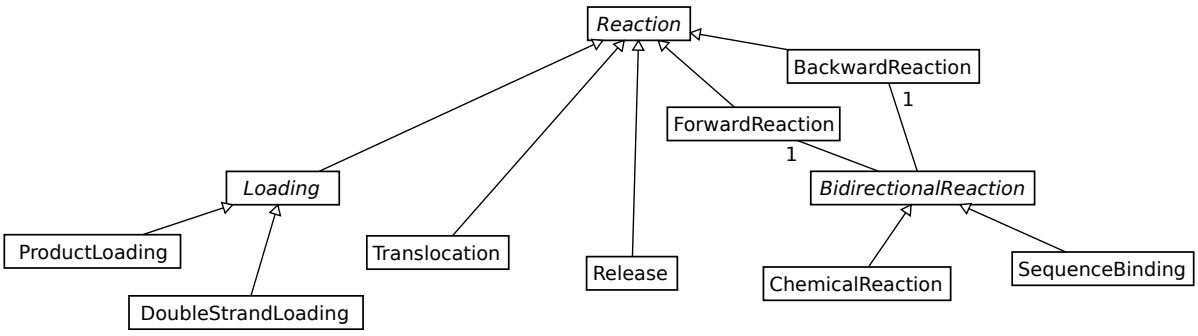
`BindingSiteFamily` is a subclass of `Reactant`. Contrary to `Chemical`, it does not represent a countable pool of molecules. Each family contains a number of related instances of `BindingSite` (*e.g.* ribosome binding sites). `BindingSiteFamily`, `BindingSite` and `ChemicalSequence` use a notification pattern (via `update` methods) to dynamically maintain the number of available sites for each binding site as well as binding rates up to date. If a binding site is used to load polymerases, a reading frame should be provided to specify where a polymerase will start reading the sequence after binding.

Input format

```
BindingSite <binding site family name> <chemical sequence> \
  <start> <end> <k_on> <k_off> [<reading frame>]
```

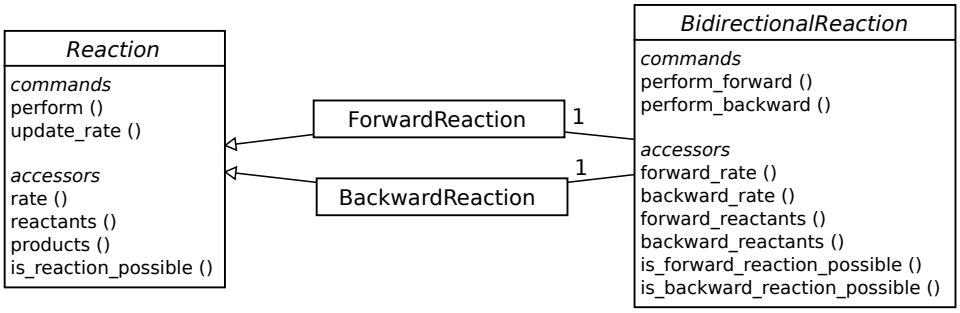
2.3 Reaction hierarchy

This section gives a quick overview of the reaction module. More details about how reactions are implemented can be found later.



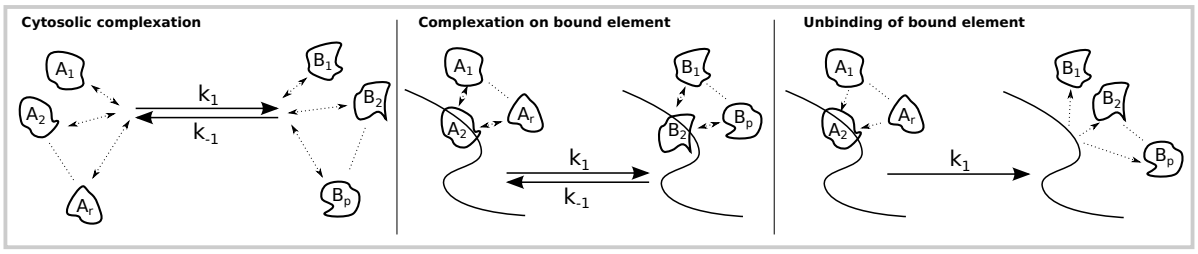
2.3.1 UML class diagram

2.3.2 Reaction



There are two abstract classes used to define reactions: `Reaction` for one-way reactions and `BidirectionalReaction` for reversible reactions. Two adapter classes `ForwardReaction` and `BackwardReaction` split reversible reactions in two one-way reactions. In the end, the solver only handles one-way reactions. A reaction can necessarily be performed, its rate updated and accessed and is composed of reactants and products.

2.3.3 ChemicalReaction



Input format

```
ChemicalReaction [<chemical> <stoichiometry>]^{1..n} rates <k_1> <k_-1>
```

Formula A `ChemicalReaction` represents association/dissociation of an arbitrary number of elements. It is defined by

$$a_1 A_1 + a_2 A_2 + ... + a_r A_r \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} b_1 B_1 + ... + b_p B_p$$

where

- A_i and B_i are of type `FreeChemical`. They can be of type `BoundChemical` in two cases: (i) a reaction containing a `BoundChemical` on each side, (ii) an *irreversible* reaction where a *reactant* is a `BoundChemical` and where there are no bound product. In both cases, the associated stoichiometric coefficient must be 1.
- a_i and b_i are stoichiometric coefficients.
- k_1 and k_{-1} are rate constants.

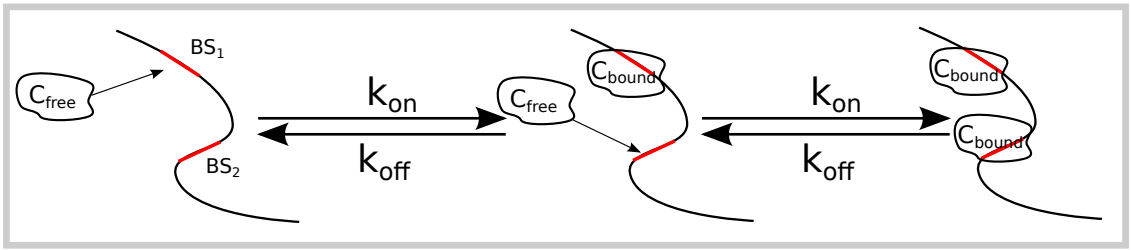
Action When the reaction is performed, the number of chemicals involved is changed according to their stoichiometric coefficient. If `BoundChemical` are involved on each side, the simulator will assume that the bound chemical that is consumed is replaced by the bound chemical on the other side of the equation (*i.e.* it will be bound at the location previously occupied by the precursor). If there is a `BoundChemical` on the reactant side of an irreversible reaction, the simulator will assume that the reaction describes the unbinding of this bound unit into the cytosol.

Rate The rates are given by

$$\lambda_{forward} = k_1 \prod_{i=1}^{r} [A_i]^{a_i}$$

$$\lambda_{backward} = k_{-1} \prod_{i=1}^{p} [B_i]^{b_i}$$

2.3.4 SequenceBinding



Input format

```
SequenceBinding <chemical> <bound form> <binding site family>
```

Formula A `SequenceBinding` represents binding of a free element on a binding site of a sequence. It is defined by

$$C_{free} + BSF \rightleftharpoons C_{bound}$$

where

- C_{free} is of type `FreeChemical`.
- BSF is of type `BindingSiteFamily`.
- C_{bound} is of type `BoundChemical`.

Action When the forward reaction is performed, a random available binding site is drawn from the binding site family (drawing is weighted by affinity). A C_{free} molecule is removed from the pool and a C_{bound} added to the `ChemicalSequence` bearing the binding site. When the backward reaction is performed, a random molecule of C_{bound} is removed from the pool (and from its sequence) and a C_{free} molecule is added.

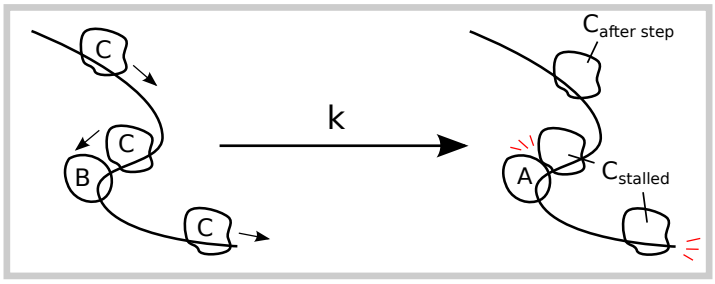
Rate The rates are given by

$$\lambda_{forward} = \frac{[C_{free}]}{V_c} \sum_{\text{sites } s \in BSF} (k_{on})_s \times \text{Number of sites } s \text{ available}$$

$$\lambda_{backward} = \frac{1}{V_c} \sum_{\text{molecules } m \in C_{bound}} (k_{off})_{\text{site on which } m \text{ is bound}}$$

- $(k_{on})_s$ is the association constant of C_{free} with binding site s.
- $(k_{off})_s$ is the dissociation constant of C_{bound} with binding site s.
- V_c is the volume of the cell.

2.3.5 Translocation



Input format

```
TerminationSite <family name> <chemical sequence> <start> <end>
Translocation <bound chemical> <form after step> <stalled form> \
  <step size> <rate> [<termination site family>]^{0..n}
```

Formula A `Translocation` represents movement of a bound element along a sequence. It is defined by

$$C \xrightarrow{k} C_{\text{after step}}$$

or

$$C \xrightarrow{k} C_{\text{stalled form}}$$

where

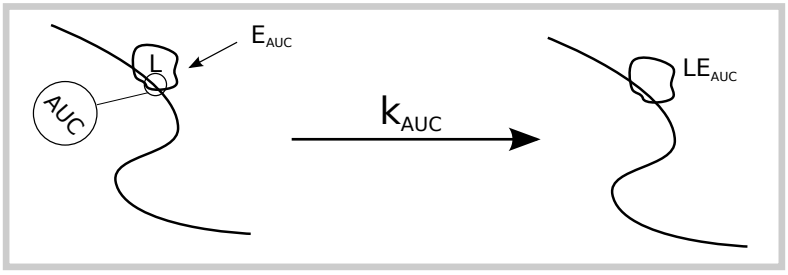
- C is of type `BoundChemical`.
- $C_{\text{after step}}$ is of type `BoundChemical`.
- $C_{\text{stalled form}}$ is of type `BoundChemical`.
- k is a rate constant.

Action When the reaction is performed, a random C is chosen. Generally, it is replaced by a $C_{\text{after step}}$, moved by a step of a given size along the sequence the original C is bound to. If the chemical cannot move because it reached the end of the sequence or it reaches a termination site, it is replaced by $C_{\text{stalled form}}$.

Rate The rate is given by

$$\lambda = k[C]$$

2.3.6 Loading



Input format

```
LoadingTable <name> \
  [<template> <element_to_load> <occupied_polymerase> <rate>,]^{1..n}
ProductLoading <bound chemical> <loading table>
DoubleStrandLoading <bound chemical> <loading table> <stalled form>
```

Formula A `Loading` typically represents loading of elements by a polymerase onto a template sequence. It is defined by

$$L + E \longrightarrow LE$$

where

- L is of type `BoundChemical`.
- E is an element to load, of type `FreeChemical`. It is defined in a `LoadingTable` associated with the reaction.
- LE is the occupied form of the loader, of type `BoundChemical`. It is defined in a `LoadingTable` associated with the reaction.

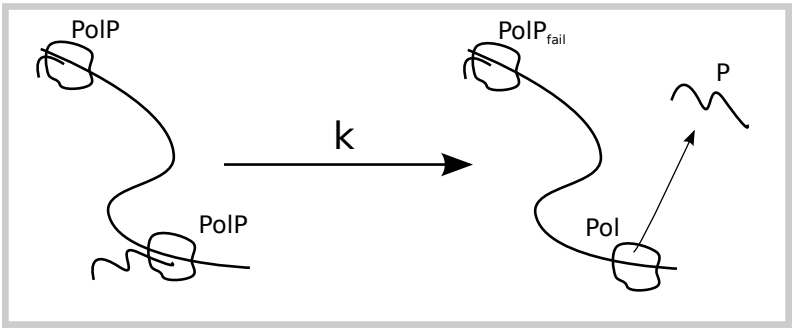
Action Each instance of L reads a specific template. Using its `LoadingTable`, we know which E it tries to load, which LE is yielded if loading occurs and the loading rate associated with the template. When the reaction is performed, a random L is chosen according to loading rates. An element to load E is removed from the pool and L is replaced with LE. A `ProductLoading` assembles loaded elements into a product that will eventually be release in the cytosol (*e.g.* RNA synthesis), while `DoubleStrandLoading` extends segments along a `DoubleStrand` (*e.g.* DNA replication). In `DoubleStrandLoading`, loading may fail because the loader met a previously synthesized segment. In the latter case, it is replaced by a `BoundChemical` representing its stalled form.

Rate The rate is given by

$$\lambda = \sum_{t \in templates} k_t [L_t][E_t]$$

where

- k_t is the loading rate associated with template t.
- L_t corresponds to loaders L reading template t.
- E_t is the chemical to load onto template t.



2.3.7 Release

Input format

```
TransformationTable <name> [<parent_letter> <product_letter>,]^{1..n}
ProductTable <name> <transformation table>
Release <polymerase> <empty_polymerase> <fail_polymerase> \
  <product table> <rate>
```

Formula A `Release` represents release of a product from a polymerase

$$PolP \xrightarrow{k} Pol + P$$

or

$$PolP \xrightarrow{k} PolP_{fail}$$

where

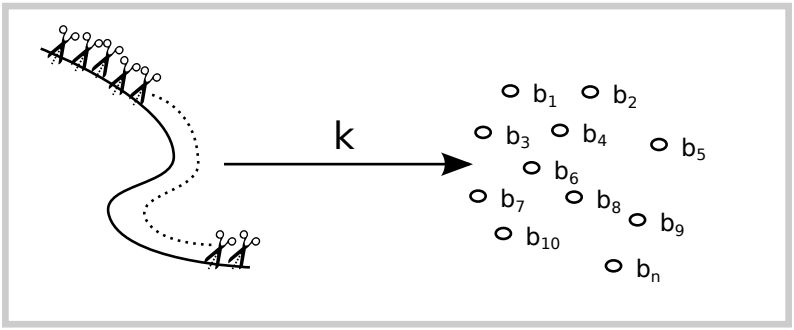
- $PolP$ is a `BoundChemical` representing a polymerase-product complex.
- P is of type `ChemicalSequence`. It is a product that is released by $PolP$ defined in a `ProductTable` associated with reaction.
- Pol is a `BoundChemical` representing an empty polymerase.
- $PolP_{fail}$ is a `BoundChemical` representing the polymerase-product complex in case release failed because P was not a valid product defined in the `ProductTable` associated with reaction.
- k is a rate constant.

Action When the reaction is performed, a random $PolP$ is chosen. A `ProductTable` uses its binding and current position to determine what product P it has synthesized. If P is defined in the product table, it is released in the cytosol and $PolP$ is replaced by an empty version of the polymerase Pol. If there is no P corresponding to current $PolP$ position, the simulator assumes that $PolP$ has not reached its actual terminator and it is replaced by $PolP_{fail}$ to enable other treatments (*e.g.* abnormal termination or continuing synthesis).

Rate The rate is given by

$$\lambda = k[PolP]$$

2.3.8 Degradation



Input format

```
CompositionTable <name> [<letter> [<chemical composing letter>]^{1..m}]^{1..n}
Degradation <chemical sequence> <composition table> <rate>
```

Formula A `Degradation` represents decomposition of a sequence into base components. It is defined by

$$CS \xrightarrow{k} b_1 + b_2 + ... + b_N$$

where

- CS is of type `ChemicalSequence`.
- b_i are of type `FreeChemical`. They are found in a `CompositionTable` specified in the reaction.
- k is the degradation constant.

Action When the reaction is performed, a CS is removed from the pool. A `CompositionTable` is specified along the reaction. It allows base-by-base conversion of the sequence of CS into components yielded by degradation. The pools of base components is updated accordingly. In the simulator, a degradation reaction is effectively implemented as a `ChemicalReaction`.

Rate The rate is given by

$$\lambda = k[CS]$$

2.4 Solver loop

Once `Reactions` and `Reactants` are defined, they must be integrated properly. We use variants of the Gillespie algorithm to provide a framework where reactions are performed according to their current reaction rate. Roughly speaking, the main hypothesis of this framework is that reaction timings are distributed according to exponential distributions. This allows for many mathematical simplifications and harmonious integration of an arbitrary number of reactions. The central point of the algorithm is that the probability that a reaction will be the next reaction in the system is proportional to its rate (mathematically speaking, the reaction is obtained by multinomial drawing according to rates).

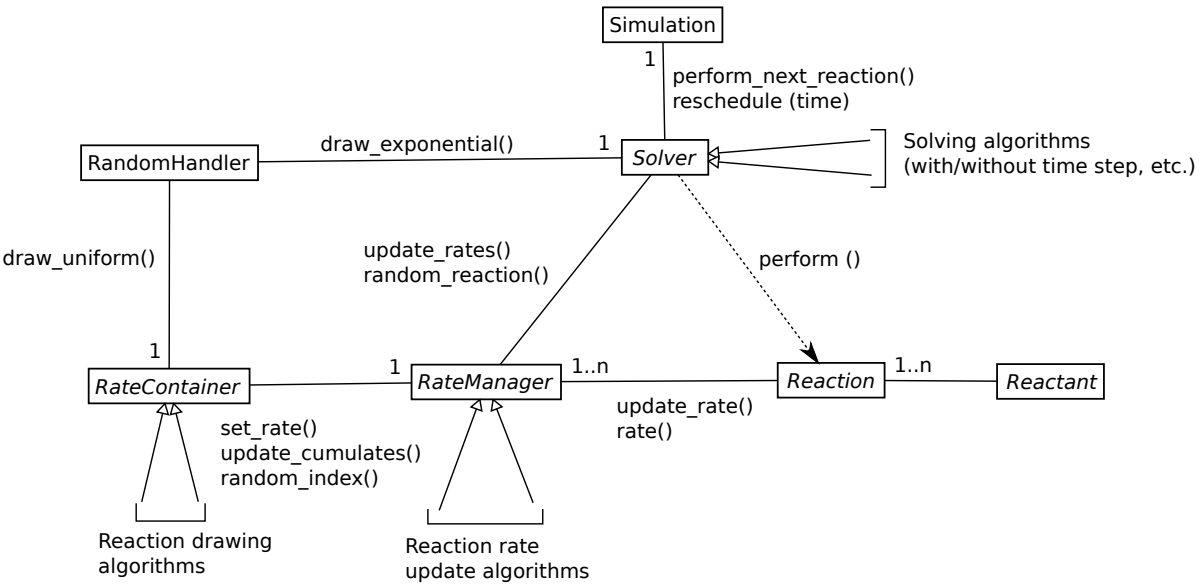


Figure 2: Solver loop. The loop is driven by the `Solver` class that defines how and when rates should be updated. The update task is performed by a `RateManager`. Once rates are known, multinomial drawing is delegated to a `RateContainer`. A central `RandomHandler` is used so that the solver only uses one seed, enabling simulation reproducibility.

The solving loop is depicted in Figure 2. The Gillespie algorithm has many variants. We decided to implement it using three *abstract* classes. By using inheritance, variants can be implemented for each step of the algorithm and combined at will by the end user. The three central classes are:

- `Solver`: Children of this class decide how and when rates should be updated, *e.g.* update rates after every reaction, only after a given time step, etc. Note that they do not perform any of these computations, they just organize how the algorithm should work.

- `RateManager`: Children of this class are responsible for updating reaction rates when prompted to by a `Solver` class. Recomputing all rates is generally inefficient,

so various implementations of this task can be used to improve the global loop speed.

- `RateContainer`: Childern of this class are responsible for storing reaction rates in a specific structure *adapted* to multinomial drawing. Again many implementations exist, their efficiency depends on the system that is integrated.

The implementations of these three classes will be described later in the document.

2.5 Events

`Event`s enable users to change molecule numbers outside of the solver loop at specific times (Fig. 3). A `Simulation` instance handles both a `Solver` instance and an `EventHandler` instance. Every time an event timing is reached, the solver loop is stopped, the event(s) is (are) performed, the solver is reinitialized and the simulation resumes. Different `Event` implementations are offered to modify molecule numbers in a convenient way.

```
################################################################################
#                              EVENT FILE                                      #
################################################################################
#
# An event should have the following format (one event per line):
#
#   TIME EVENT_TAG TARGET QUANTITY
#
# where
#  - TIME is the time at which the event should occur
#  - EVENT_TAG is the type of event among
#     + ADD: add some molecules.
#     + REMOVE: remove some molecules if possible.
#     + SET: set the number of molecules.
#  - TARGET is the target chemical
#  - QUANTITY is the quantity to add/remove/etc.
#
# example:
#  100 ADD ATP 1000
# adds 1000 ATP molecules at time t=100.
#
################################################################################

3000 SET ppGpp 10000
3000 SET GTP 0
3010 SET GTP 0
3020 SET GTP 0
3030 SET GTP 0
3040 SET GTP 0
3050 SET GTP 0
3060 SET GTP 0
3070 SET GTP 0
3080 SET GTP 0
3090 SET GTP 0
4500 ADD GTP 1000
4500 SET ppGpp 0
4510 SET ppGpp 0
4520 SET ppGpp 0
```

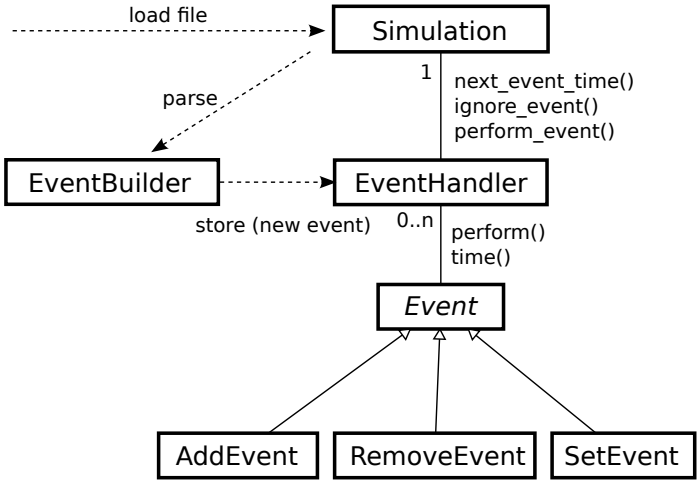


Figure 3: `Event`s: another way to modify chemical concentrations aside from reactions, *e.g.* to simulate the injection of a chemical inside a cell.

2.6 Input/Output handling

2.6.1 Simulator Input

The simulator needs the following to work:

- A general input file defining simulation parameters. A sample file is provided were all options are described (*e.g.* length of simulation, what to output, algorithm variants). One important parameter is the location of the files the simulator should open to read reactants, reactions and events.

- An arbitrary number of files where reactants, reactions and events are declared. The simulator solves dependencies across files, it is not necessary to declare reactants in the same file as or before reactions using them.

Caution:

- All reactants must be declared in some file with their appropriate type (*e.g.* `FreeChemical` or `BoundChemical`).
- Multiple declarations are forbidden, a name cannot be reused.

2.6.2 Simulator Output

Outputs provided by the simulator are:

- A general output file logging parametes used for simulation (input files used, random seed, algorithms used, etc.).
- A concentration file with the number of molecules over time (for the chemicals and at a time step defined in the parameter file).
- If a `DoubleStrand` was added in the chemicals to ouput, a replication file describing replication advancement of that `DoubleStrand`.

3 Detailed design

3.1 Reactants

3.1.1 `FreeChemical`

`FreeChemical` simply represents a pool of interchangeable molecules distributed uniformly in the cell. Computationnally, only the number of molecules in the pool is relevant.

3.1.2 `BoundChemical`

`BoundChemical` represents molecules of the same chemical species, but there are specifities for each unit of a `BoundChemical`, as all units are bound at different locations of different `ChemicalSequence` (Fig. 4). A `BoundUnitFactory` is used to recycle `BoundUnit`s, avoiding memory reallocation throughout simulation. `BoundUnitFilters` are used to sort `BoundUnit`s according to criteria useful for reactions (Fig. 4).

`BoundUnit`s are passed from one `BoundChemical` species to another through reactions, their attributes are updated if needed. They are only destroyed once they are unbound from their `ChemicalSequence`.

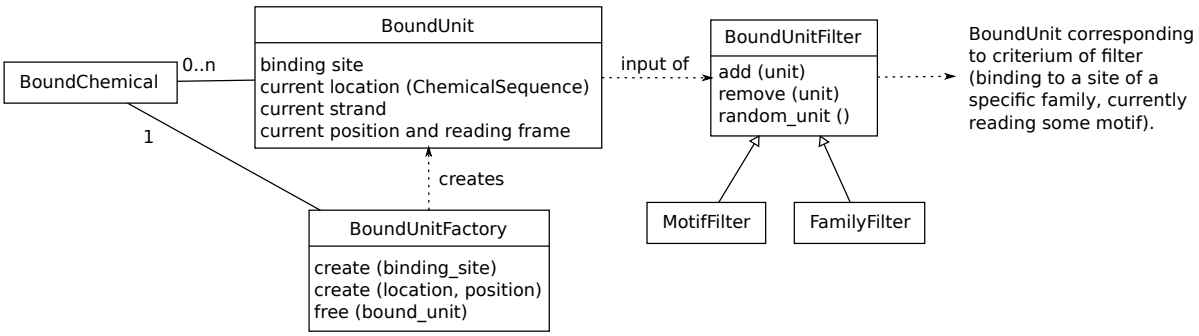


Figure 4: `BoundChemical` are in fact a pool of individual `BoundUnit` created using a `BoundUnitFactory`. A `BoundUnit` is characterized by the `ChemicalSequence` it bound to and its current position. Reaction then use `BoundUnitFilter` to sort `BoundUnit` according to some criterium of reference (*e.g.* `Loading` reactions sort `BoundUnit` according to the motif they read).

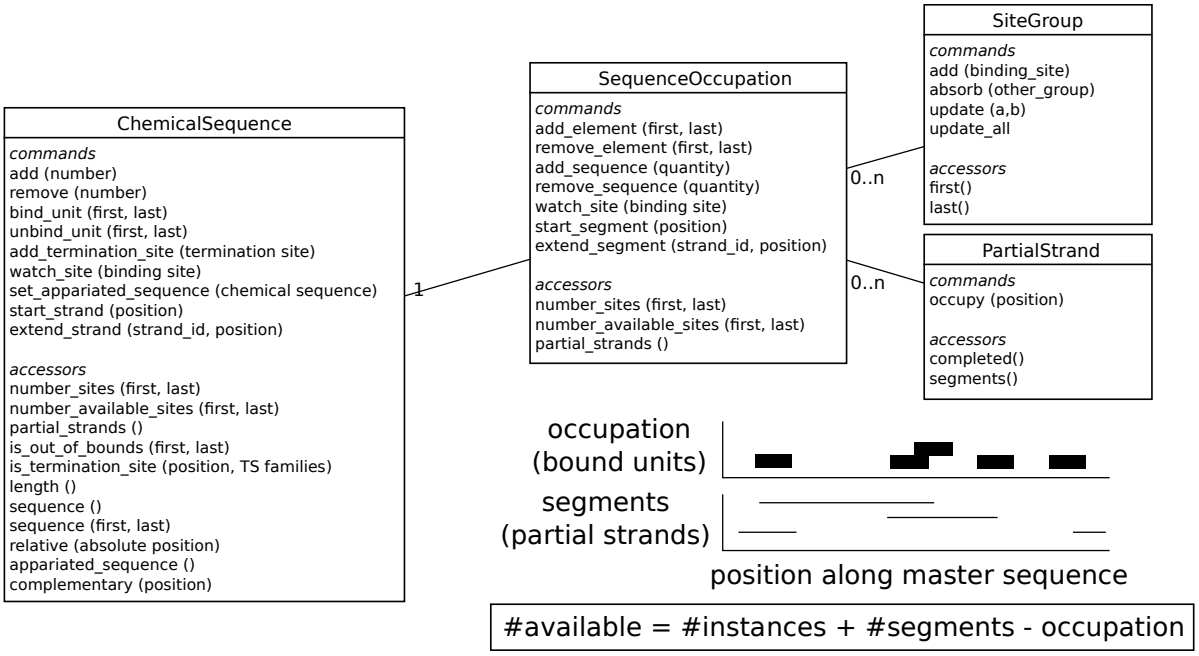


Figure 5: `ChemicalSequence` represents a pool of polymeres that can be elongated and on which `BoundUnit`s bind through `BindingSite`s. For binding to occur, availability of `BindingSite` is assessed using a utility class `SequenceOccupation` that records the number of instances of the polymer, the position of `BoundUnit`s and elongation of `PartialStrand`s. `SiteGroup` is used to notify sites of availability changes more efficiently.

3.1.3 `ChemicalSequence`

`ChemicalSequence` handles a pool of polymers. A pool is defined by a *master sequence* describing what a typical polymer looks like (*e.g.* the sequence of DnaA protein) and

the number of *instances* of the master sequence in the pool. For efficiency reason, we do the following assumptions.

Simplifying assumptions

- No deviation from master sequence, all instances are identical.
- `BoundUnit`s are not assigned to a specific instance of the sequence, they are positioned on the master sequence.

Consequences

- No direct inference of collisions is possible.
- A chemical can bind on a partial strand, yet move along the whole sequence freely.
- Degradation of an instance does not cause unbinding.

Site availability Despite our simplifying assumptions it is still possible to provide an accurate description of site availability. Availability depends of the number of sequences, number and position of bound elements, number and position of newly polymerized sequence segments (Fig. 5).

3.1.4 `BindingSiteFamily`

The task of a `BindingSiteFamily` is to regroup all the binding sites that can participate in a same `SequenceBinding` reaction. To simplify the reaction, it stores the subrate associated with each binding site. In order to update the rate properly when availability of sites changes, an *observer pattern* is used (Fig. 6).

Every `BindingSite` is viewed as an *observer* by the `ChemicalSequence` it belongs to. Every time a change occurs on the site, the `BindingSite` is notified. The latter binding site notifies its `BindingSiteFamily` using a specific identifier, letting the family know which binding rate is out of date. This information is stored in a `RateValidity` class. It is only when it is really needed (*i.e.* when a `SequenceBinding` wants to access total rate or a random site) that rates are recomputed. This avoids useless computations *e.g.* in the case of a translocation, where a bound unit is first unbound from its `ChemicalSequence` then rebound. If the bound unit does not move away from the site, two updates will be sent, but the rate will only be recomputed once at the end.

3.2 Reactions

3.2.1 `ChemicalReaction`

Nothing particular.

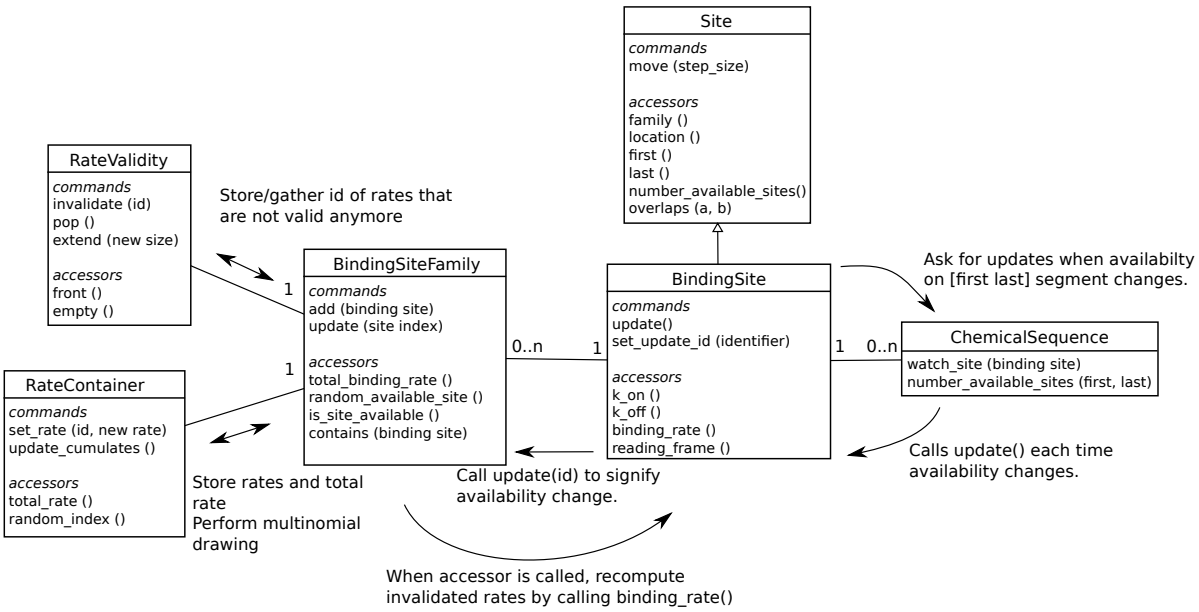


Figure 6: Schematical view of the Observer pattern used to keep availability of binding sites up to date for `SequenceBinding` reactions.

3.2.2 `SequenceBinding`

Binding Because of the way `BindingSiteFamily` is implemented, the reaction can easily and efficiently access the binding rate at all times, no matter what reactions have occured previously and how site availability changed in the meantime.

Unbinding `SequenceBinding` uses a `FamilyFilter` (see detailed description of `BoundChemical`) to filter out all `BoundUnit`s that are bound to a binding site of the `BindingSiteFamily` associated with the reaction. `BoundUnit`s that have bound to sites of a different family or that have moved away from the binding site through `Translocation` are *not* candidates for unbiding.

3.2.3 `Translocation`

Collisions For now, `Translocation` ignores collisions, making its implementation straightforward.

Stalled form

- Translocation enters stalled form if a `BoundUnit` reached the end of a sequence.
- Translocation enters stalled form if a `BoundUnit` reaches a termination site *after the translocation was completed*.

3.2.4 `Loading`

Handling each polymerase individually The main challenge with `Loading` is to maintain the subrates associated with each motif up to date. It needs to maintain a list of all `BoundUnit`s reading a specifing motif. To this end it uses a `TemplateFilter` (see detailed implementation of `BoundChemical`). Every time a `BoundUnit` becomes of the type of the `BoundChemical` associated with the reaction, the filter looks what motif defined in the `LoadingTable` it is currently reading. If the motif could not be found, an `UNKNOWN TEMPLATE` error message is displayed, the `BoundUnit` is not recorded in the filter and will not participate in the `Loading` reaction. The implementation is very similar to that used for `BindingSiteFamily` (Fig. 7).

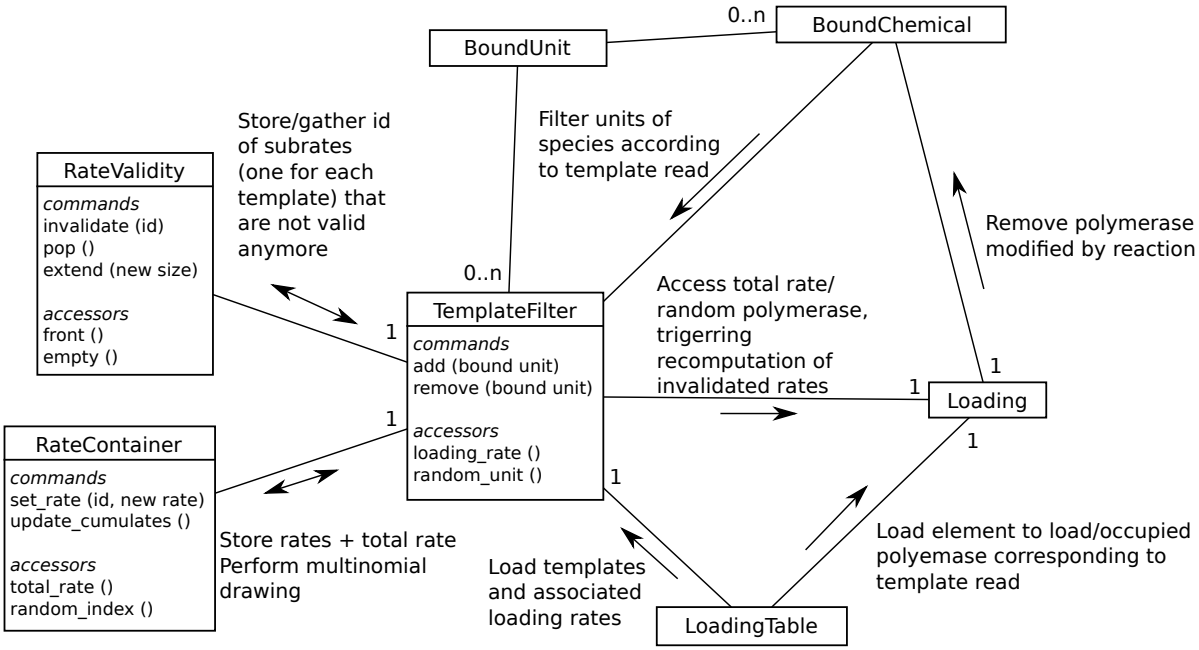


Figure 7: Schematical view of the pattern used to keep subrates associated with each template up to date in a `Loading` reaction.

`ProductLoading` **vs** `DoubleStrandLoading` There difference between the two processes is rather small. We just added a failure condition in the case of `DoubleStrandLoading` for convenience. Depending on what reactions are used to synthesize a `DoubleStrand` it might be possible that a polymerase arrives upon a position that has already been synthesized. In this case, the `DoubleStrandLoading` fails and the polymerase is replaced by the polymerase in its stalled form.

3.2.5 `Release`

Fail polymerase (unknown product) When a release is triggered, a `BoundUnit` from the `BoundChemical` associated with the `Release` reaction is randomly chosen. Because

the `BoundUnit` knows its current position and its binding site, it will assume that product it has synthesized starts the *reading frame of the binding site* and ends *at the position directly preceding its current reading frame* (we assume that the polymerase translocates onto a terminating sequence which does not contribute to product synthesis). If the product is found in the `ProductTable`, everything works normally.

If the product is not found, we display a `Unknown Product` error message but keep the simulation alive. The fail polymerase in the reaction enables the user to define a rescue pathway. If the release competes with some other reaction for the original polymerase, the fail polymerase can be the original polymerase itself. If products overlap and the polymerase was stalled due to a termination site of another product, fail polymerase can be a polymerase in a sythesizing step (*e.g.* `ProductLoading`) so synthesis will resume until the next termination site is reached.

3.3 Solver loop

Here we describe the implementations provided for each step of the algorithm. Most of the details are explained in the side document entitled CATI mi amor. We only give a quick overview here.

3.3.1 `RateContainer` classes

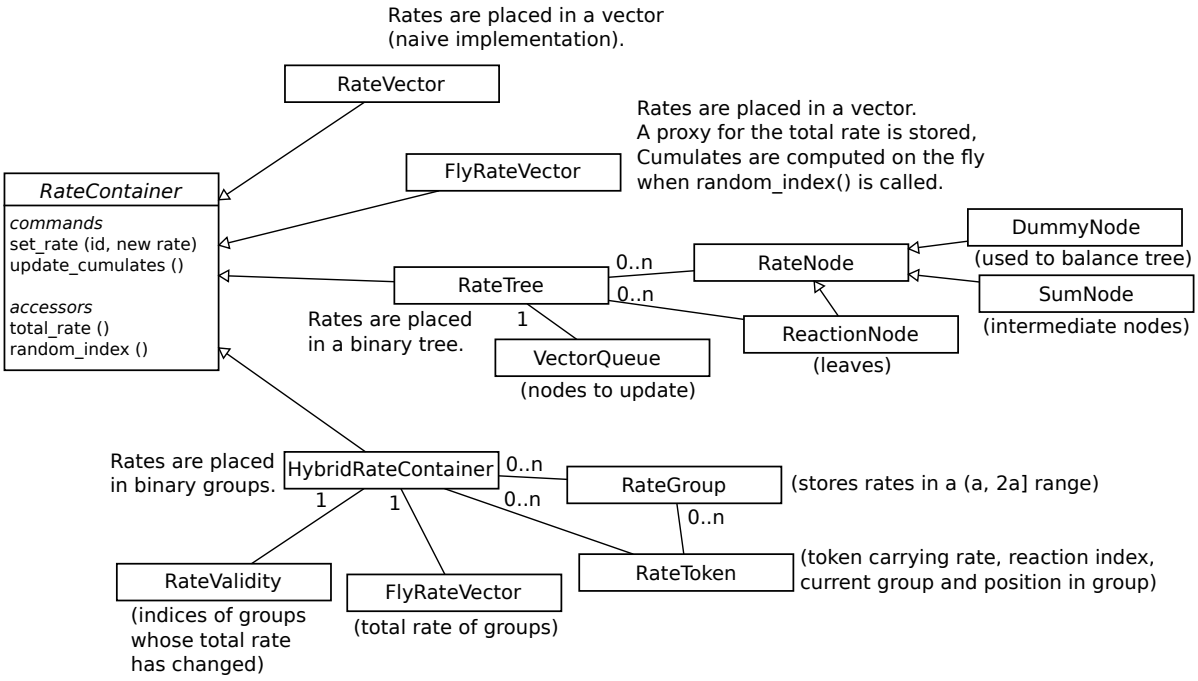


Figure 8: Implementations provided to store rates and perform a multinomial drawing. Implicitly, all theses classes use `RandomHandler` to perform their random drawings.

We start with the lowest level classes, which perform one of the central tasks of the Gillespie algorithm: drawing a reaction from reaction rates. For efficiency reasons, we propopose several implementation of the algorithm (Fig. 9). Comparison and description of theses classes are given in the side document.

Note that multinomial drawing occurs within the solver loop, but also within some reactions such as `Loading` or `SequenceBinding`, so these classes are used quite extensively throughout the simulation.

Perspectives These classes are the most sensitive classes from a numerical point of view. Stability of implementation should be checked more thouroughly (postconditions and or unit tests). `HybridRateContainer` needs a parameter to work. It is user provided for the moment but I think it should be determined automatically, probably by extending the group structure dynamically.

3.3.2 `RateManager` classes

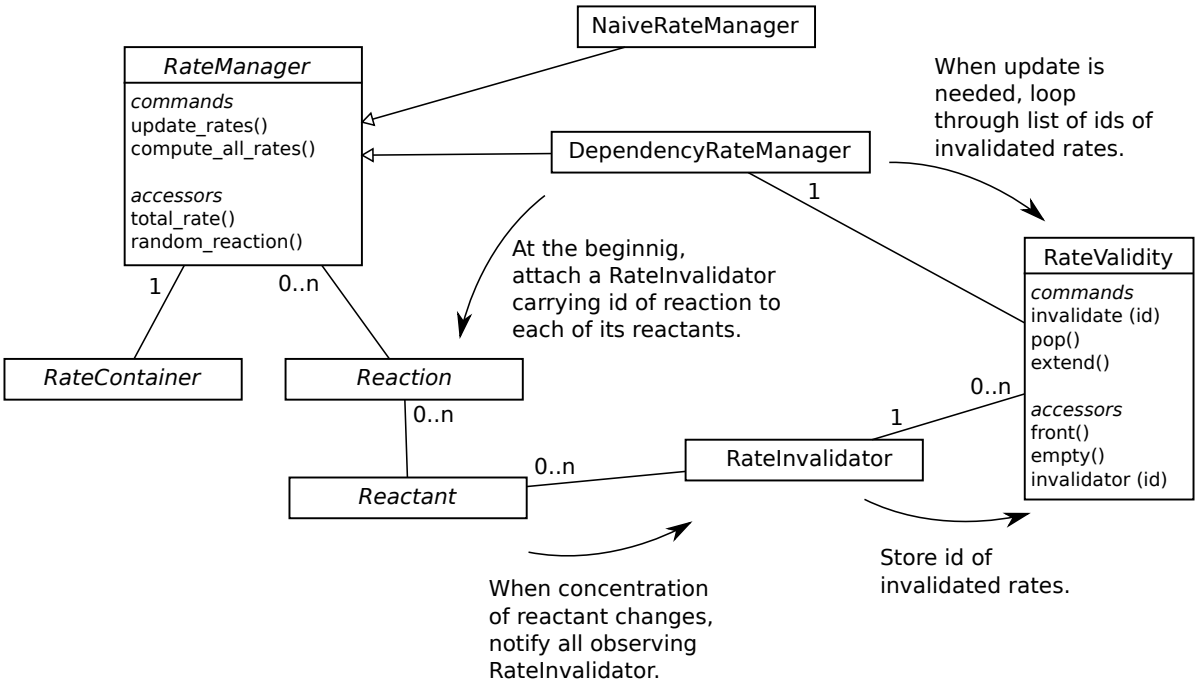


Figure 9: Implementations provided to update reaction rates. Note that the drawing part of the algorithm is always delegated to a `RateContainer`. `DependencyRateManager` uses an Observer pattern to monitor which rates have changed.

The second layer of the solver loop ensures that the rates are updated when needed to. Two implementations are proposed for this task (Fig. ??). The `NaiveRateManager` updates every rate. While it is inefficient, it can be used as a reference to test other managers.

Perspectives While `DependencyRateManager` performs fairly well in the general case. It is particularily inefficient if there is a molecule A involved in a lot of reactions because a lot of rates have to be recomputed. It could be interesting to pool the reactions involving A into a `RateContainer` of their own, the latter containing only the contribution to the rate of the *other* reactants. The total rate of reactions involving A would then be the total rate of the container multiplied by the concentration of A. This would be viewed by the system as *one* mega reaction. When the concentration of A changes, virtually *nothing* is recomputed, except the total rate of the mega reaction (we suppose the contribution of other reactants has remained constant). If the mega reaction is to be performed, the `RateContainer` is used to draw which reaction will actually be performed according to their contributions. This change is not possible with the current architecture, the definition of `RateManager` and the computation of rates would have to be changed.

3.3.3 `Solver` classes

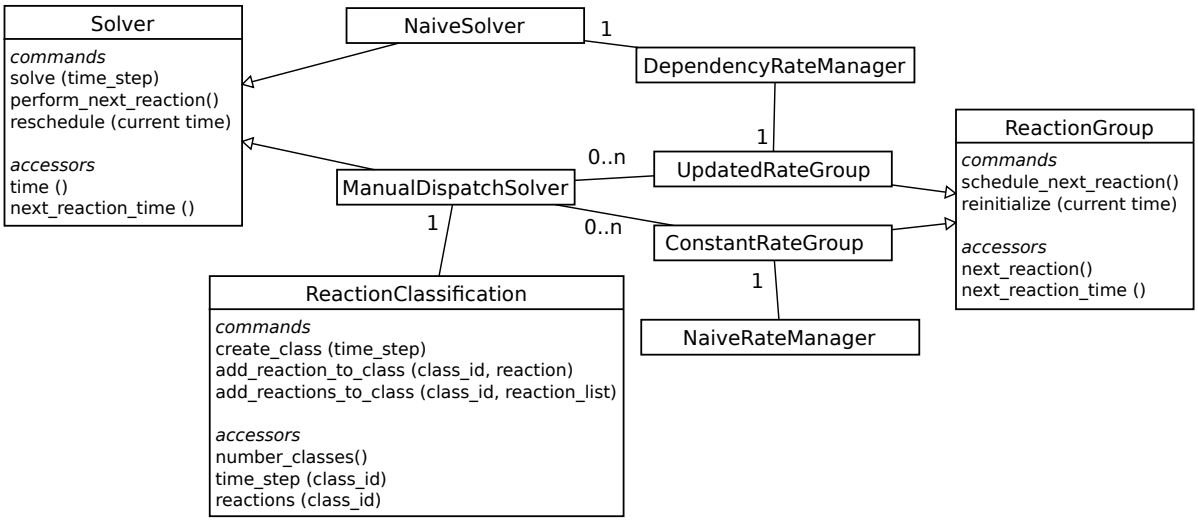


Figure 10: Two implementations of the `Solver` class organizing rate updating. `NaiveSolver` forces recomputation of rates at each time step. `ManualDispatchSolver` puts reactions into groups: reactions in `UpdatedRateGroup` are updated after every reaction while those in `ConstantRateGroup` only at user-defined steps defined in `ReactionClassification`. Note that all `Solver` classes use at leaste a variant of `RateManager` at some point to delegate storing and updating of rates.

For the moment, only one solver class is fully available to the user, `NaiveSolver`, which implements the exact Gillespie algorithm. Another variant called `ManualDispatchSolver` is implemented, were the user can assign a time step to each reaction at which its rate will be updated (Fig. 10). However, when the rate of a reaction is a constant, there is a risk that its reactants will run out and the reaction will be impossible to realize or reactant number will become negative. In the simulator, the latter case is forbid-

den, so `ManualDispatchSolver` ignores reactions impossible to perform due to reactant inavailability.

Perspectives There is no user interface to `ManualDispatchSolver` so it is not really possible to use it in practice witout changing the program. It would probably be more interesting to implement a variant or several variants of **tau-leaping**, which automatically assigns time steps to reaction to avoid reactant shortage. This has to be done carefully, the variant chosen must be effective event if the number of a chemical becomes or remains fairly low.

3.4 Input/Output handling

3.4.1 Parsing system

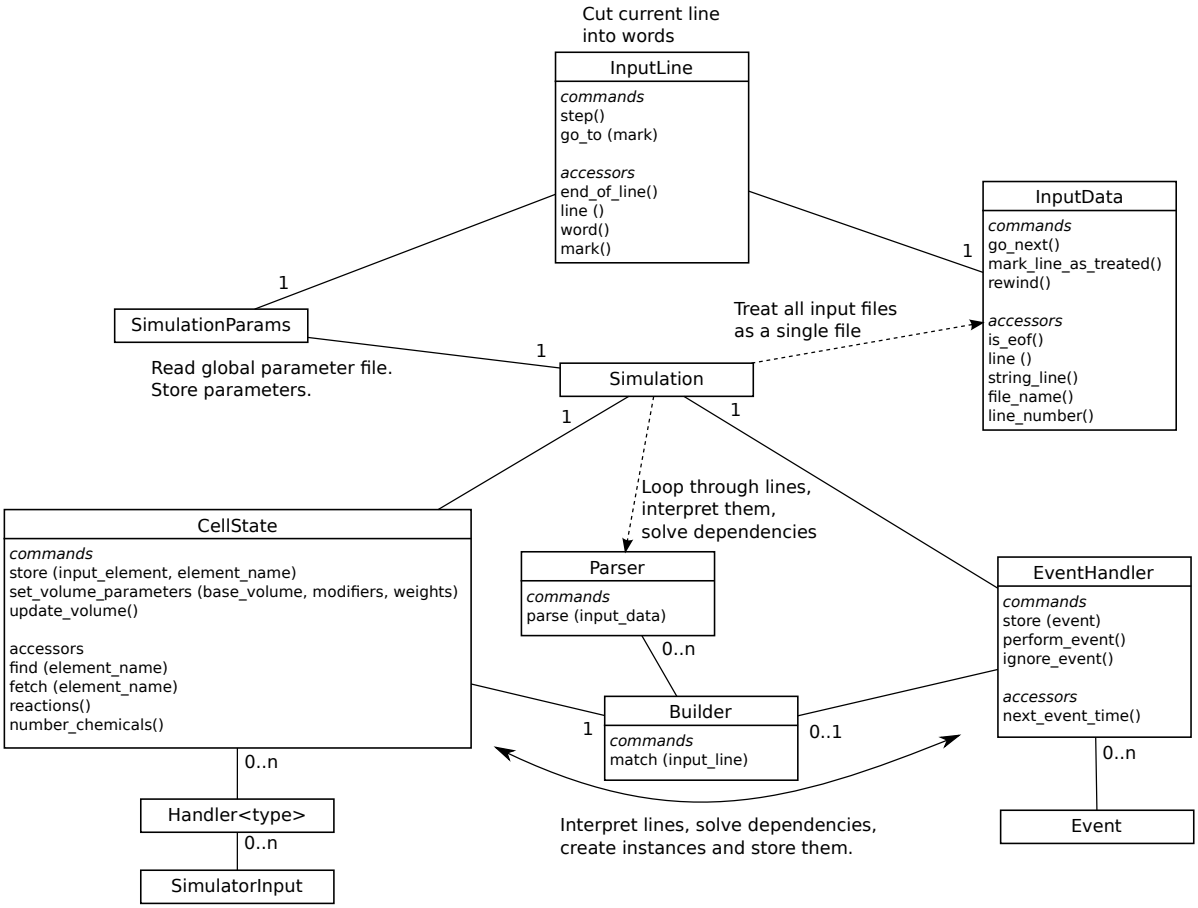


Figure 11: Architecture of the parsing system. `SimulationParams` reads the global parameter file and stores parameter values. `InputData` is used to provide a simplified interface to the input files and mark treated lines. A `Parser` and `Builders` are used to make sense of individual lines. Everything that is created is stored in `EventHandler` (for events) and `CellState` (for the rest).

The parsing system used by the simulator is pretty simple (Fig. 11). A `SimulationParams` class is used to store simulation parameters (which will be used to create and drive the `Solver`), `CellState` stores reactions to integrate and `EventHandler` stores events. At the moment, an *ad hoc* input format is used.

Perspectives Review `SimulatorInput` arborescence which seems uselessly complicated. Design an architecture that can handle multiple input formats (plain text or XML).

3.4.2 Builder and interpreter

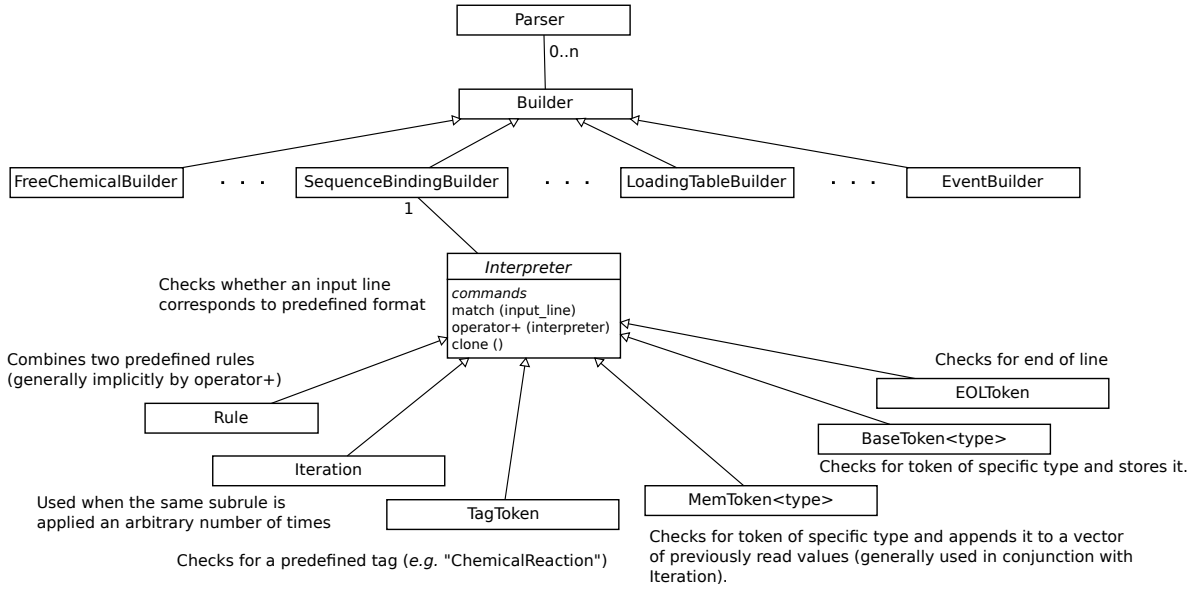


Figure 12: Interpreter system used. For each class of the simulator, a `Builder` is responsible for interpreting current line, solving dependencies, checking validity of parameters. If format is invalid or dependencies could not be resolved, exceptions are raised to warn the user.

The parsing system is designed to cut each line into words, then the words are interpreted by `Builder`s that try to create instances of each of the class of the simulator. The `Parser` loops through the `Builder`s until an instance was successfully created. Line format is checked token-wise by an `Interpreter` (Fig. **??**). If no `Builder` is able to match the line, a `FormatException` is raised. If some dependency could not be solved, a `DependencyException` is raised. In the latter case, the `Parser` will postpone the line until dependency can be successfully solved.

Perspectives Maybe create a `SimulatorToken` to automatically fetch the reference associated to a name? However, this has to be done with care because the right exceptions

have to be raised.

3.4.3 Output

Two classes are used to produce output. `ChemicalLogger` logs chemical numbers through time. `DoubleStrandLogger` logs partial strands of a `DoubleStrand`.

Perspectives A `ReactionLogger` would actually be nice...

A Tests

A.1 Testing philosophy

Tests are usually divided in several categories. Because of the size of the project, the program includes three types of tests: *programming by contract*, *unit tests*, *integration tests* (Tab. 1). They are designed to make the program fail as rapidly as possible and help find the origin of the problem.

Test type	Preconditions Postconditions Invariants	Unit Tests	Integration Tests
Test level	Implementation details	Class interface	Systemic
Time per test	a few instructions (ns)	ms to a few seconds	seconds to several minutes
Use frequency	Permanent	Very frequent	Less frequent

Table 1: Comparisons of tests used to develop the simulator

A.1.1 Programming by contract

These tests typically apply to attributes of classes and arguments of methods. They are usually divided into three subcategories: *preconditions*, *postconditions* and *invariants*. They check whether the class interact correctly with the outside world, generally other classes.

Preconditions Preconditions.

A.1.2 Unit tests

A.1.3 Integration tests

A.2 Organizing and running tests

B Utility classes

B.1 Exceptions

Programming by contract (see Section A) covers most internal errors that might happen. Exceptions are only used when user input is treated. They are used to signify inconstencies in input files during the parsing step (Fig. 14).

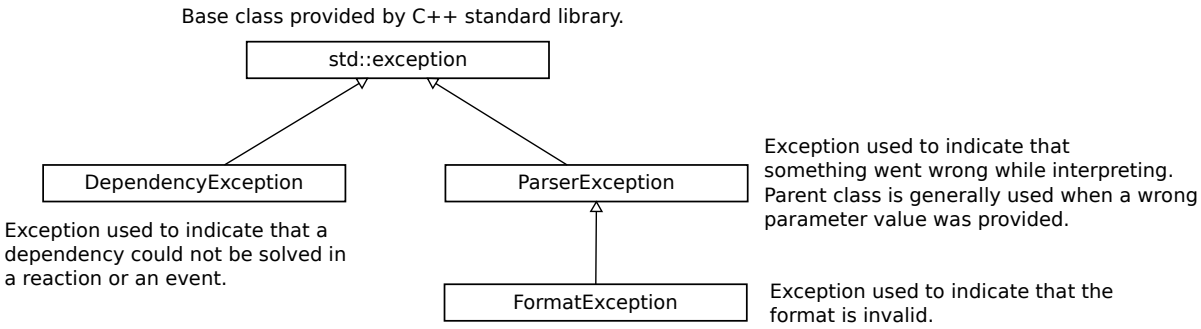


Figure 13: Exceptions used in the simulator.

Why are exceptions useful? The nice thing about exceptions is that they can be caught and treated at a level that makes sense. In the parsing system, a format error is discovered by an `Interpreter`, which has no idea where the input line comes from and could not display a useful error message on its own. It is caught at the `Parser` level, enriched with information about input file, input line and so on, before being displayed.

Perspectives Errors occurring during simulation are displayed without using exceptions and suffer the bias described above. If a `Release` fails, there is a low level message such as "Unknown product". We could throw an exception and catch it at the `Simulation` level, then use `CellState` to get the name of the `ChemicalSequence`, the `ProductTable` and the polymerase involved in the `Release`, providing useful information for the user.

B.2 Random handler

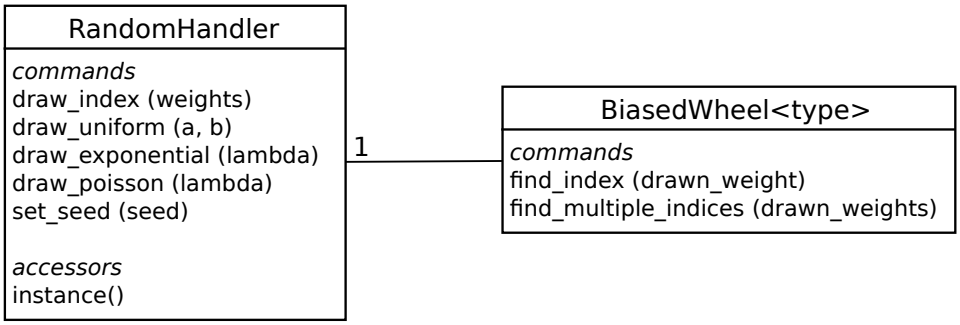


Figure 14: `RandomHandler` and `BiasedWheel` used for multinomial drawing. `RandomHandler` uses the Singleton pattern, meaning exactly one instance of the class is created and used throughout the simulator. It has to be accessed using the `instance()` accessor.

A unique `RandomHandler` is used throughout the simulation to control the random seed.

B.3 Factories

B.4 Vector-based containers

C Perspectives

C.1 Known bugs

circularity of DNA.

C.2 Collision handling