BioCRNpyler: Compiling Chemical Reaction Networks from Biomolecular Parts in Diverse Contexts

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Abstract

Biochemical interactions in systems and synthetic biology are often modeled with chemical reaction networks (CRNs). CRNs provide a principled modeling environment capable of expressing a huge range of biochemical processes. In this paper, we present a software toolbox, written in Python, that complies high-level design specifications to CRN representations. This compilation process offers four advantages. First, the building of the actual CRN representation is automatic and outputs Systems Biology Markup Language (SBML) models compatible with numerous simulators. Second, a library of modular biochemical components allows for different architectures and implementations of biochemical circuits to be represented succinctly with design choices propagated throughout the underlying CRN automatically. This prevents the often occurring mismatch between high-level designs and model dynamics. Third, high-level design specification can be embedded into diverse biomolecular environments, such as cell-free extracts and *in vivo* milieus. Finally, our software toolbox has a parameter database, which allows users to rapidly prototype large models using very few parameters which can be customized later. By using BioCRNpyler, users can easily

build, manage, and explore sophisticated biochemical models using diverse biochemical implementations, environments, and modeling assumptions.

1 Introduction

Chemical reaction networks (CRNs) are the workhorse for modeling in systems and synthetic biology.[1] The power of CRNs lies in their expressivity; CRN models can range from physically realistic descriptions of individual molecules to coarse-grained idealizations of complex multi-step processes.[2] However, this expressivity comes at a cost. Choosing the right level of detail in a model is more an art than a science. The modeling process requires careful consideration of the desired use of the model, the available data to parameterize the model, and prioritization of certain aspects of modeling or analysis over others. Additionally, biological CRN models can be incredibly complex including dozens or even hundreds or thousands of species, reactions, and parameters. Maintaining complex hand-built models is challenging and errors can quickly grow out of control for large models. Software tools can answer many of these challenges by automating and streamlining the model construction process.

Due to CRN's rich history and diverse applications, the available tools for a CRN modeler are vast and include: extensive software to generate and simulate CRNs[3,4], databases of models[5], model analysis tools [6,7], and many more. However, relatively few tools exist to aid in the automated construction of general CRN models from simple specifications. For example, even though synthetic biologists have adopted a module and part-driven approach to their laboratory work,[8] models are still typically built by hand on a case-by-case basis. Recognizing the fragile non-modular nature of hand built models, several synthetic biology design automation tools have been developed for specific purposes such as implementing transcription factor or integrase-based logic.[9,10] These tools indicate a growing need for design and simulation automation in synthetic biology, as part and design libraries are expanded.

As the name would suggest, BioCRNpyler (pronounced bio-compiler) is a Python package

that compiles CRNs from simple specifications of biological motifs and contexts. This package is inspired by the molecular compilers developed by the DNA-strand displacement community and molecular programming communities which, broadly speaking, aim to compile models of DNA circuit implementations from simpler CRN specifications[11–13] or rudimentary programming languages.[14,15] However, BioCRNpyler differs from these tools for three main reasons: first, it is not focused only on DNA implementations of chemical computation; second, it does not take the form of a traditional programming language; and third, modeling assumptions and compilation schemas can be easily redefined by the user. BioCRNpyler combines specifications consisting of synthetic biological parts and systems biology motifs that can be reused and recombined in diverse biochemical contexts at customizable levels of model complexity. In other words, BioCRNpyler compiles detailed CRN models from abstract specifications of a biochemical system. Importantly, BioCRNpyler is not a CRN simulator—models are saved in the Systems Biology Markup Language (SBML)[16] to be compatible with the user's simulator of choice. Figure 1 provides motivating examples for the utility of BioCRNpyler by demonstrating the rapid construction of diverse CRNs by reusing common parts and modifying the modeling context.

There are many existing tools that provide some of the features present in BioCRNpyler. Systems Biology Open Language (SBOL)[17] uses similar abstractions to BioCRNpyler but is fundamentally a format for sharing DNA-sequences with assigned functions and does not compile a CRN. The software package iBioSim[18,19] compiles SBOL specifications into SBML models and performs analysis and simulation. Although BioCRNpyler is capable of similar kinds of compilation into SBML, it is not a simulator. Importantly, BioCRNpyler does not hard code how models are compiled—instead it should be viewed as a customizable software compilation language that can be applied to compile many kinds of systems beyond genetic networks. The rule-based modeling framework BioNetGen[20] allows for a system to be defined via interaction rules which can then be simulated directly or compiled into a CRN. Internally, BioCRNpyler functions similarly to this rule based-modeling compilation.

Similarly to PySB[21], BioCRNpyler provides a library of parts, mechanisms, and biomolecular contexts that allow for models to be succinctly produced without having to manually specify and verify many complex rules. Finally, the MATLAB TX-TL Toolbox[22] can be seen as a prototype for BioCRNpyler but lacks the objected-oriented framework and extendability beyond cell-free extract systems.

BioCRNpyler is a purposefully suited to *in silico* workflows because it is an extendable objected-oriented framework that integrates existing software development standards and allows complete control over model compilation. Simultaneously, BioCRNpyler accelerates model construction with extensive libraries of biochemical parts, models, and examples relevant to synthetic biologists, bio-engineers, and systems biologists. The BioCRNpyler package is available on GitHub[23] and can be installed via the Python package index (PyPi).

2 Motivating Examples

This section highlights the ease-of-use of BioCRNpyler through several well-known synthetic biology examples. As a summary, Figure 1 demonstrates the utility of compiling CRNs with BioCRNpyler. The names of Python classes are highlighted `typographically` and are defined more thoroughly in later sections. Time-course simulations in Figure 1 were done with Bioscrape[24] and circuit diagrams were created with DNAplotlib.[25] Additional example models of the lac operon and an integrase circuit are depicted in Figures 3 and 5, respectively.

2.1 Inducible Repression, Toggle Switch and Repressilator

Models A, B, and C show three archetypal motifs from synthetic biology: inducible repression, a bistable toggle switch,[26] and the repressilator.[27] All three of these examples are created by reusing the same `Components` wired together in different ways as described in Section 6. The ability to reuse `Components` allows for convenient design-space exploration of different circuit architectures. Furthermore, as explained in Section 2.2 and 2.4, examples

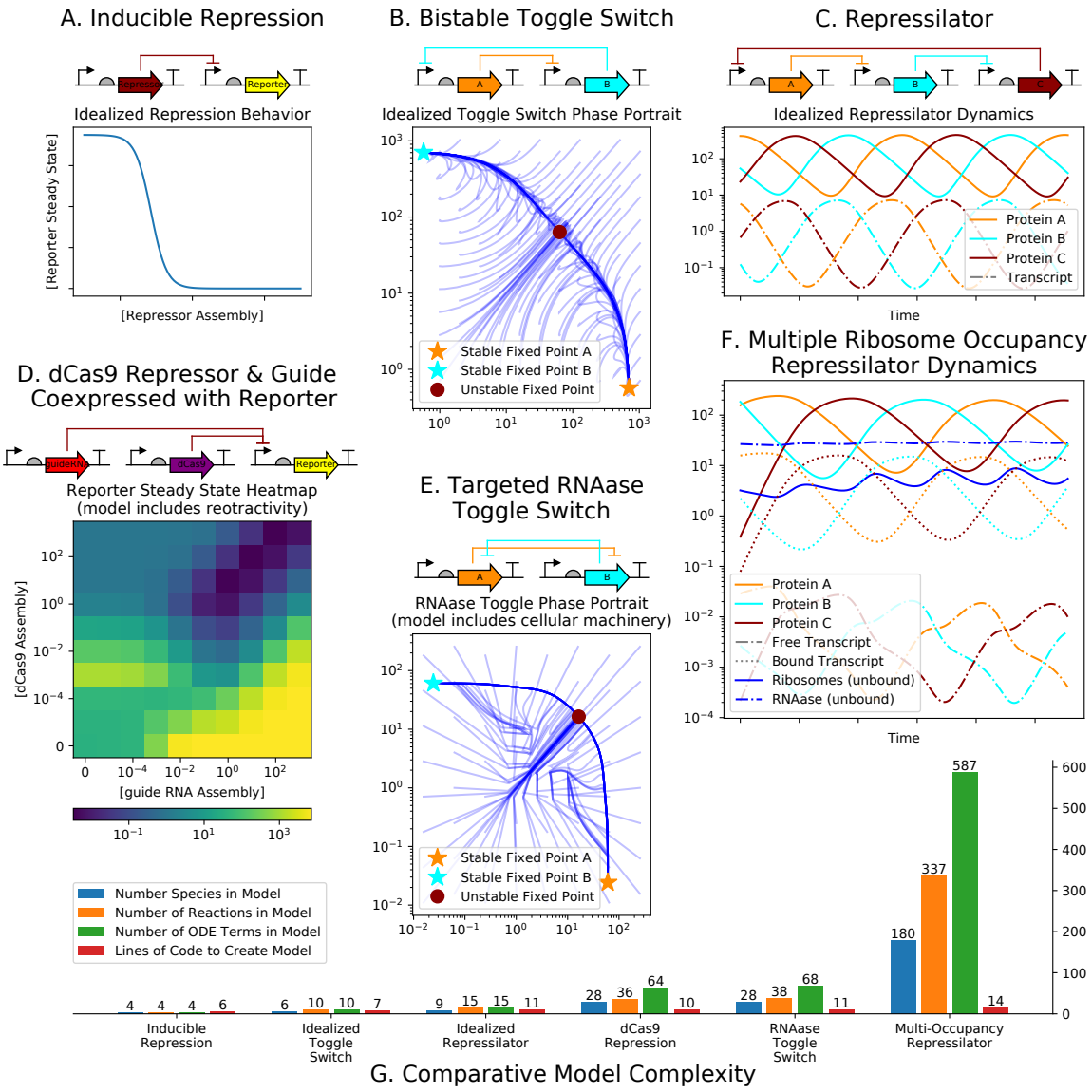

Figure 1: Motivating Examples. The idealized models (A, B, and C) do not model the cellular environment; genes and transcripts transcribe and translate catalytically. **A.** Schematic and simulation of a constiutively active repressor gene repressing a reporter. **B.** Schematic and simulations of of a toggle switch created by having two genes, A and B, mutually repress each other. **C.** Schematic and dynamics of a 3-repressor oscillator. The detailed models (D, E, & F) model the cellular environment by including ribosomes, RNAases and background resource competition for cellular resources. **D.** A dCas9-guideRNA complex binds to the promoter of a reporter and inhibiting transcription. Heatmap shows retroactivity caused by varying the amount of dCas9 and guide-RNA expressed. The sharing of transcription and translational resources gives rise to increases and decreases of reporter even when there is very little repressor. **E.** A proposed model for a non-transcriptional toggle switch formed by homodimer-RNAase; the homodimer-RNAase made from subunit A selectively degrades the mRNA producing subunit B and visa-versa. **F.** A model of the Repressillator exploring the effects of multiple ribosomes binding to the same mRNA. **G.** Histogram comparing the sizes of models A-F and the amount of BioCRNpyler code needed to generate them.

D, E and F show how these `Components` can be tested in different contexts by changing the `Mechanisms` and `Mixtures` used to compile the `Components` resulting in nuanced implementation-specific and context-specific models.

2.2 dCas9 Repressor and Guide RNA Coexpressed with Reporter

Figure 1D builds upon the repression Model A by modeling an implementation consisting of a guide-RNA and dCas9 `ChemicalComplex` that also acts as a repressor by inhibiting RNA-polymerase binding to the reporter `DNAassembly`.[28] Model A also includes more intracellular context such as nucleases and ribosomes. By including cellular machinery in the model, the co-expression of dCas9 and the guide RNA is able to influence the reporter via loading effects and retroactivity, which can cause unintended increases and decreases in reporter expression[29] even when only the guide-RNA or dCas9 is present.

2.3 Targeted RNAase Toggle Switch

Figure 1E models a hypothetical toggle switch that functions at the RNA level instead of the transcriptional level. Each `DNAassembly` expresses a subunits A and B of two homodimer-RNAase. The homodimer-RNAase made from subunit A selectively degrades the mRNA producing subunit B and visa-versa. Such a system could potentially be engineered via RNA-targeting Cas9[30] or more complex fusion proteins.[31]

2.4 Multiple Ribosome Occupancy Repressilator Dynamics

Figure 1F illustrates how BioCRNpyler can be used to easily generate more realistic and complex models of biochemical processes in order to validate if model simplifications are accurate. It is common practice in transcription and translation models to use an enzymatic process consisting of a single ribosome (R) to a transcript (T) which then produces a single protein (P). This translation `Mechanism` could be written as: $R + T \leftrightharpoons R : T \rightarrow R + T + P$.

Indeed, both example models D and E use such a simplification. However, experiments show that in fact many ribosomes can co-occupy the same mRNA.[32] By changing the underlying translation `Mechanism` to model multiple ribosomal occupancy of a single mRNA, a considerably more complex Repressilator model was created. Importantly, this model exhibits very similar behavior to the simpler model, suggesting that multi-occupancy of ribosomes on mRNA can be neglected in these kinds of genetic regulatory circuits.

2.5 Network complexity

Finally, the bottom bar chart in Figure 1G shows that even as the size of the underlying CRN grows, the amount of BioCRNpyler code that is needed to generate the model remains very small. This enables the generation of large and complex models with greater accuracy and lower chance of human error. For example, imagine writing down ODEs with hundreds of terms and then trying to systematically modify the equation: human error is nearly inevitable. By using BioCRNpyler to compile CRNs, models can be easily produced, modified, and maintained.

2.6 Parameter Database

Importantly, all these examples in this section make use of the same underlying set of 10-20 default parameters (estimated from Cell Biology by the Numbers[33]) demonstrating how BioCRNpyler's `ParameterDatabase` makes model construction and simulation possible even before detailed experiments or literature review.

3 Framework and Compilation Overview

BioCRNpyler is an open-source Python package that compiles high-level design specifications into detailed CRN models, which then are saved as an SBML files.[34] BioCRNpyler is written in Python with a flexible object-oriented design, extensive documentation, and

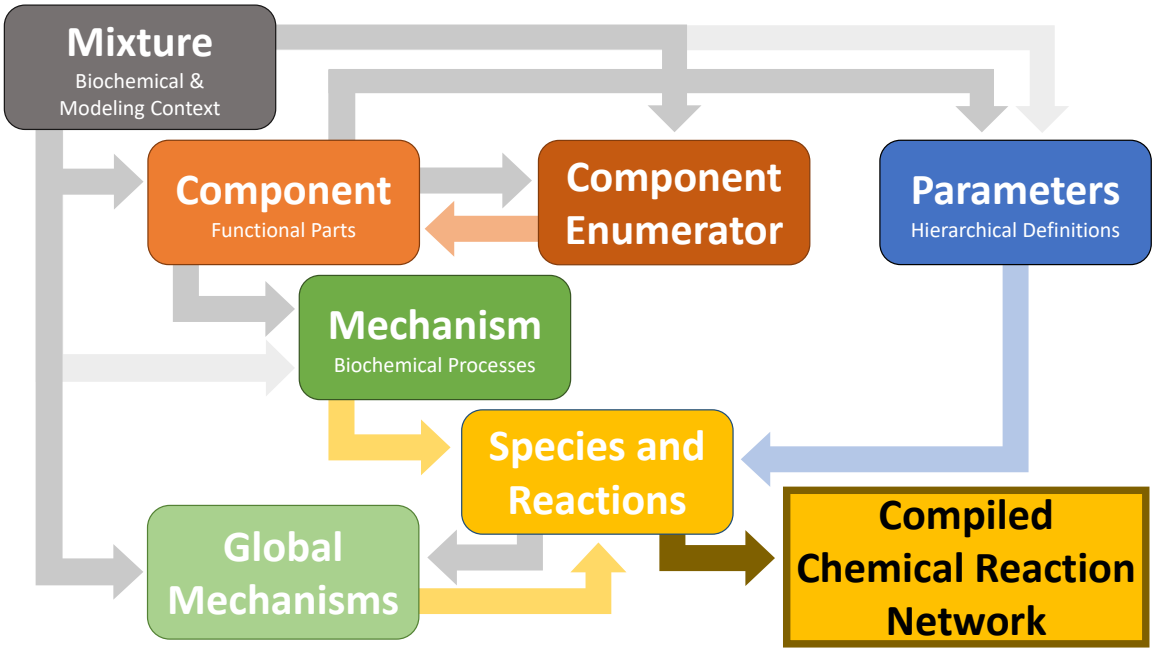

Figure 2: The hierarchical organization of Python classes in the BioCRNpyler. Dark gray arrows represent direction of compilation: from high-level design specifications (`Components`) in a modeling context (`Mixtures`) and biochemical processes (`Mechanims`) to a CRN representation. Light gray arrows represent inheritance of default `Parameters` and `Mechanims`. Yellow arrows represent `Species` and `Reaction` generation which are placed in a `ChemicalReactionNetwork` represented by the bottom right gold box. `ComponentEnumerators` are advanced objects used to automate the generation of `Components`. `GlobalMechanisms` are rules used to generate `Species` and `Reactions` at the end of compilation.

detailed examples which allow for easy model construction by modelers, customization and extension by developers, and rapid integration into data pipelines. As Figure 2 shows, underlying BioCRNpyler is a comprehensive `ChemicalReactionNetwork` class allowing for the direct creation and manipulation of `Reactions` and the participating `Species` to represent molecular interactions at many levels of complexity. For example, an entire gene may be modeled as a single `Species`, as an `OrderedPolymerSpecies` with multiple binding specific sites, or as a `PolymerConformation` which represents the secondary structure of one or more `OrderedPolymerSpecies`.

BioCRNpyler also compiles `ChemicalReactionNetwork` objects from high-level specifications defined by modular `Components` combined together in a `Mixture` representing a biochemical context (e.g. cell lysate extract). Modeling assumptions and specific knowledge of biochemical processes are defined via `Mechanisms` which can be placed inside `Components` and `Mixtures`. This class structure allows for the biochemical parts (e.g. `Components`) to be reused to quickly produce numerous different architectures and implementations, such as those described in the motivating examples. These different architectures and implementations can further be tested in different contexts providing easily customizable levels of biochemical and modeling complexity represented by `Mixtures` and `Mechanisms`.

The `Mixture`, `Component`, and `Mechanism` classes are hierarchical. `Mixtures` represent biological context by containing `Components` to represent the biochemical environment and `Mechanisms` to represent the modeling details. For example, the `TxTlExtract` subclass of `Mixture` represents bacterial cell extract and contains Ribosomes, RNA Polymerase, and RNAases `Components` as well as transcription, translation, and RNA-degredation `Mechanisms`. Additionally, `Components` can be added to a `Mixture` to produce a particular biochemical system of interest in a particular context. Figure (3) illustrates how a set of BioCRNpyler `Components` and `Mechanisms` can be joined together to produce a systems level model of the lac operon — a highly studied gene regulatory network in *E. coli* which regulates whether glucose or lactose is metabolized.[35] This model consists of around a dozen `Components` and

Mechanisms which jointly enumerate hundreds of species and reactions representing the combinatorial set of conformations of the lac operon and its associated transcription factors, transcription, translation, transport, mRNA degradation and dilution.

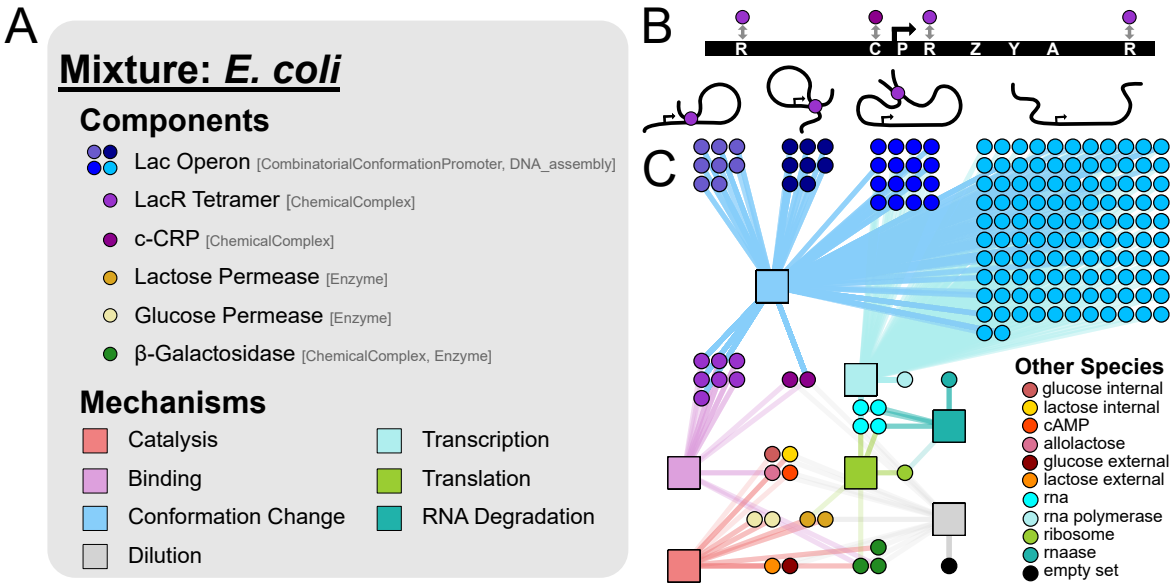

Figure 3: Compiling a model of the Lac Operon using BioCRNpyler specifications with 173 species and 343 reactions using ∼50 lines of code. **A.** A Mixture contains a set of Components and Mechanisms. The Component classes used for each element of the model are shown in brackets. **B.** A schematic of the lac operon and the three looped and one open conformation it can take. Each conformation contains a combinatoric number of states based upon the accessible binding sites: R are lac repressor binding sites; C is the activator c-CRP binding site; P is the promoter; and Z, Y, A are the three lac genes. **C.** A graph representation of the compiled CRN. Each circle is a unique chemical species. Square boxes show how chemical species interact via reactions generated by specific Mechanisms.

During compilation, Components represent biochemical functionality by calling Mechanisms to produce Species and Reactions. The Component class may use the Mechanisms contained in the Mixture or have their own custom Mechanisms to have more differentiated functionality. For example, a RepressiblePromoter (a subclass of Component) might rely on the Mixture for its translation Mechanism but use a custom transcription Mechanism. BioCRNpyler uses flexible ParameterDatabases contained in both Mixtures and Components to allow for rapid model prototyping using just a few default parameters, which can later customized for each Component and Mechanism. Specifically, Mechanisms will first search for

parameters in the `Component` that called them before defaulting to the parameters of the `Mixture`. This defaulting behavior is illustrated by the light gray arrows of Figure 2. Finally, `ComponentEnumerators` provide a highly flexible framework to automatically generate new `Components` during compilation as illustrated in the integrase example Figure 5.

3.1 Internal CRN Representation

Formally, a CRN is a set of species $S = \{S_i\}$ and reactions $R : \{I \xrightarrow{\rho(s;\theta)} O\}$ where I and O are multisets of species, ρ is the rate function or propensity, s is a vector of species' concentrations (or counts), and θ are rate parameters. Typically, CRNs are simulated using as ordinary differential equations (ODEs) and numerically integrated.[2] A stochastic semantics also allows CRNs to be simulated as continuous-time Markov chains.[36] Besides their prevalence in biological modeling, there is rich theoretical body of work related to CRNs from the mathematical,[37] computer science,[38] and physics communities.[39] Despite these theoretical foundations, many models are phenomenological in nature and lack mechanistic details of various biological processes. The challenge of constructing correct models is compounded by the difficulty in differentiating between correct and incorrect models based upon experimental data.[40–42]

BioCRNpyler allows users to easily build diverse CRNs with flexible species and reaction representations which are then saved as SBML[16] for simulation with many different simulators. The CRN classes inside BioCRNpyler provide useful functionality so that users can easily modify CRNs produced via compilation, produce entire CRNs by hand, or interface hand-produced CRNs with compiled CRNs. These functionalities include classes to represent `Species` bound together as `ComplexSpecies`, lists of species organized as `OrderedPolymerSpecies`, a multi-polymer secondary structures called a `PolymerConformation`, and many diverse propensity functoin types including mass-action, hill functions, and general user specified propensities. Additionally, user-friendly printing functionality allows for the easy visualization of CRNs in multiple text formats or as interactive reaction graphs

formatted and drawn using Bokeh and ForceAtlas2.[43,44]

3.2 Mechanisms are Reaction Schemas

When modeling biological systems, modelers frequently make use of mass-action CRN kinetics which ensure that parameters and states have clear underlying mechanistic meanings. However, for the design of synthetic biological circuits and analysis using experimental data, phenomenological or reduced-order models are commonly utilized as well.[2] Empirical phenomenological models have been successful in predicting and analyzing complex circuit behavior using simple models with only a few lumped parameters.[45–47] Bridging the connections between the different modeling abstractions is a challenging research problem. This has been explored in the literature using various approaches such as by direct mathematical comparison of mechanistic and phenomenological models[48–50] or by studying particular examples of reduced models.[2] BioCRNpyler provides a computational approach using reaction schemas to easily change the mechanisms used in compilation from detailed mass-action to coarse-grained at various level of complexity.

Reaction schemas refer to BioCRNpyler's generalization of switching between different mechanistic models: a single process can be modeled using multiple underlying motifs to generate a class of models. `Mechanisms` are the BioCRNpyler objects responsible for defining reaction schemas. In other words, various levels of abstractions and model reductions can all be represented easily by using built-in and custom `Mechanisms` in BioCRNpyler. For example, to model the process of transcription (as shown in Figure 4), BioCRNpyler allows the use of various phenomenological and mass-action kinetic models by simply changing the choice of reaction schema. Notably, this provides a unique capability to quickly compare system models across various levels of abstraction enabling a more nuanced approach to circuit design and exploring system parameter regimes.

Formally, reaction schemas are functions that produce CRN species and reactions from a set of input species and parameters: $f : (S', \theta) \rightarrow (S, R)$. Here the inputs S' are chem-

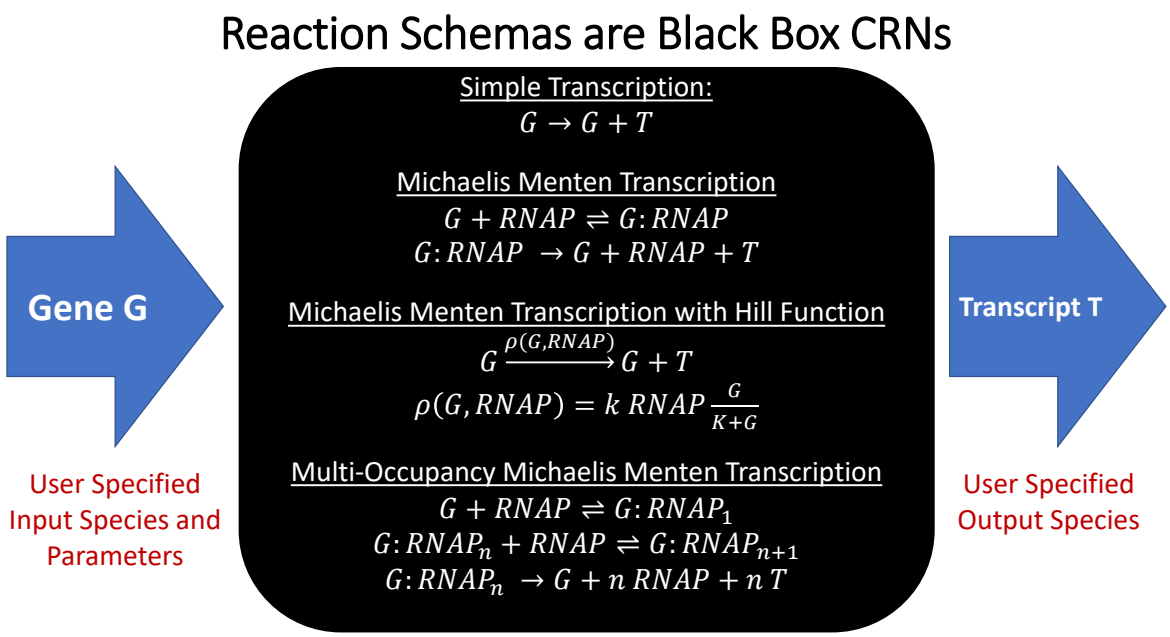

Figure 4: Mechanisms (Reaction Schemas) representing transcription.

ical species and θ are rate constants. The outputs $S \supseteq S'$ is an increased set of species and R is a set of reactions. Figure 4 gives different examples of a reaction schema's representing transcription. This functionality allows modelers to generate CRNs at different levels of complexity and reuse CRN motifs for some `Components` while customizing Mechanisms for others. Importantly, BioCRNpyler contains a large and growing library of existing `Mechanisms` extensively documented via examples making them easy to use and re-purpose without extensive coding. Internally, each `Mechanism` class has a type (e.g. transcription) which defines the input and output species it requires. `GlobalMechanisms` are a special subclass of `Mechanism` called at the end of compilation to represent processes which act on large subsets of CRN species such as dilution in cellular models. The ability to generate chemical `Species` and `Reactions` via customizable `Mechanisms` is one of the key features making BioCRNpyler distinct from other frameworks. Hierarchical SBML and supporting software provide [51] a noteable exception—however BioCRNpyler contains library of reusable chemical reaction motifs, while Hierarchical SBML is a standard for describing embedded CRN models.

3.3 Components Represent Functionality

In BioCRNpyler, `Components` are biochemical parts or motifs, such as promoters, enzymes and chemical complexes. `Components` represent biomolecular functionality; a promoter enables transcription, enzymes perform catalysis, and chemical complexes must bind together. `Components` express their functionality by calling particular `Mechanism` types during compilation. Importantly, `Components` are not the same as CRN `Species`; one `Species` might be represented by multiple `Components` and a `Component` might produce multiple `Species`! For example, the single `CombinatorialConformationPromoter Component` used in the lac operon model (shown in Figure 3) produces hundreds of unique `Species`. Conversely, the chemical species β-Galactosidase is modeled using two components: an `Enzyme` to model the metabolism of lactose and a `ChemicalComplex` to model the fact that β-Galactosidase is a homeotetramer. `Components` are flexible and can behave differently in different contexts or behave context-independently. To define dynamic-context behavior, `Components` may use mechanisms and parameters provided by the `Mixture`. To define context-independent behavior, `Components` may have their own internal `Mechanisms` and (`ParameterDatabases`). The BioCRNpyler library includes many `Component` subclasses to model enzymes (`Enzyme`), chemical complexes (`ChemicalComplexes`) formed by molecular binding, Promoters (`Promoter`), Ribosome Binding Sites (`RBS`), complex genetic architectures (such as `DNA_construct` illustrated in Figure 5), and more.

3.4 Mixtures Represent Context

`Mixtures` are collections of default `Components`, default `Mechanisms`, and user-added `Components`. `Mixtures` can represent chemical context (e.g. cell extract vs. *in vivo*), as well as modeling resolution (e.g. what level of detail to model transcription or translation at) by containing different internal `Components` and `Mechanisms`. `Mixtures` also control CRN compilation by requesting `Species` and `Reactions` for each of their `Components`. After receiving all these `Species` and `Reactions`, Mixtures then apply `GlobalMechanisms` which act on all

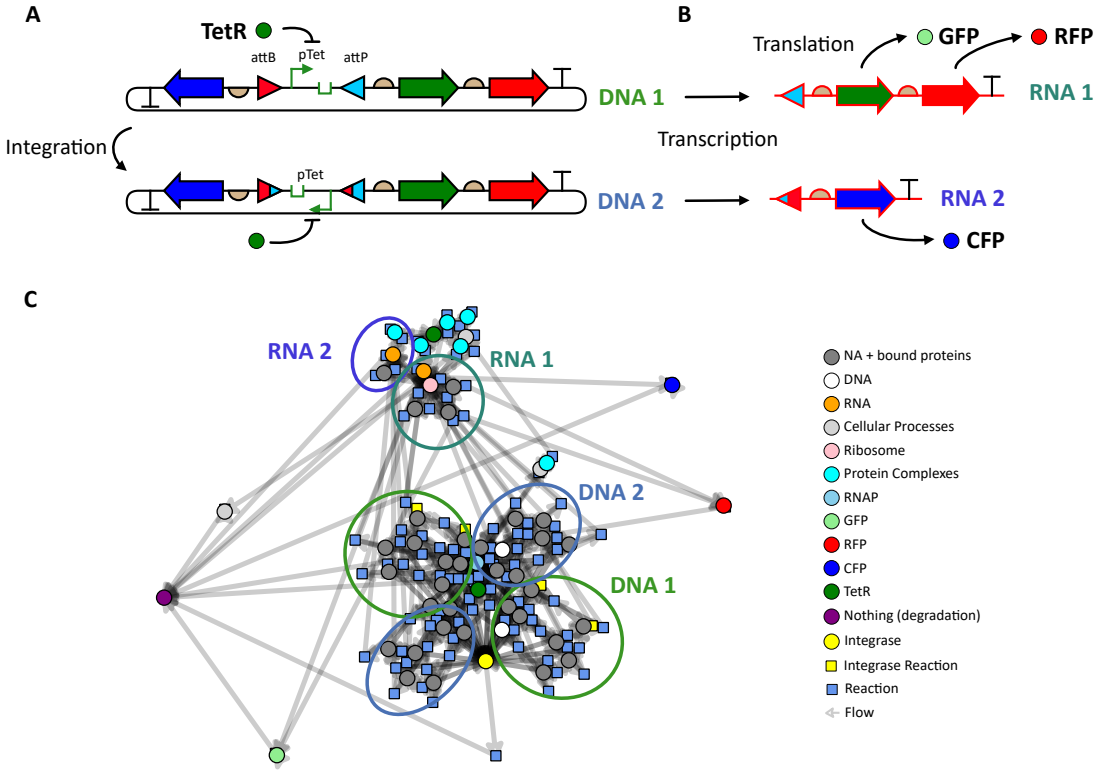

Figure 5: Using the `DNA_construct` class, `DNA_parts` (a subclass of `Component`) can be arranged in the same order as they would be in a DNA molecule and compiled into a CRN. **A.** A complicated `DNA_construct` contains three coding sequences and a single promoter which can be flipped by an integrase. Global component enumeration allows an `Integrase_Enumerator` to generate "DNA 2" from "DNA 1". **B.** Local component enumeration uses a `ConstructExplorer` to generate an `RNA_construct` from each `DNA_construct`. **C.** A directed graph representation of the compiled CRN from the `DNA_construct`. `Species` represented by circles participate in `Reactions` represented by squares. Circled groups of `Species` and `Reactions` involve the `DNA_construct` labeled "DNA 1", or the `RNA_constructs` labeled "RNA 1" or "RNA 2". The component enumeration process creates all the necessary `Species` and `Reactions` to simulate integration, transcription, and translation from linear and circular DNA, taking into account the compositional context of `DNA_parts`.

the Species produced by `Components`. BioCRNpyler comes with a variety of `Mixtures` to represent cell-extracts and cell-like systems with multiple levels of modeling complexity.

3.5 Component Enumeration allows for Arbitrary Complexity

`ComponentEnumerators` are specialized classes which allow additional `Components` to be generated dynamically during the compilation process. Local component enumeration occurs when one `Component` compiles itself into a set of many sub-components. For example, the `DNA_construct Component`, made out of an ordered list of `DNA_parts`, such as `Promoters`, `RBSs`, and `CDSs`, uses the `ConstructExplorer ComponentEnumerator` to enumerate all the possible mRNAs which could be transcribed as new `RNA_construct Components`. When the CRN is compiled, many `RNA_constructs` may be generated from a single `DNA_construct`. These `RNA_constructs` can then be coupled to `Mechanisms` automatically to compile a complex CRN.

Similarly, global component enumeration endows a `Mixture` with a `ComponentEnumerator` that generates a set of `Components` based upon all the `Components` in the `Mixture`. For example, serine integrases are enzymes which are capable of recombining strands of DNA at specific integration sites.[52] Integration events can happen within a single piece of DNA or between multiple DNA species. The `Integrase_Enumerator` is a `ComponentEnumerator` which looks at all the `DNA_constructs` present in a Mixture and enumerates all possible integration events to generate new `DNA_constructs` which are then fed back into the `Integrase_Enumerator`. Figure 5 illustrates both local and global component enumeration involving a `DNA_construct` with serine integrase attachment sites. Other integrase types (for example homotypic sites) are also supported. Due to the potential of an unbounded number of `Components` being produced by such a process, component enumeration can be called to a user specified recursion depth in order to compile arbitrarily large chemical reaction networks.

3.6 Flexible Parameter Databases

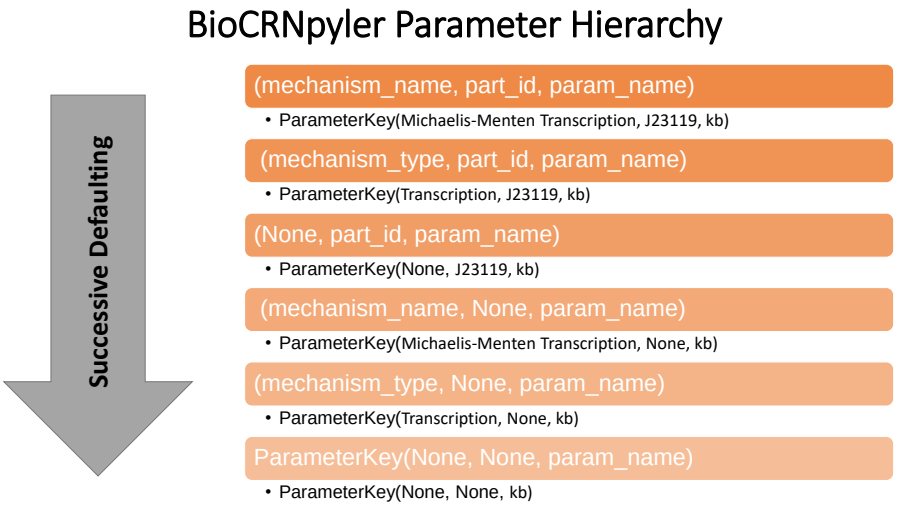

Figure 6: BioCRNpyler Parameter Defaulting Heirarchy. If a specific `ParameterKey` (orange boxes) cannot be found, the `ParameterDatabase` automatically defaults to other `ParameterKeys`. This allows for parameter sharing and rapid construction of complex models from relatively relatively few non-specific (e.g. lower in the hierarchy) parameters.

Developing models is a process that involves defining then parameterizing interactions. Often, at the early stage of model construction, exact parameter values will be unavailable. BioCRNpyler has a sophisticated parameter framework which allows for the software to search user-populated `ParameterDatabases` for the parameter that closest matches a specific `Mechanism`, `Component`, and parameter name. This allows for models to be rapidly constructed and simulated with "ball-park" parameters and then later refined with specific parameters derived from literature or experiments later. This framework also makes it easy to incorporate diverse parameter files together and share parameters between many chemical reactions.

4 Building an Open-Source Community

BioCRNpyler aims to be a piece of open-source community driven software that is easily accessible to biologists and bioengineers with varying levels of programming experience as well

as easily customizable by computational biologists and more advanced developers. Towards these ends, the software package is available via GitHub and PyPi, requires very minimal software dependencies, contains extensive examples and documentation in the form of interactive Jupyter notebooks,[23] YouTube tutorials,[53] and automated testing to ensure stability. Furthermore this software has been extensively tested via inclusion in a bio-modeling course and bootcamps with dozens of users ranging from college freshmen and sophomores with minimal coding experience to advanced computational biologists. BioCRNpyler has already been deployed to build diverse models in systems and synthetic biology.[54–57] Developing new software functionality is also a simple process documented on the GitHub contributions page.

4.1 Integrated Testing

BioCRNpyler uses Travis-CI[58] and Codecov[59] to automate testing on GitHub. Whenever the software is updated, a suite of tests is run including extensive unit tests and functional testing of tutorial and documentation notebooks. Automated testing unit testing ensures that changes to the core BioCRNpyler code preserve functionality of the package. The integration of Jupyter notebooks into testing allows users to easily define new functionality for the software and document that functionality with detailed explanations which are simultaneously tests cases.

4.2 Documentation and Tutorials

The BioCRNpyler GitHub page contains over a dozen tutorial Jupyter notebooks[23] and presentations explaining everything from the fundamental features of the code to specialized functionality for advanced models to how to add to the BioCRNpyler code-base.[53] This documentation has been used successful in multiple academic courses and is guaranteed to be up-to-date and functional due to automatic testing.

5 Future Directions

BioCRNpyler is an ongoing effort which will grow and change with the needs of its community. Extending this community via outreach, documentation, and an ever expanding suite of functionalities is central to the goals of this project. We are particularly interested in facilitating the integration of BioCRNpyler into existing laboratory pipelines in order to make modeling a central part of the design-build-test cycle in synthetic biology. One avenue towards this goal is to add compatibility to existing standards such as SBOL[17] and automation platforms such as DNA-BOT [60] so BioCRNpyler can automatically compile models of circuits as they are being designed and built. This approach will be a generalization and extension of Roehner et al.[61] In particular due to the modular BioCRNpyler compilation process, it will be possible to have programmatic control over the SBML model produced from BioCRNpyler.

We also plan on extending the library to include more realistic and diverse `Mixtures`, `Mechanisms`, and `Components` (particularly experimentally validated models of circuits in *E. coli* and in cell extracts). We hope that these models will serve as examples and inspiration for other scientists to add their own model systems in other organisms to the software library.

Finally, we believe that the Context-Part-Mechanism abstraction of model compilation used in BioCRNpyler is quite fundamental and could be extended to other non-CRN based modeling approaches. Advanced simulation techniques beyond chemical reaction networks will be required to accurately model the diversity and complexity of biological systems. New software frameworks such as Vivarium[57] have the potential to generate models which couple many simulation modalities. The abstractions used in BioCRNpyler could be extended to compile models beyond chemical reaction networks such as mechanical models, flux balance models, and statistical models derived from data. The integration of these models together will naturally depend on both detailed mechanistic descriptions as well as overarching system context. We emphasize that building extendable and reusable frameworks to enable quantitative modeling in biology will become increasingly necessary to understand and design ever

more complex biochemical systems.

Acknowledgement

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6 Supplemental: Code for Examples

This section provides code from the examples Figure 1. The first three models are idealized in the sense that they are represented by Hill functions and include no cellular machinery such as ribosomes or polymerases. Producing these models in BioCRNpyler is easy and just requires the reuse of a few parts: `DNAassembly` represents a simple transcriptional unit with a promoter, transcript and optionally an ribosome binding site (RBS) and protein product. `RepressiblePromoter` creates a promoter modeled by a hill function. `Species` creates CRN species used in the models. Notice that only `Species` which are shared between different `Components` need created by hand — BioCRNpyler takes care of the rest. For example, in inducible repression example the repressor R is created by hand because it is placed inside the `RepressiblePrmoter`. However the DNA, transcript, and protein are automatically generated from the name of the `DNAassmebly`. Finally, everything is added together into a subclass of `Mixture` and compiled into a `ChemicalReactionNetwork`. The second three models build off the general architectures of the first three but add in more complicated

context and implementation details. Instead of using `ExpressionDilutionMixture` and `SimpleTxTlDilutionMixture`, these models use the considerably more complex `TxTlDilutionMixture` which includes molecular machinery such as RNAP, ribosomes, RNAases, and background cellular processes. Additional implementation details in the form of `Components` and `Mechanisms` are also added to these models.

6.1 Inducible Repression

Here a repressor is constituitively produces from a `DNAassembly`. This repressor is then linked to a `RepressiblePromoter` which models repression using a hill function.

```
from biocrnpyler import *
# Models a piece of DNA that constitutively produces the species R
repressor = Species("R")
const_rep = DNAassembly(name="const_rep", promoter="medium", rbs="medium",
    protein=repressor)
# R represses RepressiblePromoter which is placed into another DNAassembly reporter
prom = RepressiblePromoter(name="pR", repressor=repressor)
reporter = DNAassembly(name="Reporter", promoter=prom, rbs="strong",
    initial_concentration=1)
# ExpressionDilutionMixture models gene expression without transcription/translation
mixture = ExpressionDilutionMixture(components=[reporter, const_rep],
    parameter_file="params.txt")
CRN = mixture.compile_crn()
```

6.2 Toggle Switch

In the following example, a toggle switch is created by connecting two instances of `RepressiblePromoter` together. Notice that string names passed to promoter and rbs are used to help find parameters. BioCRNpyler comes with many default parameters to enable rapid model prototyping.

```
from biocrnpyler import *
```

```
# Creates A and is repressed by B
repA = Species("A")
promA = RepressiblePromoter(name="pA", repressor=repB)
assemblyA = DNAassembly(name="A", promoter=promA, rbs="medium", protein=repA,
    initial_concentration=1)
# Creates B and is repressed by A
repB = Species("B")
promB = RepressiblePromoter(name="pB", repressor=repA)
assemblyB = DNAassembly(name="B", promoter=promB, rbs="medium", protein=repB,
    initial_concentration=1)
# SimpleTxTlDilutionMixture includes transcription and translation but no machinery
mixture = SimpleTxTlDilutionMixture(components=[assemblyA, assemblyB], parameter_file=
    "params.txt")
CRN = mixture.compile_crn()
```

6.3 Repressilator

The code to create a 3-node repession oscillator is really just adding one more unit and rewiring the toggle switch example.

```
from biocrnpyler import *
# Create Repressors
repA = Species("A")
repB = Species("B")
repC = Species("C")
# Create Promoters
promA = RepressiblePromoter(name="pA", repressor=repC)
promB = RepressiblePromoter(name="pB", repressor=repA)
promC = RepressiblePromoter(name="pC", repressor=repB)
#Create DNAassemblies
assemblyA = DNAassembly(name="A", promoter=promA, rbs="medium", protein=repA,
    initial_concentration=1)
```

```
assemblyB = DNAassembly(name="B", promoter=promB, rbs="medium", protein=repB,
    initial_concentration=1)
assemblyC = DNAassembly(name="C", promoter=promC, rbs="medium", protein=repC,
    initial_concentration=1)
# Place it all in a Mixture & Compile
mixture = SimpleTxTlDilutionMixture(components=[assemblyA, assemblyB, assemblyC],
    parameter_file="params.txt")
crn = mixture.compile_crn()
```

6.4 Cas9 Repressor and Guide RNA Coexpressed with Reporter

Modeling a dCas9-guideRNA repressor in bioCRNpyler requires that the dCAs9 and guide RNA know to bind together. This is accomplished via the `Component` subclass `ChemicalComplex` which models binding between multiple species. The resulting dCas9-guideRNA `ComplexSpecies` is used as a repressor.

```
from biocrnpyler import *
# parameter syntax: (mechanism_name, part_id, parameter_name) : value
# Only one dCas9-guideRNA complex binds to the promoter at once
params = {
    ("negativehill_transcription", None, "n"):1
}
# Create guide RNA and dCas9 Species
guide = Species("guide", material_type="rna")
dcas = Species("dCas9")
# These species will bind together by placing them in the ChemicalComplex Component
# "notdegradable" ensures that RNAases do not degrade gRNA-dCas9 complexes.
repressor = ChemicalComplex([dcas, guide], attributes=["notdegradable"])
reporter = Species("reporter")
# Constuitive Assemblies to produce dCas9 and the guideNRA
assembly_dcas = DNAassembly(name="dcas", promoter="medium", rbs="medium", protein=dcas)
assembly_guide = DNAassembly(name="guide", promoter="strong", rbs=None, transcript=guide)
```

```
# Create a repessible promoter
pReg = RepressiblePromoter(name="pA", repressor=repressor, parameters=params)
assembly_rep = DNAassembly(name="reporter", promoter=pReg, rbs="strong",
    protein=reporter, initial_concentration=1)
# Place the Components in a Mixture
extract = TxTlDilutionMixture("e coli", components=[assembly_rep, assembly_dcas,
    assembly_guide, repressor], parameter_file="params.txt")
#Compile the CRN
crn = extract.compile_crn()
```

6.5 Targeted RNAase Toggle Switch

The targeted RNAase toggle switch model is a hypothetical model similar to a normal toggle switch but with regulation at the RNA level instead of the transcriptional level. This is accomplished by creating two constitutively expressed RNAases (which are `ChemicalComplexes` made up of two subunits) and adding custom `Mechanisms` to the `Mixture` modeling the degradation of any species with the attribute "tagA" and "tagB" by RNAase A and RNAase B, respectively.

```
#Create an RNA species with degradation tag sequence tagB
TA = Species("A", attributes=["tagB"], material_type="rna")
#Create homodimer subunit A
A = Species("A", material_type="protein")
#RNAase A is a homodimer made up of two identical subunits
rnaaseA = ChemicalComplex([A]*2)
#create a DNAassembly that produces A
assemblyA = DNAassembly(name="A", promoter="strong", transcript=TA, rbs="medium",
    protein=A, initial_concentration=1)

#Same as above but for Species B
TB = Species("B", attributes=["tagA"], material_type="rna")
B = Species("B", material_type="protein")
```

```
rnaaseB = ChemicalComplex([B]*2)
assemblyB = DNAassembly(name="B", promoter="strong", transcript=TB, rbs="medium",
    protein=B, initial_concentration=1)
#add all the Components to a Mixture
mixture = TxTlDilutionMixture("e coli", components=[assemblyA, assemblyB, rnaaseA,
    rnaaseB], parameter_file="default_parameters.txt")
# Deg_Tagged_Degredation Mechanism makes rnaaseA degrade anything with attribute "tagA"
mixture.add_mechanism(Deg_Tagged_Degredation(mechanism_type="tagA_degredation",
    deg_tag="tagA", protease=rnaaseA.get_species()))
# Deg_Tagged_Degredation Mechanism makes rnaaseB degrade anything with attribute "tagB"
mixture.add_mechanism(Deg_Tagged_Degredation(mechanism_type="tagB_degredation",
    deg_tag="tagB", protease=rnaaseB.get_species()))
#Compile the CRN
CRN = mixture.compile_crn()
```

6.6 Multiple Ribosome Occupancy Repressilator Dynamics

Simulating multiple-ribosome occupancy in the Repressilator mostly reuses the code from Section 6.3 with the main addition of a new `Mechanism` to model transcription being placed into the `Mixture`.

```
#Create Repressors
repA = Species("A")
repB = Species("B")
repC = Species("C")
#Create Promoters
promA = RepressiblePromoter(name="pA", repressor=repC)
promB = RepressiblePromoter(name="pB", repressor=repA)
promC = RepressiblePromoter(name="pC", repressor=repB)
#Create DNAassemblies
assemblyA = DNAassembly(name="A", promoter=promA, rbs="strong", protein=repA,
    initial_concentration=1)
```

```
assemblyB = DNAassembly(name="B", promoter=promB, rbs="strong", protein=repB,
    initial_concentration=1)
assemblyC = DNAassembly(name="C", promoter=promC, rbs="strong", protein=repC,
    initial_concentration=1)
#Extra parameters for the Multi_tx Mechanism
extra_params = {"max_occ":10, ("multi_tx", None, "k_iso"):50, ("multi_tl", None,
    "k_iso"):50, "cooperativity":2}
#Add Everything to a Mixture
mixture = TxTlDilutionMixture("e coli", components=[assemblyA, assemblyB, assemblyC],
                            parameter_file="default_parameters.txt",
                                parameters=extra_params, overwrite_parameters=True)
#Add the multi_tl translation mechanism to the mixture, overwriting the old one.
mixture.add_mechanism(multi_tl(name="multi_tl",
    ribosome=mixture.ribosome.get_species()), overwrite=True)
#Compile the CRN
crn = mixture.compile_crn()
```

7 Supplemental: Tables of Features

This section lists many of the different `Mixture`, `Component` and `Mechanism` classes available in BioCRNpyler. For more details about these classes and examples using many of them, check out the examples folder on GitHub.

7.1 Mixtures

Mixture Name	Description
ExpressionExtract	A model for gene expression without machinery such as ribosomes, polymerases, etc. Here transcription and translation are lumped into one reaction: expression.
SimpleTxTlExtract	A model for transcription and translation in a cell-free extract without machinery such as ribosomes, polymerases, etc. RNA is degraded via a global mechanism.
TxTlExtract	A model for transcription and translation in a cell-free extract with machinery for ribosomes, polymerases, and endonucleases action. This model does not include any energy buffer.
EnergyTxTlExtract	Transcription and translation with ribosomes, polymerases, and endonucleases labelled as cellular machinery. Also includes a simple model of biochemical energy utilization involving NTPs, amino acids, and energy regeneration from a food source. Adapted from the model in [62].
ExpressionDilutionMixture	A model for *in-vivo* gene expression without any machinery such as ribosomes, polymerases, etc. Transcription and translation are lumped into one reaction and a global mechanism is used to dilute all non-DNA species.
SimpleTxTlDilutionMixture	Mixture with continuous dilution for non-DNA species. Transcription (TX) and Translation (TL) are both modeled as catalytic with no cellular machinery. mRNA is also degraded via a separate reaction to represent endonucleases.
TxTlDilutionMixture	Transcription and translation with ribosomes, polymerases, and endonucleases labelled as cellular machinery. Also includes a background load which represents innate loading effects in the cell. Effects of loading on cell growth are not modeled. It has global dilution for non-DNA and non-machinery species. This model does not include any energy.

7.2 Components

Component Type	Component Name	Description
Chemical Complex	ChemicalComplex	A complex that represents the combination of several `Species`. Takes care of binding and unbinding reactions needed to form the complex
Chemical Complex	CombinatorialComplex	A complex that represents the combination of several `Species`. Binding occurs combinatorially in many possible orders. Allowed and disallowed intermediate complexes can be specified.
Enzyme	Enzyme	An enzyme that converts substrates to products
Protein	Protein	Basic component that represents a protein
DNA	DNA	Basic component that represents a DNA sequence
DNA	DNAassembly	A relatively simple DNA sequence containing one promoter, RBS, and a product
DNA	DNA_construct	A more complex DNA sequence that can have any number of `Components` in any order
Promoter	Promoter	Constitutive $\sigma 70$ promoter
Promoter	RegulatedPromoter	Repressible or activatable promoter such as P_{tet}
Promoter	ActivatablePromoter	Activatable promoter using a positive Hill function
Promoter	RepressiblePromoter	Repressible promoter using a negative Hill function
Promoter	CombinatorialPromoter	Flexible promoter mechanism allowing various transcription factor binding configurations to allow or prevent transcription
Promoter	CombinatorialConformationPromoter	Enumerates the binding and unbinding events to represent a promoter with many regulators that can also form various secondary structures such as loops.
Ribosome Binding Site	RBS	Simple RBS using a translation mechanism
Coding Sequence	CDS	Protein coding part used for `DNA_construct`. Doesn't affect CRN
Terminator	Terminator	Transcriptional terminator used for `DNA_construct`. Doesn't affect CRN
RNA	RNA	Basic component that represents an RNA sequence
RNA	RNA_construct	A more complex RNA sequence that can have any number of `Components` in any order. Usually automatically generated by `DNA_construct`
Polymer Secondary Structure	CombinatorialConformation	Enumerates the binding and unbinding events to produce a `PolymerConformation` with different bound complexes and secondary structure.

7.3 Mechanisms

Mechanisms Type	Mechanism Name	Description
binding	Reversible_Bimolecular_Binding	$S_1 + S_2 \leftrightharpoons (S_1 : S_2)$
cooperative_binding	One_Step_Cooperative_Binding	$n\, S_1 + S_2 \leftrightharpoons (nS_1 : S_2)$
cooperative_binding	Two_Step_Cooperative_Binding	$n\, S_1 \leftrightharpoons (nS_1)$, $(nS_1) + S_2 \leftrightharpoons (nS_1 : S_2)$
cooperative_binding	Combinatorial_Cooperative_Binding	Allows a set of species S_i and cooperativities n_i to bind to a target T in any order to form $(n_1 S_1 : ... : n_k S_k : T$ along with all combinatorial intermediaries.
binding	One_Step_Binding	$S1 + S2 ... SN \leftrightharpoons S1 : S2 : ... : SN$
catalysis	BasicCatalysis	$S + C \rightarrow P + C$
catalysis	BasicProduction	$C \rightarrow P + C$
catalysis	MichaelisMenten	$Sub + Enz \leftrightharpoons Sub : Enz \rightarrow Enz + Prod$
catalysis	MichaelisMentenReversible	$Sub + Enz \leftrightharpoons Sub : Enz \leftrightharpoons Enz : Prod \leftrightharpoons Enz + Prod$
copy	MichaelisMentenCopy	$Sub + Enz \leftrightharpoons Sub : Enz \rightarrow Sub + Enz + Prod$
transcription	OneStepGeneExpression	$G \rightarrow G + P$
transcription	SimpleTranscription	$G \rightarrow G + T$
translation	SimpleTranslation	$T \rightarrow T + P$
transcription	PositiveHillTranscription	$G \rightarrow [r]G + P\ r = kG(R^n)/(K + R^n)$
transcription	NegativeHillTranscription	$G \rightarrow [r]G + P\ r = kG/(K + R^n)$
transcription	Transcription_MM	$G + RNAP \leftrightharpoons G : RNAP \rightarrow G + RNAP + mRNA$
translation	Translation_MM	$mRNA + Rib \leftrightharpoons mRNA : Rib \rightarrow mRNA + Rib + Protein$
transcription	multi_tx	$DNA : RNAp_n + RNAp \leftrightharpoons DNA : RNAp_n^{closed} \rightarrow DNA : RNAp_{n+1} DNA : RNAp_n \rightarrow DNA : RNAp_0 + nRNAp + nmRNA DNA : RNAp_n^{closed} \rightarrow DNA : RNAp_0^{closed} + nRNAp + nmRNA$ for $n = \{0, \max_{occ}\}$
translation	multi_tl	$mRNA : RBZ_n + RBZ \leftrightharpoons mRNA : RBZ_n^{closed} \rightarrow mRNA : RBZ_{n+1} mRNA : RBZ_n \rightarrow mRNA : RBZ_0 + nRBZ + nProtein mRNA : RBZ_n^{closed} \rightarrow mRNA : RBZ_0^{closed} + nRBZ + nProtein$ for $n = \{0, \max_{occ}\}$
dilution	Dilution	$s \rightarrow \emptyset$
rna_degredation_mm	Degredation_mRNA_MM	$T + Nuclease \leftrightharpoons T : Nuclease \rightarrow Nuclease$. Global mechanism effects all RNA species T. ComplexSpecies containing RNA species are broken apart via the reaction $T : X + Nuclease \leftrightharpoons T : X : Nuclease \rightarrow X + Nuclease$. for any X.
degredation	Deg_Tagged_Degredation	$X + Protease \leftrightharpoons X : Protease \rightarrow Protease$. Here X is any Species with the deg_tag attribute passed into the constructor of this GlobalMechanism.

8 Supplemental: Creating Custom BioCRNpyler Classes

BioCRNpyler is designed to be easily extendable so even non-computer scientists can add their own custom functionality. In this section we briefly show how to subclass core BioCRNpyler classes. For more details and examples, interested readers should look at the developer overview on our github.

8.1 Mechanisms

Developing custom `Mechanisms` is also as easy as making a subclass of `Mechanism` and defining three functions to produce the desired CRN:

```
class CustomMechanism(Mechanism):
    def __init__(self, args, **kwargs ):
        Mechanism.__init__(self, name="name", mechanism_type="type", **kwargs)
        # python code to set internal variables

    def update_species(self, ... ):
        # python code to create Species objects
        return species_list

    def update_reactions(self, ... ):
        # python code to create Reaction objects
        return reaction_list
```

8.2 Components

It is also straightforward to make custom `Components`: simply subclass `Component` and define three functions:

```
class CustomComponent(Component):
    def __init__(self, args, **kwargs ):
        Component.__init__(self, ... , **kwargs)
        # python code to set internal variables

    def update_species(self):
        # python code calls mechanism.update_species( ... )
        return species_list

    def update_reactions(self):
```

```
        # python code calls mechanism.update_reaction( ... )
        return reaction_list
```

8.3 Mixtures

Making custom `Mixtures` is also easy — it can be done via simple scripts by adding `Components` and `Mechanisms` to a `Mixture` object:

```
MyMixture=Mixture("customized mixture",
                  components=[List Components],
                  mechanisms=dict("mechanism_type":Mechanism))
```

The `Mixture` class can also be easily subclassed by rewriting the constructor:

```
class CustomMixture(Mixture):
    def __init__(self, args, **kwargs ):
        #python code to set up internal variables,
        # create Components, and default Mechanisms
        Mixture.__init__(self, mechanisms=dict("mechanism_type": Mechanism),
                        components=[List of Components], **kwargs)
```

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