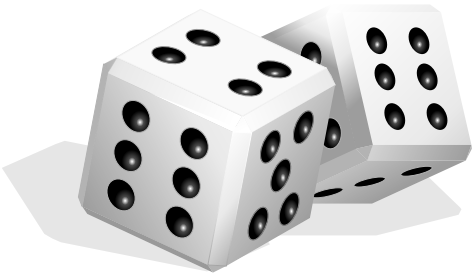
StochPy



Stochastic modeling in Python

StochPy User Guide

Release 2.3.0

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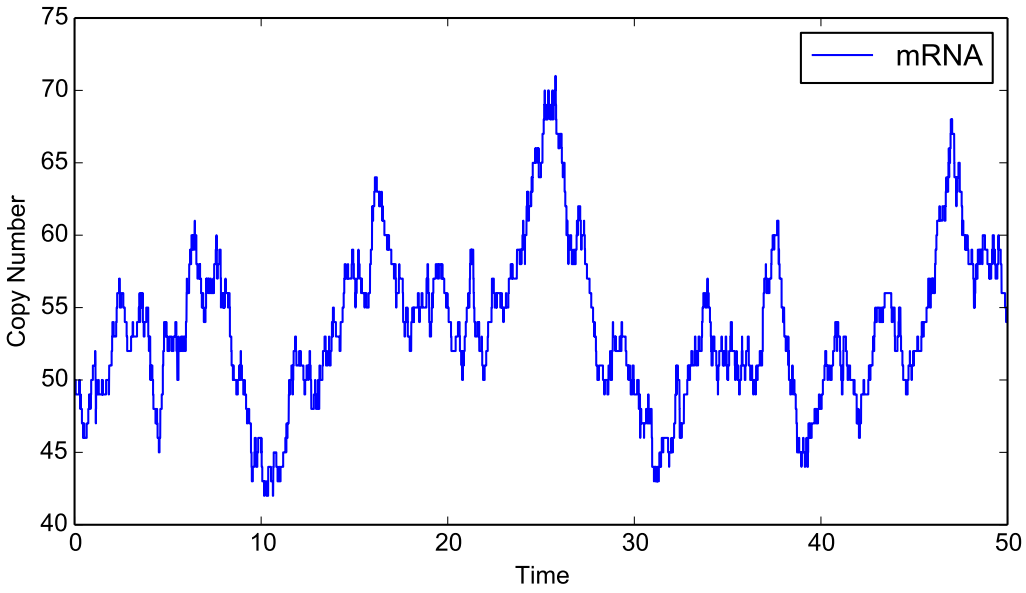
Part I

Introduction

Welcome to the user guide describing StochPy: Stochastic modeling in Python. We could start by explaining that StochPy is comprehensive and user-friendly, as we explain in our manuscript, but we decided to start this user guide with a simple, yet illustrative, example:

```
>>> import stochpy
>>> smod = stochpy.SSA()
>>> smod.DoStochSim()
>>> smod.PlotSpeciesTimeSeries()
```



'Nuff said right?

In the remainder of this user guide, we explain e.g. how to:

1. Install StochPy: *click here*,
2. Use StochPy in more detail *click here*,
3. Do stochastic simulations with cell growth and division *click here*.

Part II

Installation and Configuration

CHAPTER

ONE

INSTALLATION

1.1 Dependencies

StochPy (since version 2.1.0) is available for both Python 2 (2.6+) and Python 3 (3.4+). We recommend using Python 2.7. In addition to Python 2 or 3, the NumPy library is required before installing StochPy.

The following software is optional but recommended:

- Matplotlib
- libSBML
- libxml2
- iPython (and iPython-notebook)

Matplotlib is useful for the generation of plots and libSBML is necessary to convert models written in SBML format to the PySCeS MDL. The libSBML software requires a XML parser library (libxml2).

On Ubuntu you can use the following code to directly install all required packages on Python 2.6+:

```
sudo apt-get install python-dev python-numpy
sudo apt-get install python-scipy python-matplotlib  python-pip
sudo apt-get install ipython ipython-notebook
sudo apt-get install libxml2 libxml2-dev
sudo apt-get install zlib1g zlib1g-dev
sudo apt-get install bzip2 libbz2-dev
sudo pip install python-libsbml
```

and for Python 3.4+:

```
sudo apt-get install python3-dev python3-numpy
sudo apt-get install python3-scipy python3-matplotlib  python3-pip
sudo apt-get install ipython3 ipython3-notebook
sudo apt-get install libxml2 libxml2-dev
sudo apt-get install zlib1g zlib1g-dev
sudo apt-get install bzip2 libbz2-dev
sudo pip3 install python-libsbml
```

1.2 Windows

Download the windows installer (32-bit) or the source code and use setup.py in a terminal.

Python 2.6.x:

```
$ python setup.py install
```

Python 3.4.x:

```
$ python3 setup.py install
```

If you download the source code, you can also generate your own windows installer with the following code:

```
$ python setup.py bdist_wininst
```

1.3 Linux/MAC OS/Cygwin

Download the source code and use setup.py in the terminal:

```
$ cd /.../StochPy-2.3.0
```

Python 2.7.x:

```
$ sudo python setup.py install
```

Python 3.4.x:

```
$ sudo python3 setup.py install
```

CHAPTER

TWO

CONFIGURATION

The StochPy package contains some example models, such as:

- Immigration-Death model
- Burst model
- Decaying-Dimerizing model
- Prokaryotic auto-regulation model

The files describing these models can be found in the directory /home dir/Stochpy/pscmodels/ after importing stochpy for the first time. Most of these models are written in the PySCeS MDL format. This PySCeS MDL is explained in *the PySCeS Model Description Language section*. Besides PySCeS MDL, StochPy also accepts models written in SBML.

Part III

Using StochPy

An interactive Python shell is necessary to start interactive modeling with StochPy. There exist various options, but we recommend using iPython (and iPython notebook for teaching/code sharing). In the command-line you start IPython with the command:

```
$ ipython
```

Then, use `import stochpy` to import the installed version of StochPy:

```
>>> import stochpy

##############################################################################
#                                                                            #
#            Welcome to the interactive StochPy environment                  #
#                                                                            #
##############################################################################
#  StochPy: Stochastic modeling in Python                                    #
#  http://stochpy.sourceforge.net                                            #
#  Copyright(C) T.R Maarleveld, B.G. Olivier, F.J Bruggeman 2010-2015        #
#  DOI: 10.1371/journal.pone.0079345                                         #
#  Email: tmd200@users.sourceforge.net                                       #
#  VU University, Amsterdam, Netherlands                                     #
#  Centrum Wiskunde Informatica, Amsterdam, Netherlands                      #
#  StochPy is distributed under the BSD license.                             #
##############################################################################

Version 2.3.0
Output Directory: /home/user/Stochpy
Model Directory: /home/user/Stochpy/pscmodels
```

The message above indicates that the StochPy package is successfully imported and that StochPy is ready-to-use. Use one of the following commands to read StochPy's docstring:

```
>>> help(stochpy)
>>> stochpy?
```

Press `q` to close the help modus. This functionality is available for every high-level function implemented in StochPy:

```
>>> smod = stochpy.SSA()
>>> help(smod)
>>> help(smod.DoStochSim)
>>> smod.DoStochSim?
```

of which *DoStochSim()* is one of the key high-level functions as it runs a stochastic simulation.

CHAPTER

THREE

MODULE 1: DEMO

Before we explain in more detail how to use StochPy, we want to briefly discuss the demo and utilities modules. The StochPy Demo module currently provides 11 demo's which illustrate many features of StochPy. In each demo we show the high-level functions that are necessary to obtain the generated results. Information about the used high-level function is shown after the #. Usage is straightforward:

```
>>> import stochpy
>>> stochpy.Demo()
press any button to continue
```

Press any button to continue to the first demo:

```
>>> smod = stochpy.SSA() # start SSA module
>>> smod.DoStochSim(IsTrackPropensities=True)
>>> smod.data_stochsim.simulation_endtime
>>> smod.data_stochsim.simulation_timesteps
>>> smod.PrintWaitingtimesMeans()
Reaction     Mean Waiting times
R1           0.109
R2           0.108
>>> smod.PlotSpeciesTimeSeries()
>>> smod.PlotPropensitiesTimeSeries()
>>> smod.PlotWaitingtimesDistributions()
>>> smod.Export2File(analysis="timeseries",datatype="species")
>>> smod.Export2File(analysis="distributions",datatype="species")
>>> smod.Export2File(datatype="propensities")
```

In the first demo we determine the mean waiting times, export data to text files, and create several plots. You can continue running the remaining demo's or exit the demo session by pressing CTRL-c.

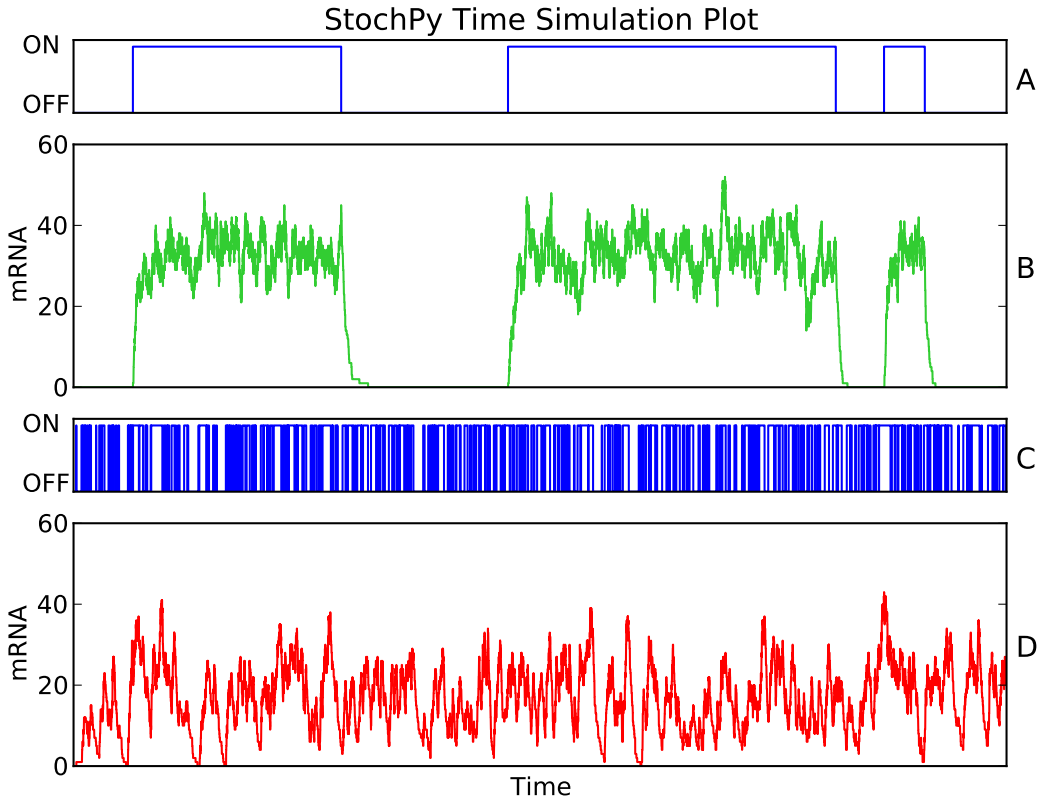
CHAPTER

FOUR

MODULE 2: UTILITIES

The StochPy Utilities module contains functions and examples created by users of StochPy:

```
>>> utils = stochpy.Utils()
>>> utils.DoExample4()
```



In the future more functions and examples are added to this module.

CHAPTER

FIVE

MODULE 3: STOCHASTIC SIMULATION ALGORITHM

The main module of this software package is the stochastic simulation algorithm module. In this module stochastic simulations and their analysis can be done. StochPy currently contains various Stochastic Simulation Algorithms (SSAs):

- Direct Method
- First Reaction Method
- Next Reaction Method (Gibson and Bruck, 1999, J. Phys. Chem.)
- Tau-leap Method (Cao et al. 2006, J. Chem. Phys.), further explained *here*
- Delayed SSAs, explained *here*
- Single Molecule Methods, explained *here*

5.1 Run a Stochastic simulation

Use the following command to start to the SSA module and to perform your first stochastic simulation with StochPy:

```
>>> import stochpy
>>> smod = stochpy.SSA()
>>> smod.DoStochSim()
>>> smod.PlotSpeciesTimeSeries()
```

StochPy uses direct method as default SSA and the immigration-death model as default stochastic model. You can therefore directly use the high-level function *DoStochSim()* to run your first stochastic simulation. The print statements are suppressed during the simulation. Use *quiet=False* to enable print statements during the stochastic simulation (see also the *Quiet modus* section):

```
>>> smod.DoStochSim(quiet=False)
Info: 1 trajectory is generated
simulation done!
Info: Number of time steps 1000 End time 50.275966611
Info: Simulation time 0.07495
```

The high-level function *DoStochSim()* accepts many arguments (e.g. method, trajectories, mode), but we first focus on selecting a stochastic model. Selecting a stochastic model of choice is done with the high-level function *Model()*:

```
>>> smod.Model("Burstmodel.psc")
>>> smod.Model(model_file="Burstmodel.psc",dir="/home/user/Stochpy/pscmodels")
```

which accepts besides models described in the PySCeS MDL, also models described in SBML if the appropriate dependencies are installed):

```
>>> smod.Model("dsmts-001-01.xml",smod.model_dir)
Info: single compartment model: locating "Death" in default compartment
Info: single compartment model: locating "Birth" in default compartment
Writing file: /home/user/Stochpy/pscmodels/dsmts-001-01.xml.psc

>>> smod.model_file
"dsmts-001-01.xml.psc"
>>> smod.model_dir
"/home/user/Stochpy/pscmodels"
```

After selecting your stochastic model of choice, you can directly use *DoStochSim()* and specify the method, number of trajectories, simulation mode etc.:

```
>>> smod.DoStochSim(method="FRM",trajectories=5,mode="time",end=50)
>>> smod.sim_method
    stochpy.implementations.FirstReactionMethod.FirstReactionMethod
>>> smod.sim_mode
    "time"
```

Here, we first used the first reaction method to do a stochastic simulation until t=50 (a.u.). Be careful with specifying a certain end time because it's difficult to predict how long the simulation is going to take. There can be ten time steps in a particular time interval, but this can be just as easily ten million time steps.

Each argument of *DoStochSim()* is treated as a "local" setting, i.e. they are forgotten if you perform a new stochastic simulation without these arguments:

```
>>> smod.DoStochSim()
>>> smod.sim_method
    stochpy.implementations.DirectMethod.DirectMethod
>>> smod.sim_mode
    "steps"
```

DoStochSim() accepts many more arguments, but we are not going to discuss each of them here in detail. The most used arguments of *DoStochSim()* are probably: *method*, *mode*, *end*, *trajectories*, *rate_selection*, *species_selection*, *IsTrackPropensities*, and *IsOnlyLastTimepoint*. The memory reduction arguments *rate_selection*, *species_selection*, and *IsOnlyLastTimepoint* are discussed *here*. We refer to the docstring of the high-level function for more information about its arguments:

```
>>> help(smod.DoStochSim)
```

Press `q` to close the docstring.

Alternatively, we can use various high-level functions that allow for specifying "global" simulation settings. These settings are stored and used until you specify them again or load a new model:

```
>>> smod.Method("Tauleap")
>>> smod.Mode("Time")
>>> smod.sim_method
    stochpy.implementations.TauLeaping.OTL
>>> smod.sim_mode
    "time"
>>> smod.DoStochSim()
>>> smod.sim_method
    stochpy.implementations.TauLeaping.OTL
>>> smod.sim_mode
    "time"
```

While we used high-level function *Mode()* in the previous example, it is probably easier to directly use one of the following high-level functions:

```
>>> smod.Endtime(50)
>>> smod.Timesteps(10**5)
```

We already showed how to select various different stochastic simulation algorithms. The high-level function *Method()* accepts the following case-insensitive strings: "Direct", "NextReactionMethod" or "NRM", "FirstReactionMethod" or "FRM", "Tauleap", "DelayedDirect" and "Delayed", "DelayedNRM" or "DelayedNextReactionMethod", "SingleMoleculeMethod" or "SMM", and "FastSingleMoleculeMethod or "fSMM".

The direct, next reaction method, and first reaction method are exact SSAs that can suffer from extensive running times. If extensive running times are an issue, we recommend using the inexact SSA: the tau-leap method (*click here*). We also provide exact SSAs with delays (*click here*) and single molecule methods (*here*).

Importantly, the high-level function *DoStochSim()* supports only the simulation of the direct, first reaction, and next reaction, and the tau-leap method. Different high-level functions are necessary for the simulation of e.g. delayed SSAs.

```
>>> smod.DoStochSim()
>>> smod.DoDelayedStochSim()
>>> smod.DoSingleMoleculeStochSim()
```

If the wrong method is provided, the high-level function switches to its default method. For example, *DoStochSim()* switches back to the direct method if e.g. the delayed direct method is provided:

```
>>> smod.DoStochSim(method="DelayedDirect")
*** WARNING ***: (DelayedDirectMethod) was selected.
                 Switching to the direct method.
```

Additionally, StochPy can modify both parameter values and initial species copy numbers with the following high-level functions:

```
>>> smod.ChangeParameter("Ksyn",5)
>>> smod.ChangeInitialSpeciesCopyNumber("mRNA",100)
```

Both *ChangeParameter()* and *ChangeInitialSpeciesCopyNumber()* change parameter values and species copy numbers in the current StochPy object. Permanent changes can be made by adjusting the model description file (in the directory where the model is stored) and reload the model with the following high-level function:

```
>>> smod.Reload()
Parsing file: /home/user/Stochpy/pscmodels/ImmigrationDeath.psc
```

We provide high-level functions that gives a summary of the status of the current settings:

```
>>> smod.ShowSpecies()
["mRNA"]
>>> smod.ShowOverview()
Current Model:        ImmigrationDeath.psc
Number of time steps: 1000
Current Algorithm:    <class "stochpy.DirectMethod.DirectMethod">
Number of trajectories:       1
Propensities are not tracked
```

Here, *ShowSpecies()* gives a list of all the species in the model and *ShowOverview()* gives all the current settings. It is also possible to save an interactive session to a Python script:

```
>>> stochpy.SaveInteractiveSession("interactiveSession1.py")
```

Reloading the interactive session is done by starting this script in iPython:

```
$ ipython interactiveSession1.py
```

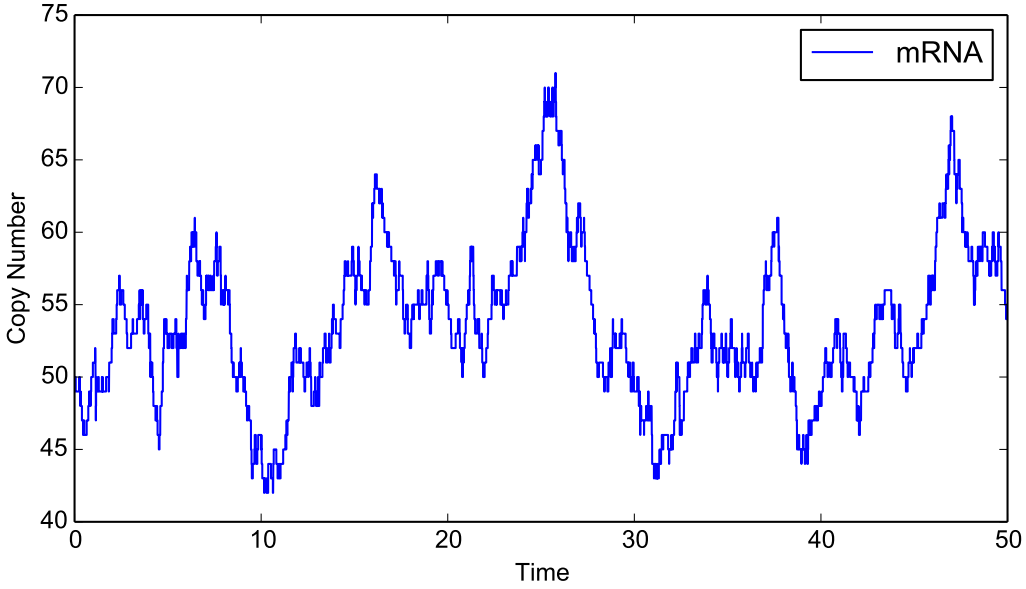
Or by running the script inside iPython (make sure you are in the correct working directory):

```
>>> run interactiveSession1.py
```

5.2 Build-in Analysis Techniques

StochPy provides many preprogrammed analysis techniques that can be used to analyze the done stochastic simulations. We start with a discrete time series plot:

```
>>> smod.Model("ImmigrationDeath.psc")
>>> smod.DoStochSim(method="direct",mode="time",end=50)
>>> smod.PlotSpeciesTimeSeries()
```



This function accepts many arguments of which species to plot and mark-up details are examples:

```
>>> smod.PlotSpeciesTimeSeries(xlabel="Time (s)",title="",
                               linestyle="dashed",linewidth=1,
                               colors="red",IsLegend=True)
```

By default, StochPy creates a figure legend. This legend can be (i) turned off with *IsLegend* =False or (ii) moved to a different location with *legend_location*. The default legend location is the upper right corner. Both *IsLegend* and *legend_location* are arguments of each plotting function. We refer to the docstring of the respective high-level function for more information.

Plots are directly shown if the interactive environment is successfully loaded. Using interactive plotting on the operating systems Windows can cause figures to freeze. If you encounter this issue, please have a look at *this* section (changing the Matplotlib backend solves typically the problem). It is also possible to turn off the interactive plotting feature:

```
>>> smod = stochpy.SSA(IsInteractive = False)
```

With the interactive modes turned off, plotting is possible but the figures are not directly shown. The first command shows the figure (if possible) and the second command saves it to a file:

```
>>> stochpy.plt.show()
>>> stochpy.plt.savefig("myfigure.pdf")
```

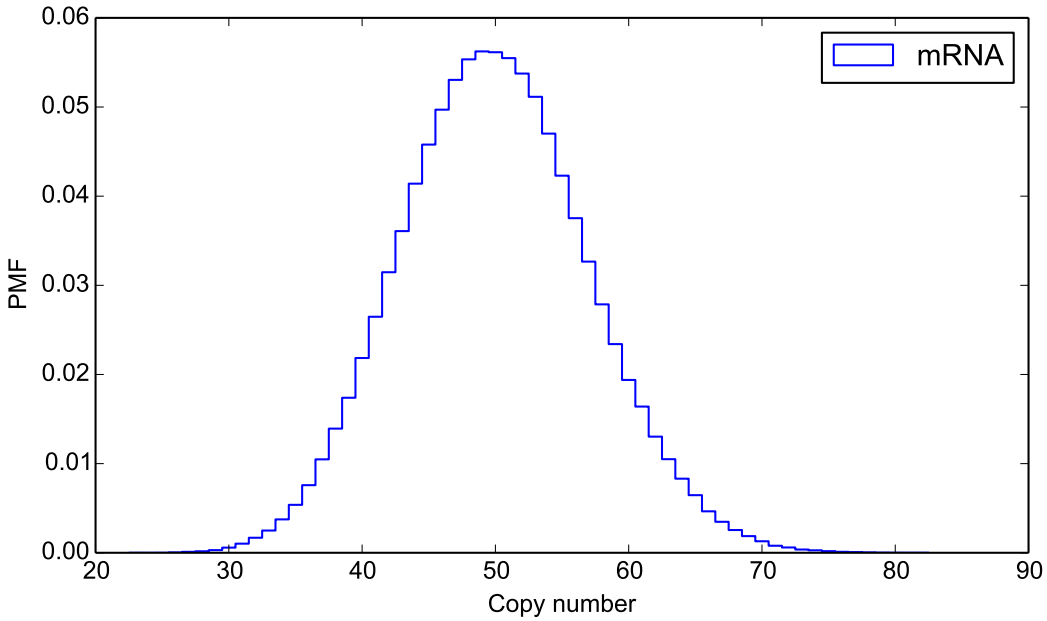
Before we continue with the remaining preprogrammed analysis techniques, we want to briefly discuss a general problem of stochastic simulations: What is the right number of time steps to get an accurate prediction of e.g. the moments or a distribution? StochPy provides a function, *DoCompleteStochSim()*, that continues until the first four moments converge within a user-specified error (default = 0.001).

```
>>> smod.DoCompleteStochSim()
```

We can use the output of this simulation to get an accurate prediction of the species probability

distribution:

```
>>> smod.PlotSpeciesDistributions()
```



We recommend using at least one million time steps before analyzing a probability distribution. Another (related) analysis feature offered by StochPy is determining both the mean and standard deviation of each species in the simulated model:

```
>>> smod.PrintSpeciesMeans()
Species   Mean
mRNA      50.086
>>> smod.PrintSpeciesStandardDeviations()
Species   Standard Deviation
mRNA      7.079
```

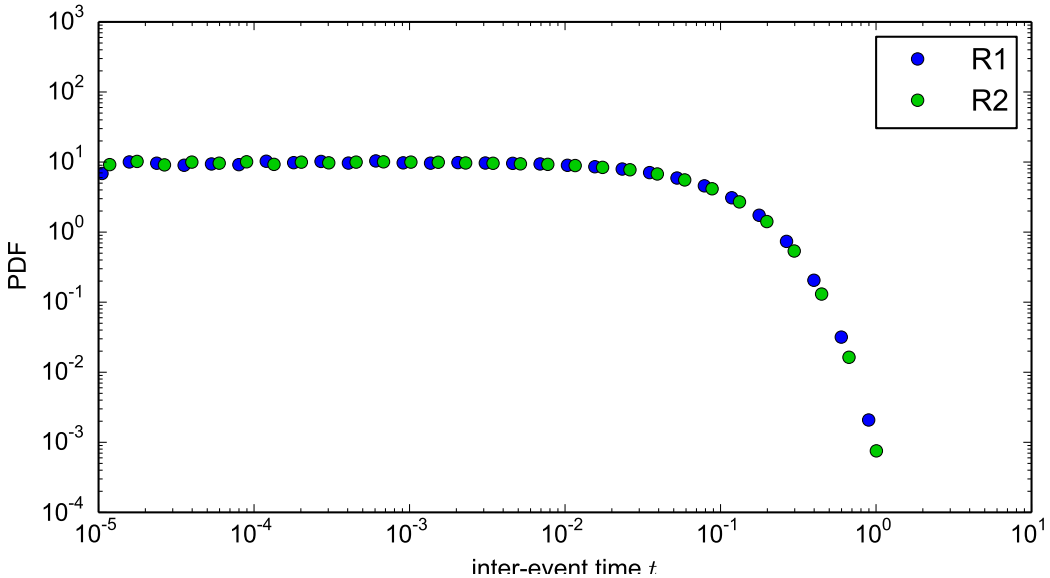
Calculating the mean and standard deviation of each species in a stochastic simulation is not as straightforward as it may sound. The time between two events is not constant, thus it is necessary to track the time spent in each state for each species. These species statistics can also be accessed via the *data_stochsim* model object as Python dictionaries:

```
>>> smod.data_stochsim.species_means
{"mRNA": 50.086035161133793}
>>> smod.data_stochsim.species_standard_deviations
{"mRNA": 7.0793421388907829}
```

Also, StochPy allows the calculation and analysis of event waiting times. Event waiting times are the times between two subsequent firings of a particular reaction. StochPy can offer this unique feature, because StochPy stores all reaction events.

```
>>> smod.GetWaitingtimes()
>>> smod.PlotWaitingtimesDistributions()
>>> stochpy.plt.xlim(xmin=10**-5)
>>> smod.PrintWaitingtimesMeans()
   Reaction Mean
```

```
R1          0.100
R2          0.100
```



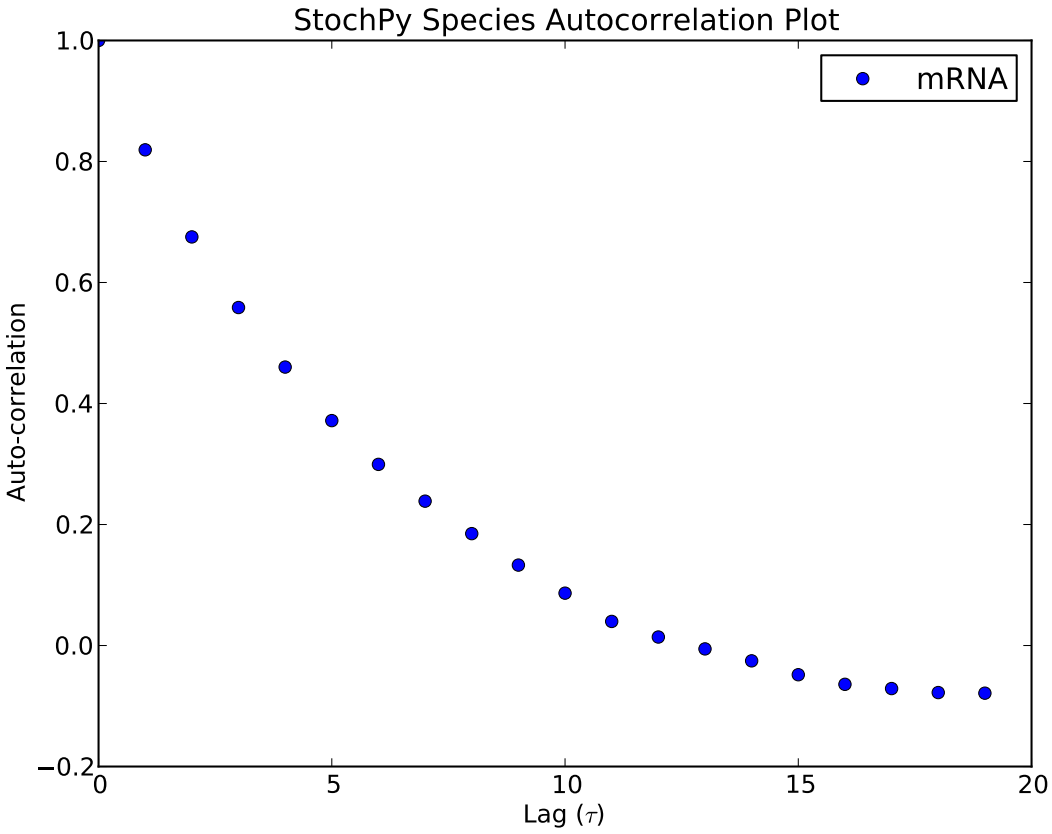
Furthermore, StochPy can determine propensities at each time point during the simulation. Propensities data is not stored by default. The boolean argument *IsTrackPropensities* of the high-level function *DoStochSim()* must be set to True:

```
>>> smod.DoStochSim(IsTrackPropensities=True)
>>> smod.PlotPropensitiesTimeSeries()
>>> smod.PlotPropensitiesDistributions()
```

This means that we can generate two types of time series data: species and propensities.

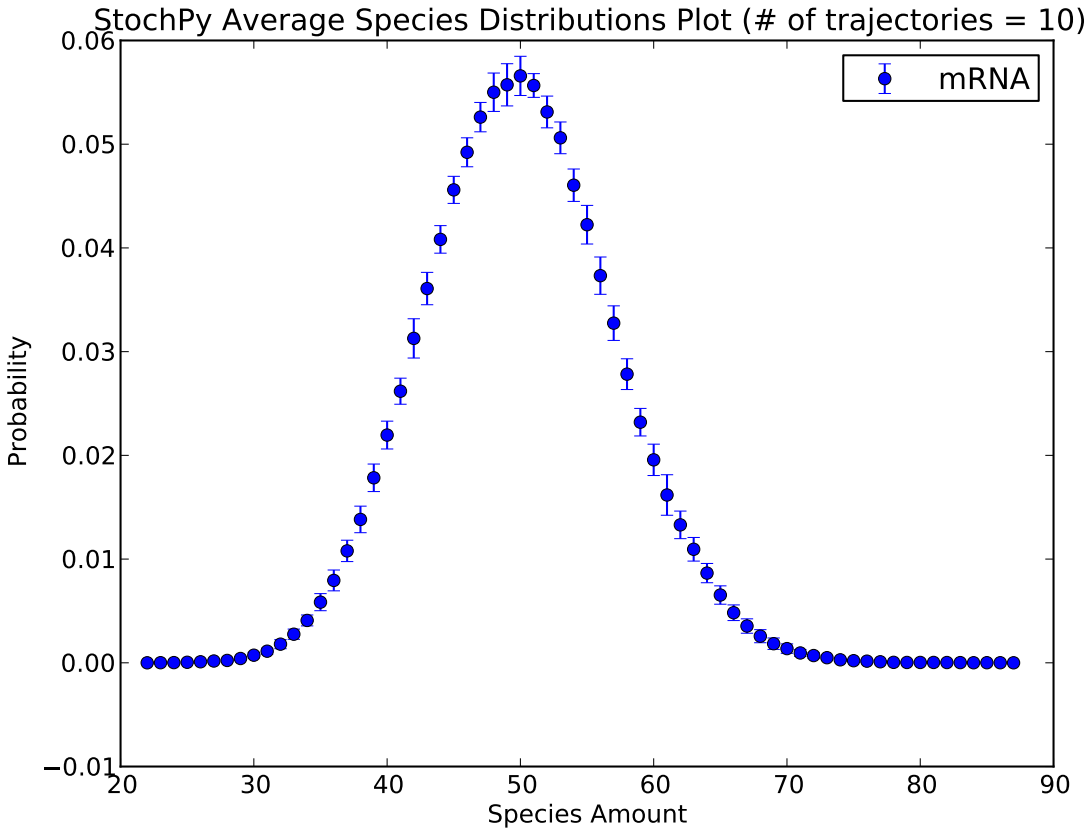
The Gillespie algorithms generate data at irregular time points, but StochPy also offers an approach which returns data on a fixed regular time grid. A grid of which the user can specify the resolution (*n_samples*). The formation of such a regular grid allows for the analysis of autocorrelation (and autocovariance) of species and/or propensities within StochPy. Autocorrelation is the cross-correlation, a measure of similarity of a signal with itself. For different time separations (lags), this similarity can be determined which results a value between 1 and -1. Here, 1 corresponds to positive correlation and -1 to negative correlation. Typically, the first 50 lags are of interest. Here, we show the first 20 lags:

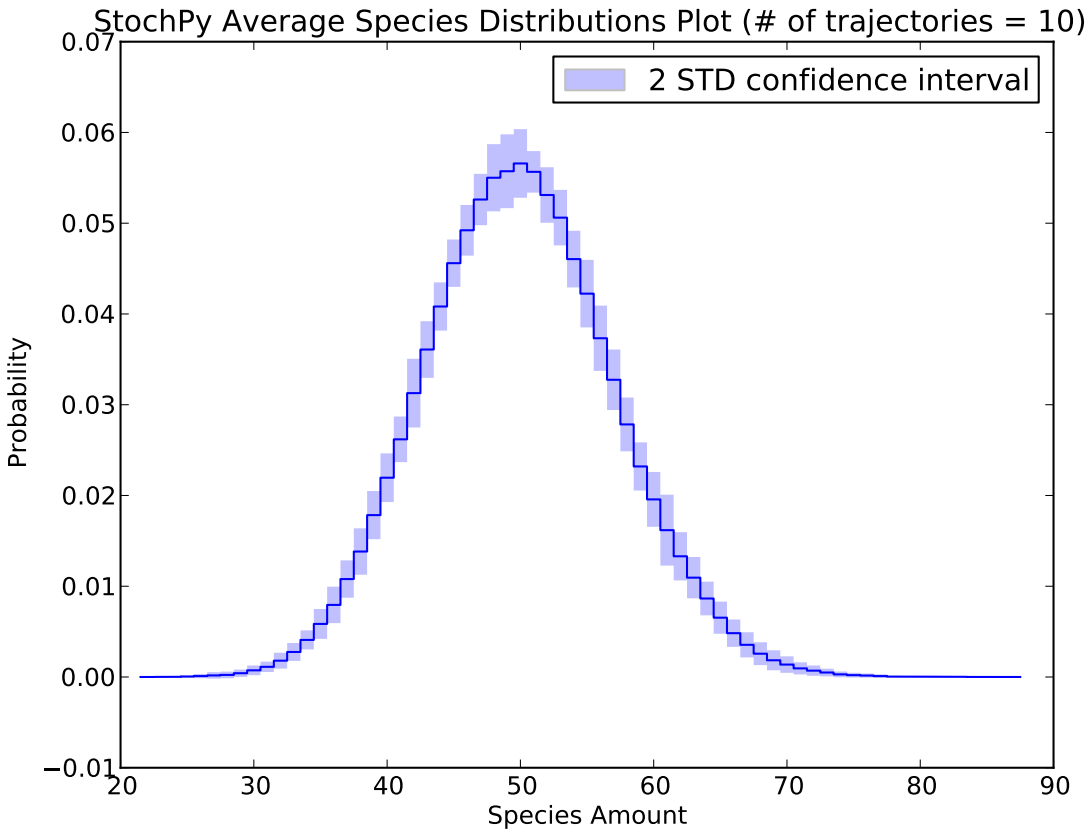
```
>>> smod = stochpy.SSA()
>>> smod.Model("ImmigrationDeath.psc")
>>> smod.DoStochSim(end=1000,mode="time",IsTrackPropensities=True)
>>> smod.GetSpeciesAutocorrelations(n_samples=1001)
>>> smod.PlotSpeciesAutocorrelations(nlags=20)
>>> smod.GetPropensitiesAutocorrelations()
>>> smod.PlotPropensitiesAutocorrelations(nlags=20)
```



Additionally, we can use the regular grid for analyzing multiple generated trajectories. Via StochPy, we can generate e.g. average time series plots for both species and propensities. Here, we discuss two different plotting functionalities for analyzing probability distributions of species averaged over multiple trajectories. The argument *nstd* can be used to set the number of standard deviations of the error bars:

```
>>> smod.DoStochSim(trajectories=10,end=10**5)
>>> smod.GetRegularGrid(n_samples=51)
>>> smod.PlotAverageSpeciesDistributions()
>>> smod.PlotAverageSpeciesDistributionsConfidenceIntervals(nstd=2)
```


StochPy Average Species Distributions Plot (# of trajectories = 10)
mRNA
Probability
Species Amount
0.06
0.05
0.04
0.03
0.02
0.01
0.00
−0.01
20
30
40
50
60
70
80
90


StochPy Average Species Distributions Plot (# of trajectories = 10)
2 STD confidence interval
Probability
Species Amount
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
−0.01
20
30
40
50
60
70
80
90

We already explained that we do not have to store the molecule copy numbers of every species. As a consequence, we can only plot data for "stored" species:

```
>>> smod.PlotSpeciesTimeSeries(species2plot = ["S1","S2"])
>>> smod.PlotPropensitiesTimeSeries(rates2plot = "R1")
>>> smod.PlotWaitingtimesDistributions(rates2plot = "R2")
>>> smod.PlotSpeciesDistributions("S4")
AssertionError: Species S4 is not in the model
```

StochPy also offers *stochpy.plt* (a renamed version of matplotlib.pyplot) to manipulate generated plots or to generate your own plots:

```
>>> smod.PlotSpeciesTimeSeries(marker = "v")
>>> stochpy.plt.title("Your own title")
>>> stochpy.plt.xlabel("Time (s)")
>>> stochpy.plt.xlim([0,10**3])
>>> stochpy.plt.savefig("stochpy_plot.pdf")
```

Besides plotting features StochPy allows printing and exporting of generated data sets. Printing data is only useful for small datasets, so we prefer using *Export2File()* which allows data storage in a text file for e.g. external manipulation:

```
>>> smod.Export2File()
>>> smod.Export2File(analysis="timeseries",datatype="species")
>>> smod.Export2File(analysis="timeseries",datatype="propensities")
>>> smod.Export2File(analysis="distributions",datatype="species")
>>> smod.Export2File(analysis="distributions",datatype="waitingtimes")
>>> smod.Export2File(analysis="distributions",datatype="autocorrelations")
>>> smod.Export2File(analysis="timeseries",datatype="species",IsAverage=True)
```

StochPy also supports the import of previously exported time series data:

```
>>> smod.Import2StochPy("myfile","myfiledir",delimiter="\t")
```

Finally, one can access and manipulate data objects that store the simulation data to perform your own type of analysis in StochPy. See *Using Stochpy as a Library*.

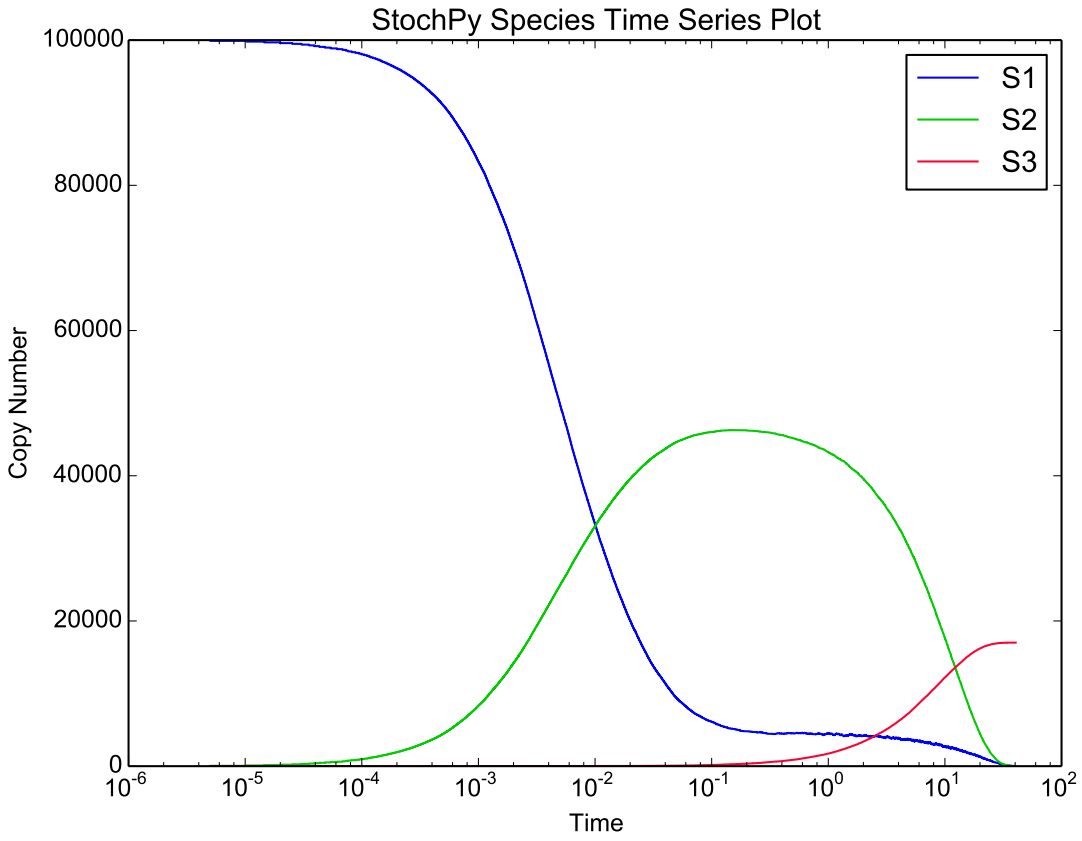
5.3 Inexact Stochastic Algorithms

Here, we use the "decaying-dimerizing" model to illustrate the potential advantage of inexact SSA solvers. While exact solvers always simulate one reaction per time step, inexact solvers such as the tau-leap method try to simulate more than one reaction per time step. They do this by asking the following question: How often does each reaction fire in the next specified time interval tau. The larger the value of this tau the more reactions can fire within this time period, but this tau value must be small enough to make sure that no propensity function suffers from a significant change.

A significant speed advantage can be obtained for models where molecules are present in large quantities. An example of such a system is the decaying-dimerizing model, where S1(t=0)= 100000. We simulate this model with first the tau-leap and secondly the direct method of

which the results are shown below. Information about exact firing times (and therefore also event waiting times) cannot be obtained with this tau-leap method:

```
>>> smod.Model("DecayingDimerizing.psc")
>>> smod.DoStochSim(method="Tauleap",end=50,mode="time")  # sim. time 0.51 s
>>> smod.PlotSpeciesTimeSeries()
>>> smod.DoStochSim(method="Direct",end=50,mode="time")   # sim. time 27.9 s
>>> smod.PlotSpeciesTimeSeries()
>>> stochpy.plt.xscale("log")
```



Tau-leap methods exploit a error-control parameter, epsilon, that bounds the expected change in the propensity functions. This epsilon value ranges between 0-1 where smaller values result in more accurate simulations (but also a smaller tau size and longer simulations). We set this value, by default, to 0.03 (as is also done by Cao et al. 2006). For the decaying-dimerizing model, the tau-leap method is about 50 times faster than the direct method. StochPy allows users to manipulate this setting with the argument *epsilon*, as we show next.

We now set *epsilon* to 0.05:

```
>>> smod.DoStochSim(method="Tauleap",end=50,mode="time",epsilon=0.05)
```

The tau-leap method is now about 100 times faster than the direct method. Too large values of epsilon give incorrect simulation results. We therefore recommend verifying the outcome of the tau-leap method against that of an exact solver. A more strict value can be necessary if you want an accurate prediction of species quantities at different time points. An example is the dsmts-001-05 model of the test suite from Evans et al. 2008. We demanded an accurate prediction at t=0,1, ... 50 for which an epsilon value of 0.01 was required.

*** Warning ***

The tau-leap method automatically reduces the epsilon value for second and third-order reactions. StochPy, therefore, tries to tries to determine the order of each reaction, the "highest order of reaction" (HOR) for each species and its contribution. This works properly for mass-action kinetics, but is likely to fail for non mass-action kinetics. Use the following commands to access the automatically generated results of StochPy:

```
>>> smod.Model("DecayingDimerizing.psc")
>>> smod.Method("Tauleap")
>>> smod.SSA.parse.reaction_orders       # for each reaction
[1,2,1,1]
>>> smod.SSA.parse.species_HORs          # for each species
[2,1,0]
>>> smod.SSA.parse.species_max_influence # for each species
[2,1,0]
```

These results are correct (the model is also described by mass-action kinetics).

1. All reactions are first order, except the second reaction. This is a dimerization reaction of species S1.
2. The highest order of reaction (HOR) of S1 is thus 2. Species S2 acts as reactant in a first-order reaction (HOR, S2 = 1) and S3 is not a reactant (HOR, S3 = 0).
3. *species_max_influence* gives the highest contribution of a species to its HOR. The second reaction is, as said, a dimerization reaction of species S1, so the contribution of S1 for this reaction is also 2. In a similar manner, we can conclude that the highest contribution of species S2 to its HOR is 1 (it's a first-order reaction).

StochPy allows users to modify the three lists shown above directly, but one can also provide them as arguments to the *DoStochSim()* function:

```
>>> smod.DoStochSim(end=50,mode="time",method="Tauleap",
                    reaction_orders=[1,2,1,1],
                    species_HORs=[2,1,0],
                    species_max_influence=[2,1,0])
```

Finally, StochPy"s implementation of the tau-leap method defines "critical reactions", i.e. reactions that are within a few firings of exhausting (one of) its reactants. These critical reactions are used to reduce the probability for a negative population by allowing these reactions to fire only once per time step. The automated determination of critical reactions works fine for most rate equations, but could go wrong for some very specific rate equations. StochPy allows specification of reactions that can fire no more than once during a single time step for the entire simulation. While it is not necessary for the decaying-dimerizing model, we now set R4 as a critical reaction for the entire simulation:

```
>>> smod.DoStochSim(end=50,mode="time",method="Tauleap",
                    critical_reactions=["R4"])
```

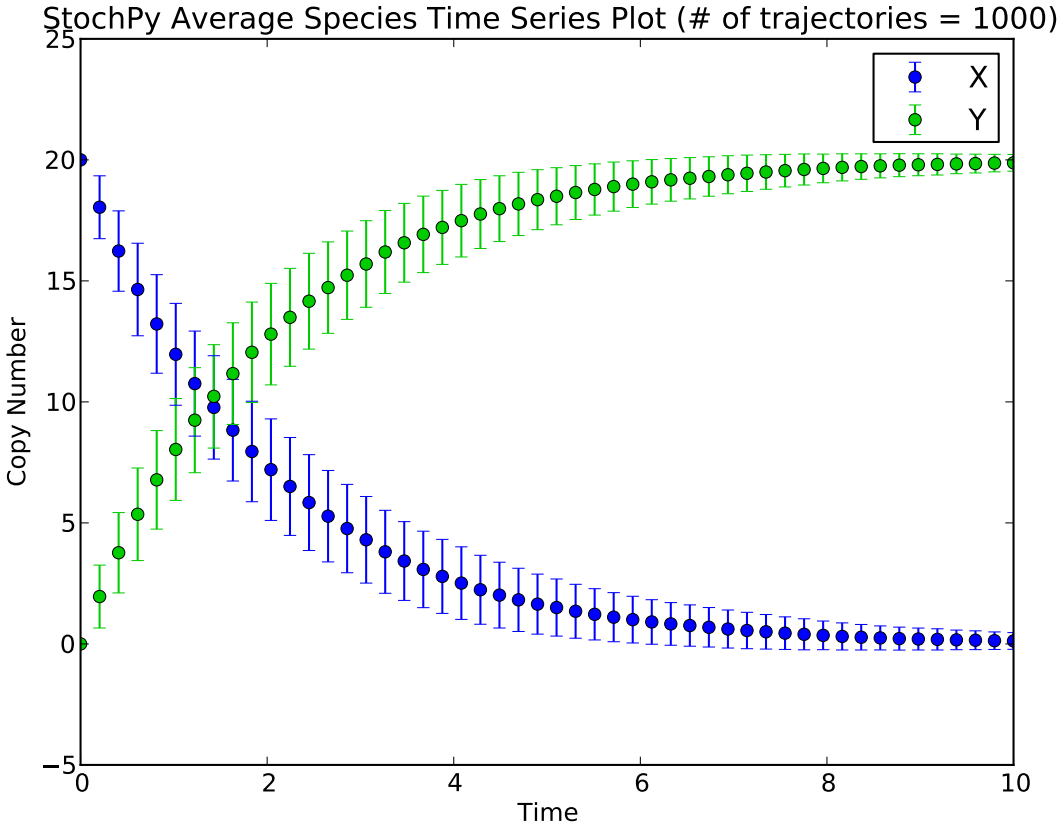
Because it is not necessary for this model, the simulation time increases notably (only five times faster than the direct method).

5.4 Delayed Stochastic Algorithms

Delayed stochastic algorithm extent the Gillespie stochastic simulation algorithm (SSA) to a system with delays. A delayed reaction consists of an exponential waiting time as initiation step with a subsequent delay time. We implemented both the Delayed Direct Method (Cai 2007, J. Chem. Phys) and the Delayed Next Reaction Method (Anderson 2007, J. Chem. Phys).

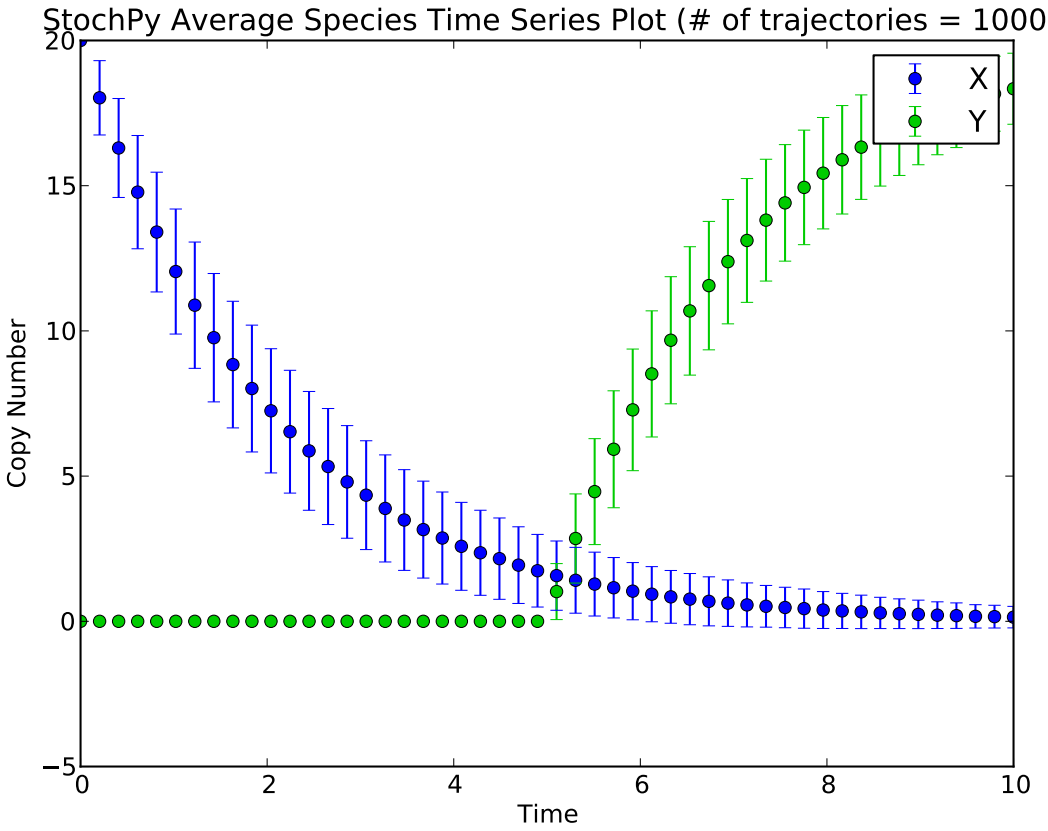
To illustrate the difference between "normal" and "delayed" SSAs, we start by running a normal stochastic simulation with a simple model where X molecules are converted to Y molecules:

```
>>> smod.Model("Isomerization.psc")
>>> smod.DoStochSim(mode="time",end=10,trajectories=1000)
>>> smod.GetRegularGrid(n_samples=50)
>>> smod.PlotAverageSpeciesTimeSeries()
```



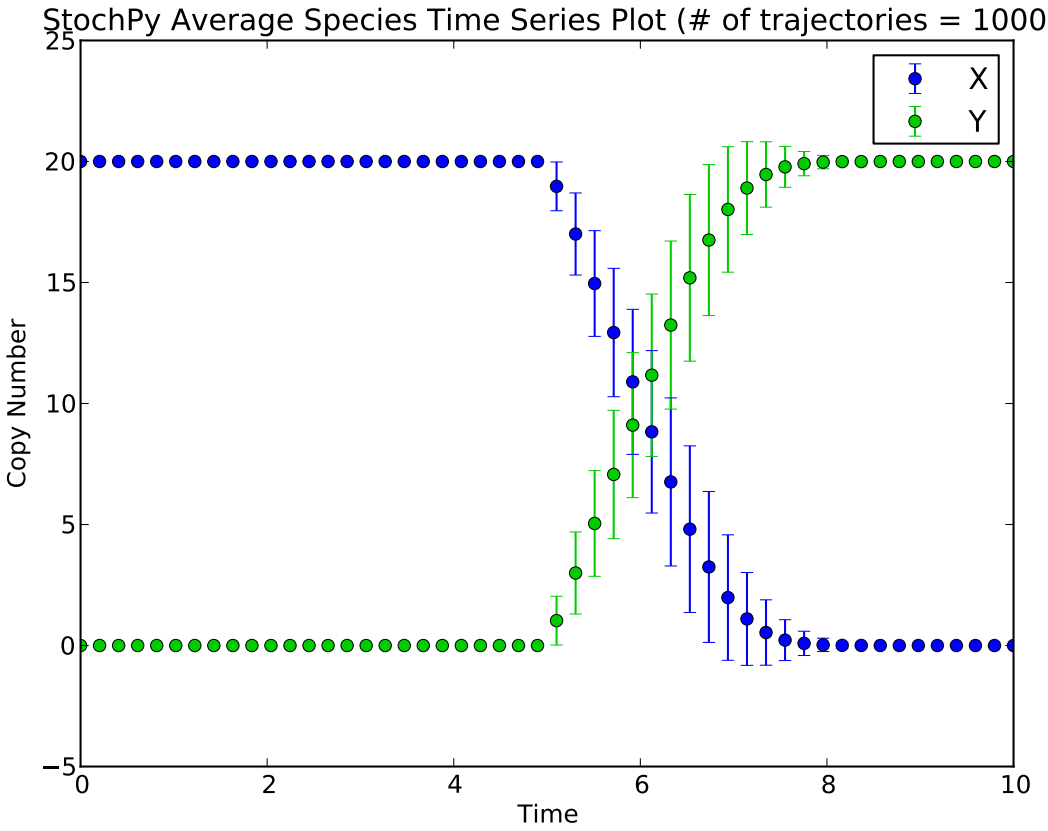
Next, we set a fixed delay of five seconds on reaction R1. This means that after R1 fires, a X molecule is consumed and it takes exactly five seconds before a Y molecule is produced:

```
>>> smod.SetDelayParameters({"R1":("fixed",5)})
>>> smod.DoDelayedStochSim(mode="time",end=10,trajectories=1000)
>>> smod.GetRegularGrid(n_samples=50)
>>> smod.PlotAverageSpeciesTimeSeries()
```



There exist two types of delayed reactions: consuming and nonconsuming delayed reactions. For consuming delayed reactions, the reactants are consumed at initiation and for nonconsuming delayed reactions, the reactants are consumed at completion. In both cases, the products are updated at completion. By default, delayed reactions are "consuming delayed reactions". Nonconsuming delayed reactions have to be specified specifically with the argument *nonconsuming_reactions*. In this example, after reaction R1 fires, it takes exactly five seconds before a particular X is degraded and a particular Y is produced:

```
>>> smod.SetDelayParameters({"R1":("fixed",5)},nonconsuming_reactions=["R1"])
>>> smod.DoDelayedStochSim(mode="time",end=10,trajectories=1000)
>>> smod.GetRegularGrid(n_samples=50)
>>> smod.PlotAverageSpeciesTimeSeries()
```



Performing a delayed stochastic simulation is not allowed without setting delayed parameters:

```
>>> smod.Model("ImmigrationDeath.psc")
>>> smod.DoDelayedStochSim()
AttributeError: No delay parameters have been set.
Please first use the function .SetDelayParameters().
```

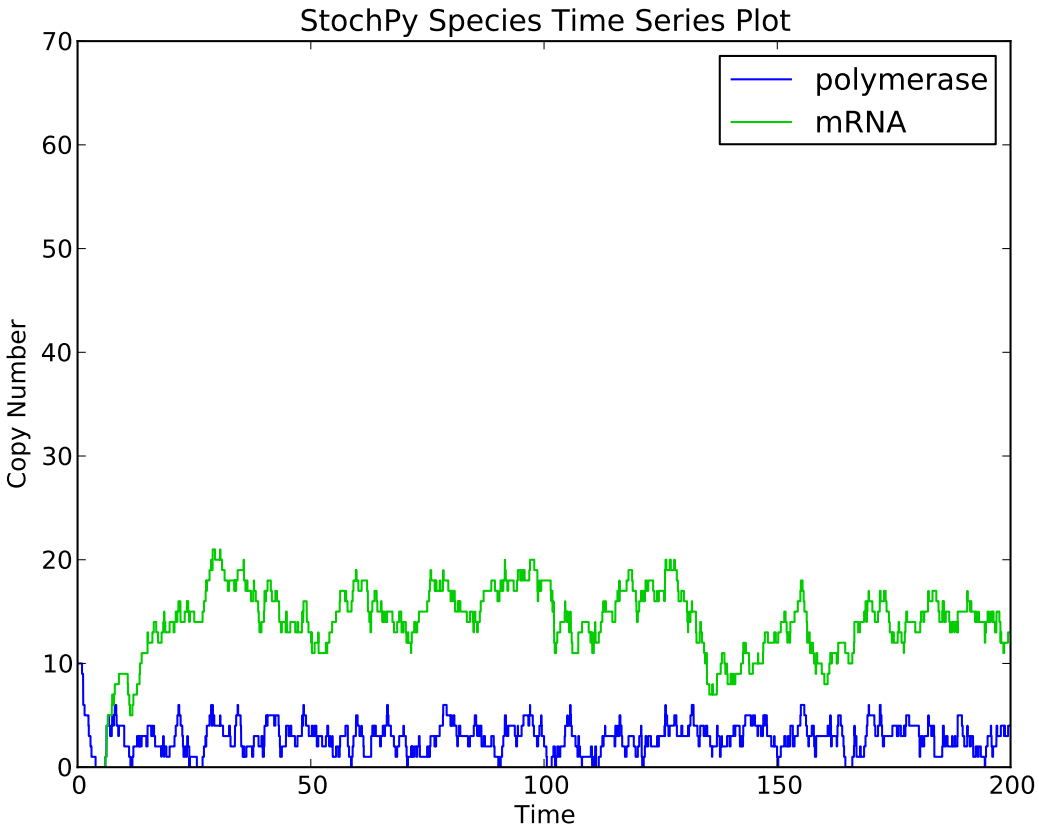
An empty set of delayed parameters is sufficient, but realize that this is similar to doing a non-delayed stochastic simulation:

```
>>> smod.SetDelayParameters({})
>>> smod.DoDelayedStochSim()
```

Next, we use a small model of gene transcription to illustrate the difference between consuming and nonconsuming delayed reactions. RNA polymerase can bind to the promoter region of a gene, which starts the transcription process. When the RNA polymerase leaves the promoter region and elongation phase of the transcription process starts, a next RNA polymerase can bind to the gene. This means that, if we assume that the number of RNA polymerases is relatively large, gene transcription can be viewed as a nonconsuming reaction.

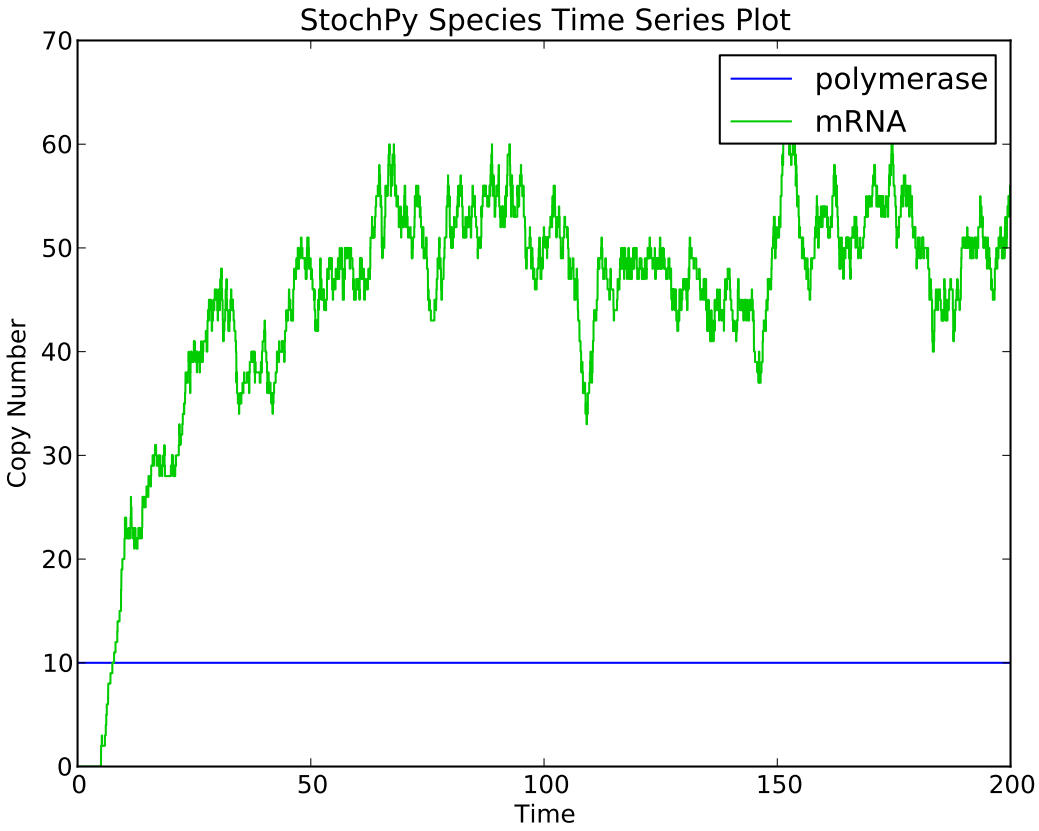
Modeling this process as a nonconsuming reactions gives completely different results as is shown here with two simple simulations. First, we simulate this process as a consuming reaction. The available RNA polymerase pool depletes quickly which reduces the formation rate of mRNA chains:

```
>>> smod.Model("Polymerase.psc")
>>> smod.Endtime(200)

>>> smod.SetDelayParameters({"R1":("fixed",5)})
>>> smod.DoDelayedStochSim()
>>> smod.PlotSpeciesTimeSeries()
>>> stochpy.plt.ylim(0,70)
```



And second, we model this process as a nonconsuming reaction. Now, the available RNA polymerase stays constant, and therefore, significantly more mRNA chains are produced.

```
>>> smod.SetDelayParameters({"R1":("fixed",5)},nonconsuming_reactions="R1")
>>> smod.DoDelayedStochSim()
>>> smod.PlotSpeciesTimeSeries()
>>> stochpy.plt.ylim(0,70)
```



5.5 Single Molecule Method

In stochastic simulations, reaction times are exponential random variables with mean and standard deviation 1/a0; a0 is the sum of all propensities. For certain processes, reaction times can be distributed differently (think of e.g. lumped reactions). One way to circumvent this is to exploit delays. We developed a method called the Single Molecule Method (SMM) that approaches reactions differently. Here, both the exponential step and a potential delay are replaced by a reaction time for the whole reaction. More importantly, the reaction time does not have to be drawn from an exponential distribution, whereas can be drawn from any distribution.

The propensity is typically a rate parameter multiplied with one or more species. For example, for the first-order reaction X -> Y, the propensity function is k1*X. Hence, the mean reaction time decreases with increasing X and vice versa. The SMM treats the copy number differently: Putative reaction times are determined for each set of substrate molecules individually. This means that, for our example model X -> Y, we determine a putative reaction time for each X molecule. These putative reactions times can be fixed or drawn from a distribution of choice. Of course, negative values are not allowed so be careful with e.g. normal distributions. If a molecule acts as a reactant in multiple reactions, it has a putative reaction time for each of these reactions. The shortest putative reaction time "wins" and the molecule reacts in this reaction channel. This is similar to the Next Reaction Method developed by Gibson and Bruck, with the difference that we look at single molecule rather than single reactions.

The SMM can be rather slow, especially for models with many second-order reactions with high

reactant copy numbers. For second-order reactions, every combination of molecules should be considered. We use a simple example to illustrate this. Consider the reaction A + B > C, with ten molecules of A and hundred molecules of B. There are in total thousand different reactions possible, i.e. each molecule of A can react with hundred different molecules of species B. For each of these thousand reactions, a putative reaction time is generated. After selecting the reaction with the shortest putative reaction time, many modifications have to be made to the system. For instance, all reactions that belong to the selected A and B molecules have to be deleted. Imagine what happens if multiple reactions can use these compounds as reactants.

Often, the majority of reactions in a model are regular exponential reactions. We, therefore developed the fast Single Molecule Method (fSMM) to speed-up the SMM simulation time. This method combines a regular Next Reaction Method and the SMM. The main advantage is that only reactions which are given a particular putative reaction time distribution, the single molecule reactions, are treated as in the SMM. The other reactions have a exponential putative time distribution and are simulated as in a normal Next Reaction Method. Hence, the number of putative reaction times stored are significantly decreased, resulting in a faster search for the shortest putative reaction time.

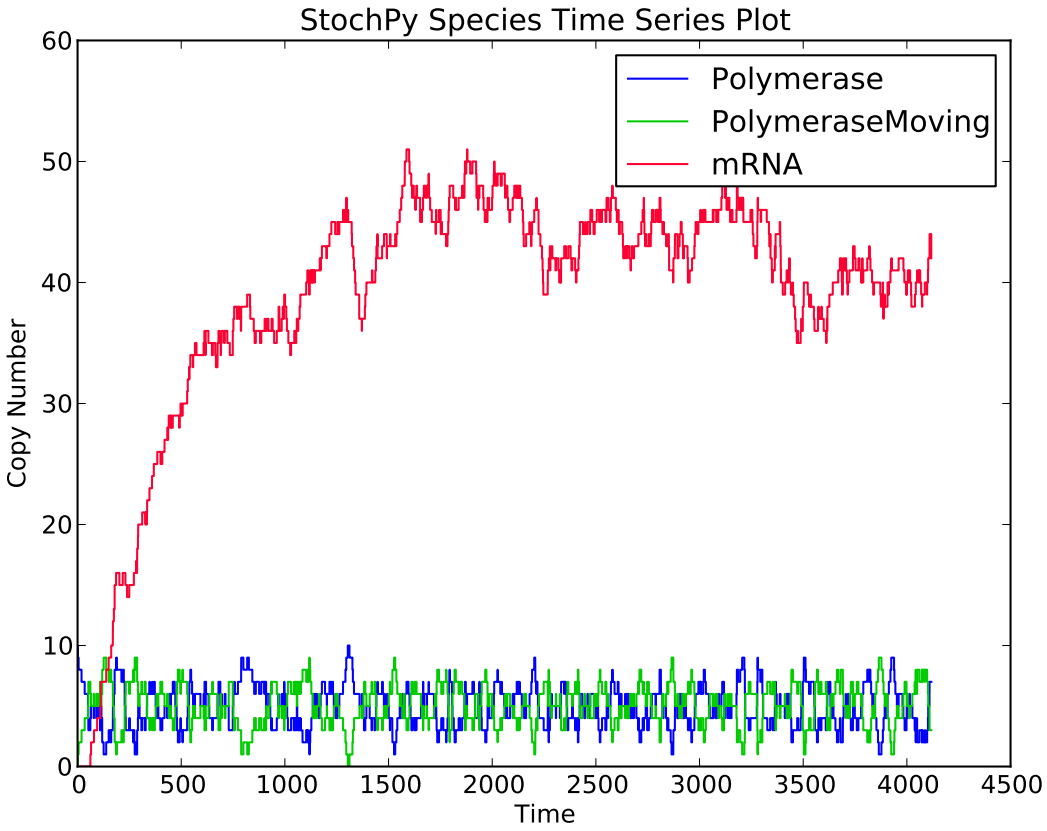
Before we use a simple example to illustrate the single molecule method, we first discuss a few important points:

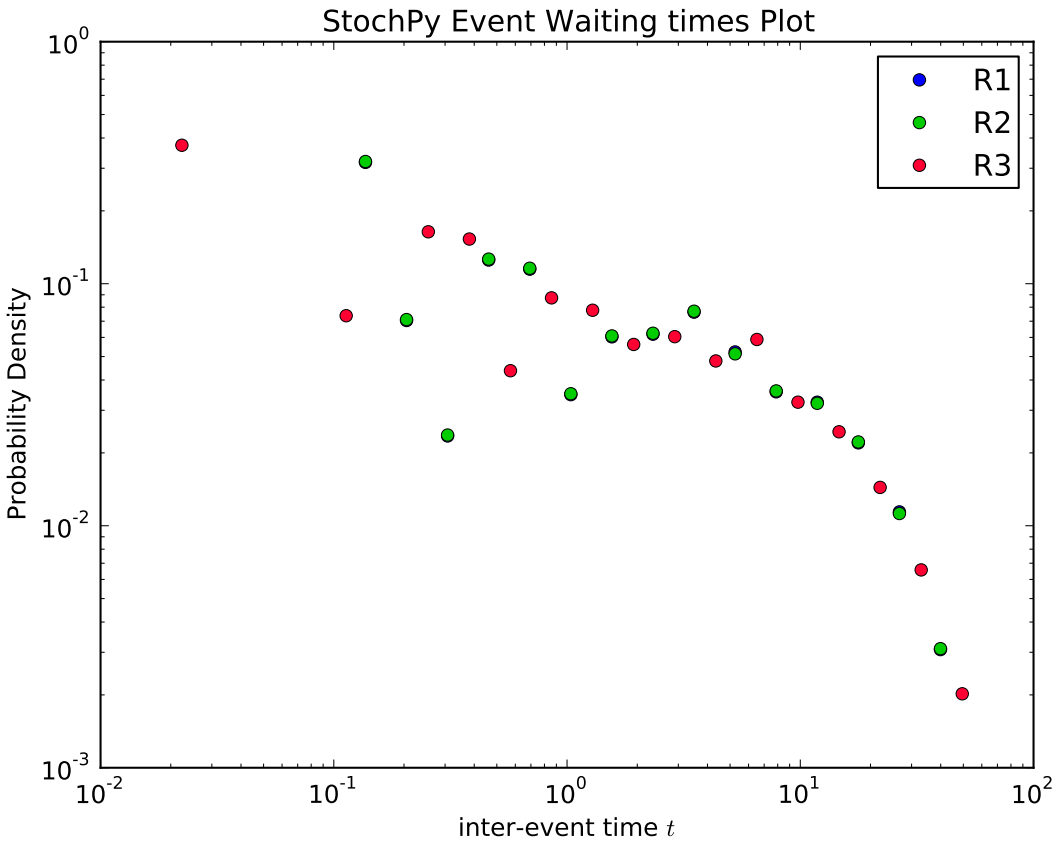
1. Do not use rate equations different than mass-action kinetics if you use the SMM method (e.g. dsmts-003-05 fails). The fSMM does support rate equations different than mass-action kinetics.
2. Do not use third-order rate equations in for both the SMM and the fSMM.

In this first example model, we explicitly model a intermediate form of the polymerase, polymerase moving. After exactly 60 seconds, the moving polymerase produces mRNA and is free again.

```
>>> smod.Model("TranscriptionIntermediate.psc")
>>> smod.SetPutativeReactionTimes({"R2":("fixed",60)})
>>> smod.DoSingleMoleculeStochSim(method = "fSMM")
>>> smod.PlotSpeciesTimeSeries()
>>> smod.PlotWaitingtimesDistributions()
```

Interestingly, the waiting times between subsequent firings of R1 and R2 match; R2 fires exactly 60 time units after R1 fires. In a delayed-SSA, the intermediate form is not modeled explicitly.

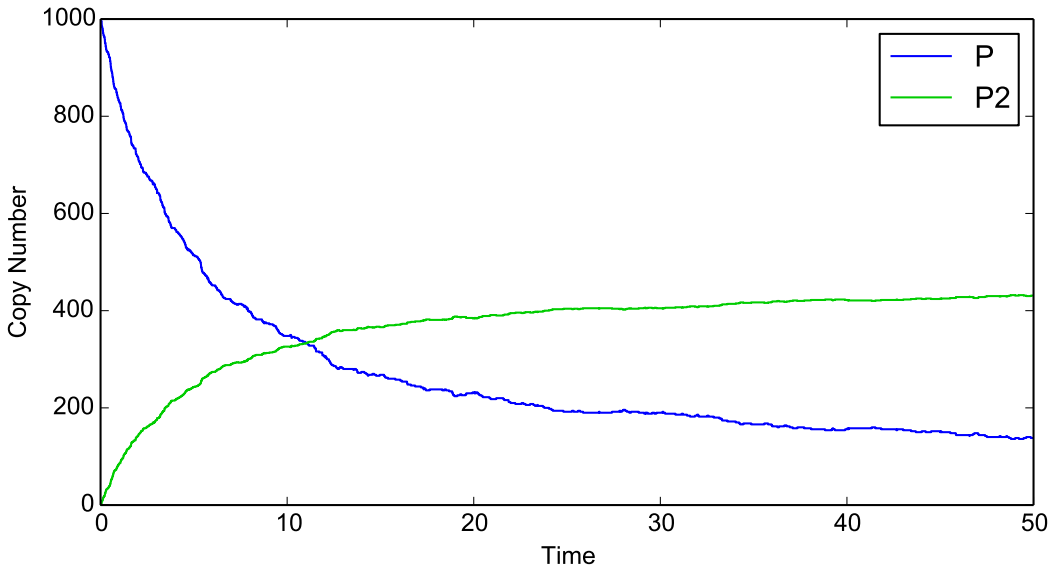

StochPy Species Time Series Plot
Polymerase
PolymeraseMoving
mRNA
Copy Number
Time
0
10
20
30
40
50
60
0
500
1000
1500
2000
2500
3000
3500
4000
4500

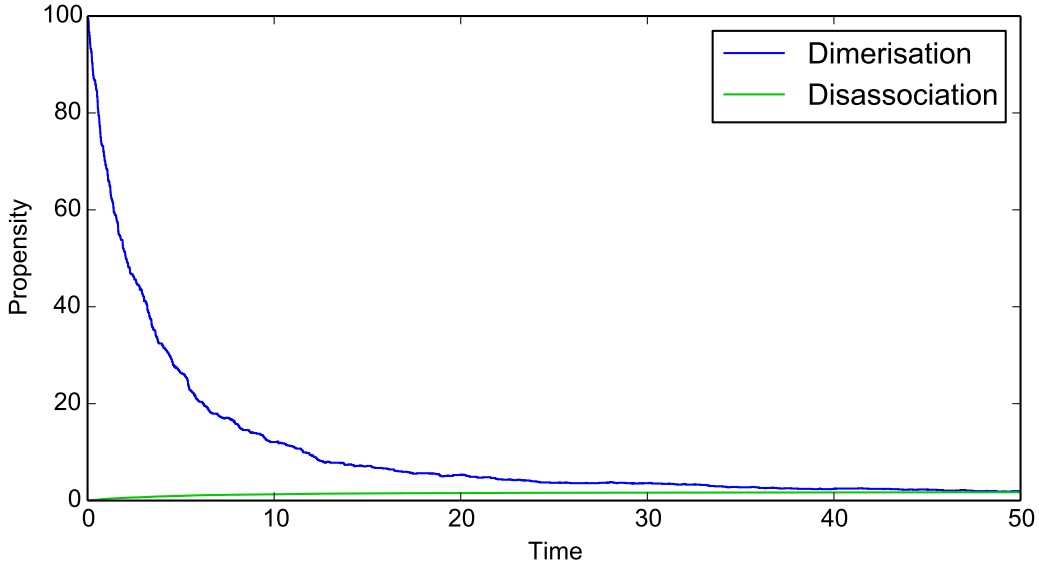

StochPy Event Waiting times Plot
R1
R2
R3
Probability Density
inter-event time t
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}

5.6 Examples

Stochastic simulations are done with a dimerization model to illustrate how you can use the stochastic simulation algorithm module. This model contains two species, denoted by P and P2:

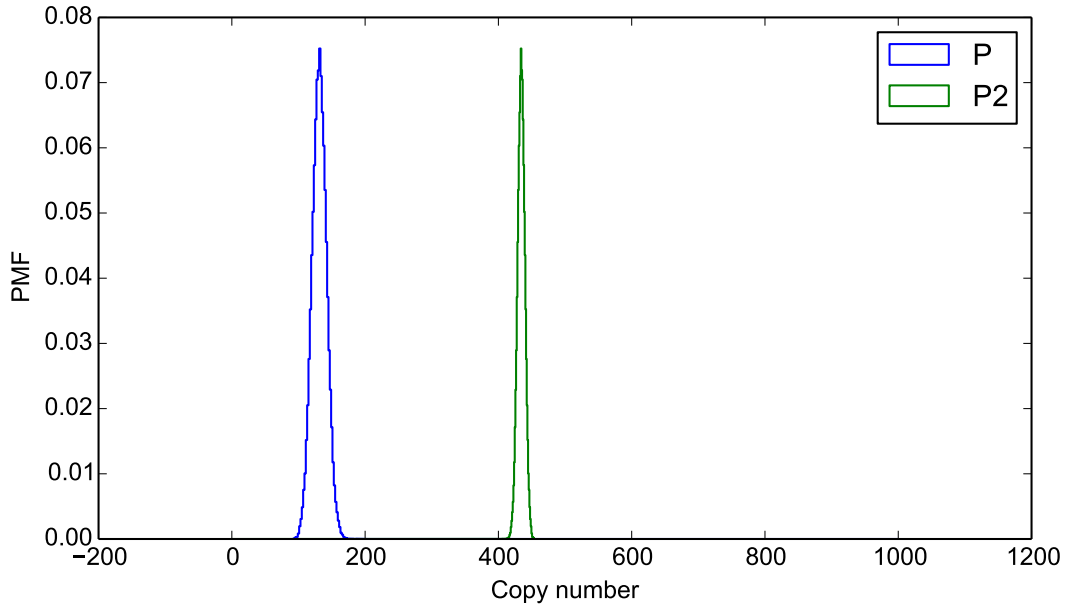
```
>>> import stochpy
>>> smod = stochpy.SSA()
>>> smod.Model("dsmts-003-02.xml.psc")
>>> smod.DoStochSim(end = 50,mode = "time",IsTrackPropensities=True)
>>> smod.PlotSpeciesTimeSeries()
>>> smod.PlotPropensitiesTimeSeries()
```

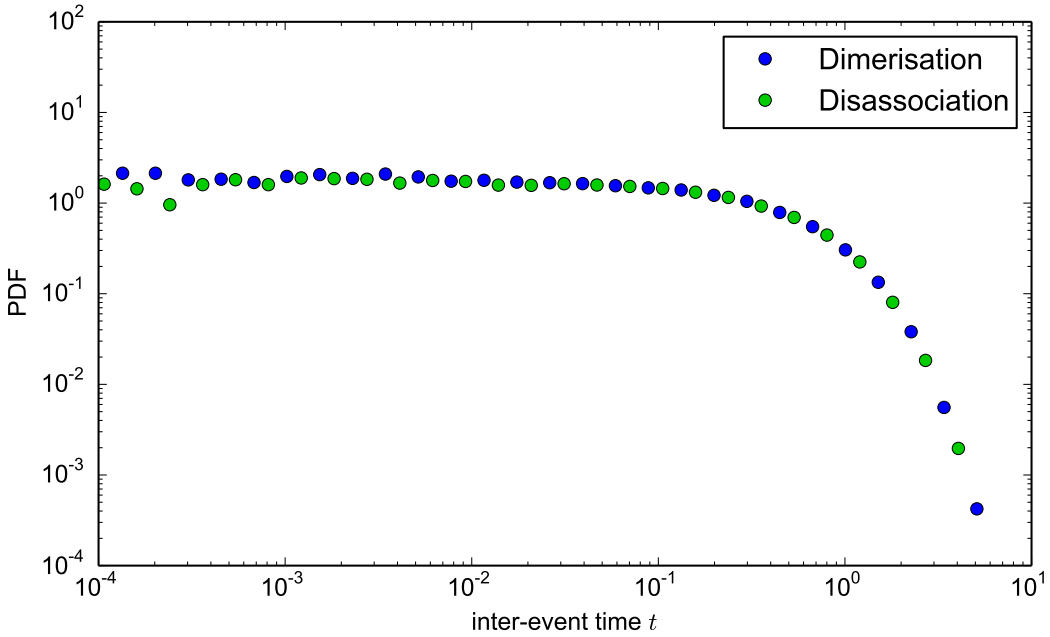




Doing a simulation for more time steps for allows the calculation of more accurate species distributions and event waiting time distributions:

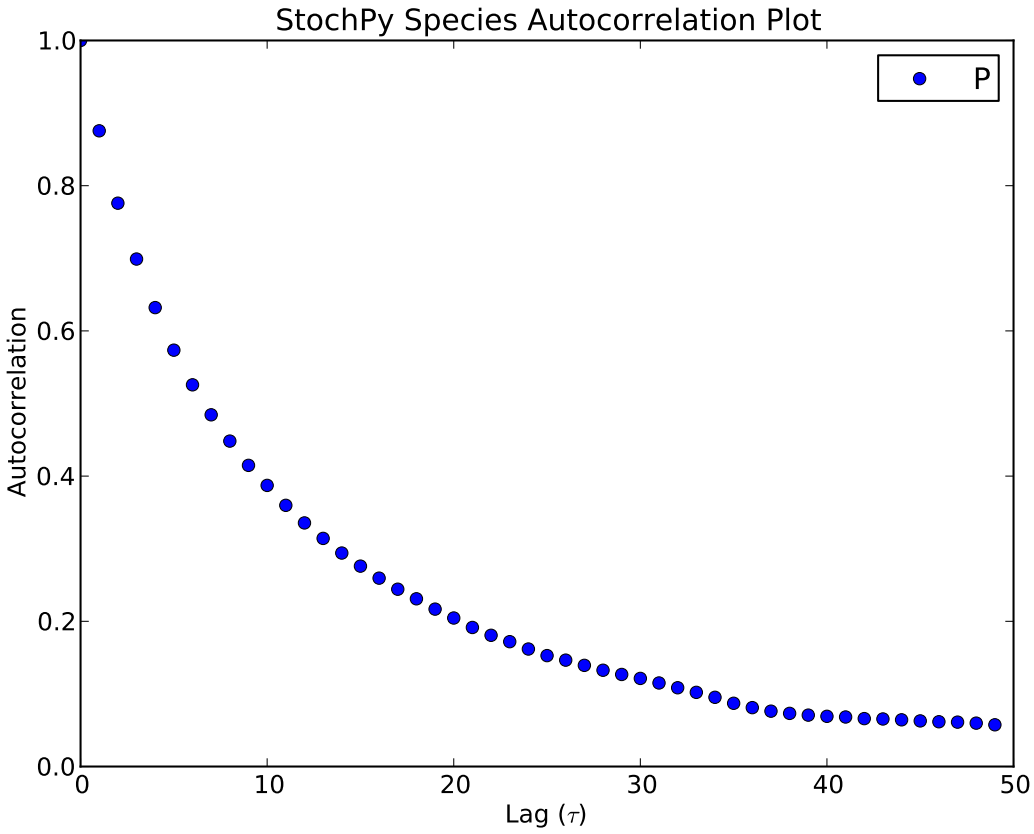
```
>>> smod.DoStochSim(end=5000,mode="time",IsTrackPropensities=True)
>>> smod.PlotSpeciesDistributions(species2plot="P",bin_size=2,colors="blue")
>>> smod.plot.plotnum-=1  # reset figure number
>>> smod.PlotSpeciesDistributions(species2plot="P2",bin_size=1,colors="green")
>>> stochpy.plt.legend(['P','P2'])
>>> smod.PlotWaitingtimesDistributions()
```

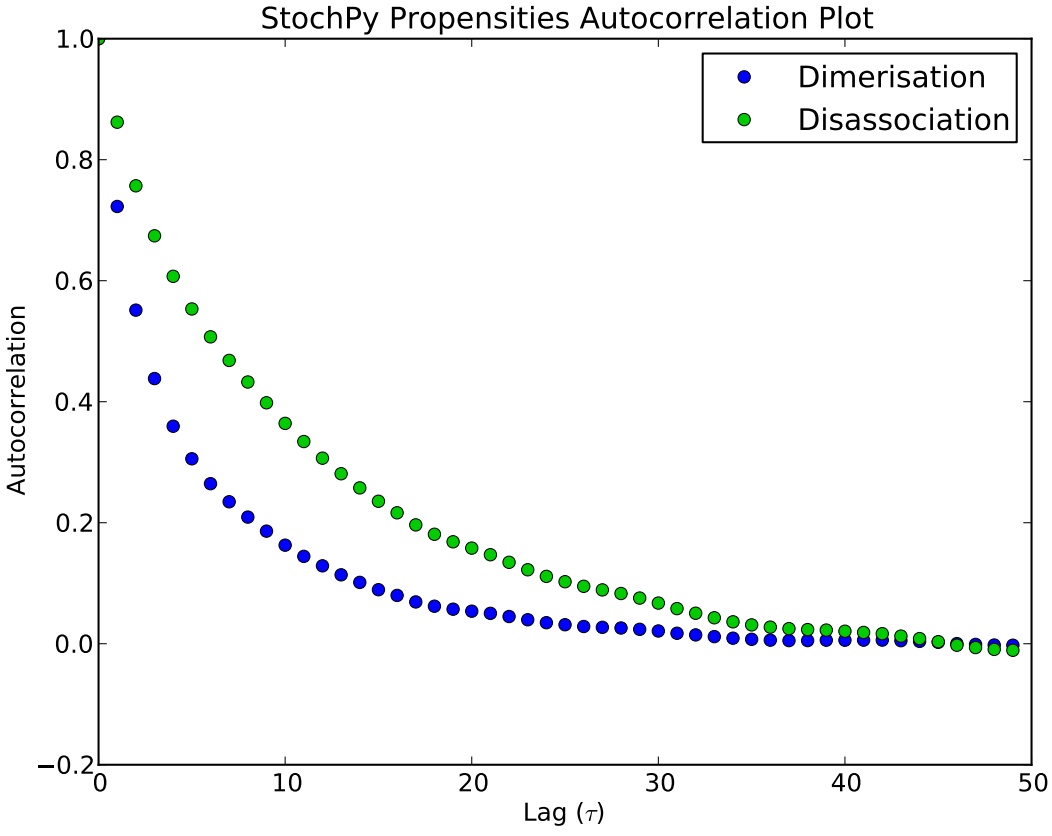




Finally, autocorrelations for both species and propensities can be determined:

```
>>> smod.GetSpeciesAutocorrelations()
>>> smod.GetPropensitiesAutocorrelations()
>>> smod.PlotSpeciesAutocorrelations(species2plot="P",nlags=50)
>>> smod.PlotPropensitiesAutocorrelations(nlags=50)
```


StochPy Species Autocorrelation Plot
P
Autocorrelation
Lag (τ)
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50


StochPy Propensities Autocorrelation Plot
Dimerisation
Disassociation
Autocorrelation
Lag (τ)
−0.2
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50

5.7 Reducing Memory Usage

StochPy returns the "raw" stochastic simulation output, i.e. for each firing time we store the copy number of each species and if desired also the propensity of each reaction. This can result in memory issues for models with many species (and reactions). For this reason, StochPy provides several ways to reduce the amount of stored data. We provide two arguments: *species_selection* and *rate_selection* which can be used to specify for which species and rates we store its copy number and propensity at each firing time. By default, we store this information for each species and for none of the rates. Adding *IsTrackPropensities* =True as argument results in storing this information for every rate. Here, we provide an example of a model that consists of 50 species and 98 reactions. We store two species and the propensities of two reactions.

```
>>> smod.Trajectories(1)
>>> smod.Model("chain50.psc")
>>> smod.DoStochSim(species_selection=["S1","S5"],rate_selection=["R1","R15"])
>>> smod.data_stochsim.species_labels
 ["S1", "S5"]
>>> smod.data_stochsim.species
array([[ 1.,  0.],
    [ 0.,  0.],
    [ 0.,  0.],
    ...,
    [ 0.,  0.],
    [ 0.,  0.],
    [ 0.,  0.]])
>>>  smod.data_stochsim.propensities
array([[ 1.,  0.],
    [ 0.,  0.],
    [ 0.,  0.],
    ...,
    [ 0.,  0.],
    [ 0.,  0.],
    [ 0.,  0.]])
>>>  smod.data_stochsim.propensities_labels
 ["R1", "R15"]
```

In addition, the boolean *IsOnlyLastTimepoint* can be used to store only the last time point of the simulation. When *IsOnlyLastTimepoint* =True, many functionalities such as plotting and determining statistics are disabled.

```
>>> smod.Model("ImmigrationDeath.psc")
>>> smod.DoStochSim(end=50,mode="time",IsOnlyLastTimepoint = True)
>>> smod.data_stochsim.species # molecule copy number at (t=50)
    array([[55.]])
>>> smod.data_stochsim.time # stored time points (t=50)
    array([[50.]])
```

5.8 Quiet Modus

The StochPy high-level functionalities can print information which can be useful for (inexperienced) StochPy users.

```
>>> smod = stochpy.SSA(IsQuiet=False)
Info: Direct method is selected to perform
Parsing file: /home/timo/Stochpy/pscmodels/ImmigrationDeath.psc
Info: No reagents have been fixed
>>> smod.DoStochSim()
Info: 1 trajectory is generated
simulation done!
Info: Number of time steps 1000 End time 510.780391803
Info: Simulation time 0.08498
```

StochPy 2.3 provides a quiet modus (default = True). In the quiet modus, only essential information is printed:

```
>>> smod = stochpy.SSA()
>>> smod.SetQuiet()
>>> smod.DoStochSim()

>>> smod = stochpy.SSA(IsQuiet=True)
>>> smod.DoStochSim()
```

or alternatively:

```
>>> smod = stochpy.SSA()
>>> smod.DoStochSim(quiet=True)
```

This functionality also works in different StochPy modules.

5.9 Experiencing plotting problems

StochPy uses Matplotlib for plotting. If Windows is your operating system, interactive plotting with Matplotlib can result in figure freezing. If you encounter figure freezing, we suggest to try out different back-ends (http://matplotlib.org/faq/usage_faq.html#what-is-a-backend). TkAgg is probably the best for interactive plotting, but is not available on every operating system and/or python environment:

```
>>> stochpy.plt.switch_backend("TkAgg")
```

Different non-interactive back-ends are available (e.g. the "Agg"-backend):

```
>>> stochpy.plt.switch_backend("Agg")
>>> smod.DoStochSim()
>>> smod.PlotSpeciesTimeSeries() # no plot is shown
>>> stochpy.plt.savefig(stochpy.os.path.join(stochpy.output_dir,"test1.png")
```

If you switch to a non-interactive back-end, make sure to initialize the SSA object with *IsInteractive= False*:

```
>>> smod = stochpy.SSA(IsInteractive=False)
>>> smod.DoStochSim()
>>> smod.PlotSpeciesTimeSeries()
>>> stochpy.plt.savefig(stochpy.os.path.join(stochpy.output_dir,"test1.png")
>>> cmod = stochpy.CellDivision(IsInteractive=False)
```

Plotting in Windows:

- Canopy command prompt: switch to TkAgg
- Canopy interactive data-analysis environment: works nicely with the default Qt4Agg
- Spyder: works nicely with the default Qt4Agg
- Anaconda command prompt: interactive plotting does not work. Use Agg and/or *IsInteractive* =False.

CHAPTER

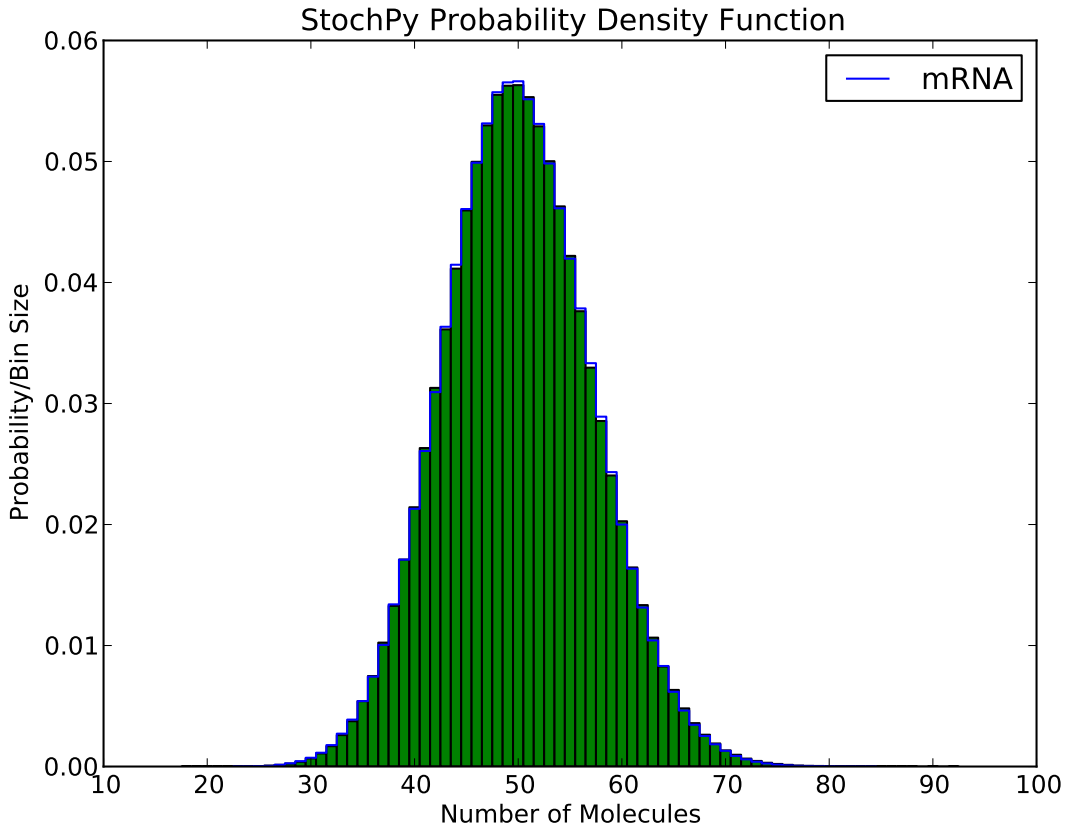
SIX

COMBINING STOCHPY WITH PYTHON SCIENTIFIC LIBRARIES

StochPy is written in Python, so users of StochPy can take advantage of e.g. the scientific libraries that are available in Python. We illustrate this here with the immigration-death model. This immigration-death model contains one species, mRNA. mRNA is synthesized with a constant rate and degraded with a first-order reaction. The immigration-death model is one of the simplest examples of a Poisson process: A stochastic process where events occur continuously and independently of one another.

One of the characteristics of a Poisson process is that the mean and variance are identical. In this example, we use a synthesis rate of 10 and a degradation rate of 0.2, thus both the mean and variance are 50. Here, we first draw N samples from a Poisson distribution with mean = 50. Secondly, we perform a stochastic simulation for N time steps. The initial mRNA copy number is 50. After the simulation, we create a probability density function of the done stochastic simulation. Finally, we create a histogram of the randomly generated Poisson data and add this to the existing plot of the mRNA probability density function. If N is large enough (we use N = 2.5 million), both distributions should be almost identical.

```
>>> import numpy as np
>>> lambda_ = 50
>>> N = 2500000
>>> data = np.random.poisson(lambda_,N)
>>> smod.ChangeParameter("Ksyn",10)
>>> smod.ChangeParameter("Kdeg",0.2)
>>> smod.Model("ImmigrationDeath.psc")
>>> smod.DoStochSim(end=N,mode="steps")
>>> smod.PlotSpeciesDistributions(linestyle= "solid")
>>> n, bins, patches = stochpy.plt.hist(data, max(data)-min(data),
    normed=1, facecolor="green",align="left")
>>> smod.PrintSpeciesMeans()
mRNA  49.912
>>> smod.PrintSpeciesStandardDeviations()
Species       Standard Deviation
mRNA  7.073
```


StochPy Probability Density Function
mRNA
Probability/Bin Size
Number of Molecules
0.00
0.01
0.02
0.03
0.04
0.05
0.06
10
20
30
40
50
60
70
80
90
100

CHAPTER

SEVEN

USING STOCHPY AS A LIBRARY

It is straightforward to use StochPy as a library in your project. A data object, data_stochsim, is available for those that want to use StochPy as a library or for those that want to do their own analysis. Species, distributions, propensities, simulation time, and waiting times are stored in NumPy arrays and lists. Labels are stored for each of these data types in separate lists. Furthermore, species, propensities, and waiting time statistics are stored in dictionaries and information about the stochastic simulation such as the number of time steps, the simulation end time, and the trajectory is stored. Data is, of course, only available if it is already calculated.

Returning explicit output can result in large data sets. Hence, if multiple time trajectories are generated the data object of each generated trajectory is dumped in a different file on the hard drive by using *pickle*, an algorithm for serializing and de-serializing a Python object structure. Once necessary for analysis, these data objects are automatically reloaded into StochPy. To improve performance propensities data is not stored by default. Time series visualization can be time-consuming if large data sets are generated. To improve the visualization performance of time series data, by default, not more than 10000 time series points are shown (but you are free to modify this). The high-level function *GetTrajectoryData()* can be used to access the simulation data of a specific trajectory. By default the latest generated trajectory is not written to disk space, thus accessible without using the high-level function *GetTrajectoryData()*:

```
>>> import stochpy
>>> smod = stochpy.SSA()
>>> smod.DoStochSim(trajectories = 10,mode = "steps",end = 1000)
>>> smod.data_stochsim
<stochpy.PyscesMiniModel.IntegrationStochasticDataObj object at 0x32cd750>
>>> smod.data_stochsim.simulation_trajectory
10
>>> smod.data_stochsim.time          # time array (not shown)
>>> smod.data_stochsim.species       # species array (not shown)
>>> smod.data_stochsim.species_labels
>>> smod.data_stochsim.getSpecies()  # time + species array (not shown)
>>> smod.GetSpeciesMeans()           # for each species
>>> smod.data_stochsim.species_means
>>> smod.data_stochsim.species_distributions # for each species (not shown)
>>> smod.GetWaitingtimes()
>>> smod.data_stochsim.waiting_times # for each reaction (not shown)
>>> smod.GetTrajectoryData(5)
>>> smod.data_stochsim.simulation_trajectory
5
```

```
>>> smod.data_stochsim.getDataInTimeInterval(10,5)
Searching (5:10:15)
Out[10]:
array([[   5.00059882,  224.        ],
       [   5.01493943,  223.        ],
       [   5.0151249 ,  224.        ],
       ...,
       [  14.95863992,  221.        ],
       [  14.96716153,  220.        ],
       [  14.99282164,  219.        ]])
```

A second data object (data_stochsim_grid) is available that stores data of multiple trajectories:

```
>>> smod.GetRegularGrid()
>>> smod.data_stochsim_grid.time  # time array (not shown)
>>> smod.data_stochsim_grid.species_means # at every t (not shown)
>>> smod.data_stochsim_grid.species_standard_deviations  # STDs (not shown)
```

CHAPTER

EIGHT

GETTING FIXED-INTERVAL OUTPUT

StochPy's solvers return the raw stochastic simulation output which makes it slower than simulators that return fixed-interval output. Cain and StochKit are examples of stochastic simulators that return fixed-interval output. Returning fixed-interval output results in losing all information about the time between events. Therefore, it is not possible to get information about, for instance, event waiting times.

The number of fixed-intervals, chosen by the user, determines the accuracy of the simulation results. As an example, creating accurate probability distributions of molecule copy numbers requires a number of fixed-intervals similar to the number of time steps in the simulation. Many simulations are required to determine the right number of fixed-interval to obtain accurate probability distributions. On the other hand, fixed-interval output is useful to get an indication of the behavior of your model or to quickly obtain an accurate estimate of the species means and standard deviations.

Because of the flexible design of StochPy, we decided to offer users of StochPy interfaces to both Cain and StochKit solvers (successfully tested with StochKit version 2.0.11). Users can benefit from the speed advantage of the Cain and StochKit solvers in the interactive modeling environment of StochPy. Subsequently, analysis can be done within StochPy. These solvers give wrong output if net stoichiometric coefficients are used in the model description.

To use Cain and StochKit inside of StochPy, one needs to download and install the interfaces InterfaceCain and InterfaceStochKit, respectively. StochKit solvers cannot be used without an installation of StochKit. In addition, usage of StochKit in StochPy requires modification of "InterfaceStochKit.ini" (before installation or after installation in the installation directory of StochPy). After installation of these interfaces, one can use the high-levels functions *DoCainStochSim()* and *DoStochKitStochSim()* to use the faster fixed-interval solvers of Cain and StochKit. *DoCainStochSim()* accepts the following arguments:

```
>>> smod.DoCainStochSim(endtime=100,frames=10000,trajectories=False,
                        solver="HomogeneousDirect2DSearch",
                        IsTrackPropensities=False)
```

The interface to Cain provides the solvers HomogeneousDirect2DSearch and HomogeneousFirstReaction, but in principle other solvers could be used too. *DoStochKitStochSim()* accepts the following arguments:

```
>>> smod.DoStochKitStochSim(endtime=100,frames=10**4,trajectories=False,
                    IsTrackPropensities=True,customized_reactions=None,
                    solver=None,keep_stats=False,keep_histograms=False)
```

```
>>> smod.PlotSpeciesTimeSeries()
>>> smod.PlotPropensitiesTimeSeries()
```

This means that we can save the statistics and histograms information that is created by StochKit, but StochPy does not use this data. StochPy offers both the direct method and the tau-leap method of StochKit. These high-level functions can be used in the same way as *DoStochSim()*:

```
>>> smod.DoCainStochSim()
>>> smod.DoStochKitStochSim()
StochKit MESSAGE: determining appropriate driver...
running $STOCHKIT_HOME/bin/  ssa_direct_small...
StochKit MESSAGE: created output directory
"/home/user/Stochpy/temp/out/ImmigrationDeath_stochkit.xml"...
running simulation...finished (simulation time approx. 0.0330269 s)
>>> smod.DoStochKitStochSim(solver="tau_leaping")
StochKit MESSAGE: determining appropriate driver...
running $STOCHKIT_HOME/bin/tau_leaping_exp_adapt...
StochKit MESSAGE: created output directory
"/home/user/Stochpy/temp/out/ImmigrationDeath_stochkit.xml"...
running simulation...finished (simulation time approx. 0.02966 s)
done!
```

After a simulation is performed with the Cain or StochKit solvers, StochPy offers almost all data analysis functionalities. Remember that the data is fixed-interval so information about the time between events is completely lost. Both simulators, however, lack full support of events, assignments, SBML format, and reactions more complicated than mass-action kinetics. StochPy returns warnings and errors if problems arise with these simulators:

```
>>> smod.Model("CellDivision.psc")
>>> smod.DoStochKitStochSim(endtime=3,frames=29019,trajectories=1)
AssertionError:  Reaction R6 is not recognized as mass-action,
so add this reaction to the customized_reactions (list) flag
>>> smod.DoStochKitStochSim(endtime=3,frames=29019,customized_reactions=["R6"])
StochKit MESSAGE: determining appropriate driver...
running "c:\model\StochKit2.0.4_WINDOWS\bin\ssa_direct_mixed_small"...
StochKit MESSAGE: compiling generated code...
this will take a few moments...
StochKit MESSAGE: created output directory
"c:\Stochpy\stochkit_out\out\CellDivision_stochkit.xml"..
running simulation...finished (simulation time approx. 0.623178 seconds)
done!
```

In this example, compilation time of reactions that are not described by mass-action kinetics costs so much time that StochPy is almost five times faster:

```
>>> smod.DoStochSim(end=3,mode="time",trajectories=1) # Sim. time: 1.77 s
```

CHAPTER

NINE

STOCHASTIC TEST SUITE

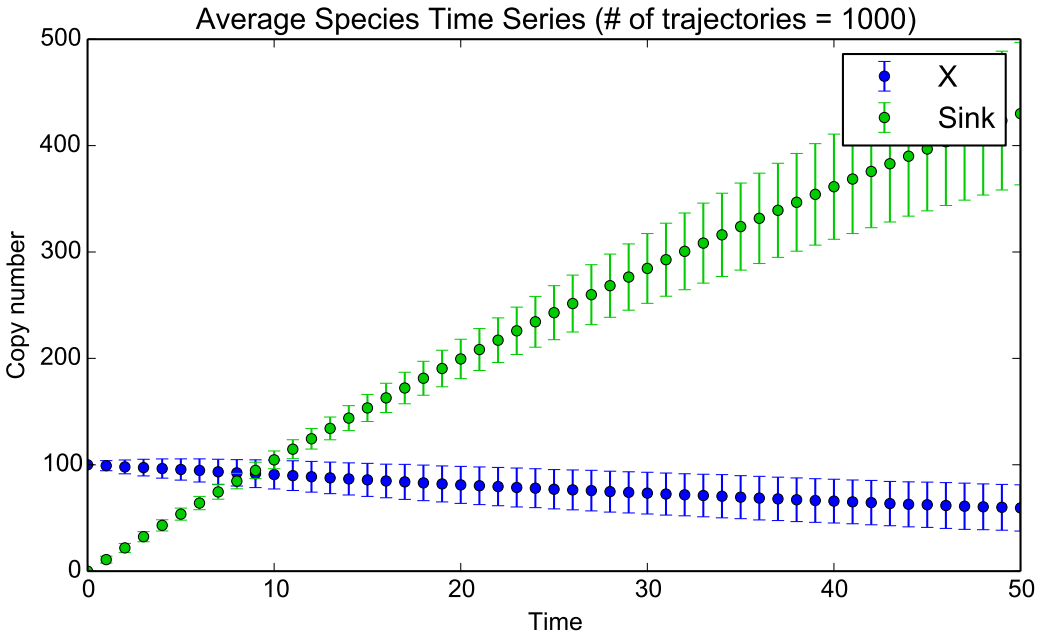
The stochastic test suite from Evans et al. 2008 (The SBML discrete stochastic models test suite) was used to test the algorithms implemented in StochPy. This test suite tests stochastic simulation software on the following points:

- local and global parameters (parameter overloading)
- boundary conditions
- cell compartment volume
- hasOnlySubstanceUnits flag
- math expression parsing
- compartment volume explicitly including in the rate laws
- assignment rules
- time events
- species population events

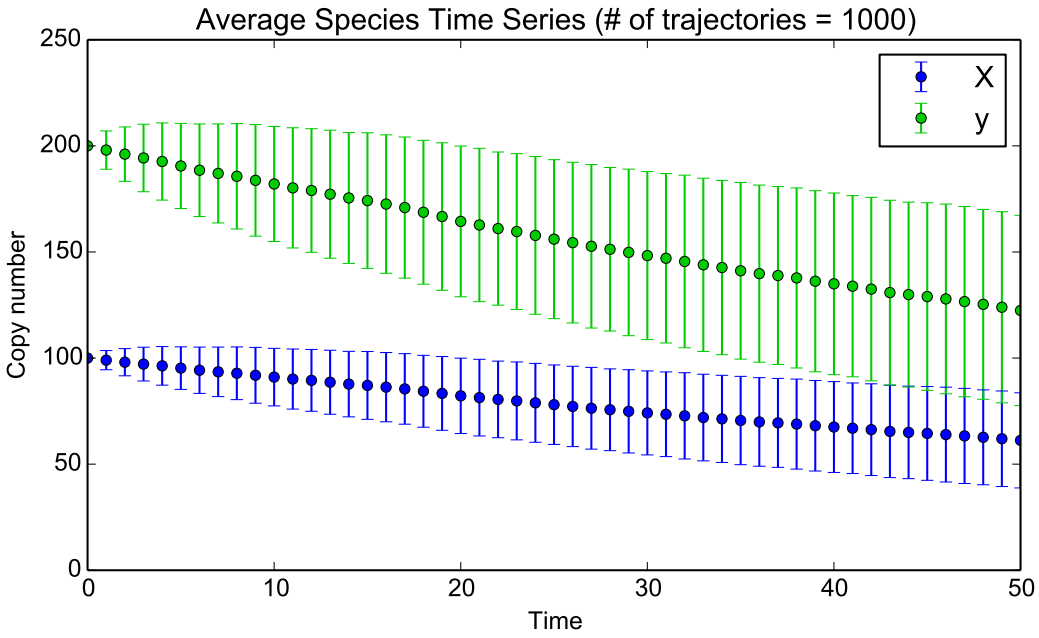
All 36 tests were successfully passed by StochPy, except test 3.4 where molecule copy numbers are reset whenever the copy number threshold is reached. A similar observation was done by Erhard et al. (2008). StochPy generates also different output for test 1.11. The reason is that StochPy converts species concentrations (HasOnlySubstanceUnits = True) to species copy numbers; species concentrations are multiplied by the volume of the compartment. Different rate equations are therefore obtained which results in different (but correct) output.

Some examples (1.7, 1.19, 3.3, and 3.4) of the stochastic test suite are shown below. These models test the stochastic simulator on multiple species, assignment rules, time events, and species copy number events respectively. A smaller epsilon value (0.01 rather than 0.03) was necessary to get an accurate prediction with the tau-leap method for 1.5.

```
>>> smod.Model("dsmts-001-07.xml.psc")
>>> smod.DoStochSim(end=50,mode="time",trajectories=1000)
>>> smod.GetRegularGrid()
>>> smod.PlotAverageSpeciesTimeSeries()
```

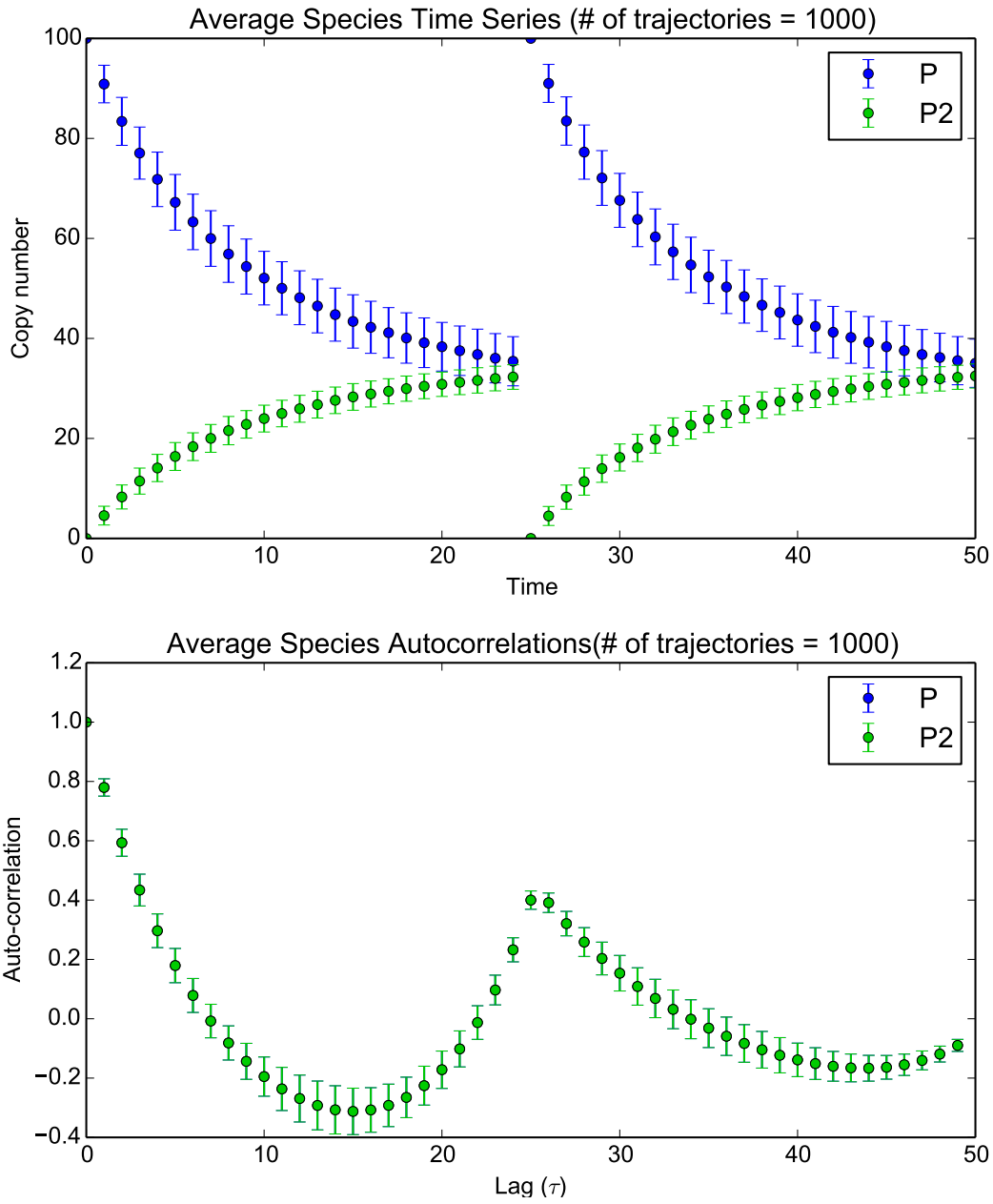



```
>>> smod.Model("dsmts-001-19.xml.psc")
>>> smod.DoStochSim(end=50,mode="time",trajectories=1000)
>>> smod.GetRegularGrid()
>>> smod.PlotAverageSpeciesTimeSeries()
```



StochPy can also calculate average autocorrelations. An example is shown for the models 3.3:

```
>>> smod.Model("dsmts-003-03.xml.psc")
>>> smod.DoStochSim(end=50,mode="time",trajectories=1000)
>>> smod.GetRegularGrid()
>>> smod.PlotAverageSpeciesTimeSeries()
>>> smod.GetSpeciesAutocorrelations()
>>> smod.PlotAverageSpeciesAutocorrelations()
```

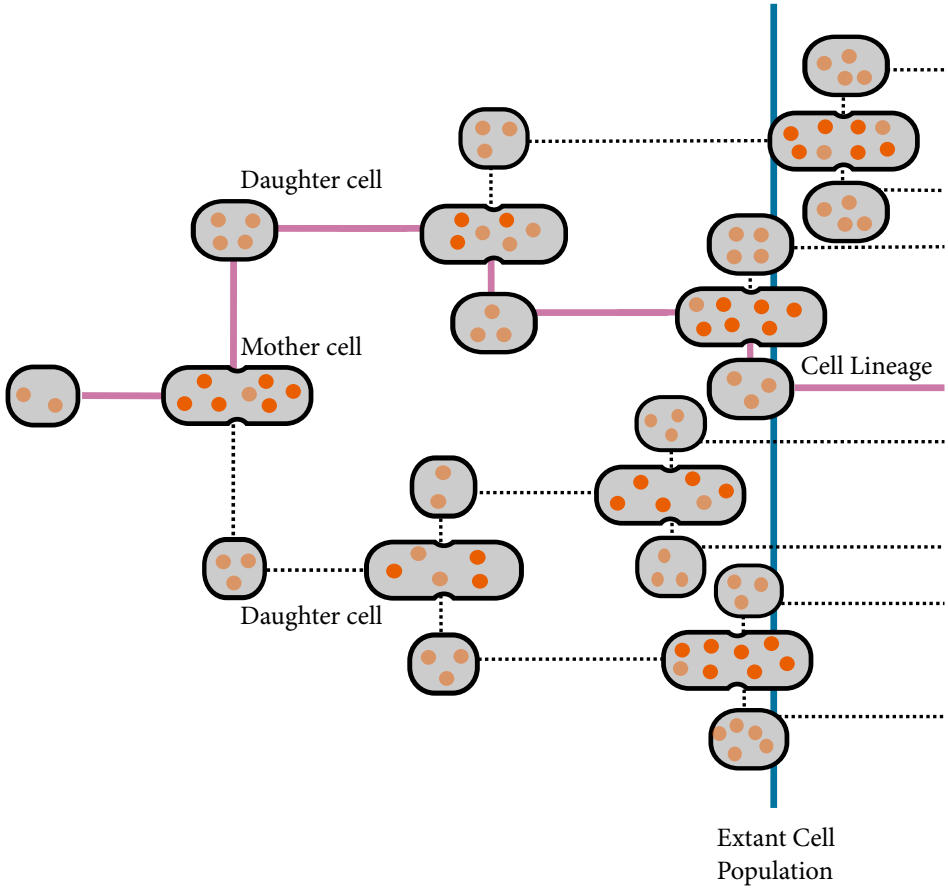

Average Species Time Series (# of trajectories = 1000)
Copy number
Time
P
P2
0
20
40
60
80
100
0
10
20
30
40
50
Average Species Autocorrelations(# of trajectories = 1000)
Auto-correlation
Lag (τ)
P
P2
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
0
10
20
30
40
50

CHAPTER

TEN

MODULE 4: CELL DIVISION

Cell-to-cell variability arises from the inherent stochasticity of biochemical reactions and of cell growth and division. We developed a stochastic simulation algorithm with cell growth and division which are available in StochPy's cell division module. While the number of cells increases with 2^n, our algorithm simulates the time evolution of one particular cell, i.e. we track one cell lineage over time. The simulated lineage must reflect a well-defined sample of cells in order to relate the statistical properties of this particular lineage to the statistical properties of the whole three or a well defined sample of cells.



Three different types of samples can be distinguished: samples of extant, baby and mother cells (see also Painter and Marr, 1968). Extant cells are all cells that exist at a specific moment in time, a sample of baby cells consists of all cells that are born within a defined time interval, and sample of mother cells consists of all cells that divide within a defined time interval. In our simulation, we simulate a lineage that represents a sample of mother cells.

StochPy's cell division module can be started with the following command:

```
>>> cmod = stochpy.CellDivision()
```

By default, StochPy uses the model "CellDivision.psc" which describes protein synthesis inside the cell. The StochPy cell division module continues simulating one cell lineage until the number of specified generations, time steps or end time is reached. Modeling of cell division is an example of sequential simulations where each generation starts with the output of the previous generation. This makes these sequential simulations more tricky than doing parallel simulations which is generally easy for most stochastic simulator software packages.

10.1 Specifying growth and division properties

Doing a stochastic simulation with cell growth and division requires setting, amongst others, growth and division properties. An overview of these settings is shown in the table below. Without specifying these settings, StochPy exploits the default settings shown in the last column.

Description	StochPy Function	Mandatory Arguments (defaults)
Growth Function	SetGrowthFunction(growth_rate,growth_type)	(1.0, "exponential")
Initial Cell Volume	SetInitialVolume(initial_volume)	1.0
Exact Dividing Species	SetExactDividingSpecies(species)	[]
Non Dividing Species	SetNonDividingSpecies(species)	[]
Set Volume Dependencies	SetVolumeDependencies(IsVolumeDependent, VolumeDependencies,SpeciesExtracellular)	True, [],[]
Division properties	SetVolumeDistributions(Phi,K)	("beta",5,5), ("beta",2,2),2)

Most functionalities are relatively straightforward to use, but we discuss each of them here extensively.

- **SetGrowthFunction()** sets the specific volume growth rate and the type of growth (exponential or linear).
- **SetInitialVolume()** sets the initial cell volume (float).
- **SetExactDividingSpecies()** sets exact dividing species. An example of exact dividing species are chromosomes: In a regular cell division event both daughter cells get one.
- ***SetNonDividingSpecies()** sets non-dividing species. Specifying non-dividing species can be useful for modeling e.g. DNA.
- **SetVolumeDependencies()** can be used to override volume dependencies (StochPy determines the order of each reaction automatically). Additionally, this function can be

used to set extracellular species. That is, species that are not volume dependent at all and for which no concentrations can be calculated. By default, all species are assumed to be intracellular (and therefore volume dependent if they are second or higher-order reactions).

- **SetVolumeDistributions()** sets division properties of the cell division volumes and the partitioning distribution between both daughter cells. *Phi* corresponds to the cell volume distribution at division for a sample of mother cells and *K* to the partition distribution (Vdaughter1 = Vmother*K and Vdaughter2 = Vmother - Vdaugther1).

The behavior of *Phi* and *K* arguments used in *SetVolumeDistributions()* depends on what type of distribution is provided.

1. In the case where both distributions are not beta distributions (all probability distributions from NumPy are available):

- *Phi* = ("normal",3,0.1): division volume is normally distributed around 3 with a standard deviation of 0.1;
- *Phi* = ("fixed,2): division volume is fixed at 2; and
- *K* = ("fixed,0.75): the volume of the first daughter is always 3/4 of the volume at dvivision and the volume of the second daughter is always 1/4 of the volume at division.

This means that, for instance, *Phi* = ("fixed", 2) and *K* = ("fixed",0.5) yields a cell lineage where each cells grows from a volume of 1 to 2 (assuming that we also have a initial cell volume of 1). For both distributions, we use by default a beta distribution which is defined on the interval [0,1]. This beta-distribution is symmetrical around 0.5 if both shape parameters are identical.

2. In the case where *Phi* or *K* are beta distributions:

- *Phi* = ("beta",1,1): uniform distribution around the specified mean;
- *Phi* = ("beta",2,2): relatively broad distribution around the specified mean; and
- *K* = ("beta",100,100): relatively narrow partitioning distribution.

3. If both *Phi* and *K* are beta distributions, *SetVolumeDistributions()* requires a third argument that represents the mean of the division volume of the mother cell.

- *Phi_beta_mean* = 2: a desired volume at division of 2; and
- *Phi_beta_mean* = 5: a desired volume at division of 5.

We now illustrate how we use these beta distributions:

```
>>> SetVolumeDistributions(Phi=("beta",5,5),K=("beta",2,2),Phi_beta_mean=2)
```

In this example, we set a broad distribution for both *Phi* and *K*, and specified a mean of 2. The latter means that the cell division event is triggered on average when the cell volume equals 2. We first calculate the Phi_shift: Phi_shift = *Phi_beta_mean* - 0.5 = 2 -0.5 = 1.5. Next, we can determine the distributions for the mother volume at cell division

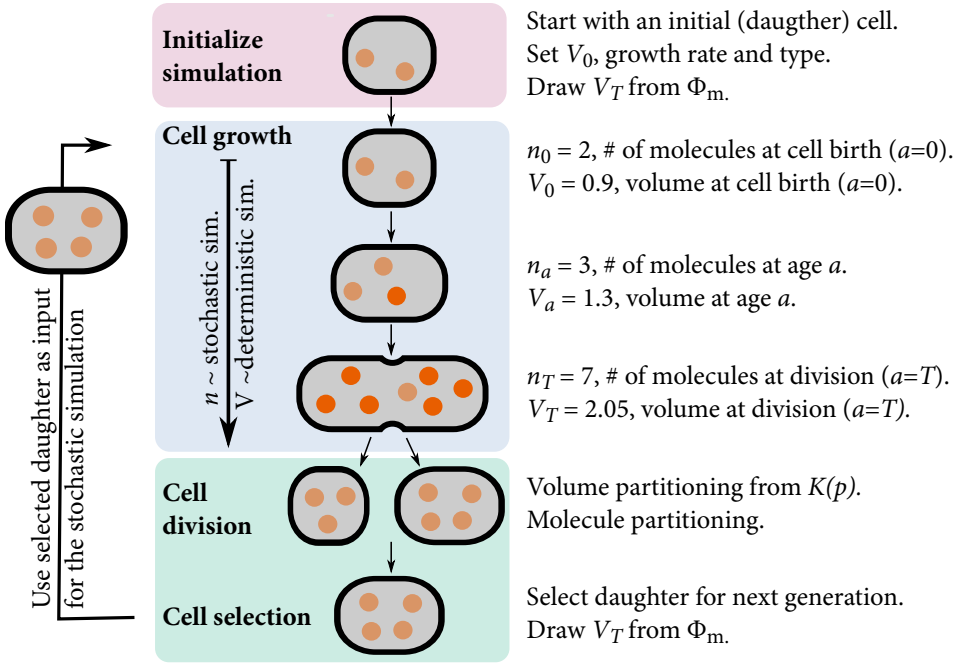
and the partitioning distribution

From this, we can calculate the daughter volume just after the cell division event.

In summary, the cell divides at a volume between [1.5,2.5] and the volume at birth is between [0.6,1.5] for these settings.

10.2 Run a SSA with cell growth and division

After specifying the (default) settings, we can start a stochastic simulation algorithm with explicit cell division in. An overview of this approach is shown in the following figure.



This simulation starts with drawing a next division volume from the specified volume distribution for a sample of mother cells. StochPy subsequently calculates the interdivision time (T) which follows from the chosen volume growth rate, type of growth function, the volume at birth and the next division volume. Assuming that we run a stochastic simulation for a number of generations, we next run a stochastic simulation with cell growth until the cell division event. During the stochastic simulation, the cell volume increases which can have an effect on the likelihood of reactions to fire. When the cell volume at division (drawn from *Phi*) is reached, StochPy starts with partitioning the cell volume into two daughters. In addition, each species is binomially distributed (weighted by daughter cell volume) between both daughter cells. Then, a next division volume is drawn from *Psi* and one of the daughters is selected for the next generation. Here, the largest daughter cell is more likely to be selected, because this cell reaches the next division volume faster and is therefore likely to have more descendants. This process continues until StochPy reaches the specified number of generations, time steps or end time. An overview of method is shown above.

10.3 From a single lineage simulation to extant-cell population distributions

To generate a lineage that is representative for a sample of mother cells, at each division the daughter to be followed by the simulation is chosen with a probability according to the fraction of descendants it can be expected to contribute to the population. Additionally, the following two conditions should be satisfied:

1. The first condition requires that cell volume distributions at division and birth are independent. Dependency occurs when a volume at birth is larger than a volume at division, and thus not all volumes from volume distribution at division can be chosen. Independence can be achieved by choosing *Phi* and *K* such that there is no overlap possible between cell volumes at division and birth. The simplest way to do this is by choosing a beta distribution for both *Phi* and *K*, because this distribution is bounded between 0 and 1 (in contrast to a normal distribution, which is has no bounds).
2. The growth law for a single cell is deterministic and independent of concentrations. This is guaranteed by the algorithm.

After simulating the single lineage that is representative for a sample of mother cells, statistical properties of other defined samples (extant, baby) can be calculated with the known relationships of interdivision times and cell age between these samples (more details can be found in our manuscript). In the next section, we provide three examples. In the second and third example, we illustrate analyzing statistical properties for especially the extant cell distribution.

10.4 Examples

Example 1

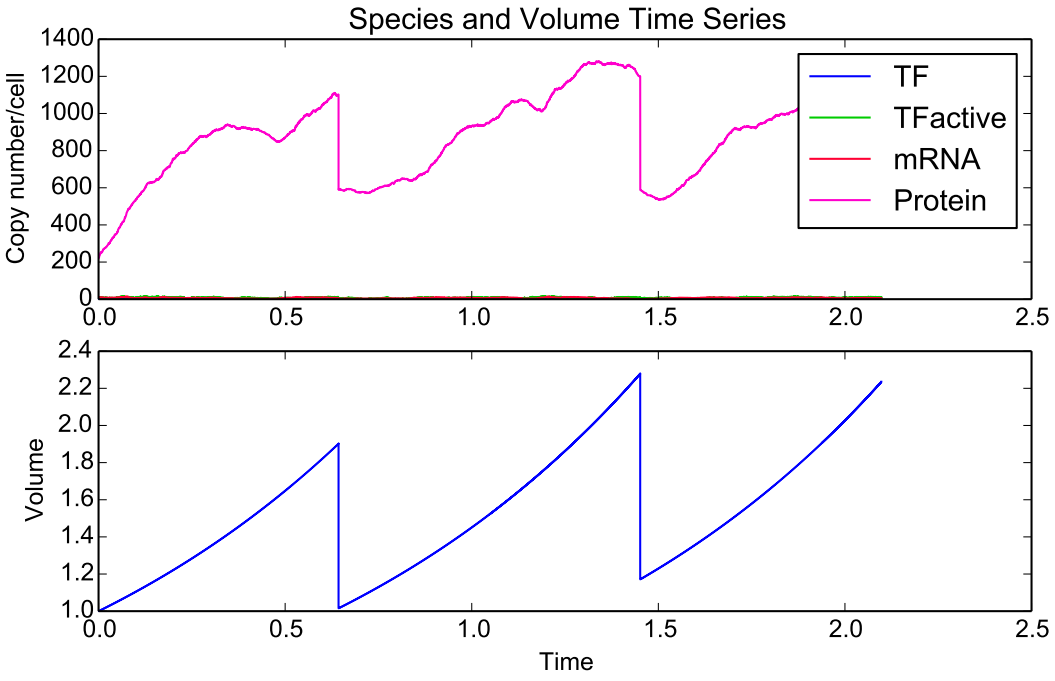
We demonstrate the incorporation of cell division for different example models. The first example considers the model "CellDivision.psc" with all default specifications (growth rate, initial cell volume etc.).:

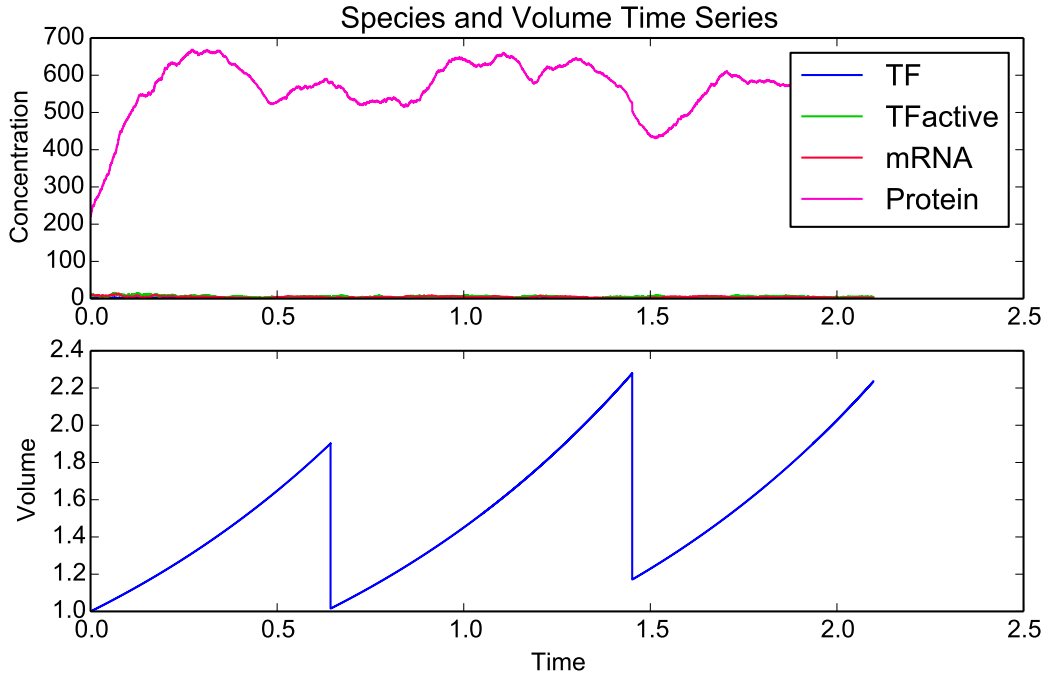
```
>>> cmod = stochpy.CellDivision()
>>> cmod.DoCellDivisionStochSim()
```

This model consists of four species, TF, TFactive, mRNA, and Protein. In addition to the existing high-level functions of the stochastic simulation module, several new functionalities are provided within the cell division module. An example is plotting of both species and volume time series. StochPy allows plotting molecule copy numbers and concentrations:

```
>>> cmod.PlotSpeciesVolumeTimeSeries()
>>> cmod.PlotSpeciesVolumeTimeSeries("concentrations")
```

The molecule copy numbers plot nicely illustrates illustrates the effect of cell division where each species is binomially distributed between two daughter cells.





In the volume time series plot, we show the cell volume you expect based on a deterministic growth rate. Importantly, in the stochastic simulation cell volume is only updated if a reaction fires. This means that we underestimate the time until a volume-dependent reaction fires. The reason is that we calculate the volume-dependent propensities with a volume smaller than the volume at the time of the reaction. If we correct for additional volume growth, we would get on average an increased time until firing for these volume-dependent reactions. However, this error is typically very small, because most models have at least one reaction that fires frequently. The error can even be zero if there are no second or higher-order reactions (zero and first order reactions are volume independent).

We can do any type of analysis that is also possible in the *SSA module*.

Example 2

Now, we use the "ImmigrationDeath.psc" model to illustrate some more functionalities of the cell division module. First, we select the model and modify it interactively:

```
>>> cmod = stochpy.CellDivision()
>>> cmod.Model("ImmigrationDeath.psc")
>>> cmod.ChangeInitialSpeciesCopyNumber("mRNA",11)
>>> cmod.ChangeParameter("Ksyn",2)
>>> cmod.ChangeParameter("Kdeg",0.1)
```

Second, we set volume and growth statistics:

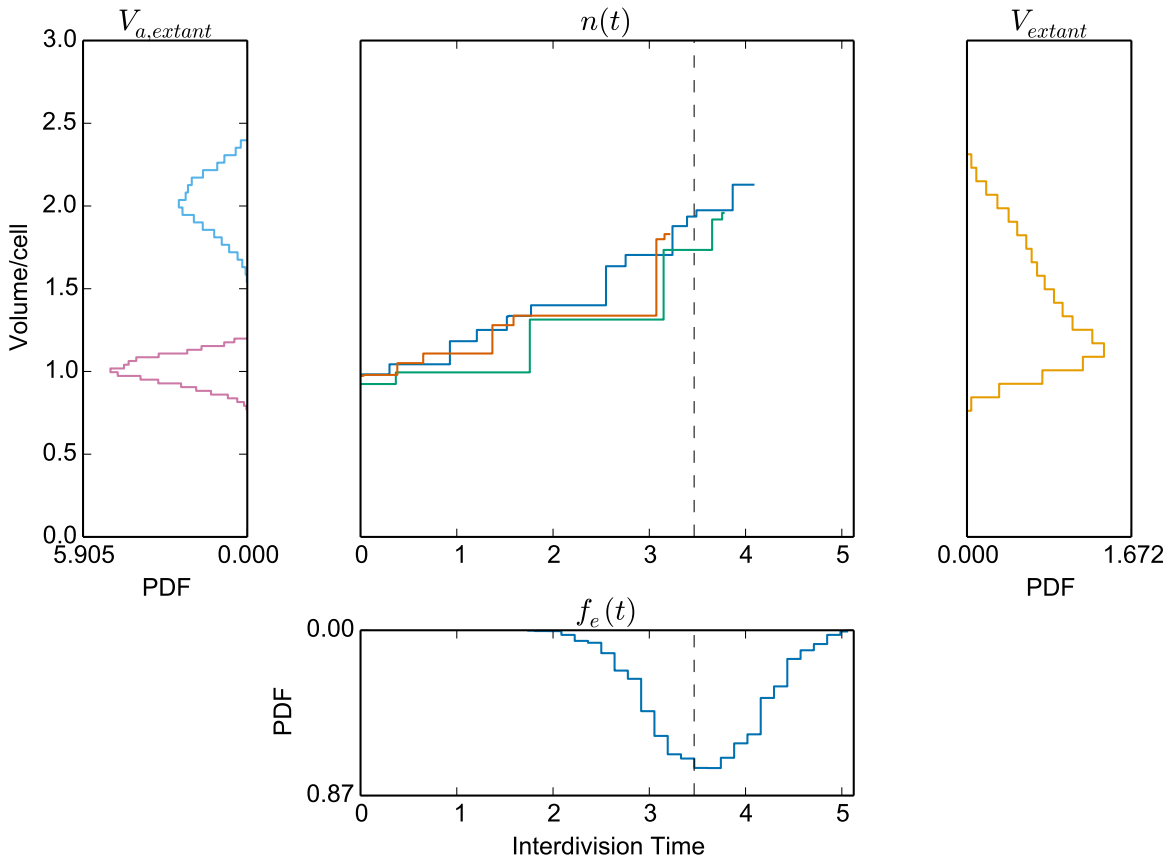
```
>>> cmod.SetVolumeDistributions(Phi=("beta",5,5), K=("fixed",0.5),
                                Phi_beta_mean=2)
>>> cmod.SetGrowthFunction(growth_rate=0.2, growth_type="exponential")
```

And thirdly, we perform a stochastic simulation with growth and cell division:

```
>>> cmod.DoCellDivisionStochSim(end=4000, mode="generations")
```

We generated 4000 generations to get relatively accurate statistical properties (we recommend using 10000 generations). The cell division module offers the analysis of both species and volume statistics. Here, we start with volume statistics:

```
>>> cmod.PlotVolumeOverview()
```



Here, we plot the volume distributions at birth and division for a sample of extant cells (left top

panel), the volume time series (center top panel), the volume distribution for a sample of extant cells (right top panel), and the interdivision times for a sample of extant cells (center bottom panel).

Binning and numerical integration is used to relate the sample of mother cells to different samples. StochPy prints a warning if the numerical integration was not accurate enough. Using *AnalyzeExtantCells()* with a different number of bins for both age and interdivision times should solve this issue.

```
>>> cmod.AnalyzeExtantCells(n_bins_age=20,n_bins_IDT=20,n_bins_volume=20)
```

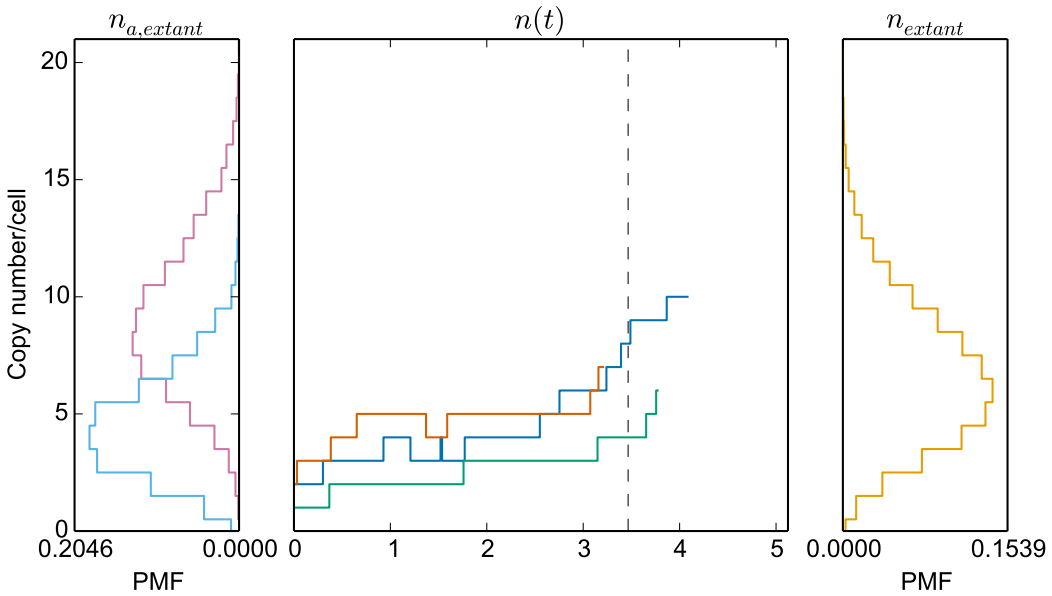
By specifying a different sample, the same plot is generated whereas for a different sample:

```
>>> cmod.PlotVolumeOverview(sample="mother")
>>> cmod.PlotVolumeOverview(sample="baby")
```

Plotting functions also exist to plot each of these panels individually: *PlotVolumeAtBirthDistribution()* , *PlotVolumeAtDivisionDistribution()*, *PlotVolumeTimeSeries()*, *PlotVolumeDistribution()*, and *PlotInterdivisionTimeDistribution()*.

For species, a similar high-level function exists:

```
>>> cmod.PlotSpeciesOverview()
```

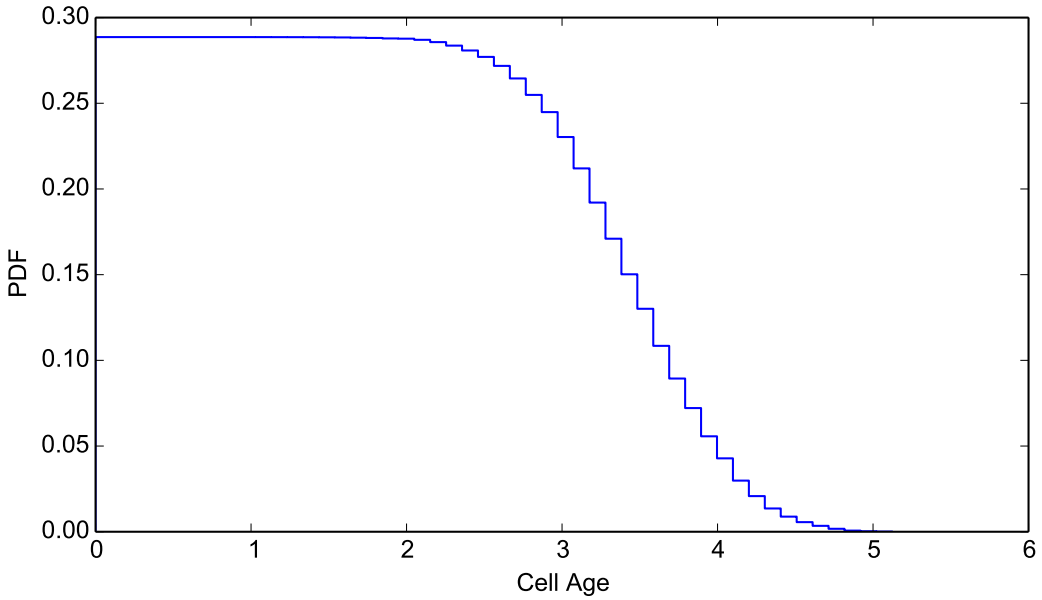


Here, we plot the species distribution at birth and division for a sample of extant cells (left panel), the species time series (center panel), and the species distribution for a sample of extant cells (right panel). Again, by specifying a different sample, the same plot is generated whereas for a different sample:

```
>>> cmod.PlotSpeciesOverview(sample="mother")
>>> cmod.PlotSpeciesOverview(sample="baby")
```

Also, plotting function exist to plot each of these panels individually: *PlotSpeciesAtBirthDistributions()* , *PlotSpeciesAtDivisionDistributions()*, *PlotSpeciesTimeSeries()*, *PlotSpeciesDistributions()*. Note that volume plots are singular and species plot plural; there is only one cell volume and there can be different molecular species inside the cell.

```
>>> cmod.PlotCellAgeDistribution()
```



We can also calculate the mean and standard deviation of each species for samples of extant and mother cells. Again, StochPy returns by default the statistics for a sample of extant cells:

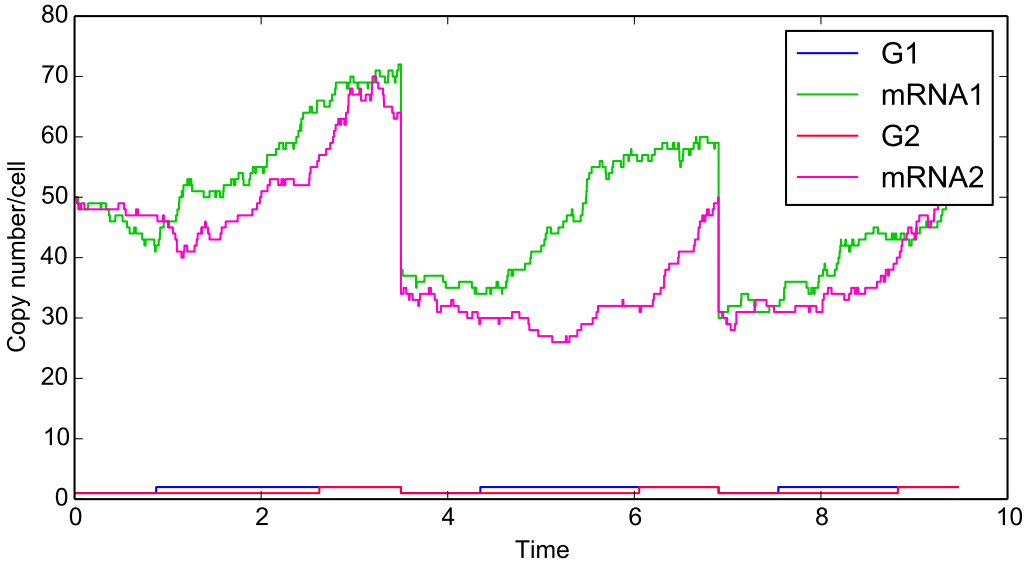
```
>>> cmod.PrintSpeciesMeans()
mRNA            6.86
>>> cmod.PrintSpeciesStandardDeviations()
mRNA            2.82
>>> cmod.PrintSpeciesMeans(sample="mother")
mRNA            6.93
```

In this particular case, they are slightly different, i.e. the mean of the cell lineage is slightly greater.

Example 3

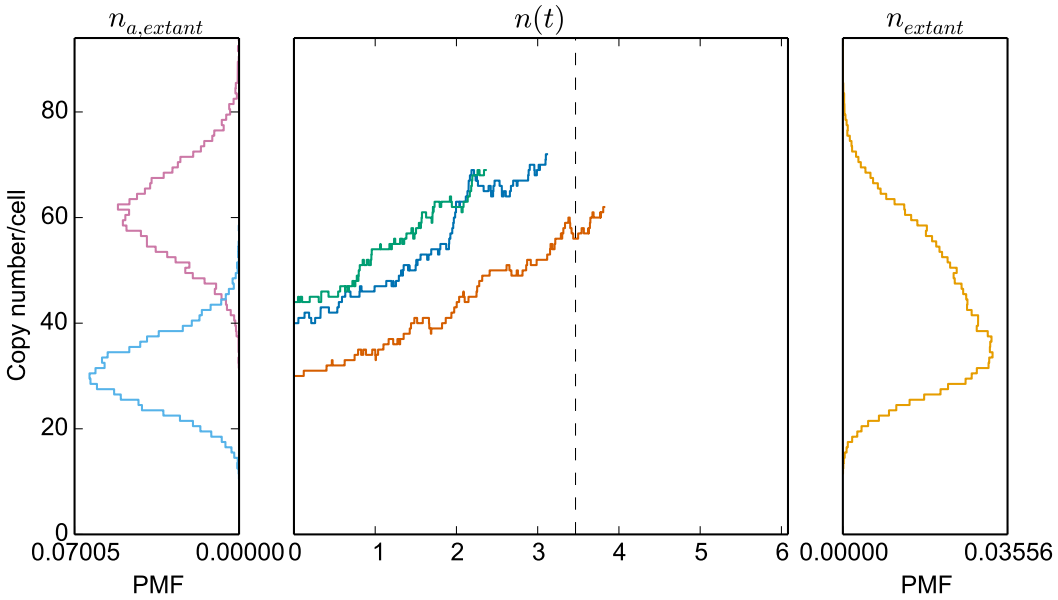
In the third and final example of the cell division module, we illustrate the effect gene duplications. We use an extended immigration-death model which consists of two different mRNAs that are both transcribed from a different part of the genome. Gene duplication doubles the transcription rate of the corresponding mRNA. We add gene duplication for mRNA1 and mRNA2 which occur as 25% and 75% of the interdivision time, respectively. We expect that in steady state that mRNA1 > mRNA2.

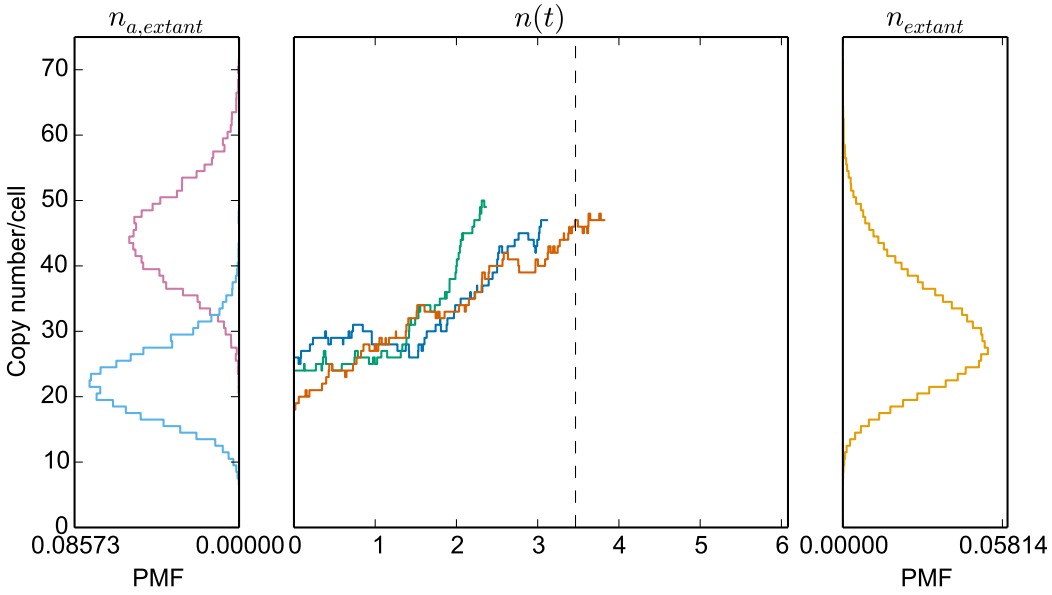
```
>>> cmod = stochpy.CellDivision()
>>> cmod.Model("GeneDuplication.psc")
>>> cmod.SetGrowthFunction(0.2)
>>> cmod.SetGeneDuplications(["G1","G2"],[0.25,0.75]
>>> cmod.DoCellDivisionStochSim(end=3,method="direct")
>>> cmod.PlotSpeciesTimeSeries()
```



The time series plot confirms that G1 and G2 are duplicated after 25% and 75% of the interdivision time, respectively. Next, we perform a simulation for 4000 generations to illustrate the differences in mRNA1 and MRNA2 copy numbers:

```
>>> cmod.DoCellDivisionStochSim(end=4000,mode="generations")
>>> cmod.PrintSpeciesMeans()
G1     1.757
mRNA1  44.849
G2     1.294
mRNA2  31.762

>>> cmod.PlotSpeciesOverview("mRNA1")
>>> cmod.PlotSpeciesOverview("mRNA2")
```




$n_{a,extant}$
$n(t)$
n_{extant}
Copy number/cell
70
60
50
40
30
20
10
0
0.08573
0.00000
PMF
0
1
2
3
4
5
6
0.00000
0.05814
PMF

CHAPTER

ELEVEN

MODULE 5: SBML2PSC

Models are often described in the SBML format, which makes it difficult to interpret. StochPy offers the SBML2PSC module that can used to convert models written in SBML to models written in the human interpretable PySCeS MDL:

```
>>> sbml2psc_mod = stochpy.SBML2PSC()
>>> sbml2psc_mod.SBML2PSC(sbmlfile="dsmts-003-03.xml",
sbmldir=stochpy.model_dir, pscfile=None, pscdir=None)
csymbol time defined as "t" in event
Info: single compartment model: locating "Dimerisation" in def. compartment
Info: single compartment model: locating "Disassociation" in def. compartment
Writing file: /home/user/Stochpy/pscmodels/dsmts-003-03.xml.psc

SBML2PSC
in : /home/user/Stochpy/pscmodels/dsmts-003-03.xml
out: /home/user/Stochpy/pscmodels/dsmts-003-03.xml.psc
```

The PySCeS MDL (see *the PySCeS Model Description Language section*) is the default model description format, but StochPy can parse SBML models.

Part IV

Modeling Input

CHAPTER

TWELVE

MODELS

In the previous sections, we used existing models to illustrate how StochPy can be used for stochastic modeling. Here, we briefly discuss what kind of models StochPy accepts. Deterministic rate equations have normally been used to describe a system of biochemical reactions, whereas these equations are often not valid for stochastic modeling. We advice potential users of StochPy that are unaware of the differences between deterministic and stochastic rate equations to read section *Stochastic Rate Equations* carefully.

In short, for stochastic simulators the input consists of *irreversible rate equations* and initial *copy numbers*. StochPy automatically converts concentrations into molecule copy numbers. We advice users of StochPy to do not use net stoichiometric coefficients in the model description. While the StochPy solvers handle them correctly, the *fixed-interval solvers* that we use of other software packages cannot handle them correctly.

StochPy supports models written in both PySCeS MDL and SBML and therefore also SBML event facilities (see *here*) and assignments (see *here*). The PySCeS MDL is used as default format which is further explained in *the PySCeS Model Description Language section*. Not all functionalities are supported by StochPy (e.g. piecewise functions). Correct installation of libSBML is required before StochPy supports parsing of SBML models. More details can be found in the *Installation* section. Models written in SBML are automatically converted into the PySCeS MDL format which are subsequently used by the stochastic simulators.

StochPy provides many example models, which can be used as a reference for building your own models. We show here two examples:

```
# Reactions
R1:
    $pool > mRNA
    Ksyn

R2:
    mRNA > $pool
    Kdeg*mRNA

# Variable species
mRNA = 50.0

# Parameters
Ksyn = 10
Kdeg = 0.2
```

We call this the Immigration-Death model which we use as the default model in StochPy. First, we define two reactions: a zero-order reaction R1 synthesizing the species mRNA and a first-order reaction R1 degrading mRNA. Second, we set an initial mRNA copy number. And third, we set the parameter values of the synthesis and degradation rate. The next example is the Birth-Death model:

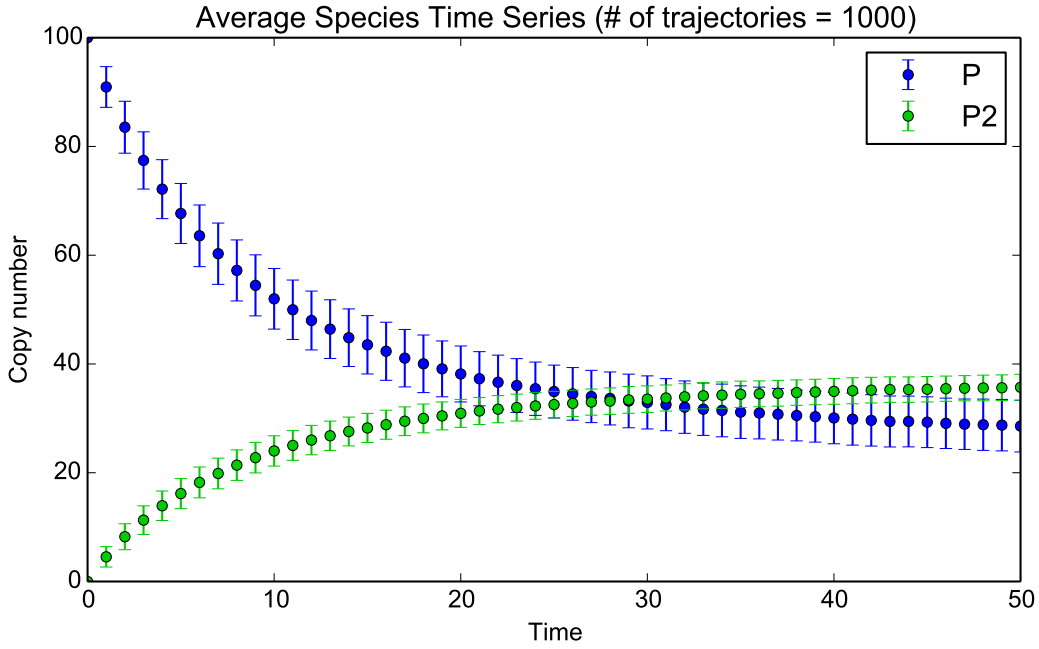
```
# Compartments
Compartment: Cell, 1.0, 3

# Reactions
Death@Cell:
    X > $pool
    Mu*X

Birth@Cell:
    X > {2} X
    Lambda*X

# Fixed species

# Variable species
X@Cell = 100.0

# Parameters
Mu = 0.11
Lambda = 0.1
```

We first define a compartment called "Cell" where all reactions take place. Now, both reactions are first order reactions. The birth reaction is an example where we can use a net stoichiometry ($pool > X).

12.1 Events

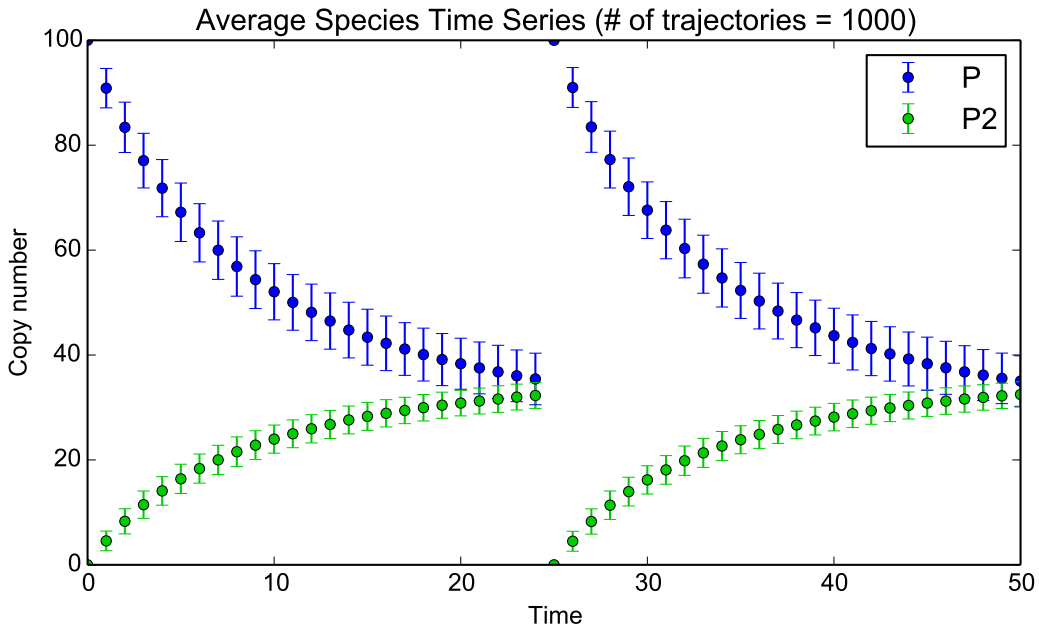
StochPy supports both time and species copy number events (for more details see *here*). Here, we illustrate these events with a model of dimerization of P to P2. The initial numbers of molecules of P and P2 are 100 and 0 respectively. We use model 3.3 from the dsmts test suite, but to illustrate the difference with and without (time) event we first **remove** the time event:

```
>>> smod.Model("dsmts-003-03.xml.psc")
>>> smod.DoStochSim(end=50,mode="time",trajectories=1000)
>>> smod.GetRegularGrid()
>>> smod.PlotAverageSpeciesTimeSeries()
```



The figure above shows the outcome of our simulations. We now put back the time event in model 3.3 of the dsmts test suite. This time event resets the molecule numbers of P and P2 at t>=25. Events have access to the "current" simulation time using the *_TIME_* symbol, which gives the following description of an event in the PySCeS MDL:

```
# Event definitions
Event: reset1, _TIME_ >= 25, 0.0
{
P2 = 0
P = 100
}
```



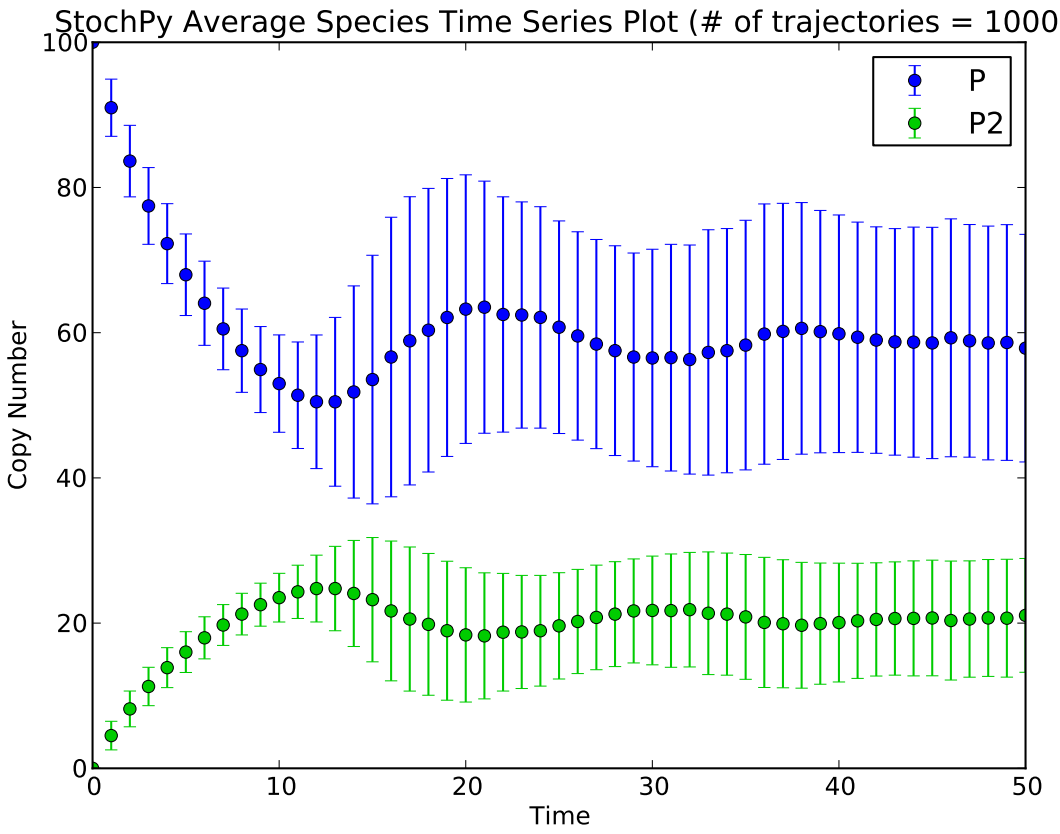
The figure above shows the effect of adding the time event: the copy numbers of both molecular species are reset at t=25.

A second example is a species event where we reset the molecule numbers once molecule P is greater than 30:

```
# Event definitions
Event: reset2, P2 > 30, 0.0
{
P2 = 0
P = 100
}
```

This event is incorporated in the model 3.4 from the dsmts test suite. Stochastic simulation with StochPy yields the following result:

```
>>> smod.Model("dsmts-003-04.xml.psc")
>>> smod.DoStochSim(end=50,mode="time",trajectories=1000)
>>> smod.GetRegularGrid()
>>> smod.PlotAverageSpeciesTimeSeries()
```



So far, we used a time or species trigger but we can also combine both type of events:

```
# Event definitions
Event: reset3, P2 > 30 and _TIME_ > 20, 0.0
{
P2 = 0
P = 100
}
```

Species are now reset if P2 > 30 and if t>20. This event can also occur multiple times.

Warning: do not use *or* specifications in event triggers, but use separate events instead.

StochPy also allows for the mutation of parameters. Define this parameter as a "fixed species" (StochPy gives a warning that this "fixed species" does not occur in any reaction). This warning message is correct (it's a parameter, not a species). Next, you can add a time event to modify the parameter during the simulation:

```
Event: jump1, _TIME_ >= 25, 0.0
{
k1 = 0.01
}
```

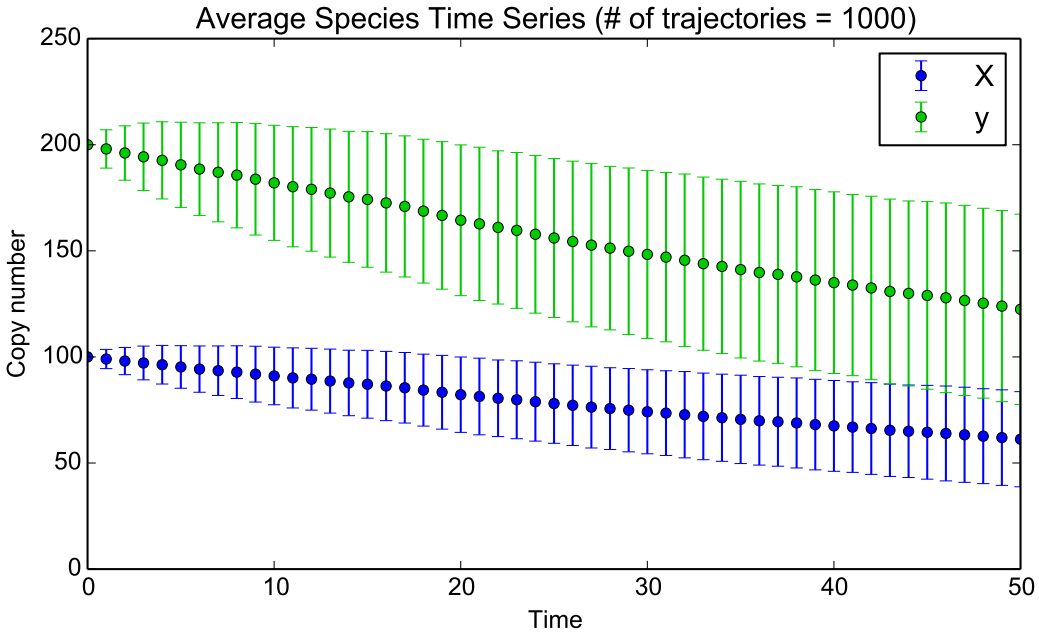
In this example, k1 is set to 0.01 at t=25 without any delay.

12.2 Assignments

Now, we shortly describe one example of an assignment rule for which we use a simple birth-death process of metabolite X. The assignment rules defines that a new species y = 2 * X:

```
# Assignment rules
!F y = 2.0*X
```

This assignment is incorporated in the dsmts-001-19.xml.psc file and simulation yields the following result.



CHAPTER

THIRTEEN

STOCHASTIC RATE EQUATIONS

In this section, we compare deterministic and stochastic rate equations. In short, they are identical for zero and first-order reactions, but not for second and higher-order reactions (if a two or more of the same species are necessary for the reaction to take place, e.g. 2X > Y).

Deterministic models represent species often by concentration, while stochastic models represent species often by molecule copy numbers. The molecule copy numbers are identical to:

```
Na*[X]*Volume(L)
```

Here, Na is the number of Avegadro (6.02*10e23). This is the first step of the conversion of deterministic rate constants (k) to stochastic rate constants (c).

13.1 Zero-order reaction

Assume the following reaction:

```
--> X
```

The deterministic rate equation of this reaction is:

```
k (Ms^-1)
```

The stochastic rate equation of this reaction is:

```
c (s^-1)
```

13.2 First-order reaction

Consider the following reaction:

```
X --> Y
```

The deterministic rate equation of this reaction is:

```
k*[X]
```

The stochastic rate equation of this reaction is:

```
c*x (s^-1)
```

Here, x corresponds to Na*[X]*Volume(L) particles. Therefore, x changes k*Na*[X]*Volume(L) = k*x molecules per second, which means that k=c. This means that first order stochastic and deterministic rate equations are identical.

13.3 Second-order reaction

There exist several types of second-order reactions. First, consider the next reaction:

```
X+Y --> Z
```

The deterministic rate equation of this reaction is:

```
k*[X]*[Y] (Ms^-1)
```

The stochastic rate equation of this reaction is:

```
c*x*y (s^-1)
```

Again, [X] corresponds to Na*[X]*Volume(L) particles and the same is true for [Y]. Therefore, the following relationship holds:

```
k*[X]*[Y] (Ms^-1) = (k*x*y)/(Na*V)^2, hence c = k/(Na*V)
```

Secondly, consider a dimerization reaction:

```
2X --> Y
```

The deterministic rate equation of this reaction is:

```
k*X^2
```

The stochastic rate equation of this reaction is:

```
0.5*c*x*(x-1)
```

The first important concept to understand is that a dimerization reaction can only occur if there are at least two molecules of species X available. For this reason, the stochastic rate equation must be zero if there is only one X molecule available.

13.4 Third-order reaction

This type of reactions does usually not occur in chemical reactions, but they are described just for the sake of completeness.

There exist three types of third-order reactions

1) X+Y+Z –> A

The deterministic rate equation of this reaction is:

```
k*[X]*[Y]*[Z]
```

The stochastic rate equation of this reaction is:

```
c*x*y*z
```

2) X+2Y –> Z

The deterministic rate equation of this reaction is:

```
k*[X]*([Y]^2)
```

The stochastic rate equation of this reaction is:

```
0.5*c*x*y*(y-1)
```

3) 3X –> Y

The deterministic rate equation of this reaction is:

```
k*[X]^3
```

The stochastic rate equation of this reaction is:

```
(1/6)*x*(x-1)*(x-2)
```

For the last reaction three x molecules are necessary, thus this rate equation can not fire if there are less than three molecules of x available.

Part V

The PySCeS Model Description Language

StochPy uses the PySCeS MDL, an ASCII text based *input file* to describe a cellular system in terms of it's stoichiometry, kinetics, compartments and parameters. Input files may have any filename with the single restriction that, for cross platform compatibility, they must end with the extension *.psc*. In this document we describe the PySCeS Model Description Language (MDL).

CHAPTER

FOURTEEN

DEFINING A PYSCES MODEL

14.1 A kinetic model

The basic description of a kinetic model in the PySCeS MDL contains the following information:

- whether any fixed (boundary) species are present
- the reaction network stoichiometry
- rate equations for each reaction step
- parameter and boundary species initial values
- the initial values of the variable species

Although it is in principle possible to define a model without reactions or free species, for practical purposes StochPy requires a minimum of a single reaction. Once this information is obtained it can be organised and written as a StochPy input file. While this list is the minimum information required for a StochPy input file the MDL allows the definition of advanced models that contain compartments, global units, functions, rate and assignment rules.

14.2 Model keywords

In StochPy it is now possible to define keywords that specify model information. Keywords have the general form

```
<keyword>:  <value>
```

The *Modelname* (optional) keyword, containing only alphanumeric characters (or _), describes the model filename (typically used when the model is exported via the PySCeS interface module) while the *Description* keyword is a (short) single line model description.

```
Modelname: rohwer_sucrose1
Description: Sucrose metabolism in sugar cane (Johann M. Rohwer)
```

Two keywords are available for use (optional) with models that have one or more compartments defined. Both take a boolean (True/False) as their value:

- *Species_In_Conc* specifies whether the species symbols used in the rate equations represent a concentration (True) or an amount (False, default).
- *Output_In_Conc* tells StochPy to output the results of numerical operations in concentrations (True, not supported) or in amounts (False, default).

```
Species_In_Conc: False
Output_In_Conc: False
```

14.3 Global unit definition

StochPy supports the (optional) definition of a set of global units. In doing so we have chosen to follow the general approach used in the Systems Biology Modelling Language (SBML L2V3) specification. The general definition of a PySCeS unit is: `'<UnitType>: <kind>, <multiplier>, <scale>, <exponent>'` where *kind* is a string describing the base unit (for SBML compatibility this should be an SI unit) e.g. mole, litre, second or metre. The base unit is modified by the multiplier, scale and index using the following relationship: *<multiplier> * (<kind> * 10**<scale>)**<index>*. The default unit definitions are:

```
UnitSubstance: mole, 1, 0, 1
UnitVolume: litre, 1, 0, 1
UnitTime: second, 1, 0, 1
UnitLength: metre, 1, 0, 1
UnitArea: metre, 1, 0, 2
```

Please note that defining these values does not affect the numerical analysis of the model in any way.

14.4 Symbol names and comments

Symbol names (i.e. reaction, species, compartment, function, rule and parameter names etc.) must start with either an underscore or letter and be followed by any combination of alpha-numeric characters or an underscore. Like all other elements of the input file names are case sensitive:

```
R1
_subA
par1b
ext_1
```

Explicit access to the "current" time in a time simulation is provided by the special symbol `_TIME_`. This is useful in the definition of events and rules (see chapter on advanced model construction for more details).

Comments can be placed anywhere in the input file in one of two ways, as single line comment starting with a # or as a multi-line triple quoted comment *"""<comment>"""*:

```
# everything after this is ignored

"""
This is a comment
spread over a
few lines.
"""
```

14.5 Compartment definition

By default StochPy assumes that the model exists in a single unit volume compartment. In this case it is **not** necessary to define a compartment and the ODE's therefore describe changes in concentration per time. However, if a compartment is defined, StochPy assumes that the propensities describe changes in substance amount per time. Doing this affects how the model is defined in the input file (especially with respect to the definitions of rate equations and species) and the user is **strongly** advised to read the Users Guide before building models in this way. The compartment definition is as follows `Compartment: <name>, <size>, <dimensions>`, where <*name*> is the unique compartment id, <*size*> is the size of the compartment (i.e. length, volume or area) defined by the number of <*dimensions*> (e.g. 1,2,3):

```
Compartment: Cell, 2.0, 3
Compartment: Memb, 1.0, 2
```

14.6 Function definitions

A new addition to the PySCeS MDL is the ability to define SBML styled functions. Simply put these are code substitutions that can be used in rate equation definitions to, for example, simplify the kinetic law. The general syntax for a function is `Function: <name>, <args> {<formula>}` where <*name*> is the unique function id, <*arglist*> is one or more comma separated function arguments. The <*formula*> field, enclosed in curly brackets, may only make use of arguments listed in the <*arglist*> and therefore **cannot** reference model attributes directly. If this functionality is required a forcing function (assignment rule) may be what you are looking for.

```
Function: rmm_num, Vf, s, p, Keq {
Vf*(s - p/Keq)
}

Function: rmm_den, s, p, Ks, Kp {
s + Ks*(1.0 + p/Kp)
}
```

The syntax for function definitions has been adapted from Frank Bergmann and Herbert Sauro's "Human Readable Model Definition Language" (Draft 1).

14.7 Defining fixed species

Boundary species, also known as fixed or external species, are a special class of parameter used when modelling biological systems. The PySCeS MDL fixed species are declared on a single line as `FIX: <fixedlist>`. The <*fixedlist*> is a space separated list of symbol names which should be initialised like any other species or parameter:

```
FIX: Fru_ex Glc_ex ATP ADP UDP phos glycolysis Suc_vac
```

If no fixed species are present in the model then this declaration should be omitted entirely.

14.8 Reaction stoichiometry and rate equations

The reaction stoichiometry and rate equation are defined together as a single reaction step. Each step in the system is defined as having a name (identifier), a stoichiometry (substrates are converted to products) and rate equation (the catalytic activity, described in terms of species and parameters). All reaction definitions should be separated by an empty line. The general format of a reaction in a model with no compartments is:

```
<name>:
        <stoichiometry>
        <rate equation>
```

The <*name*> argument follows the syntax as discussed in a previous section, however, when more than one compartment has been defined it is important to locate the reaction in its specific compartment. This is done using the `@` operator:

```
<name>@<compartment>:
                      <stoichiometry>
                      <rate equation>
```

Where <*compartment*> is a valid compartment name. In either case this then followed either directly (or on the next line) by the reaction stoichiometry.

Each <*stoichiometry*> argument is defined in terms of reaction substrates, appearing on the left hand side and products on the right hand side of an identifier which labels the reaction as either reversible (=) or irreversible (>). While the PySCeS MDL supports both reversible and irreversible reactions, StochPy accepts only irreversible reactions. If required each reagent's stoichiometric coefficient (StochPy accepts both integer and floating point) should be included in curly braces *{}* immediately preceding the reagent name. If these are omitted a coefficient of one is assumed:

```
{2.0}Hex_P = Suc6P + UDP  # reversible reaction (not supported within StochPy)
Fru_ex > Fru              # irreversible reaction
species_5 > $pool         # a reaction to a sink
```

The PySCeS MDL also allows the use of the *$pool* token that represents a placeholder reagent for reactions that have no net substrate or product. Reversibility of a reaction is only used when exporting the model to other formats (such as SBML) and in the calculation of elementary modes. It does not affect the numerical evaluation of the rate equations in any way.

Central to any reaction definition is the <*rate equation*> (SBML kinetic law). This should be written as valid Python expression and may fall across more than one line. Standard Python operators + − ⋆ / ⋆⋆ are supported (note the Python power e.g. *2^4* is written as *2**4*). There is no shorthand for multiplication with a bracket so *-2(a+b)^h* would be written as *-2*(a+b)**h}* and normal operator precedence applies:

+, -	addition, subtraction
*, /	multiplication, division
+x,-x	positive, negative
**	exponentiation

Operator precedence increase from top to bottom and left to right (adapted from the Python Reference Manual).

The PySCeS MDL parser has been developed to parse and translate different styles of infix into Python/Numpy based expressions, the following functions are supported in any mathematical expression:

- log, log10, ln, abs
- pow, exp, root, sqrt
- sin, cos, tan, sinh, cosh, tanh
- arccos, arccosh, arcsin, arcsinh, arctan, arctanh
- floor, ceil, ceiling, piecewise
- notanumber, pi, infinity, exponentiale

Logical operators are supported in rules, events etc but *not* in rate equation definitions. The PySCeS MDL parser understands Python infix as well as libSBML and NumPy prefix notation.

- and or xor not
- > gt(x,y) greater(x,y)
- < lt(x,y) less(x,y)
- >= ge(x,y) geq(x,y) greater_equal(x,y)
- <= le(x,y) leq(x,y) less_equal(x,y)
- == eq(x,y) equal(x,y)
- != neq(x,y) not_equal(x,y)

Note that currently the MathML *delay and factorial* functions are not supported. Delay is handled by simply removing it from any expression, e.g. *delay(f(x), delay)* would be parsed as *f(x)*.

A reaction definition when no compartments are defined:

```
R1:
   X > $pool
   Mu*X
```

When compartments are defined note how now the reaction is now given a location and that because the propensities formed from these reactions must be in changes in substance per time the rate equation is multiplied by its compartment size:

```
R1@Cell:
   X > $pool
   Mu*X
```

If *Species_In_Conc: True* the location of the species is defined when it is initialised and will be explained later in this manual.

14.9 Species and parameter initialisation

The general form of any species (fixed, free) and parameter is simply:

```
property = value
```

Initialisations can be written in any order anywhere in the input file but for human readability purposes these are usually placed after the reaction that uses them or grouped at the end of the input file. Both decimal and scientific notation is allowed with the following provisions that neither floating point *(1.)* nor scientific shorthand *(1.e-3)* syntax should be used, instead use the full form *(1.0e-3)*, *(0.001)* or *(1.0)*.

Variable or free species are initialised differently depending on whether compartments are present in the model. While in essence the variables are set by the system parameters the

Although the variable species concentrations are determined by the parameters of the system, their initial values are used in various places, calculating total moiety concentrations (if present), time simulation initial values (e.g. time=zero) and as initial guesses for the steady-state algorithms. If an empty initial species pool is required it is not recommended to initialise these values to zero (in order to prevent potential divide-by-zero errors) but rather to a small value (e.g. 10**-8).

For a model with no compartments these initial values assumed to be concentrations:

```
NADH = 0.001
ATP  = 2.3e-3
sucrose = 1
```

In a model with compartments it is expected that the species are located in a compartment (even if *Species_In_Conc: False*) this is done useing the @ symbol:

```
s1@Memb = 0.01
s2@Cell = 2.0e-4
```

A word of warning, the user is responsible for making sure that the units of the initialised species match those of the model. Please keep in mind that **all** species (and anything that depends on them) is defined in terms of the *Species_In_Conc* keyword. For example, if the preceding initialisations were for *R1* (see Reaction section) then they would be concentrations (as *Species_In_Conc: True*). However, in the next example, we are initialising species for *R4* and they are therefore in amounts (*Species_In_Conc: False*):

```
s3@Memb = 1.0
s4@Cell = 2.0
```

Fixed species are defined in a similar way and although technically a parameter, they should be given a location in compartmental models:

```
# InitExt
X0 = 10.0
X4@Cell = 1.0
```

However, fixed species are true parameters in the sense that their associated compartment size does not affect their value when it changes size. If compartment size dependent behaviour is required an assignment or rate rule should be considered.

Finally, the parameters should be initialised. StochPy checks if a parameter is defined that is not present in the rate equations and if such parameter initialisations are detected a harmless warning is generated. If, on the other hand, an uninitialised parameter is detected a warning is generated and a value of 1.0 assigned:

```
# InitPar
Vf2 = 10.0
Ks4 = 1.0
```

CHAPTER

FIFTEEN

ADVANCED MODEL CONSTRUCTION

15.1 Assignment rules

Assignment rules or forcing functions are used to set the value of a model attribute before the ODE's are evaluated. This model attribute can either be a parameter used in the rate equations (this is traditionally used to describe an equilibrium block) a compartment or an arbitrary parameter (commonly used to define some sort of tracking function). Assignment rules can access other model attributes directly and have the generic form `!F <par> = <formula>`. Where <*par*> is the parameter assigned the result of <*formula*>. Assignment rules can be defined anywhere in the input file:

```
!F S_V_Ratio = Mem_Area/Vcyt
!F sigma_test = sigma_P*Pmem + sigma_L*Lmem
```

These rules would set the value of <*par*> which whose value can be followed with using the simulation and steady state extra_data functionality.

15.2 Events

Time dependant events may now be defined whose definition follows the event framework described in the SBML L2V1 specification. The general form of an event is *Event:* <*name*>, <*trigger*>, <*delay*> *{* <*assignments*> *}*. As can be seen an event consists of essentially three parts, a conditional <*trigger*>, a set of one or more <*assignments*> and a <*delay*> between when the trigger is fired (and the assignments are evaluated) and the eventual assignment to the model. Assignments have the general form <*par*> = <*formula*>. Events have access to the "current" simulation time using the *_TIME_* symbol:

```
Event: event1, _TIME_ > 10 and A > 150.0, 0 {
V1 = V1*vfact
V2 = V2*vfact
}
```

The following event illustrates the use of a delay of ten time units as well as the prefix notation (used by libSBML) for the trigger (StochPy understands both notations):

```
Event: event2, geq(_TIME_, 15.0), 10 {
V3 = V3*vfact2
}

# Event definitions
```

Event: reset, P2 > 30, 0.0 { P2 = 0 P = 100 }

15.3 Reagent placeholder

Some models contain reactions which are defined as only have substrates or products:

```
R1: A + B >

R2: > C + D
```

The implication is that the relevant reagents appear or disappear from or into a constant pool. Unfortunately the *PySCeS* parser does not accept such an unbalanced reaction definition and requires these pools to be represented as a `$pool` token:

```
R1: A + B > $pool

R2: $pool > C + D
```

`$pool` is neither counted as a reagent nor does it ever appear in the stoichiometry (think of it as dev/null) and no other $<str> tokens are allowed.

CHAPTER

SIXTEEN

EXAMPLE STOCHPY INPUT FILES

16.1 Basic model definition

StochPy test model *BirthDeath.psc*:

```
# Stochastic Simulation Algorithm input file
# --> mRNA -->

# Reactions
R1:
    mRNA > {2} mRNA
    Ksyn*mRNA

R2:
    mRNA > $pool
    Kdeg*mRNA

# Fixed species

# Variable species
mRNA = 100

# Parameters
Ksyn = 2.9
Kdeg = 3
```

16.2 Advanced example

Test suite model *dsmts-003-04.xml.psc*:

```
# Generated by PySCeS 0.8.0 (2012-02-28 14:09)

# Keywords
Description: Dimerisation model (003), variant 04
Modelname: Dimerisation04
Output_In_Conc: False
```

```
Species_In_Conc: False

# GlobalUnitDefinitions
UnitVolume: litre, 1.0, 0, 1
UnitLength: metre, 1.0, 0, 1
UnitSubstance: item, 1.0, 0, 1
UnitArea: metre, 1.0, 0, 2
UnitTime: second, 1.0, 0, 1

# Compartments
Compartment: Cell, 1.0, 3

# Reactions
Dimerisation@Cell:
    {2.0}P > P2
    k1*P*(P-1.0)/2.0

Disassociation@Cell:
    P2 > {2.0}P
    k2*P2

# Event definitions
Event: reset, gt(P2, 30), 0.0
{
P2 = 0
P = 100
}

# Fixed species

# Variable species
P2@Cell = 0.0
P@Cell = 100.0

# Parameters
k1 = 0.001
k2 = 0.01
```

Although it may be slightly more complicated than the basic model described above it is still, by our definition, certainly human readable.