Quasi Steady State Petri Net (QSSPN) tutorial.

This tutorial introduces QSSPN model building in Snoopy Petri Net editor and subsequent simulation in MUFINS. We use kinetic model of cortisol signaling integrated with dFBA simulation of human Genome Scale Metabolic Network (GSMN) as an example (Use Case 3 of MUFINS manuscript). This model involves all new features of QSSPN available in MUFINS. The Petri Net part is composed of fully parameterized Ordinary Differential Equation (ODE) model of cortisol signaling and external metabolite concentrations in physiological compartments. To learn how to use QSSPN for non-parametric simulation with Monte Carlo sampling of qualitative dynamic trajectories, see examples in original QSSPN publication [1]. All features of original QSSPN method are retained in new version.

Install Snoopy Petri Net tool.
MUFINS uses Snoopy Petri Net tool [2] as a graphical editor for Dynamic Transitions part of QSSPN model. The QSSPN models can also be edited in spreadsheet interface of JyMet (see below) or directly in text files. However, we recommend editing models in Snoopy to take full advantage of Petri Net graphical notation.

Snoopy distribution for multiple platforms is available at:
http://www-dssz.informatik.tu-cottbus.de/DSSZ/Software/Snoopy#downloads

The model.
Download `MUFINS**_Examples.zip` file from software distribution and uncompress in location of your choice. Enter `NR_Recon2` directory with the following files:

`NR_Recon2.v3.1.spept` – The model of cortisol signaling integrated with dFBA simulation of human Genome Scale Metabolic Network (GSMN). This is Snoopy file used to build Dynamic Transition (DT) part of the model.

`recon2_xt.PIPES.CORE.v1.sfba` – The SurreyFBA reaction table file containing Quasi Steady State Fluxes (QSSF) part of the model. The human GSMN Recon 2 [3] has been parameterized as described in Use Case 3. The constraint and objective places connecting DT and QSSF parts of the model are defined in Snoopy file.

`NR_Recon2.v3.1.ctrl` – qsspn simulation control file. See `MUFINS**_Doc/qsspn.pdf` for format description.

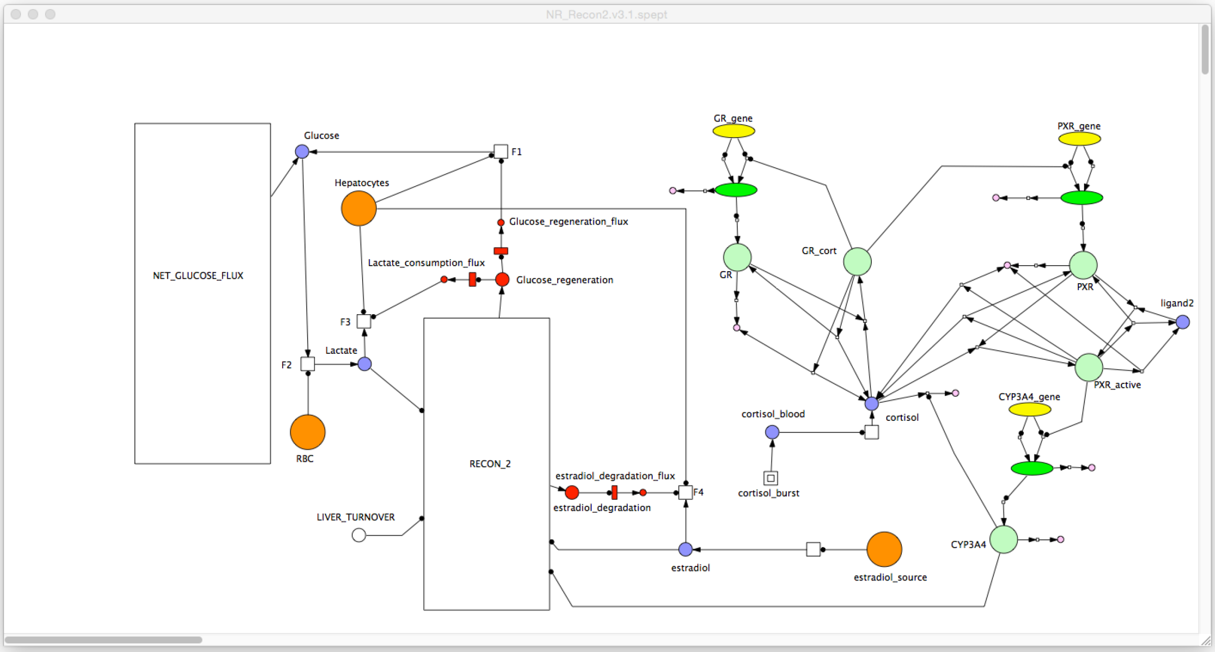
`/bin/qsspn` – qsspn solver for Mac copied here to create stand alone example distribution. The solver can be run in command line mode or started from JyMet interface or other scripts.

`/bin/qsspn.exe` - windows binary of qsspn solver
`/bin/qsspn-linux` – linux binary of qsspn solver. If this binary does not work on your version of Unix go to `MUFINS**_Source/QSSPN/` and run compile.sh , install.sh to create new binary in `MUFINS**_Source/QSSPN/bin`.

`/bin/spept2qsspn` – python script converting Snoopy *.spept file to QSSPN model file. This script provides “semantics” for Petri Net model build in Snoopy. It interprets graphical symbols and annotations in comments section to construct Petri Net part of QSSPN model to be executed with qsspn solver.

`RUN_Mac` – script that run simulation of this example on MacOSX using binaries in bin/
`CLEAN_Mac` – scripts that clears simulation results on MacOSX.

Open the model in Snoopy Petri net editor.
Click on file name to see graphical representation of the model and check if Snoopy has been installed correctly. When the file opens use View->Zoom out in Snoopy to see display entire network:



Before discussing this representation in detail, lets run the model first to see the results.

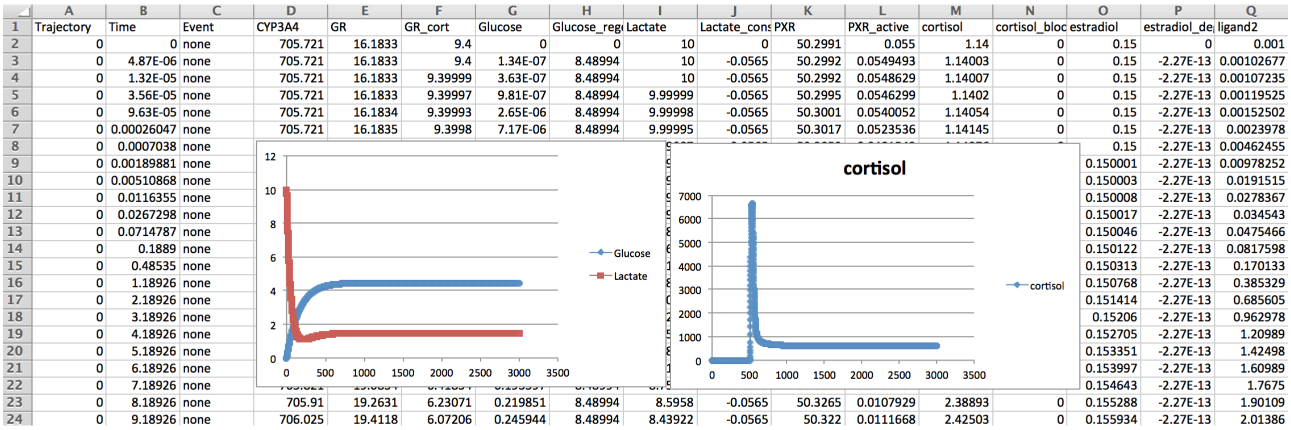
Run model in command line.
If you are Mac user, open NR_Recon2 directory and click `RUN_Mac`. This script will execute the following commands:

```
./bin/spept2qsspn NR_Recon2.v3.1.spept 1e9 > NR_Recon2.v3.1.qsspn
./bin/qsspn NR_Recon2.v3.1.qsspn NR_Recon2.v3.1.ctrl.txt
```

These two commands can also be executed in Mac terminal. To run simulation on **Windows** execute commands above in Windows command line terminal with Windows syntax. You will need python interpreter to run spept2qsspn. We recommend to use JyMet for running qsspn simulations on Windows operating system (see "**Run Model in JyMet**" below). **Linux** users should execute two command lines using either binaries available in bin or go to MUFINS**_Source/QSSPN/ and run compile.sh , install.sh to create new binary in MUFINS**_Source/QSSPN/bin . Both qsspn and spept2qsspn do not require any libraries or environmental variables and can be moved to any convenient location on the system, such as /usr/local/bin .

The spept2qsspn converts Snoopy file given as first parameter into QSSPN model file. The second parameter of the command line is default maximal state of PN place. In the case of this ODE model is set to very large number. In the case of discrete, qualitative models this parameter can be used to set maximal number of activity levels.

The qsspn is a solver that takes qsspn file name as first argument and control file name as second argument. The qsspn command executes simulation, which in the case of this example will take about 30 seconds. Simulation results are saved to output.xls file specified in the control file. Open output.xls file to see results. Create scatter plots of Glucose, lactate and cortisol as a function of time.



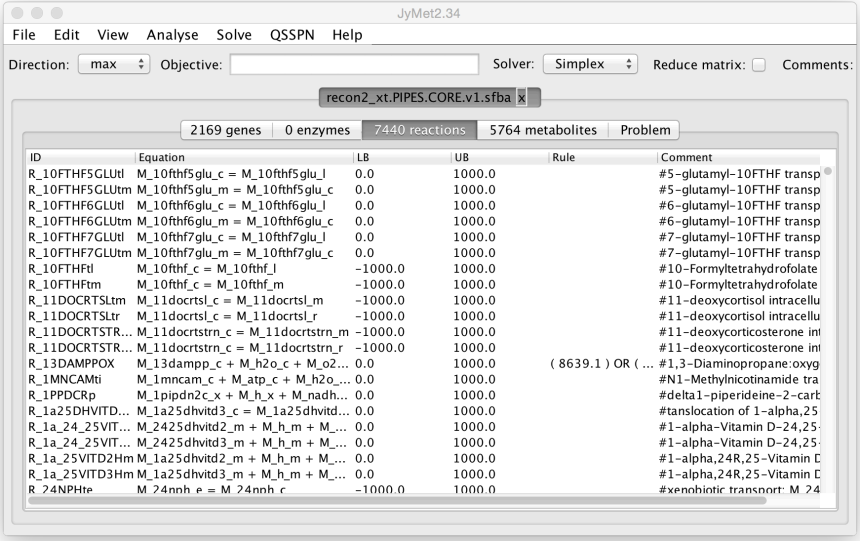
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Trajectory	Time	Event	CYP3A4	GR	GR_cort	Glucose	Glucose_regı	Lactate	Lactate_cons	PXR	PXR_active	cortisol	cortisol_bloc	estradiol	estradiol_de	ligand2
2	0	0	none	705.721	16.1833	9.4	0	0	10	0	50.2991	0.055	1.14	0	0.15	0	0.001
3	0	4.87E-06	none	705.721	16.1833	9.4	1.34E-07	8.48994	10	-0.0565	50.2992	0.0549493	1.14003	0	0.15	-2.27E-13	0.00102677
4	0	1.32E-05	none	705.721	16.1833	9.39999	3.63E-07	8.48994	10	-0.0565	50.2992	0.0548629	1.14007	0	0.15	-2.27E-13	0.00107235
5	0	3.56E-05	none	705.721	16.1833	9.39997	9.81E-07	8.48994	9.99999	-0.0565	50.2995	0.0546299	1.1402	0	0.15	-2.27E-13	0.00119525
6	0	9.63E-05	none	705.721	16.1834	9.39993	2.65E-06	8.48994	9.99998	-0.0565	50.3001	0.0540052	1.14054	0	0.15	-2.27E-13	0.00152502
7	0	0.00026047	none	705.721	16.1835	9.3998	7.17E-06	8.48994	9.99995	-0.0565	50.3017	0.0523536	1.14145	0	0.15	-2.27E-13	0.0023978
8	0	0.0007038	none												0.15	-2.27E-13	0.00462455
9	0	0.00189881	none												0.150001	-2.27E-13	0.00978252
10	0	0.00510868	none												0.150003	-2.27E-13	0.0191515
11	0	0.0116355	none												0.150008	-2.27E-13	0.0278367
12	0	0.0267298	none												0.150017	-2.27E-13	0.034543
13	0	0.0714787	none												0.150046	-2.27E-13	0.0475466
14	0	0.1889	none												0.150122	-2.27E-13	0.0817598
15	0	0.48535	none												0.150313	-2.27E-13	0.170133
16	0	1.18926	none												0.150768	-2.27E-13	0.385329
17	0	2.18926	none												0.151414	-2.27E-13	0.685605
18	0	3.18926	none												0.15206	-2.27E-13	0.962978
19	0	4.18926	none												0.152705	-2.27E-13	1.20989
20	0	5.18926	none												0.153351	-2.27E-13	1.42498
21	0	6.18926	none												0.153997	-2.27E-13	1.60989
22	0	7.18926	none												0.154643	-2.27E-13	1.7675
23	0	8.18926	none	705.91	19.2631	6.23071	0.219851	8.48994	8.5958	-0.0565	50.3265	0.0107929	2.38893	0	0.155288	-2.27E-13	1.90109
24	0	9.18926	none	706.025	19.4118	6.07206	0.245944	8.48994	8.43922	-0.0565	50.322	0.0111668	2.42503	0	0.155934	-2.27E-13	2.01386

First column of the file contains trajectory number. This is relevant for stochastic simulations where multiple trajectories starting from the same conditions are simulated. Second column is simulation time. The units of time are determined by model parameters. Here time is in minutes. Third column contains transition name that fired in particular simulation step or "none" if multiple transitions were fired in synchronous update step. Here we use continuous transitions only so simulation is deterministic, all transitions are synchronously updated in every step and only one trajectory is needed.

The following columns of the file contain marking of PN places. The list of places to be included in output files is specified in `PETRI_NET_MONITORS` section of the control file. In this quantitative model marking is interpreted as concentration. See description of Use Case 3 for model parameter description.

Run model in JyMet

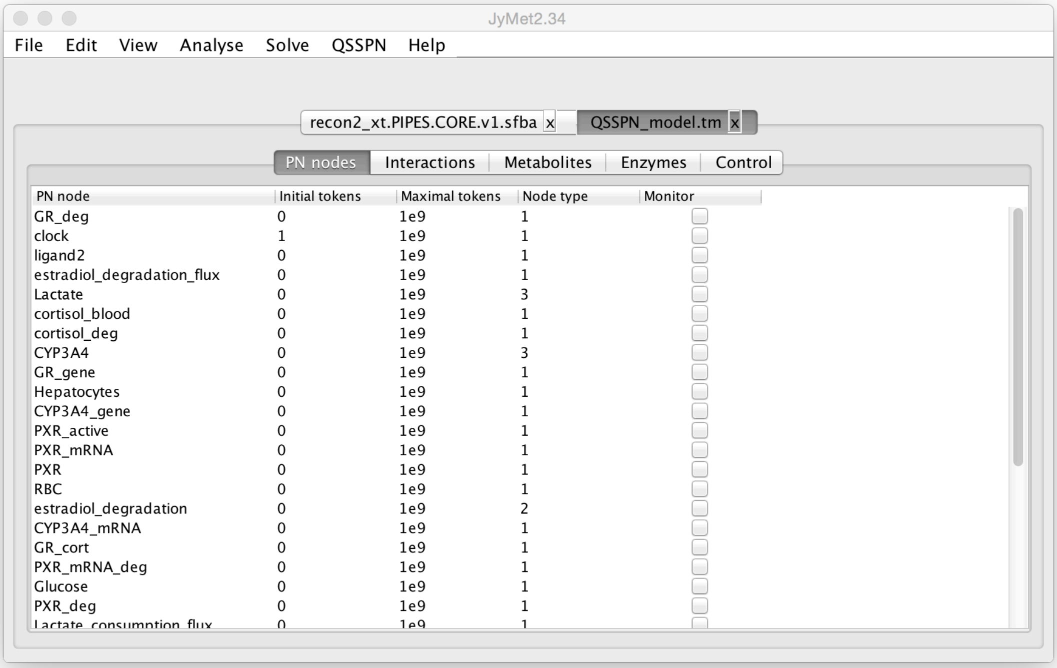
The JyMet interface to QSSPN simulations is major new feature of MUFINS. Start JyMet from MUFINS** (see GETTING_STARTED.pdf in MUFINS**_Docs). First open the GSMN model. Use "File->Open model" in JyMet to open `recon2_xt.PIPES.CORE.v1.sfba.`



Direction: max Objective: Solver: Simplex Reduce matrix: □ Comments:

ID	Equation	LB	UB	Rule	Comment
R_10FTHF5GLUtl	M_10fthf5glu_c = M_10fthf5glu_l	0.0	1000.0		#5-glutamyl-10FTHF transp
R_10FTHF5GLUtm	M_10fthf5glu_m = M_10fthf5glu_c	0.0	1000.0		#5-glutamyl-10FTHF transp
R_10FTHF6GLUtl	M_10fthf6glu_c = M_10fthf6glu_l	0.0	1000.0		#6-glutamyl-10FTHF transp
R_10FTHF6GLUtm	M_10fthf6glu_m = M_10fthf6glu_c	0.0	1000.0		#6-glutamyl-10FTHF transp
R_10FTHF7GLUtl	M_10fthf7glu_c = M_10fthf7glu_l	0.0	1000.0		#7-glutamyl-10FTHF transp
R_10FTHF7GLUtm	M_10fthf7glu_m = M_10fthf7glu_c	0.0	1000.0		#7-glutamyl-10FTHF transp
R_10FTHFtl	M_10fthf_c = M_10fthf_l	-1000.0	1000.0		#10-Formyltetrahydrofolate
R_10FTHFtm	M_10fthf_c = M_10fthf_m	-1000.0	1000.0		#10-Formyltetrahydrofolate
R_11DOCRTSLtm	M_11docrtsl_c = M_11docrtsl_m	-1000.0	1000.0		#11-deoxycortisol intracellu
R_11DOCRTSLtr	M_11docrtsl_c = M_11docrtsl_r	-1000.0	1000.0		#11-deoxycortisol intracellu
R_11DOCRTSTR...	M_11docrtstrn_c = M_11docrtstrn_m	-1000.0	1000.0		#11-deoxycorticosterone int
R_11DOCRTSTR...	M_11docrtstrn_c = M_11docrtstrn_r	-1000.0	1000.0		#11-deoxycorticosterone int
R_13DAMPPOX	M_13dampp_c + M_h2o_c + M_o2...	0.0	1000.0	(8639.1) OR (...	#1,3-Diaminopropane:oxyg
R_1MNCAMti	M_1mncam_c + M_atp_c + M_h2o_...	0.0	1000.0		#N1-Methylnicotinamide tra
R_1PPDCRp	M_1pipdn2c_x + M_h_x + M_nadh...	0.0	1000.0		#delta1-piperideine-2-carb
R_1a25DHVITD...	M_1a25dhvitd3_c = M_1a25dhvitd...	0.0	1000.0		#tanslocation of 1-alpha,25
R_1a_24_25VIT...	M_2425dhvitd2_m + M_h_m + M_...	0.0	1000.0		#1-alpha-Vitamin D-24,25
R_1a_24_25VIT...	M_2425dhvitd3_m + M_h_m + M_...	0.0	1000.0		#1-alpha-Vitamin D-24,25
R_1a_25VITD2Hm	M_1a25dhvitd2_m + M_h_m + M_...	0.0	1000.0		#1-alpha,24R,25-Vitamin D
R_1a_25VITD3Hm	M_1a25dhvitd3_m + M_h_m + M_...	0.0	1000.0		#1-alpha,24R,25-Vitamin D
R_24NPHte	M_24nph_e = M_24nph_c	-1000.0	1000.0		#xenobiotic transport: M_24

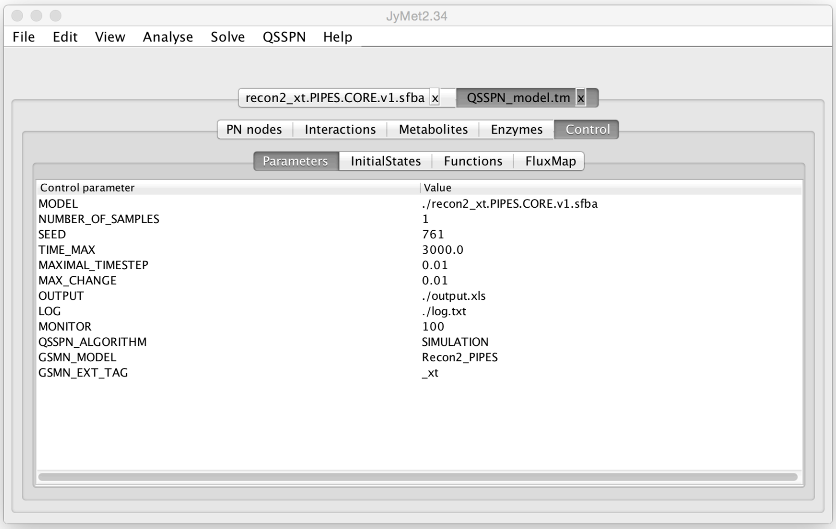
Import Snoopy file containing Dynamic Transitions part of the model. Use "QSSPN->Import SPEPT" to import `NR_Recon2.v3.1.spept` file. During the import you will be asked to "Set default maximal number of tokens”. Type 1e9 to dialog box. This dialog sets maximal state of Petri Net place. This is important for qualitative simulations [1], where molecular amounts are discretised to a few levels. Here, we use quantitative models where Petri Net place marking is interpreted as concentration. Setting 1e9 is very large number representing infinity. If the import was successful you will see the following screen:



PN node	Initial tokens	Maximal tokens	Node type	Monitor
GR_deg	0	1e9	1	☐
clock	1	1e9	1	☐
ligand2	0	1e9	1	☐
estradiol_degradation_flux	0	1e9	1	☐
Lactate	0	1e9	3	☐
cortisol_blood	0	1e9	1	☐
cortisol_deg	0	1e9	1	☐
CYP3A4	0	1e9	3	☐
GR_gene	0	1e9	1	☐
Hepatocytes	0	1e9	1	☐
CYP3A4_gene	0	1e9	1	☐
PXR_active	0	1e9	1	☐
PXR_mRNA	0	1e9	1	☐
PXR	0	1e9	1	☐
RBC	0	1e9	1	☐
estradiol_degradation	0	1e9	2	☐
CYP3A4_mRNA	0	1e9	1	☐
GR_cort	0	1e9	1	☐
PXR_mRNA_deg	0	1e9	1	☐
Glucose	0	1e9	1	☐
PXR_deg	0	1e9	1	☐
Lactate_consumption_flux	0	1e9	1	☐

Note, setting of maximal number of tokens to 1e9. The tabs “PN nodes”, “Interactions”, provide interface to Petri Net places and Transitions. The “Metabolites” and “Enzymes” tabs contain lookup tables linking Petri Net model to GSMN. All parameters are defined in SurreyFBA**_Docs qsspn.pdf and in Additional File 1 of this manuscript (QSSPN algorithm). The following chapters of this tutorial explain editing of model parameters in Snoopy. For now, lets proceed to running simulation.

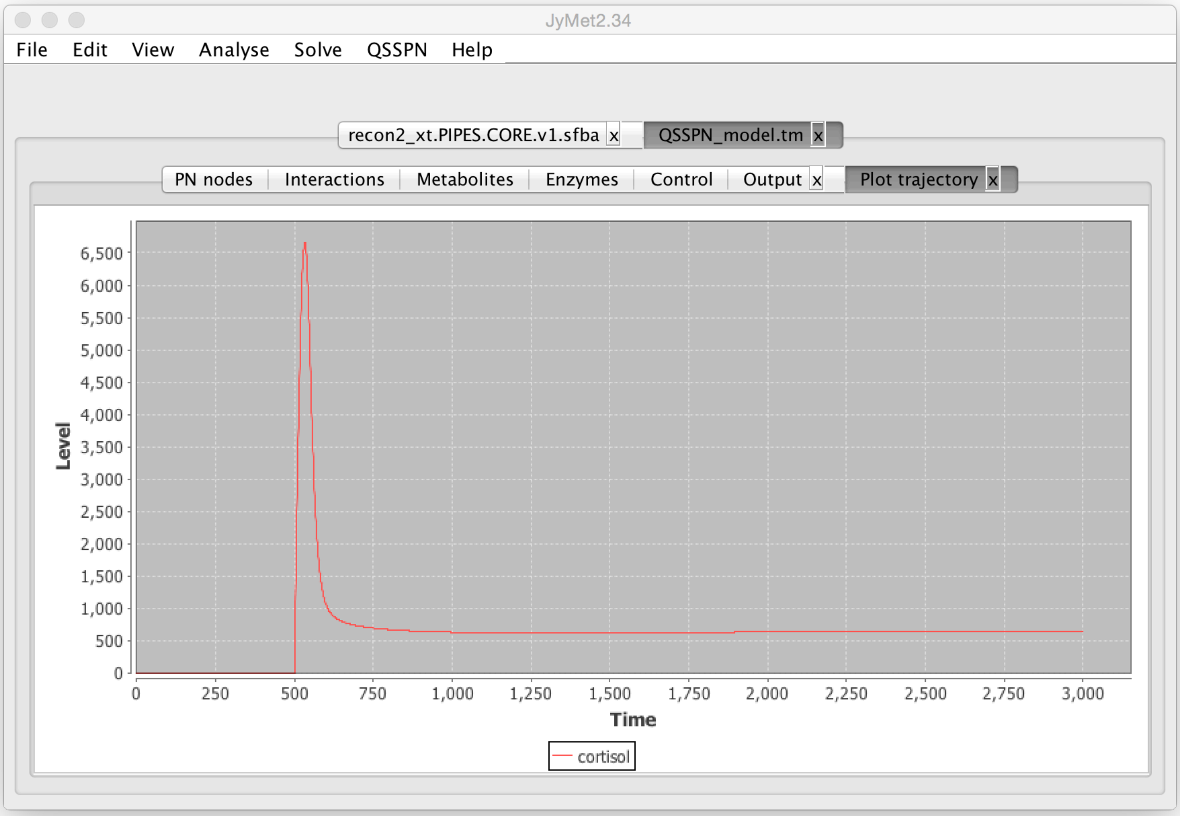
Load control file containing simulation parameters. Use “QSSPN->Load control” to open `NR_Recon2.v3.1.ctrl.txt` file. Click “Control” tab to see the following screen.



Control parameter	Value
MODEL	./recon2_xt.PIPES.CORE.v1.sfba
NUMBER_OF_SAMPLES	1
SEED	761
TIME_MAX	3000.0
MAXIMAL_TIMESTEP	0.01
MAX_CHANGE	0.01
OUTPUT	./output.xls
LOG	./log.txt
MONITOR	100
QSSPN_ALGORITHM	SIMULATION
GSMN_MODEL	Recon2_PIPES
GSMN_EXT_TAG	_xt

The simulation parameters are shown. Tabs “InitialStates” allows setting of PN place initial marking, which overrides settings in SPEPT file. The “Functions” tab allows editing of algebraic formulas for transition propensity functions. The “Flux Map” tab provides interface to flux transitions, which are used to access individual fluxes in FBA solutions.

To Run simulation click “QSSPN->Run”. Simulation will take a couple of minutes. Note that the MODEL tag changed to gsmn.tmp. The JyMet interface uses currently opened GSMN model and overrides settings in QSSPN control file. The “Output” tab will appear when simulation is finished. Select all numbers in “cortisol” column of output file and use “QSSPN->Plot trajectory” to plot the timecourse of cortisol concentration:

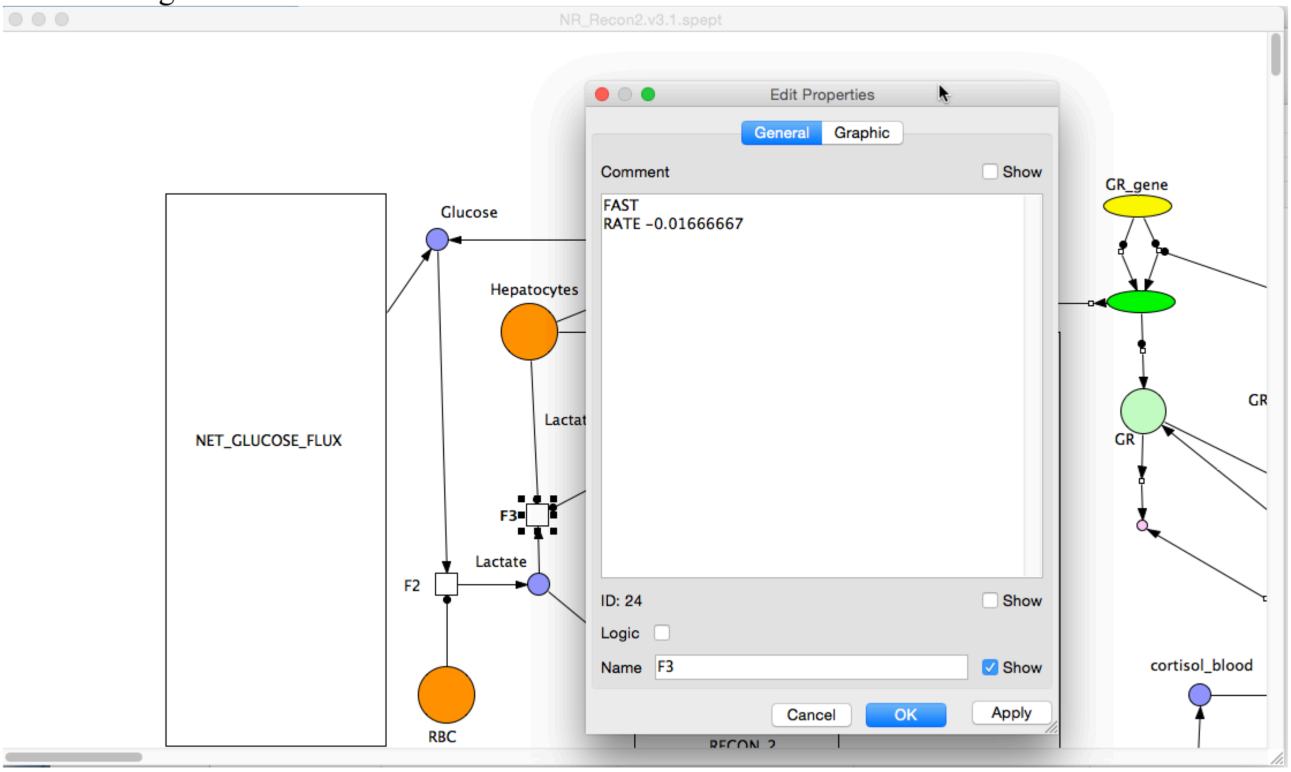


You can now examine timecourses of all Petri Net places, which were indicated by “Monitor” flag in PN node tab.

Understand format of spept and control files

The Dynamic Transitions (DT) part of QSSPN model can be graphically constructed in Snoopy Petri Net editor. There are many Petri Net classes in Snoopy, the QSSPN uses Extended Petri Net, which are saved in the file with default extension “.spept”. Install Snoopy (see above) and click on `NR_Recon2.v3.1.spept` to open the file. You will see the screen shown in section “`Open model in Snoopy Petri Net editor`” above. All rectangular symbols denote transitions. The oval symbols are places. Common, read (activator) and inhibitor edges have the same meaning as in Extended Petri Net. Additional symbols size and colour coding is applied to highlight different place and transition classes as well as different types of biological objects. Colour and size notation does not influence simulation. It is used solely for the sake of visual representation. We note that colour and size coding can be used to represent symbols from established graphical notations such as SBGN [4].

The QSSPN specific parameters are input into Comments sections of PN places, transitions and edges. We will now review all parameters used in this example. First click on transition F3 to see the following screen:



Comment □ Show

```
FAST
RATE -0.01666667
```

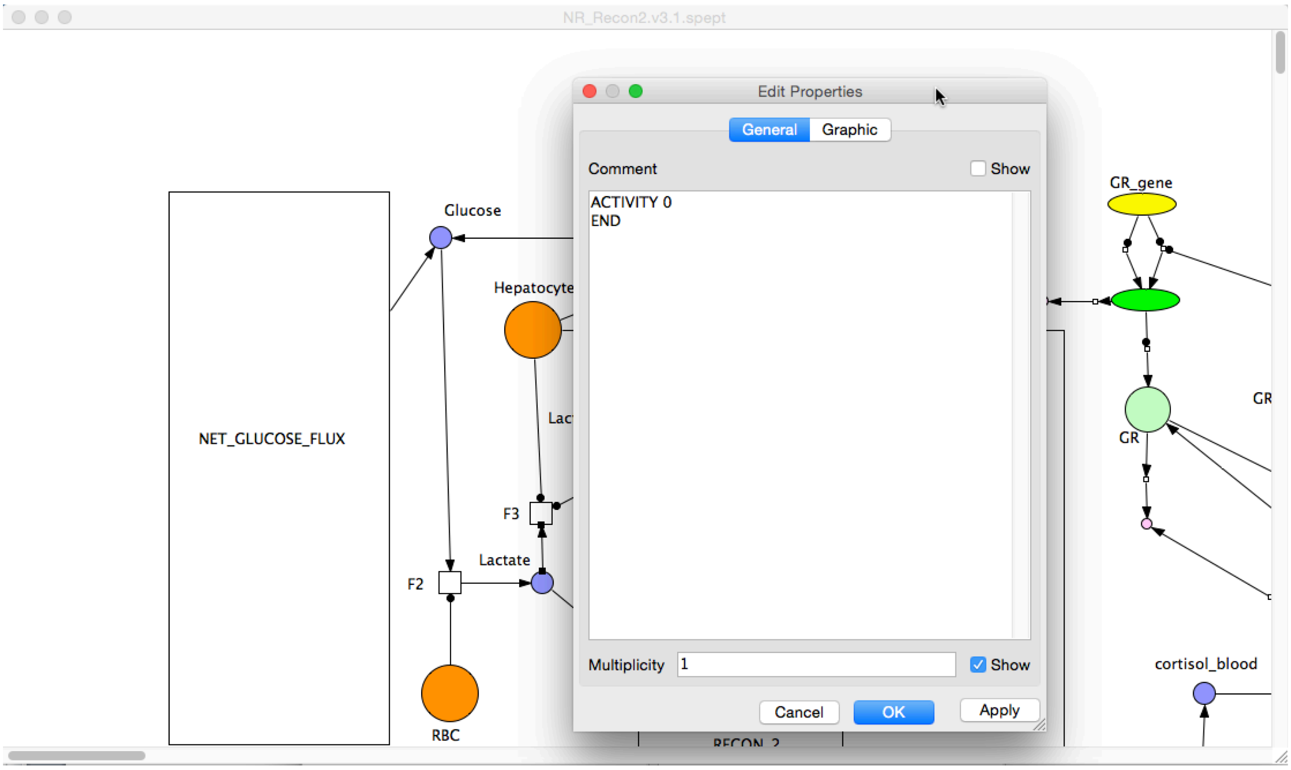
ID: 24 □ Show

Logic □

Name F3 ☑ Show

Cancel OK Apply

The tag "FAST" is used to specify that this is continuous transition. The tag "RATE" is subsequently used to set transition to -0.01666667 1/min. Now click the edge linking "Lactate" to F3.



Comment □ Show

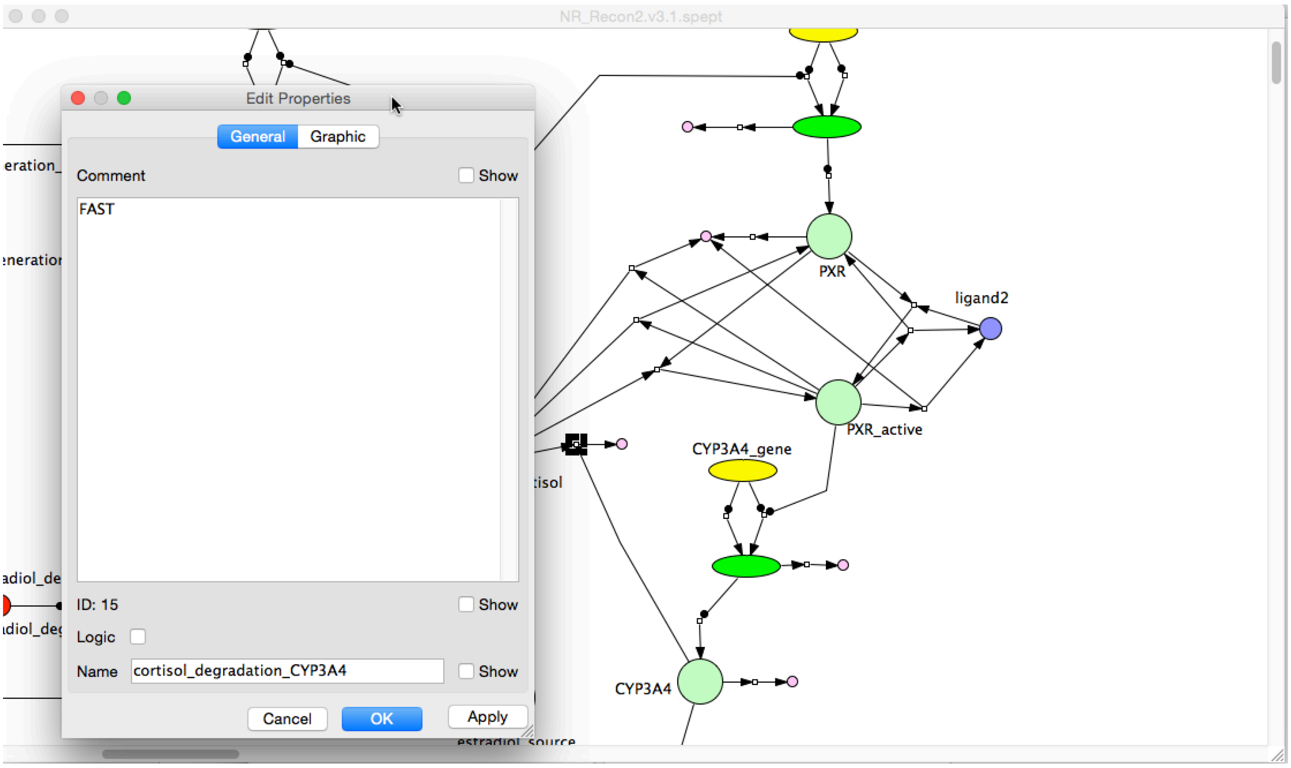
```
ACTIVITY 0
END
```

Multiplicity 1 ☑ Show

Cancel OK Apply

The comment section of an edge specifies activity of the pre-place, in this case Lactate in the transition propensity function. The “`ACTIVITY 0`” followed by “`END`” denotes mass action activity i.e. the marking of the pre-place is used. The `ACTIVITY` tag can be used to specify lookup tables mapping marking to activity in arbitrary way with multiple thresholds. This is useful for qualitative models (e.g. [1]) or linking of qualitative and quantitative models. To summarise, transition F3 is a continuous transition with mass action rate law equal to the product of rate constant and “Lactate”, “Hepatocytes”, “Lactate_consumption_flux” place markings.

The extended version of QSSPN available in MUFINS allows definition of transition propensity functions by algebraic formulas. This allows representation of kinetic models where complex rate laws are used. Here, we use our model of cortisol signalling [5, 6] available in BioModels database [7] as BIOMD0000000576. Click on transition representing cortisol degradation by CYP3A4:



Edit Properties

Comment □ Show

FAST

ID: 15 □ Show

Logic □

Name cortisol_degradation_CYP3A4 □ Show

The transition “cortisol_degradation_CYP3A4” is set to continuous (FAST tag), but no other information about its RATE is given. The propensity function of this transition is defined in the control file. Open `NR_Recon2.v3.1.ctrl.txt` file in text editor (Notepad, TextEdit, pico) and search for “cortisol_degradation_CYP3A4”. The propensity function of cortisol degradation transition is defined as follows:

```
PROPENSITY_FUNCTION cortisol_degradation_CYP3A4
CYP3A4*(0.083*cortisol/15000)/(1 + cortisol/15000)
END
```

The “PROPENSITY_FUNCTION” tag is followed by transition name. The following line contains algebraic formula. All variable names must correspond to the pre-place names of this transition. Parameters are given as numbers. To use parameter name, one needs to create a PN place connected to the transition by read edge. The parameter value can be then set and varied within “INITIAL_STATE” section of the control file. The formula definition is then closed by END tag.

The “RECON_2” transition represents the Quasi Steady State Flux (QSSF) part of the model, which in this example is human GSMN Recon 2 [3]. Click on RECON_2 transition to see the following comment section:

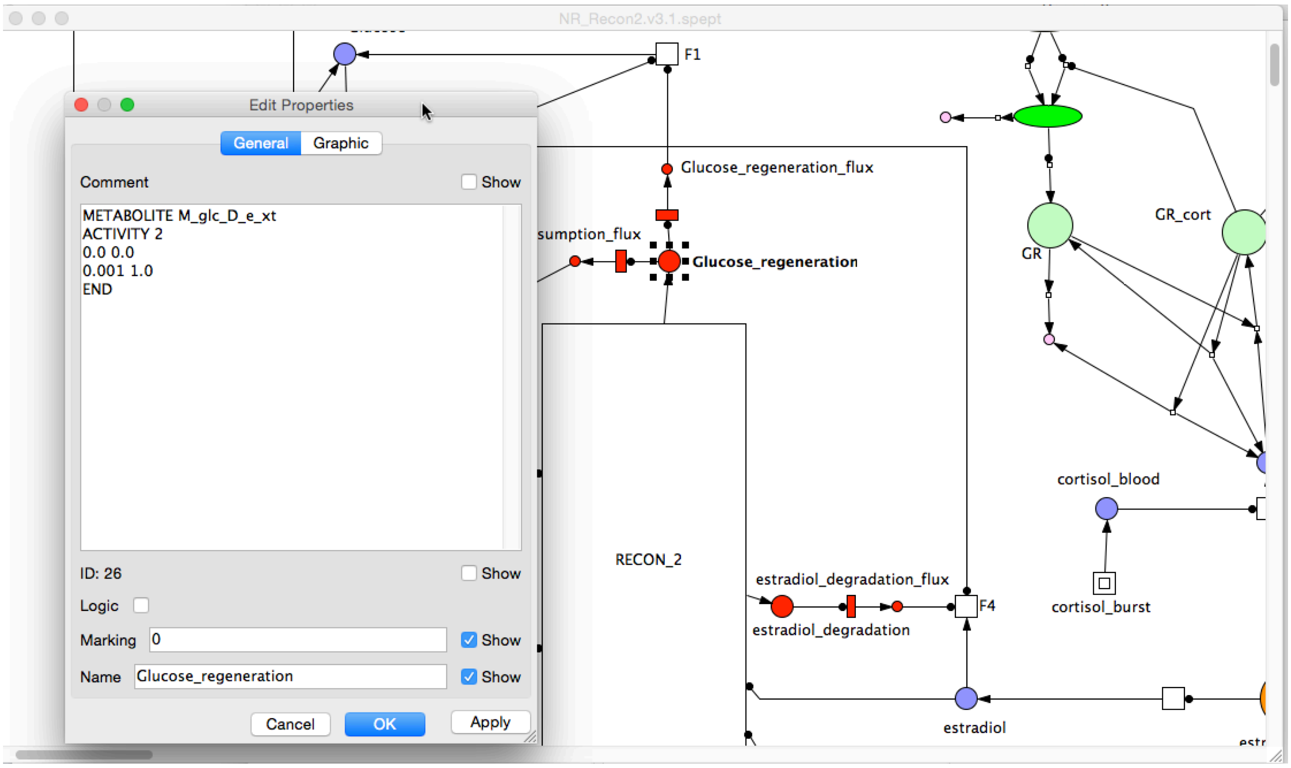
```
MODEL Recon2_PIPES
EXT_TAG _xt
```

The QSSF part of the model is specified in SurreyFBA reaction table file `recon2_xt.PIPES.CORE.v1.sfba`. The “EXT_TAG” specifies the “externality tag” used in SurreyFBA file, “_xt” in this case. The string is “_xt” is added at the end of each external metabolite name in metabolic model (see GETTING_STARTED tutorial). The MODEL tag can be used to give name of the SurreyFBA file. However, in this case we override this setting by using MODEL tag in the control file. The first line in NR_Recon2.v3.1.ctrl.txt reads:

```
MODEL ./recon2_xt.PIPES.CORE.v1.sfba
```

The name following MODEL tag in RECON_2 transition comment section is just a placeholder. We find it more convenient to name SurreyFBA files in control file, rather than in *.spept file.

We will now describe constraint and objective places used to connect the DT and QSSF parts of the model. Click on “Glucose_regeneration” place:



Edit Properties

General | Graphic

Comment ☐ Show

```
METABOLITE M_glc_D_e_xt
ACTIVITY 2
0.0 0.0
0.001 1.0
END
```

ID: 26 ☐ Show

Logic ☐

Marking 0 ☑ Show

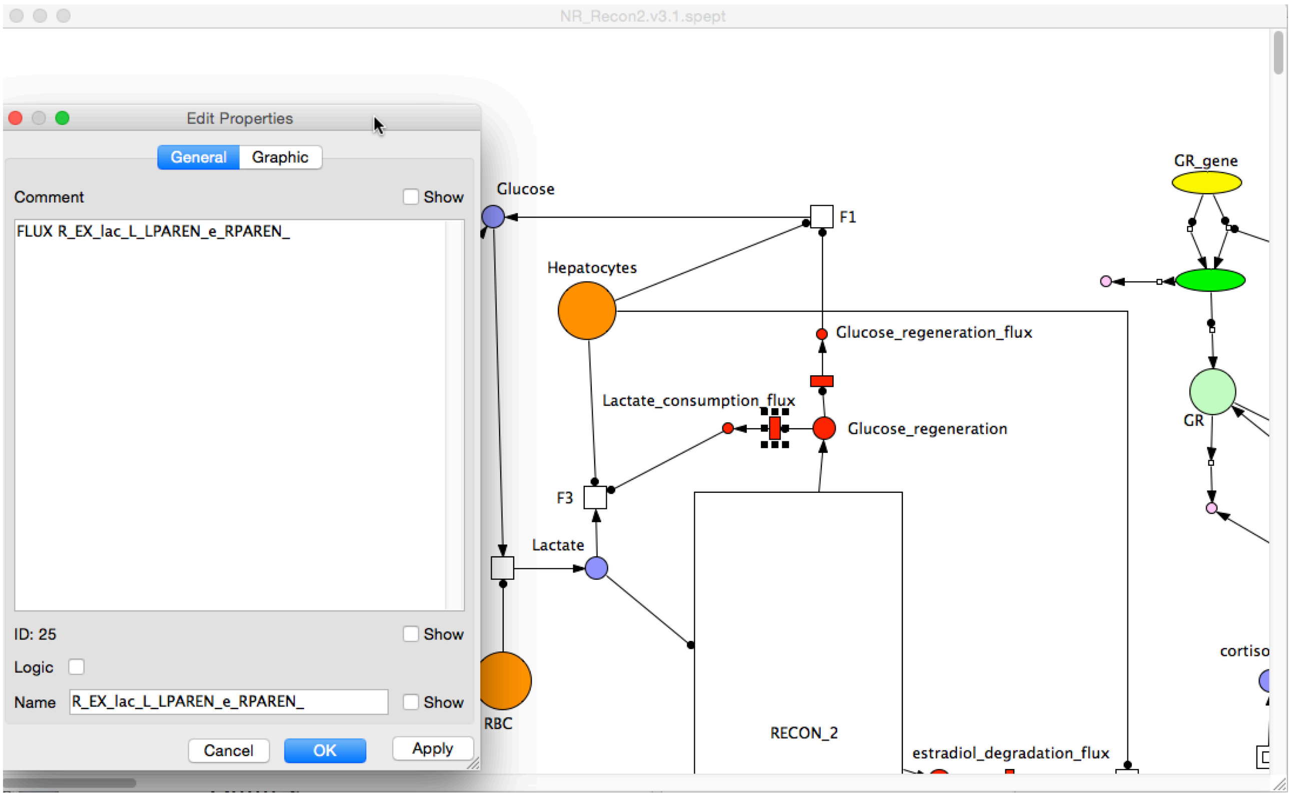
Name Glucose_regeneration ☑ Show

Cancel | OK | Apply

The “Glucose_regeneration” is an objective place. The METABOLITE tag specifies objective function. Here, the “M_glc_D_e_xt” is a name of metabolite representing external glucose. If metabolite name is given, the objective function is set to metabolite producibility [8] defined as the sum of fluxes producing the metabolite. Reaction names can be also used here. The “ACTIVITY”, “END” tag enclose lookup table that maps objective function value to marking of the objective place. Here we set marking to “1” if flux is larger than 0.001.

The mapping of objective function value to objective place marking is useful in qualitative models [1]. In extended version of QSSPN algorithm we provide FLUX transitions to extract actual flux values from FBA solution. This enables dFBA simulations [9] and thus improves integration of quantitative models. The flux transitions can have only one pre-place and one postplace. The pre-place must be an objective place. Upon evaluation of objective function FLUX transition extracts one flux from the FBA solution and sets marking of the post-place to this flux. Note, that marking of flux transition postplace can be a negative number, since FBA solution fluxes can be negative.

In this model we use graphical notation where objective places are red and default size, flux transitions are red rectangles, connected to objective places and flux transition postplaces are small circles. Click on flux transition linking "Glucose_regeneration" objective with "Lactate_consumption_flux".



Edit Properties

Comment □ Show

FLUX R_EX_lac_L_LPAREN_e_RPAREN_

ID: 25 □ Show

Logic □

Name R_EX_lac_L_LPAREN_e_RPAREN_ □ Show

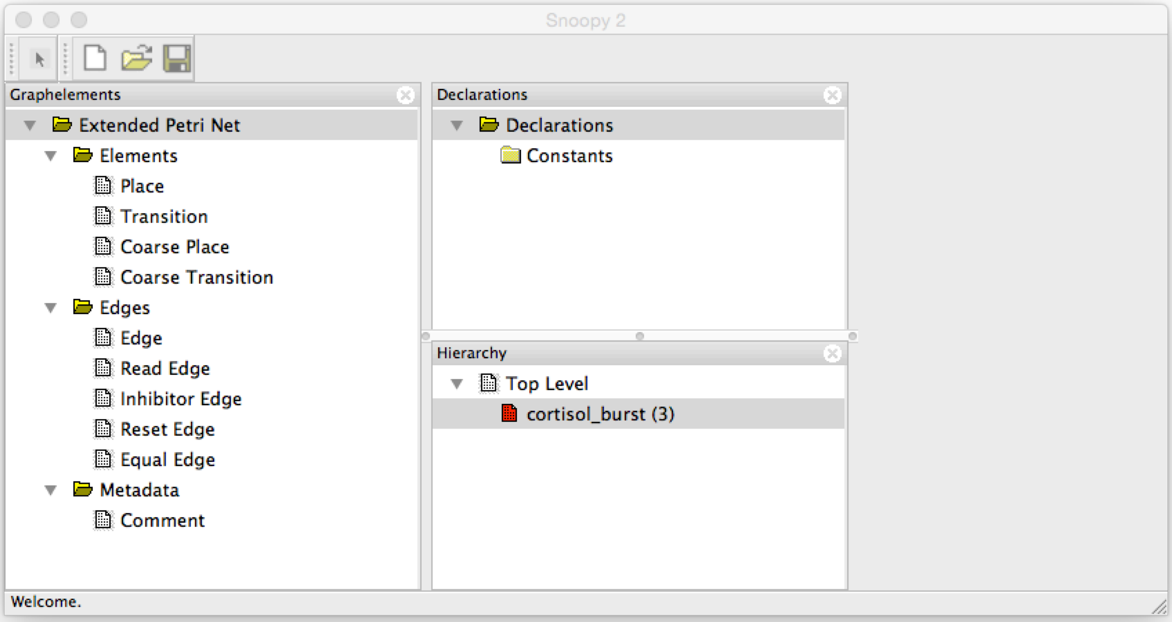
The comment section of flux transition contains FLUX tag followed by the reaction name. Flux transition shown here extracts lactate consumption flux from the FBA solution corresponding to maximal glucose regeneration flux. When objective value is calculated this transition extracts flux of reaction "R_EX_lac_L_LPAREN_e_PAREN_" and sets marking of "Lactate_consumption_flux" place to this value. Second flux transition connected to "Glucose_regeneration_objective" extracts flux of glucose exchange reaction "R_EX_glc_LPAREN_e_RPAREN_". Given that objective function is set to external glucose (M_glc_D_e_xt), glucose exchange reaction flux is equal to objective function value.

The "Glucose_regeneration_flux" and "Lactate_consumption_flux" places are connected by read edges to transitions F1 and F3. Thus, propensity function formulas of both transitions use FBA solution fluxes as variables. For example, propensity function of transition F3 is a product of lactate consumption flux, lactate concentration, number of hepatocytes and rate constant. The rate constant is negative (-0.01666667) because lactate consumption flux has negative values. If lactate is consumed, the propensity function of transition F3 has positive value. Petri Net connectivity than determines that F3 consumes lactate with the rate equal to its propensity function. In ODE semantics used here, transition propensity function is interpreted as a kinetic model reaction rate.

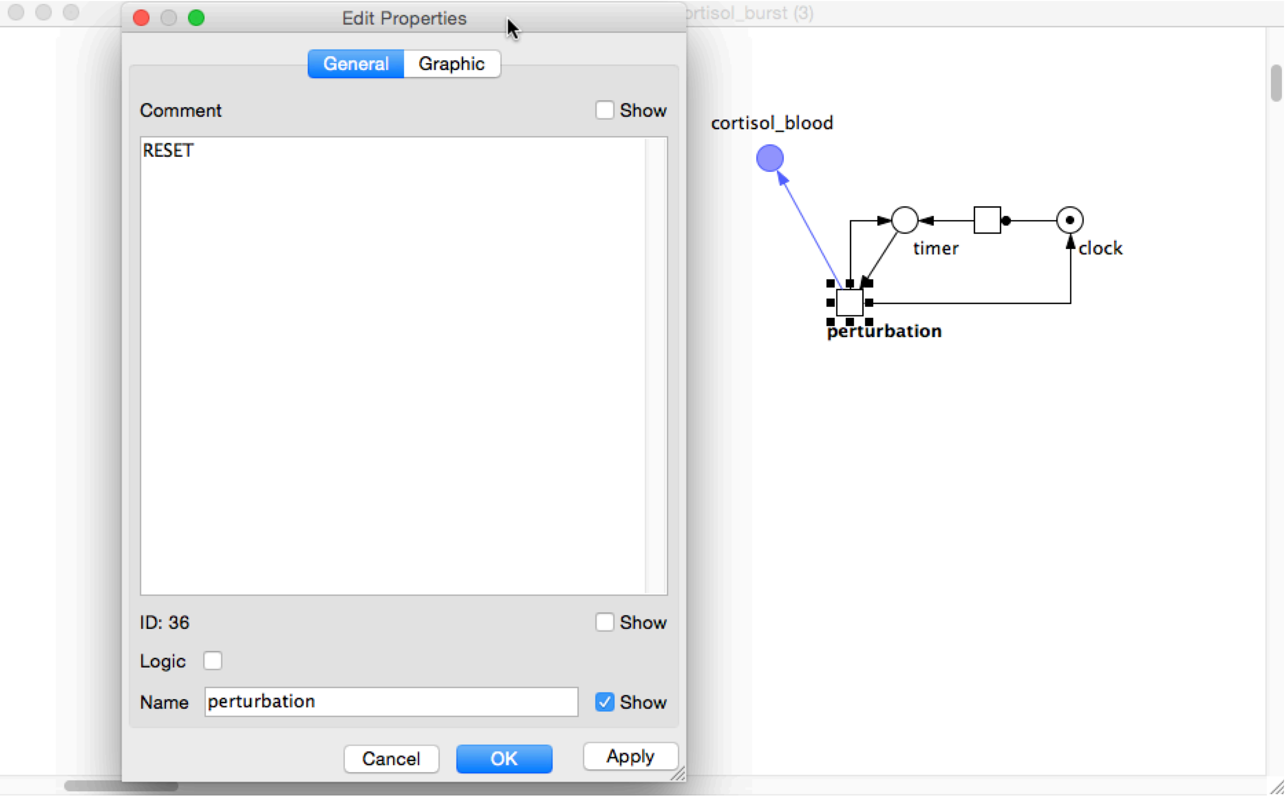
The spep2qsspn script can interpret hierarchical PNs build with Snoopy. Hierarchy allows encapsulation of modules into coarse places or transitions, which clarifies network diagrams.

Transition "cortisol_burst" encapsulates sub-network, which generates burst of cortisol after 500 minutes of simulation time. Since connectivity of this sub-network represents experimental perturbation rather than molecules in the system, encapsulation in coarse transition module clarifies the picture.

In Snoopy window find Hierarchy section and click on "cortisol_burst" sub-network.



When the new window appears, click "perturbation" transition:



Comment □ Show

RESET

ID: 36 □ Show

Logic □

Name perturbation ☑ Show

Transition “perturbation” is a “RESET” transition. It fires immediately after being enabled and sets marking of its postplaces to specific values. Here, marking of a timer is increased by continuous transition with propensity function of 1. Thus marking of place “timer” equals simulation time. When timer reaches 500, perturbation transition sets “cortisol_blood” to 0.5 and sets both “timer” and “clock” to 0. Therefore, a single burst of cortisol is generated. Threshold of 500 and reset values are set in the comments section of edges. The edge linking “timer” to “perturbation” has the following comment section:

```
ACTIVITY 2
    0.0 0.0
  500.0 1.0
END
```

The activity of “timer” pre-place in propensity function of “perturbation” transition is 0, if marking of timer is less than 500.0 and 1 otherwise. Thus, while marking of timer is less than 500, the propensity of “perturbation” is multiplied by 0. The “perturbation” rate constant is 1 by default and thus propensity of this transition equals 0 when marking of timer is less than 500. Transition does not fire. When marking of “timer” exceeds 500 for the first time, propensity of “perturbation” becomes 1, transition is enabled and fires immediately.

Edges linking reset transition with postplaces can be used to specify values to which postplaces are reset. We re-use STOICHIOMETRY tag for this purpose as this tag is also used in other context to associate coefficients with edges. Click on edges connecting perturbation to post-places to see how this is done.

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