Slides, videos, links and more:
https://github.com/physicell-training/ws2022

Session 12: Intracellular with libRoadrunner

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PhysiCell Project

July 27, 2022

What we have learned so far

- PhysiCell folder structure
- PhysiCell model domain
  - Different dts (diffusion_dt, mechanic_dt, phenotype_dt)
- Using PMB to create config file (XML)
- PhysiCell C++ functions
- And much more!

What we will learn

- Basics of Kinetic Modeling (ODE Model)
- How to integrate kinetic models to ABM
- How to control phenotype based on intracellular model
- Kinetic ODE solver (libRR) related functions
- How to save intracellular data
- How to change intracellular_dt

What we will see

- An example of how to create spheroid
- Main file addings
- How to iterate all over cells in C++
- Coloring Function

Agenda:

- First Session
  - ⓾ PhysiCell Intracellular Class
  - ⓾ Kinetic SBML
  - ⓾ PhysiCell Integration
    - Model Design
    - Results
    - Convergence Tests
  - ⓾ libRR Add-on
    - Functions
    - Phenotypic Changes
  - ⓾ Sample Model
    - Description
    - Basic Domain Creation (If we can)

PhysiCell Intracellular Class

- PhysiCell 1.9.0 = Intracellular Integrations
  - Released: 12 July 2021
- Major new features
  - Includes three intracellular modeling approaches
    - Boolean Network => PhysiBoSS
    - Kinetic Modeling (ODEs) => libroadrunner
    - Flux Balance Analysis => PhysidFBA
  - New Intracellular Object in Phenotype
    - All intracellular packages basic generic functions with same syntax
      - Start(), Update(), get_parameter(), set_parameter(), …
    - Some special functions specific to packages
      - get_boolean_variable_value(PhysiBoSS) validate_SBML_species (ODE), …

3 Sample Projects

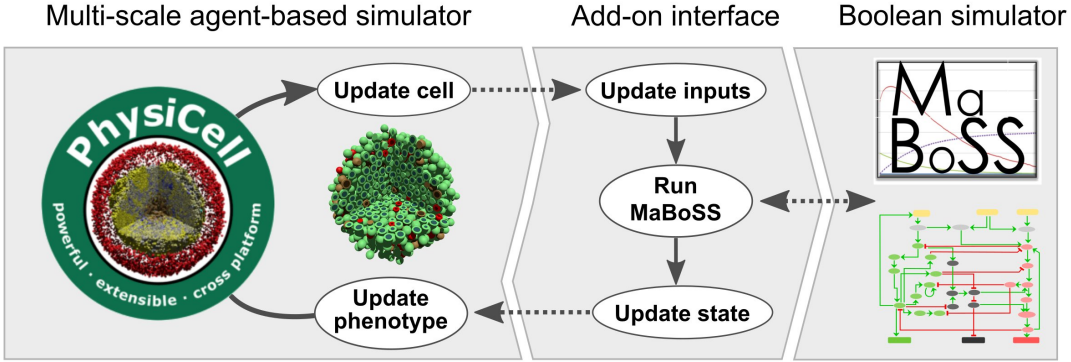
- PhysiBoSS
  - physiboss-cell-lines-sample
- Libroadrunner
  - ode-sample-project
- PhysidFBA
  - cancer-metabolism

Solvers & Installation

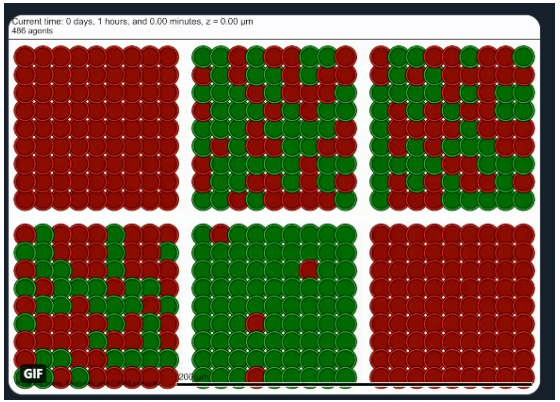
- Each add-on requires related solver
  - PhysiBoSS – MaBoSS
  - PhysidFBA – coin-clp
  - Libroadrunner – Libroadrunner (no surprise!)
- To install related solver, you need to populate sample-project, first
  - make ode-energy-sample
- Then, compile once.
  - make

PhysiBoSS

- Boolean Network
  - MaBoSS
- SysBioCurie & BSC

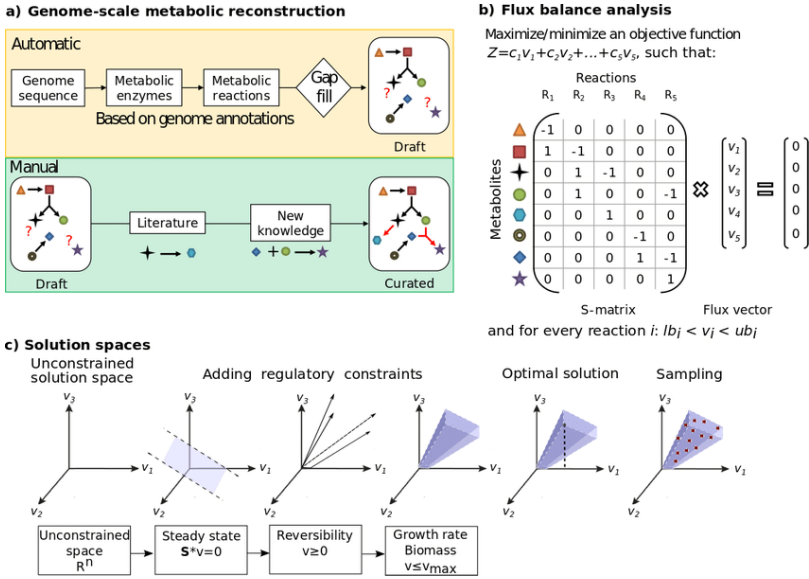


- https://github.com/gletort/PhysiBoSS
  - Fully integrated as “add-on” to PhysiCell
- Session 10-11:
  - Please visit agenda for slides and video



PhysidFBA

- Aims to couple ABM and FBA.
- Miguel Ponce de Leon
- Approach will be explained in future slides.
- https://github.com/migp11/PhysiCelldFBA
  - Added alpha version as “add-on” to PhysiCell.



Heirendt et al, 2017

Libroadrunner

- Integrating intracellular kinetic models to individual agents.
- Individual phenotype changes according to molecular simulations
  - Intracellular values (Molecular Concentrations, Signal Transductions, i.e.)
    - Intracellular oxygen deficiency in cell leads to change necrosis rate.
    - Intracellular amino acids levels define the cellular growth / cycle rate.
- Opens new opportunities for PhysiCell syntax
  - Users can utilize SBML to model phenotypic behaviors.
  - Since molecular values can edit phenotypic parameters in SBML, PhysiCell interface is getting changed.
- LibRR is developed by
  - Herbert Sauro and Andy Somogyi
  - C++ API

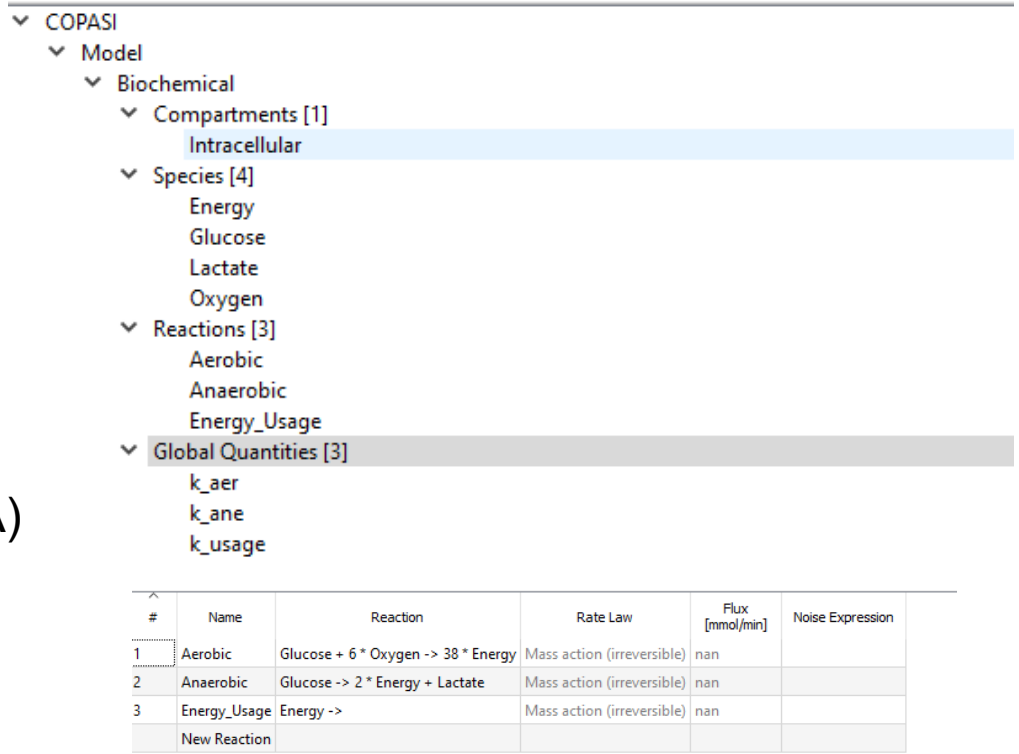
SBML

- Systems Biology Mark-up Language
- Reaction – Stoichiometry
- Kinetic – Pseudo Steady State
  - Kinetic – Ordinary Differential Equations
  - Pseudo Steady State – Optimization (Flux Balance Analysis)
- Hard to read for Human (xml file)
- Parsed according to some rules.

```
[...]
<species metaid="heme"
      id="heme"
      compartment="Comp01"
      initialConcentration="0">
   <annotation>
      <rdf:RDF xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#"
               xmlns:bqbiol="http://biomodels.net/biology-qualifiers/">
         <rdf:Description rdf:about="#heme">
            <bqbiol:hasPart>
               <rdf:Bag>
                  <rdf:li rdf:resource="urn:miriam:uniprot:P69905" />
                  <rdf:li rdf:resource="urn:miriam:uniprot:P68871" />
                  <rdf:li rdf:resource="urn:miriam:obo.chebi:CHEBI%3A17627" />
               </rdf:Bag>
            </bqbiol:hasPart>
         </rdf:Description>
      </rdf:RDF>
   </annotation>
</species>
[...]
```

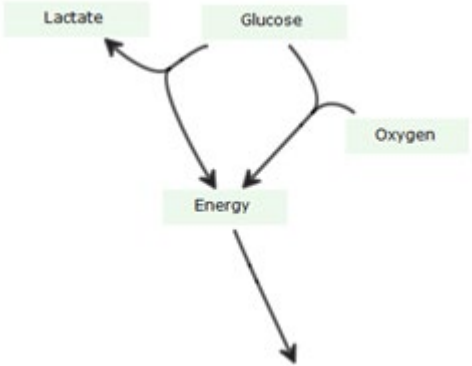
SBML

- Compartments
  - Volumetric Entities
- Species
  - Chemicals
- Reactions
  - Stoichiometric Relations
  - Boundaries (Lower and Upper) (FBA)
- Global Quantities
  - Parameters
  - Constants



#	Name	Reaction	Rate Law	Flux [mmol/min]	Noise Expression
1	Aerobic	Glucose + 6 * Oxygen -> 38 * Energy	Mass action (irreversible)	nan	
2	Anaerobic	Glucose -> 2 * Energy + Lactate	Mass action (irreversible)	nan	
3	Energy_Usage	Energy ->	Mass action (irreversible)	nan	
	New Reaction				

Kinetic Modeling



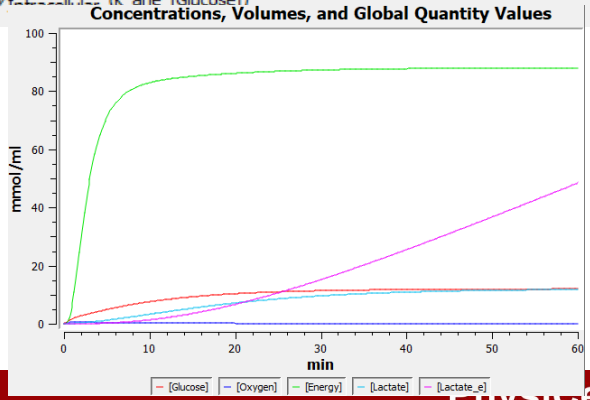
#	Name	Reaction
1	Aerobic	Glucose + 6 * Oxygen -> 38 * Energy
2	Anaerobic	Glucose -> 2 * Energy + Lactate
3	Energy_Usage	Energy ->
	New Reaction	

$$\frac{d([Glucose] \cdot V_{Intracellular})}{dt} = -V_{Intracellular} \cdot (k\_aer \cdot [Glucose] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen]) - V_{Intracellular} \cdot (k\_ane \cdot [Glucose])$$

$$\frac{d([Oxygen] \cdot V_{Intracellular})}{dt} = -6 \cdot V_{Intracellular} \cdot (k\_aer \cdot [Glucose] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen])$$

$$\frac{d([Energy] \cdot V_{Intracellular})}{dt} = +38 \cdot V_{Intracellular} \cdot (k\_aer \cdot [Glucose] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen]) + 2 \cdot V_{Intracellular} \cdot (k\_ane \cdot [Glucose]) - V_{Intracellular} \cdot (k\_usage \cdot [Energy])$$

$$\frac{d([Lactate] \cdot V_{Intracellular})}{dt} = +V_{Intracellular} \cdot (k\_ane \cdot [Glucose])$$

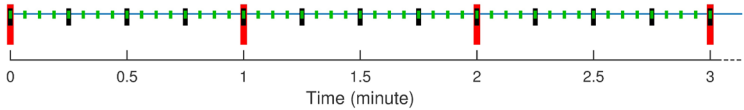


SBML

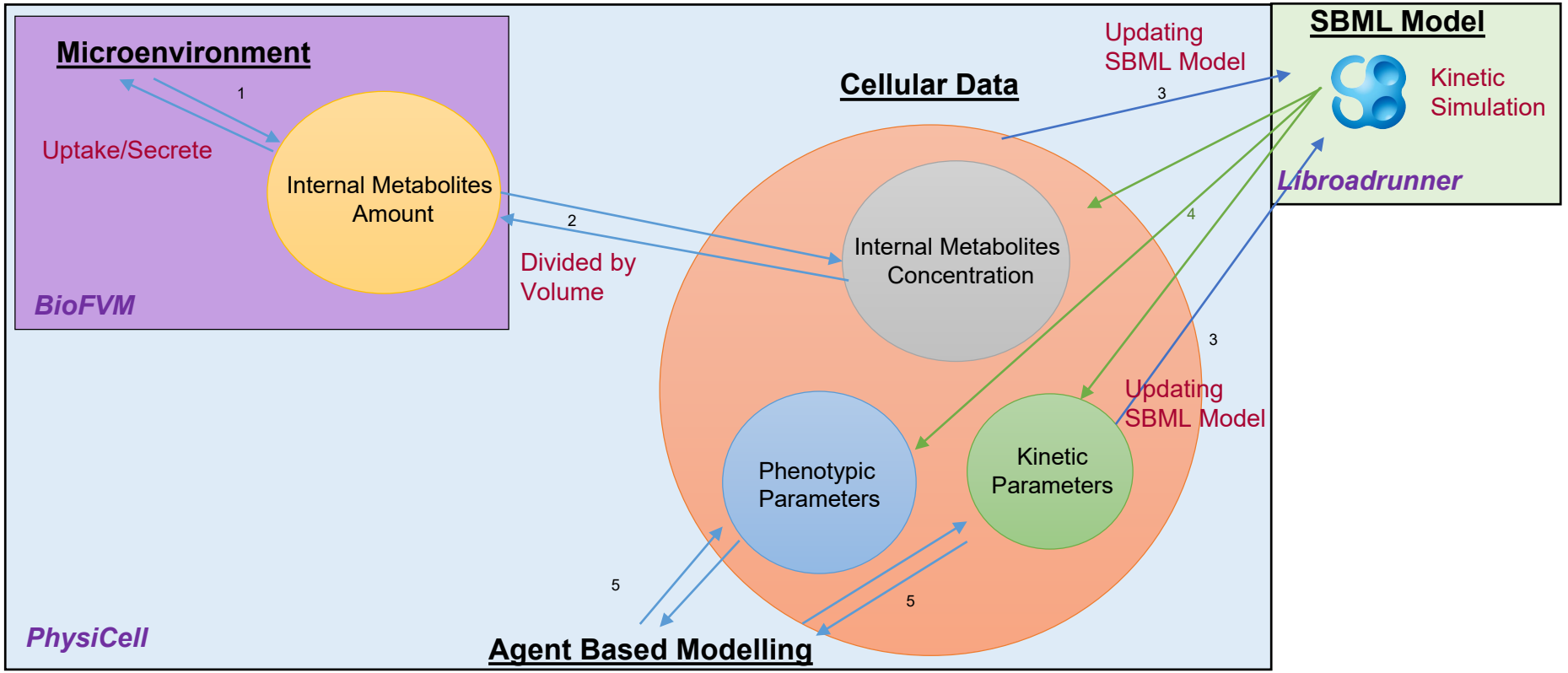
- General SBMLs have more than one compartment
  - Extracellular
  - Intracellular
  - Mitochondria (Sometimes)
  - Nucleus (?)
- And have two or more species for one substrate
  - Glucose[e] => extracellular
  - Glucose[i] => intracellular
- Transfer reaction between compartments
  - Glucose[e] = Glucose[i]

Assumptions

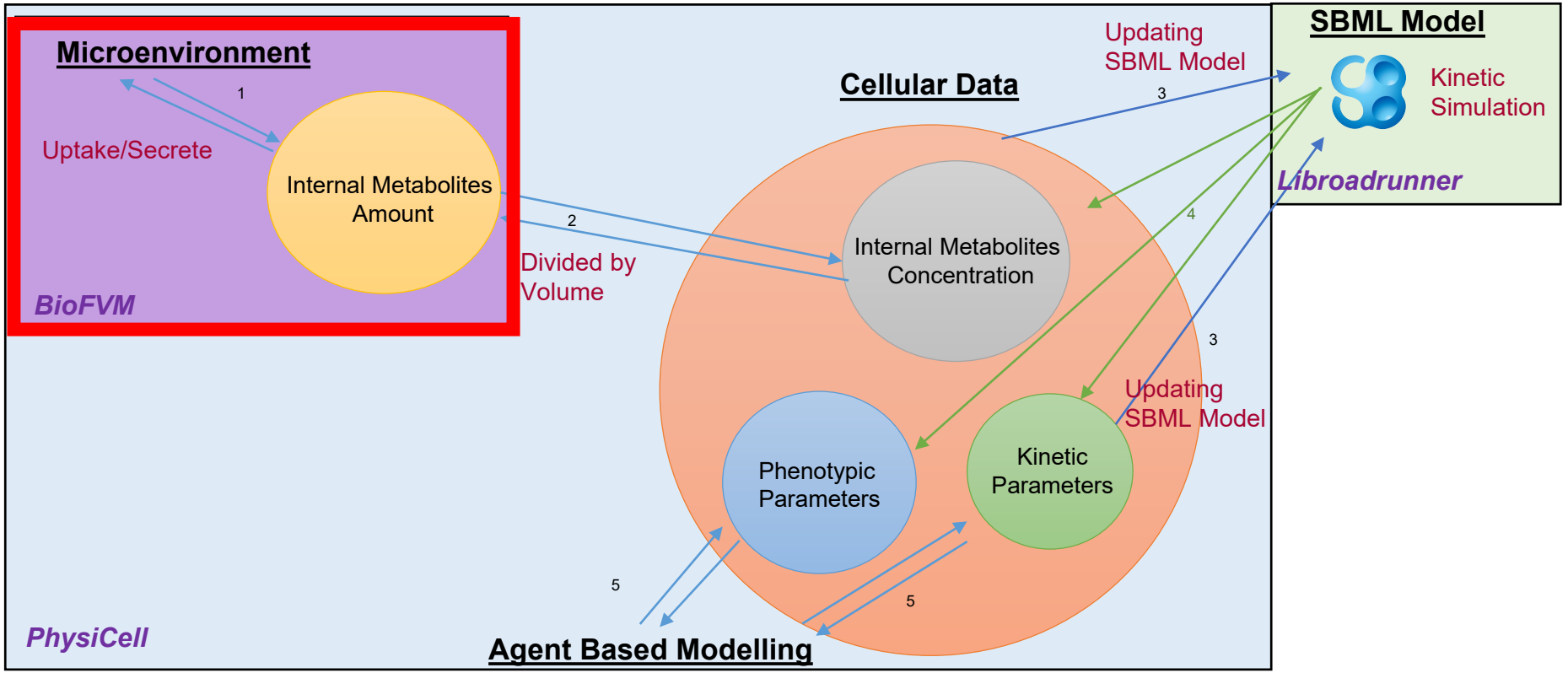
- **But …**
- PhysiCell has transfer reactions through
  - BioFVM
- So, we can support specific type of SBMLs
  - Only Intracellular (might have more than one compartment – Mitochondria, Nucleus)
- Well-Structured SBMLs
  - **Not all SBMLs are supported!!!**
    - **No External Compartment and Transfer Reactions in SBML!**
    - **Mapping is needed between SBML & PhysiCell**
  - Works on each diffusion_dt (0.01 min)
    - (as default for best convergence/can be changed)



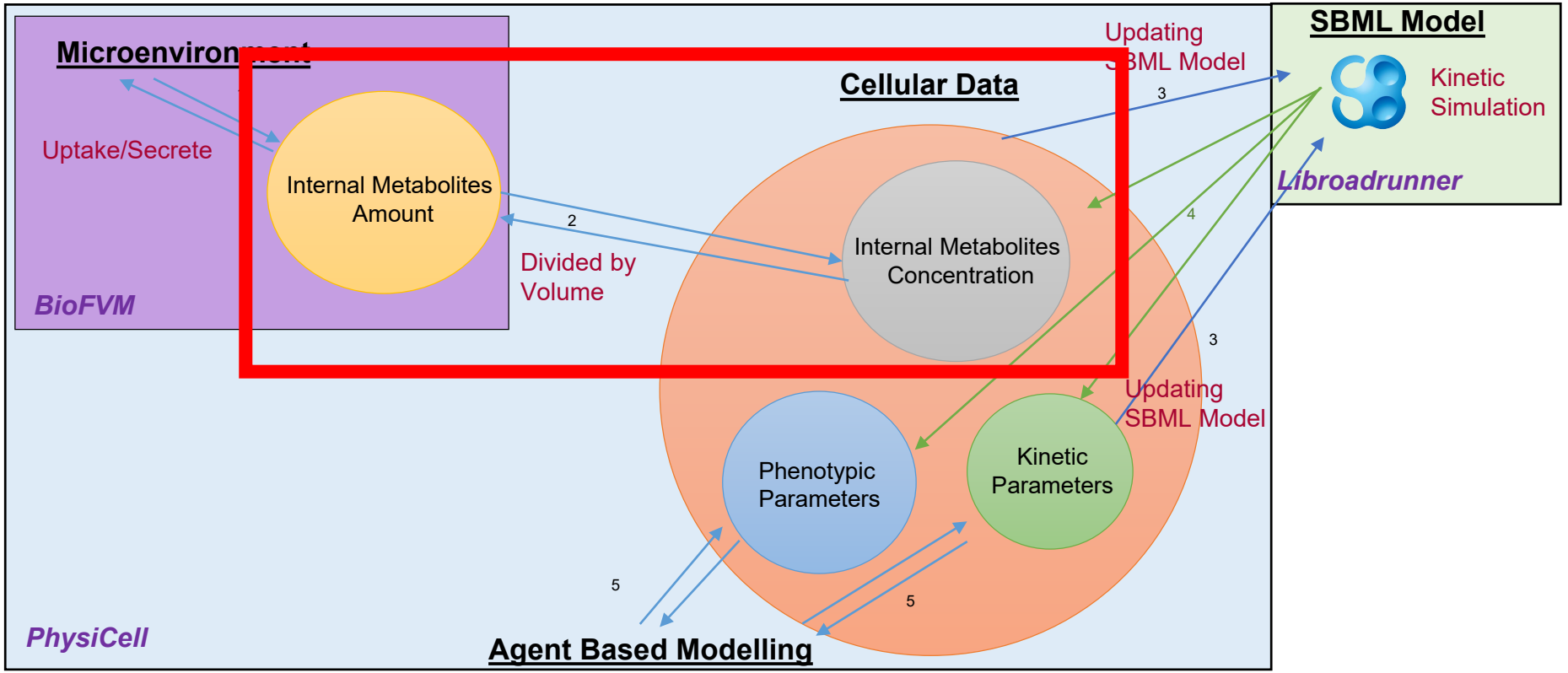
Integration Design



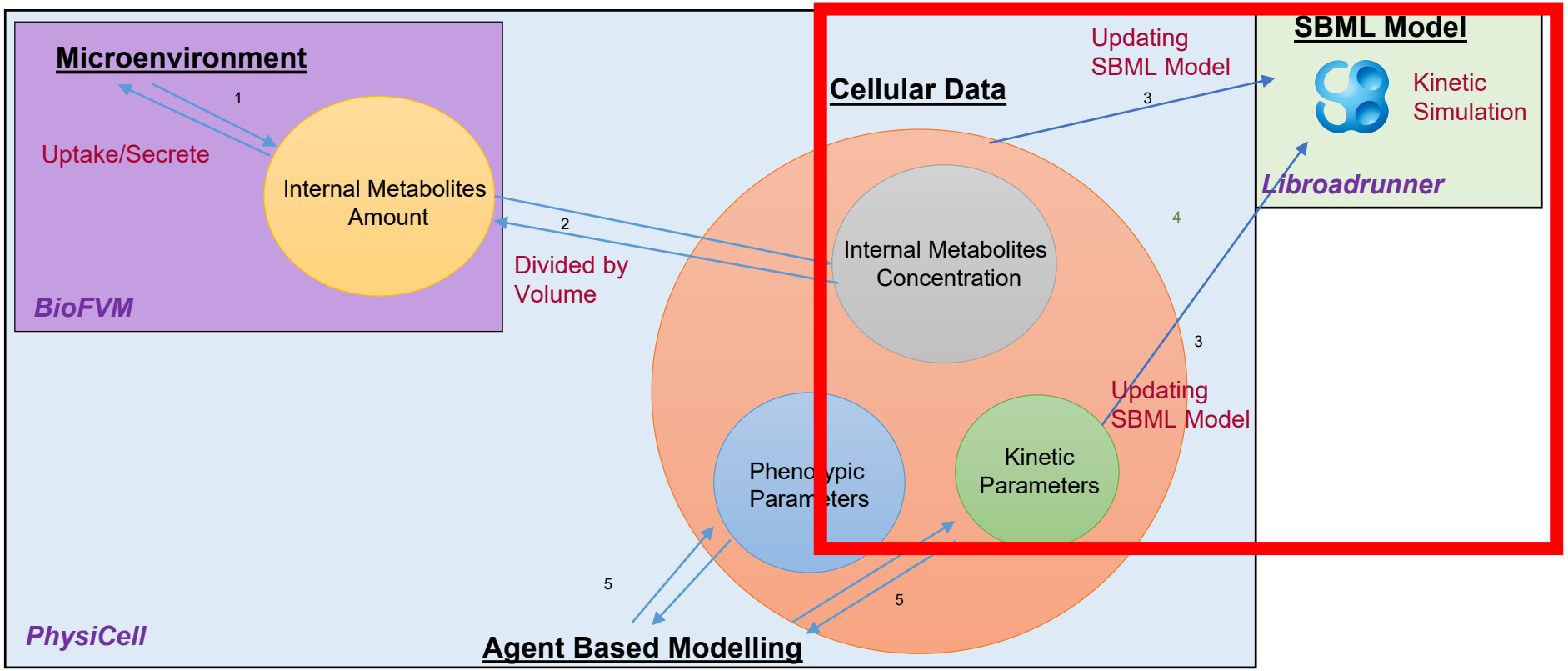
Integration Design



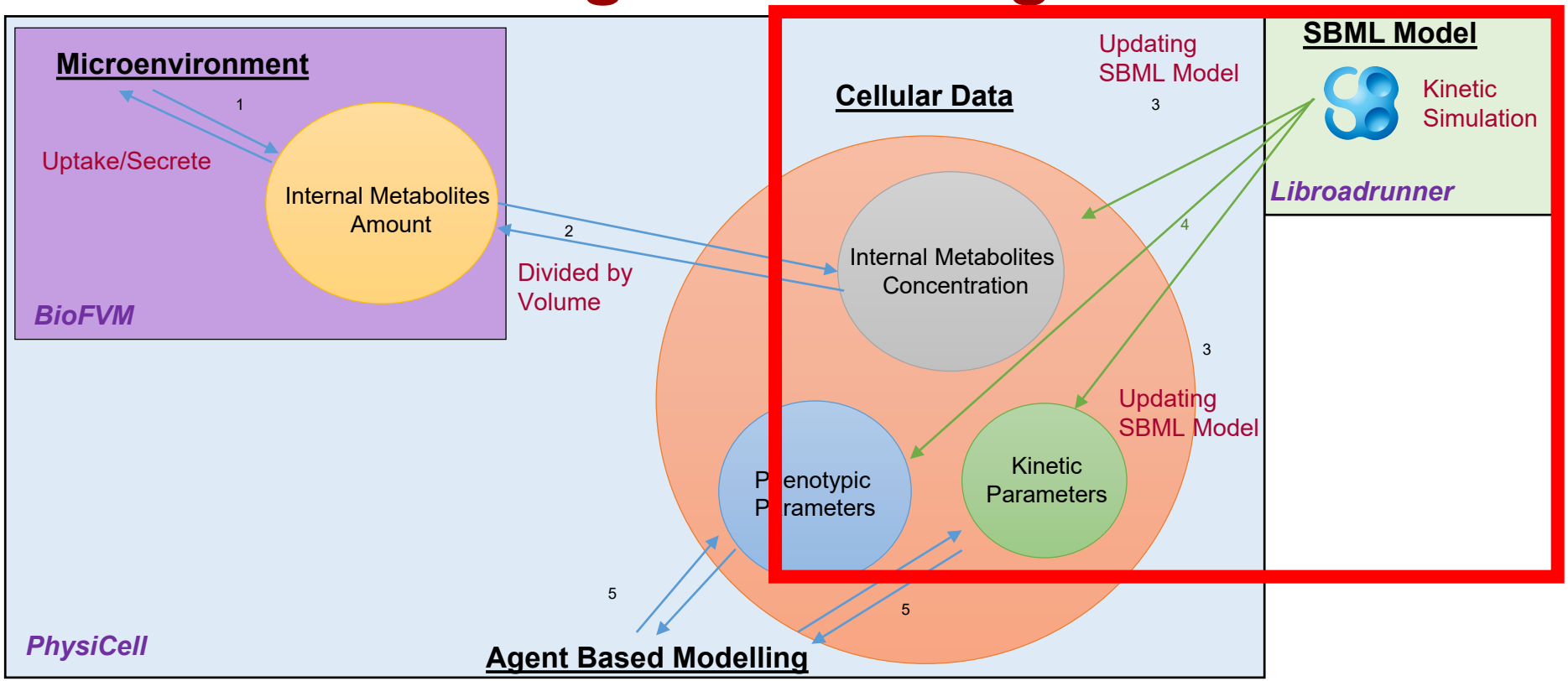
Integration Design



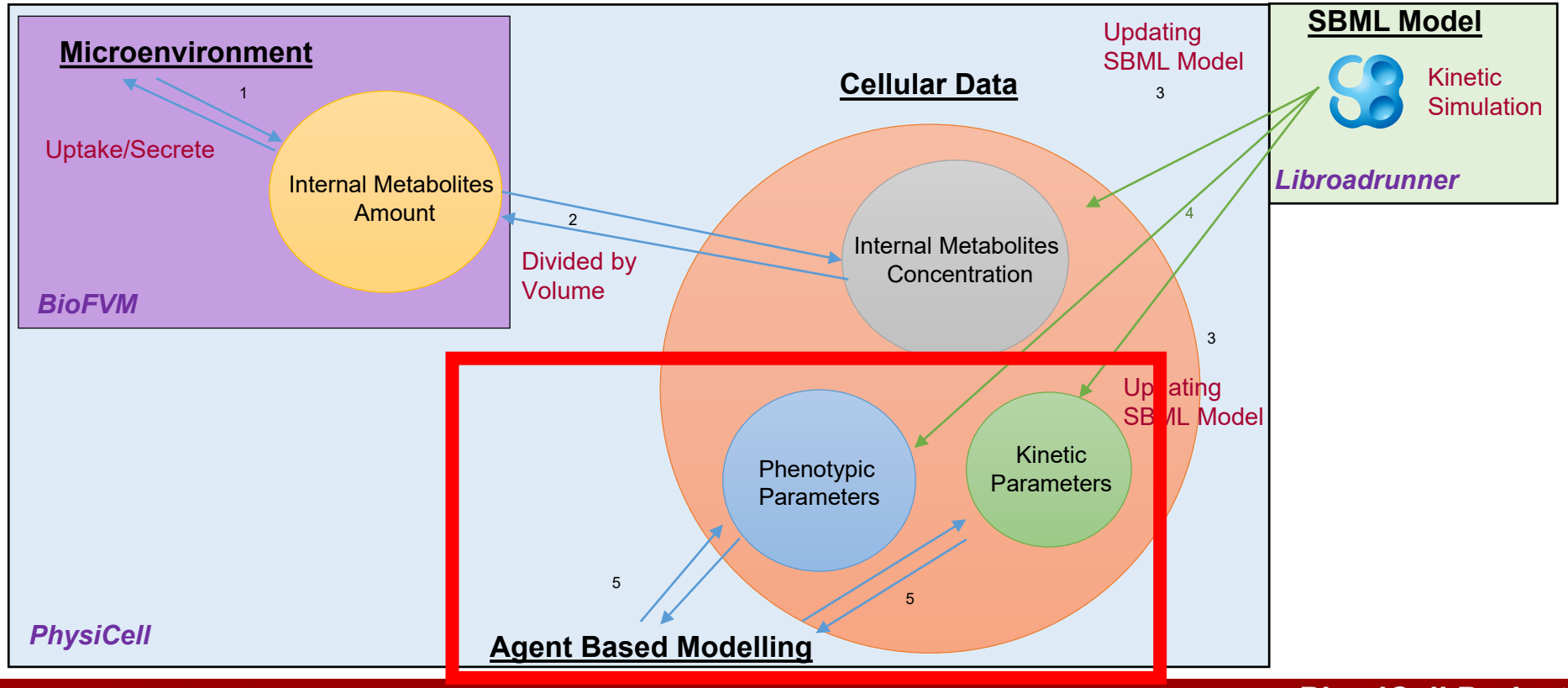
Integration Design



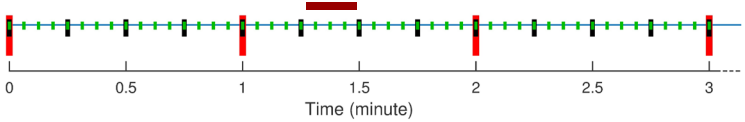
Integration Design



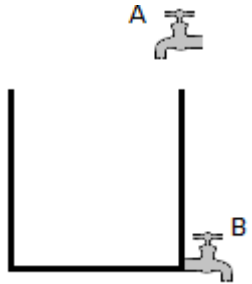
Integration Design



How about intracellular_dt



- Default 0.01 min but…
- We can change intracellular_dt
  - As we are working with rate, you don't have to change the parameters
  - Time units will match
- We can change intracellular_dt but updating less can produce some convergence errors.



How it looks

- Config File

```
<cell_definitions>
    <cell_definition name="default" ID="0">
        <phenotype>
            <cycle code="5" name="live">
                <!-- using higher than normal significant digits to match divisions in default code -->
                <transition_rates units="1/min">
                    <rate start_index="0" end_index="0" fixed_duration="false">0.0</rate>
                </transition_rates>
            </cycle>
            <death>
            <volume>
            <motility>
                <speed units="micron/min">0.0</speed>
                <persistence_time units="min">0.1</persistence_time>
                <migration_bias units="dimensionless">.9</migration_bias>
                <options>
                    <enabled>true</enabled>
                    <use_2D>true</use_2D>
                    <chemotaxis>
                        <enabled>false</enabled>
                        <substrate>oxygen</substrate>
                        <direction>1</direction>
                    </chemotaxis>
                </options>
            </motility>
            <secretion>
            <intracellular type="roadrunner">
                <sbml_filename>./config/Toy_oxy_mms_tr_01.xml</sbml_filename>
                <map PC_substrate="oxygen" sbml_species="Oxy"></map >
                <map PC_custom_data="PC_Test_CD" sbml_species="death_rate"></map>
                <map PC_phenotype="ctr00" sbml_species="transition_rate_0_1"></map>
                <map PC_phenotype="mms" sbml_species="migration_speed"></map>
                <map PC_phenotype="da" sbml_species="death_rate"></map>
                <map PC_phenotype="ssr1" sbml_species="secretion_rate_Lactate"></map>
                <map PC_phenotype="test" sbml_species="test"></map>
            </intracellular>
        </phenotype>
```

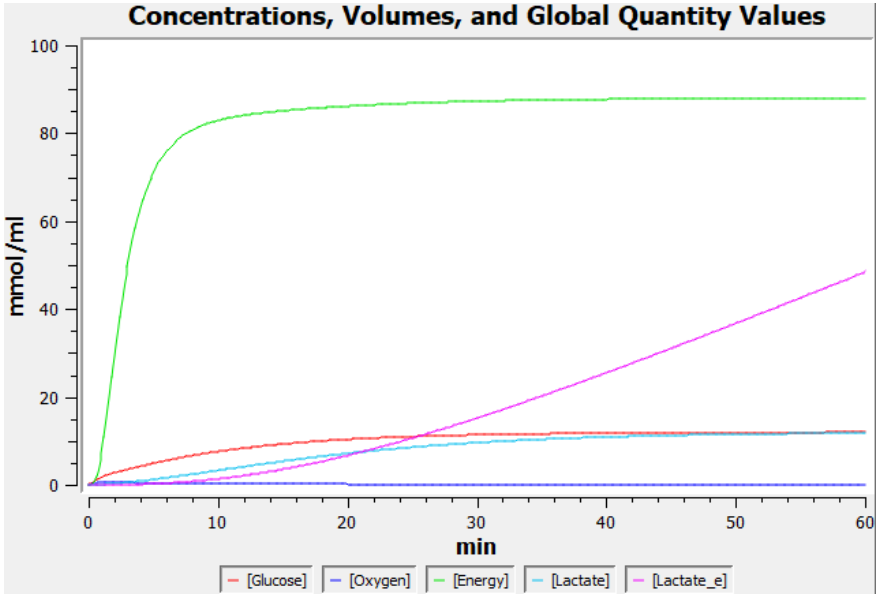
SBML-Phenotypic Parameters

PhysiCell Phenotype Parameter	First letter	phenotype_token	example
Phase Transition Rate	c	ctr_*_*	ctr_0_1
Death Rate	d	da,dn	da,dn
Persistence Time	m	mpt	mpt
Migration Speed	m	mms	mms
Migration Bias	m	mmb	mmb
Uptake rate	s	sur_*	sur_oxygen
Secretion rate	s	ssr_*	ssr_glucose
Saturation density	s	ssd_*	ssd_oxygen
Export rate	s	ser_*	ser_lactate
Target solid cytoplasmic	v	vtsc	vtsc
Target solid nuclear	v	vtsn	vtsn
Target fluid fraction	v	vff	vff

Some Results

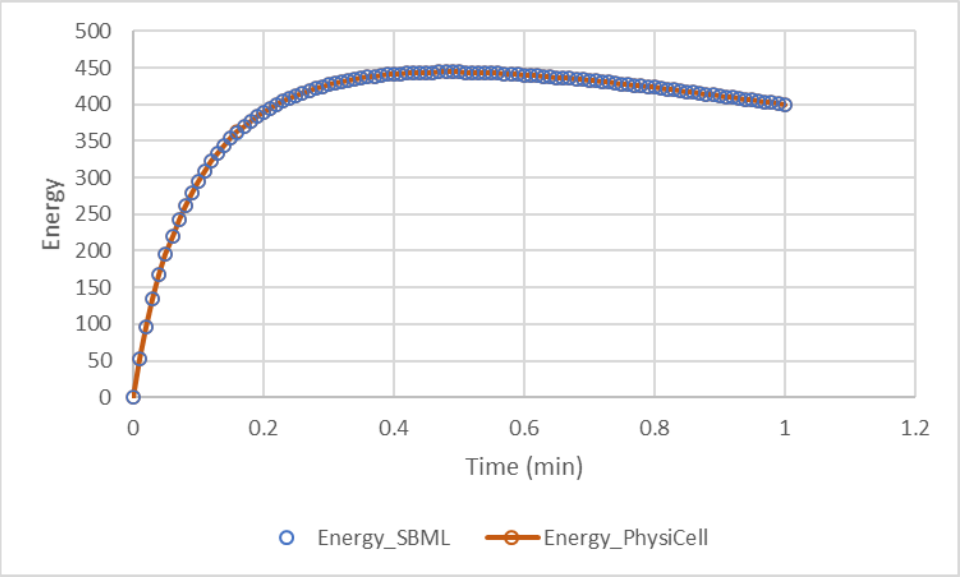
Model 0 - SBML Model

- 4 Species
  - Oxygen
  - Glucose
  - Lactate
  - Energy
- 3 Internal Reactions
  - ♦ Aerobic reaction
    - » Glucose + Oxygen -> Energy
  - ♦ Anaerobic reaction
    - » Glucose -> Energy + Lactate
  - ♦ Energy Usage
    - » Energy ->



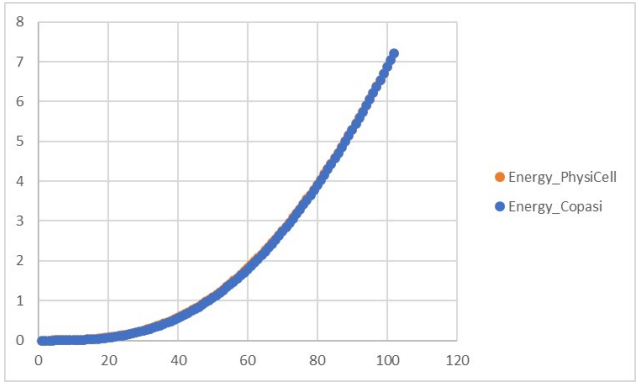
Model 0

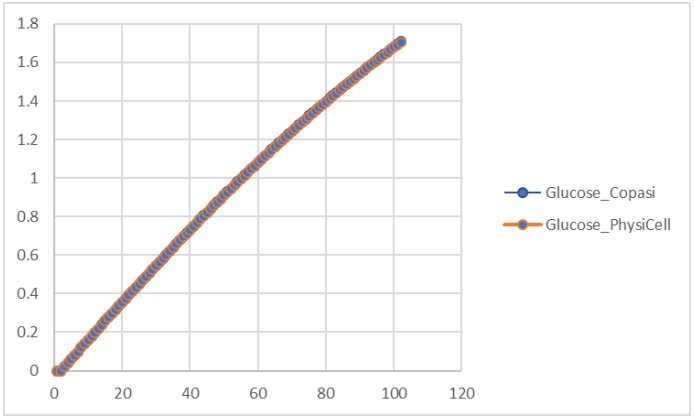
- Very Simple SBML toy model
- Both simulated in PhysiCell and Copasi
- No Transfer Reaction in SBML
- No Uptake Rate
- Only solving SBML



Model 1

- Uptake glucose, oxygen
- Produces Energy internally.

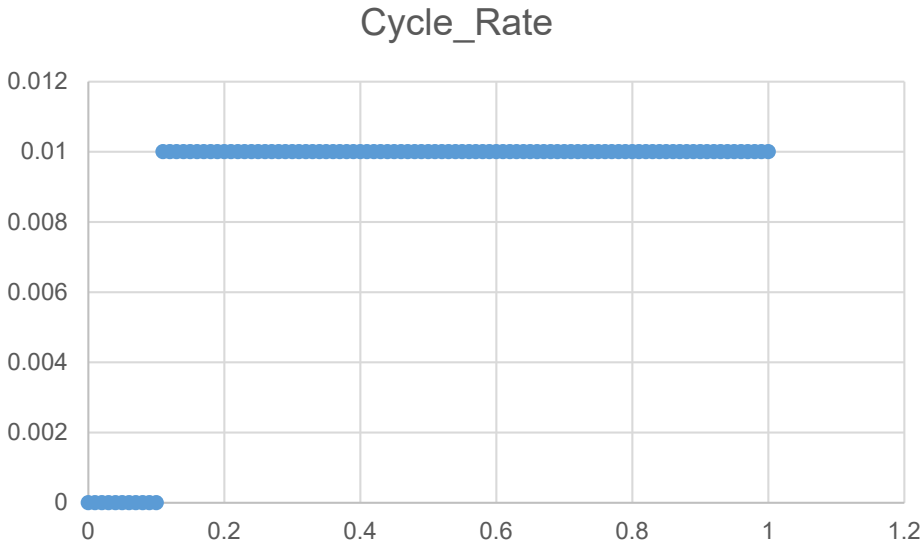




SBML Events

Cycle Rate

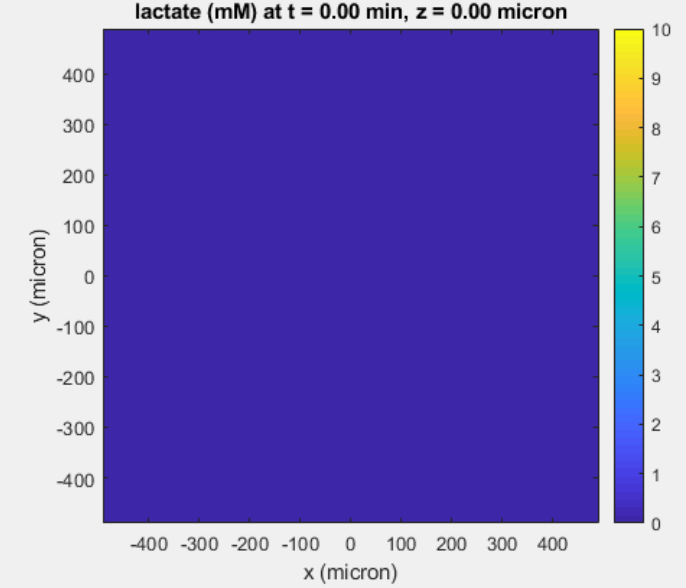
- If Energy level is smaller than 50 a.u.
  - Equals to zero 1/min
- If Energy level is greater than 50 a.u.
  - Equals to 0.01 1/min

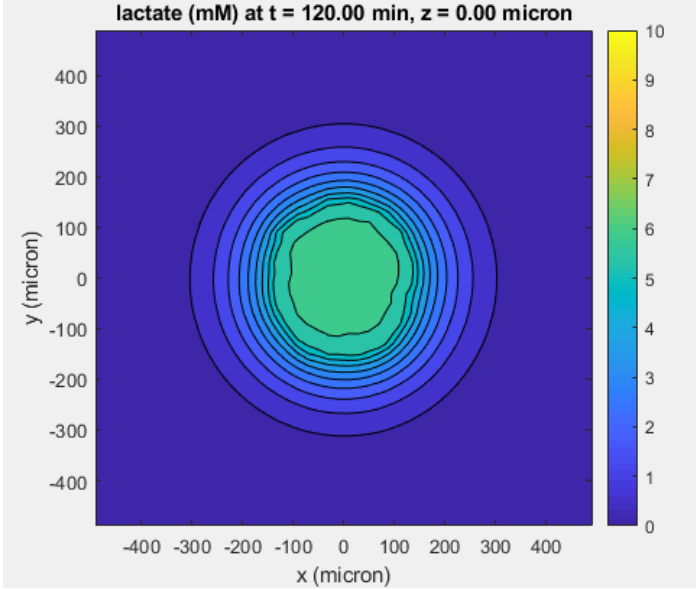


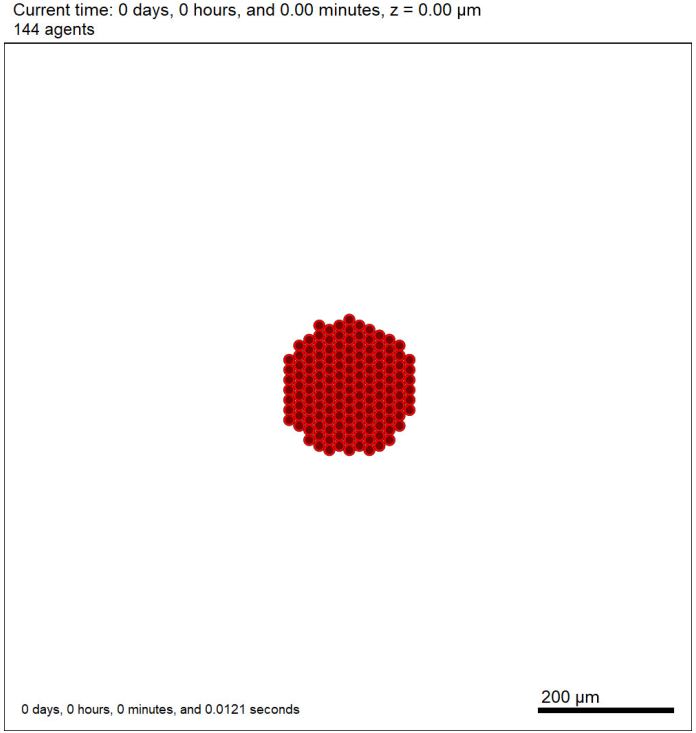
Model 2

- Phenotypic changes according to SBML species
- Organoid (2D) seeding.
- Lactate Secretion Rate increases
  - Lactate Concentration
- If oxygen level is less than threshold
  - Cells increase their migration speed
- If Energy level is less than threshold
  - Cells go apoptosis

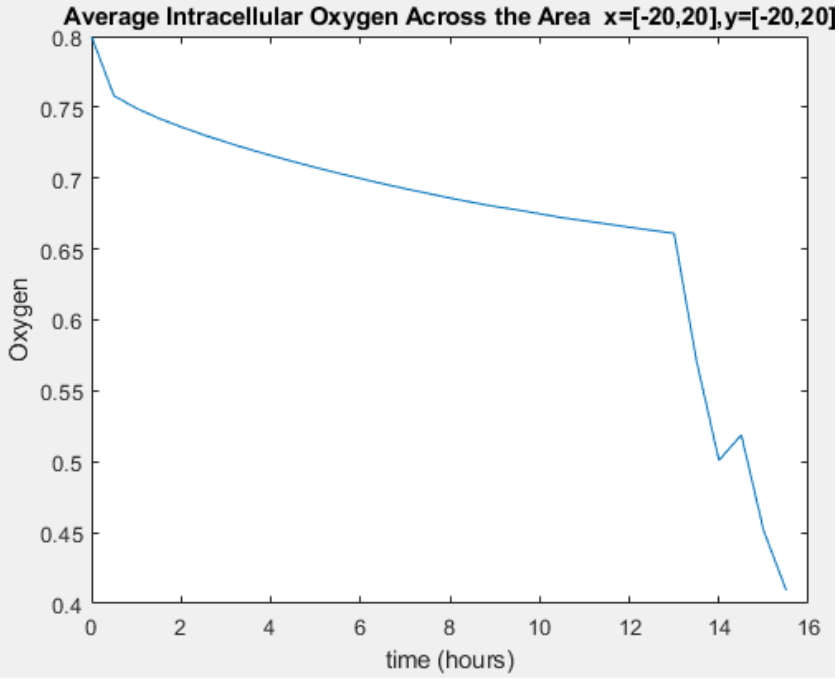
Lactate Secretion

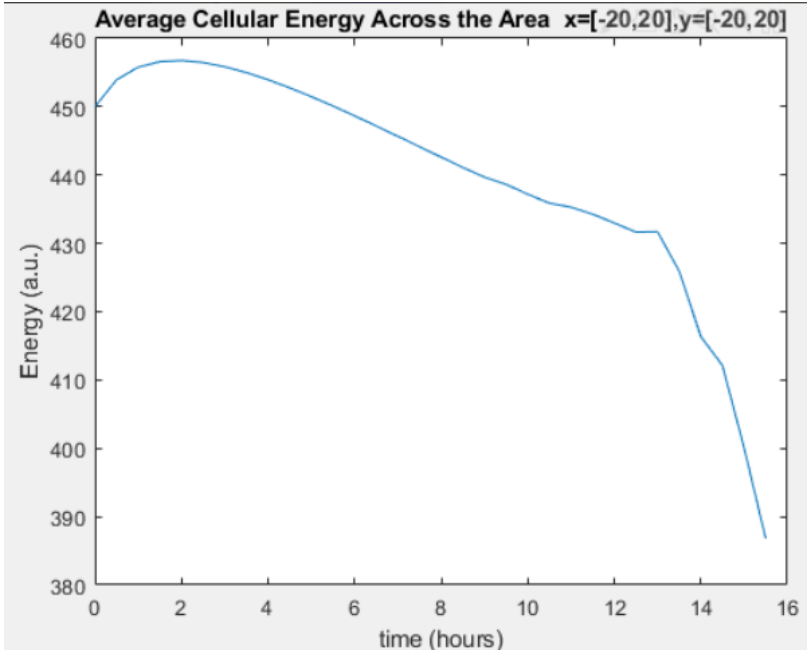





Current time: 0 days, 0 hours, and 0.00 minutes, z = 0.00 μm
144 agents
0 days, 0 hours, 0 minutes, and 0.0121 seconds
200 μm

Intracellular

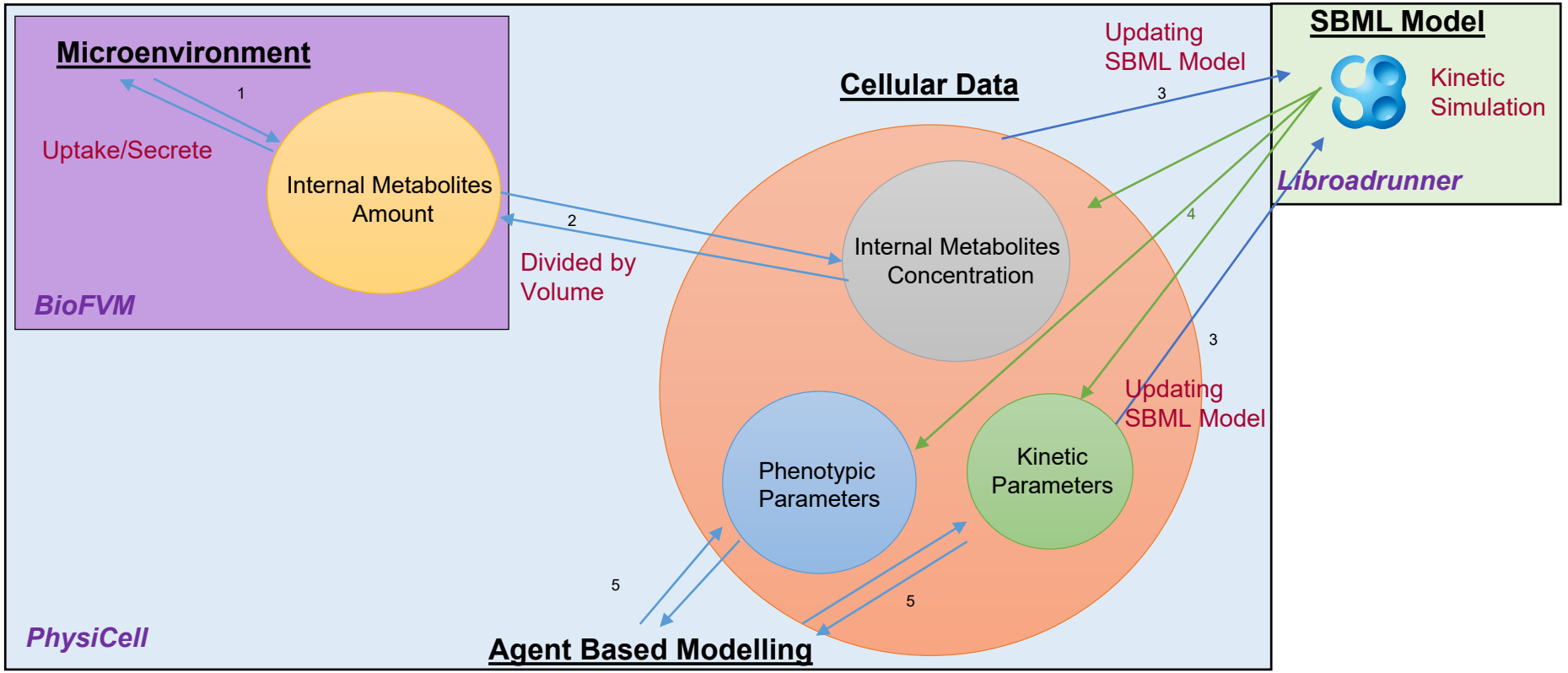




Libroadrunner Addon

- Same format for intracellular addons. (PhysiFBA, PhysiBoSS)
- Libroadrunner
  - **start()** = start intracellular in cell (it should be used after seeding and is called after proliferation)
  - **Initialize_SBML()** = to read SBML (users will not use)
  - **get_values()** = to get value from SBML
  - **set_values()** = to set value in SBML
  - **update()** = to simulate SBML
  - **get_state()** = to get the name of SBML
  - **update_phenotypic_parameters()** = to update phenotype according to given tokens
  - **validate_tokens()** = to validate given token
  - **validate_species()** = to validate SBML species

Integration Design



SBML-Phenotypic Parameters

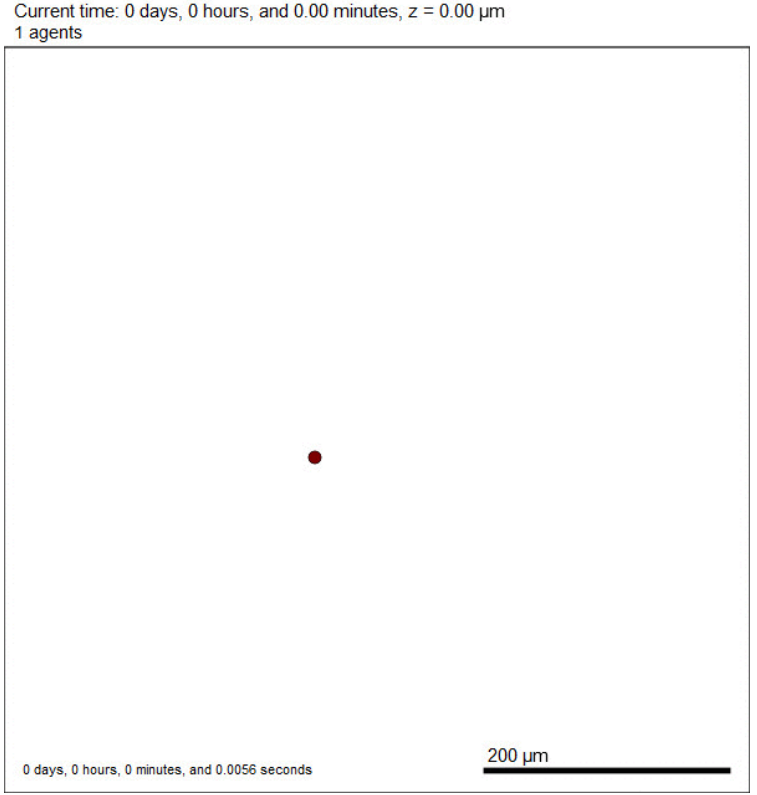
PhysiCell Phenotype Parameter	First letter	phenotype_token	example
Phase Transition Rate	c	ctr_*_*	ctr_0_1
Death Rate	d	da,dn	da,dn
Persistence Time	m	mpt	mpt
Migration Speed	m	mms	mms
Migration Bias	m	mmb	mmb
Uptake rate	s	sur_*	sur_oxygen
Secretion rate	s	ssr_*	ssr_glucose
Saturation density	s	ssd_*	ssd_oxygen
Export rate	s	ser_*	ser_lactate
Target solid cytoplasmic	v	vtsc	vtsc
Target solid nuclear	v	vtsn	vtsn
Target fluid fraction	v	vff	vff

Migration Speed

Cell Definition

```
<motility>
    <speed units="micron/min">0.0</speed>
    <persistence_time units="min">0.1</persistence_time>
    <migration_bias units="dimensionless">.9</migration_bias>
    <options>
```

```
<intracellular type="roadrunner">
    <sbml_filename>./config/Toy_oxy_mms_tr_01.xml</sbml_filename>
    <map PC_substrate="oxygen" sbml_species="Oxy"></map >
    <map PC_custom_data="PC_Test_CD" sbml_species="death_rate"></map>
    <map PC_phenotype="mms" sbml_species="migration_speed"></map>
</intracellular>
```

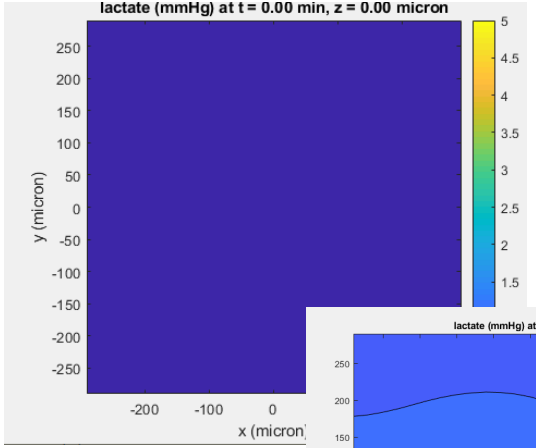


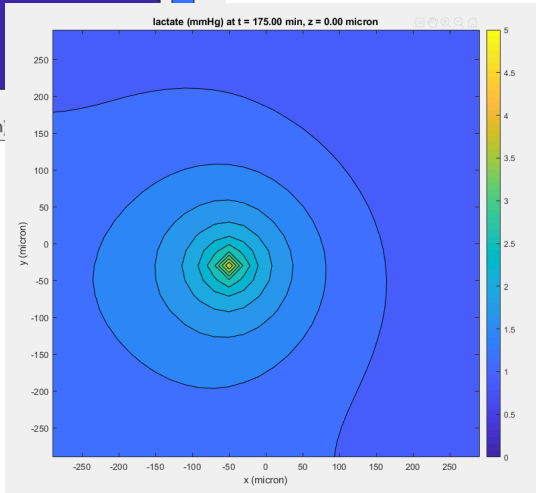
Lactate Distribution

Cell Definition

```
        <secretion_rate units="1/min">0</secretion_rate>
        <secretion_target units="substrate density">10</secretion_target>
        <uptake_rate units="1/min">0.0</uptake_rate>
        <net_export_rate units="total substrate/min">0</net_export_rate>
    </substrate>
```

```
<intracellular type="roadrunner">
    <sbml_filename>./config/Toy_oxy_mms_tr_01.xml</sbml_filename>
    <map PC_substrate="oxygen" sbml_species="Oxy"></map >
    <map PC_phenotype="ssr1" sbml_species="secretion_rate_Lactate"></map>
</intracellular>
```





Wrong Tokens

```
<intracellular type="roadrunner">
    <sbml_filename>./config/Toy_oxy_mms_tr_01.xml</sbml_filename>
    <map PC_substrate="oxygen" sbml_species="Oxy"></map >
    <map PC_custom_data="PC_Test_CD" sbml_species="death_rate"></map>
    <map PC_phenotype="ctr00" sbml_species="transition_rate_0_1"></map>
    <map PC_phenotype="ssr1" sbml_species="secretion_rate_Lactate"></map>
    <map PC_phenotype="mtr1" sbml_species="test"></map>
    <map PC_phenotype="WRONG_TOKEN" sbml_species="test"></map>
</intracellular>
```

```
ERROR: There is no specified token parameters in the name of "mtr1" at motility parameters. Please take a look token specifications.

PS C:\Users\Furkan\Documents\GitHub\PhysiCell-SBML-trials\PhysiCell_intracellular_Phenotype>
```

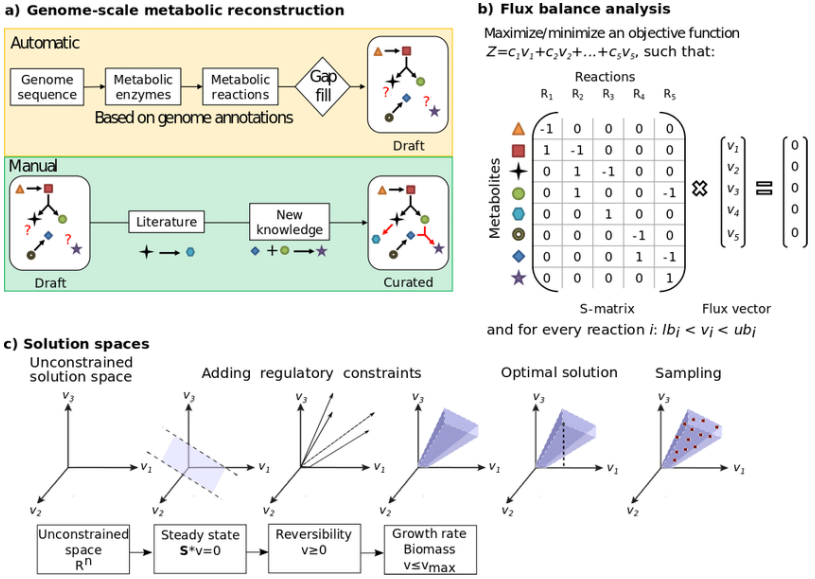
```
ERROR: There is no specified token parameters in the name of "WRONG_TOKEN" at phenotypic parameters. Please take a look token specifications.
```

Code walk

- Let's do code walk together…

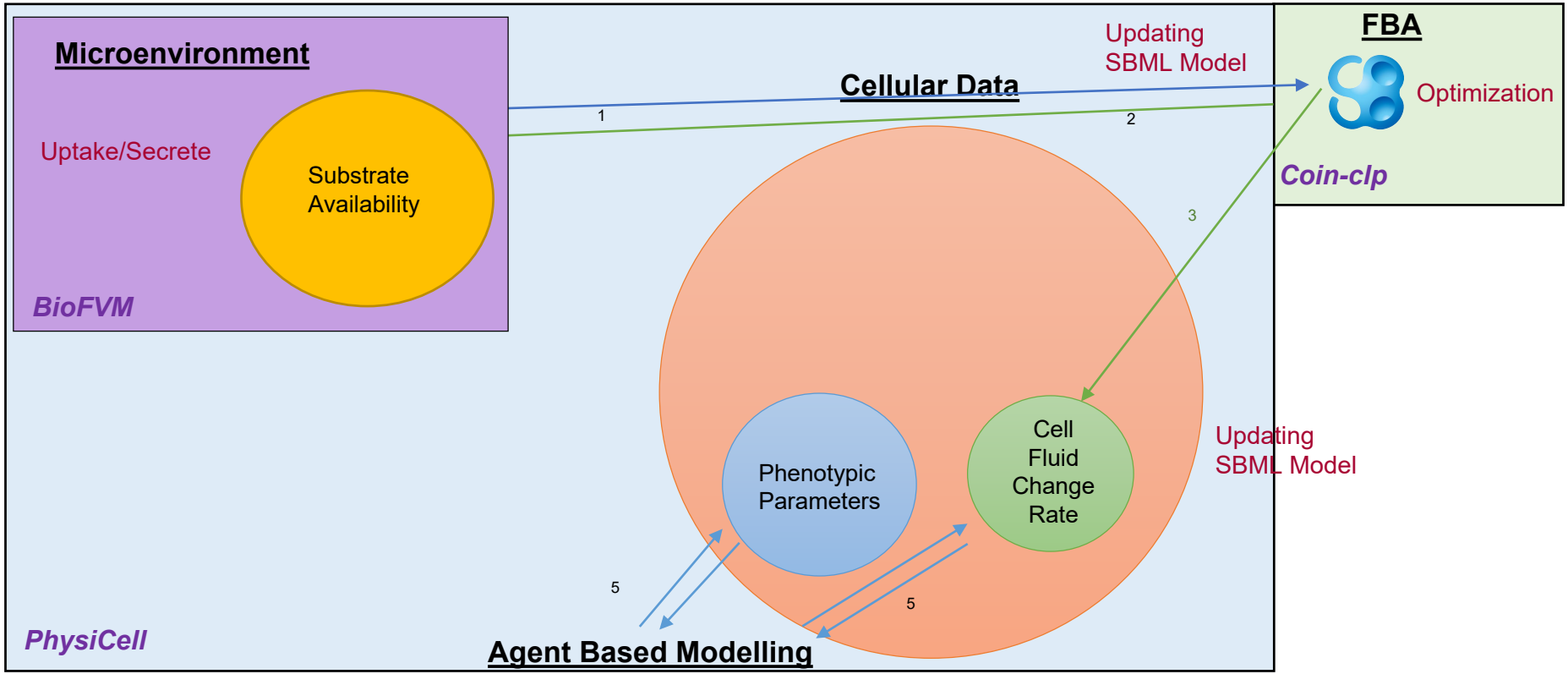
PhysidFBA

Flux Balance Analysis



Heirendt et al, 2017

Integration Design



LibRoadRunner Interactive Demo

Model

- Three Substrates
  - Oxygen, Glucose, Lactate
  - Energy is created with two reactions
    - Glucose + Oxygen -> 38 * Energy (Aerobic)
    - Glucose -> 4 * Energy + Lactate (Anaerobic)
  - Energy consumes
    - Energy -> (Energy_Usage)
- Phenotypic Tokens
  - migration speed, apoptosis_rate, lac_Secretion_Rate, Transition_Rate

Model Rules

- Initial Energy = 450
- If Energy > 445
  - Cycle
- If Energy < 445
  - Don’t Cycle
- If Energy < 440
  - Move
- If Energy < 430
  - Die

Populate together

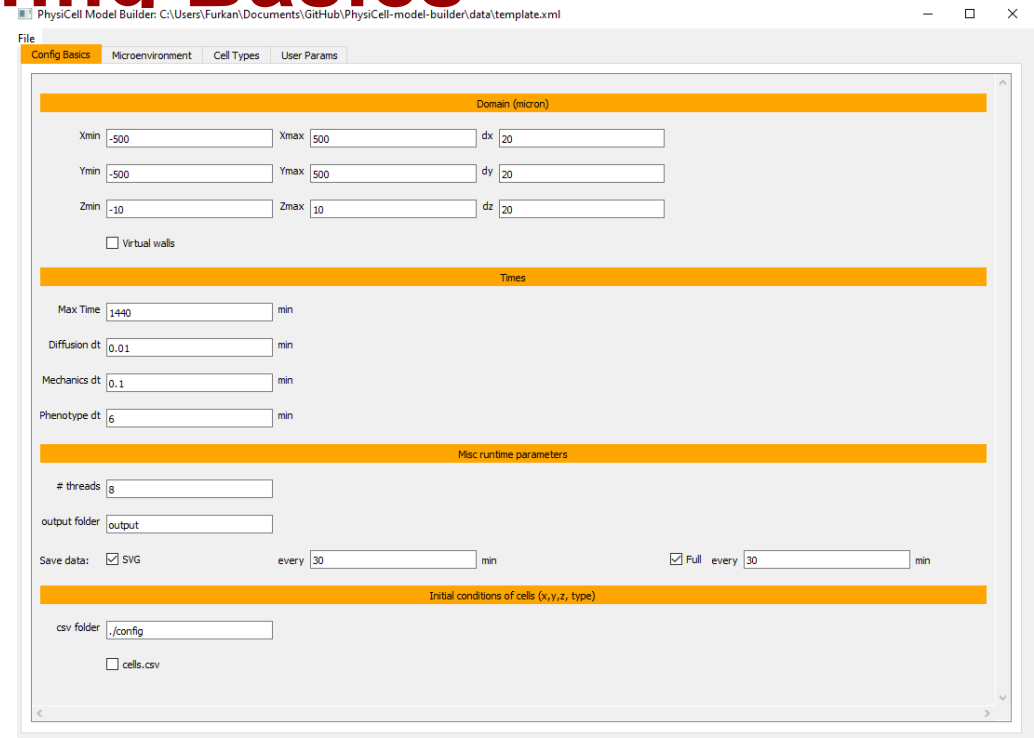
- **PhysiCell folder**
- make clean
- make data-cleanup
- make reset
- make list-projects
- make ode-energy-sample
- make

The Roadmap

- First, we will create the modal domain in the PMB
  - Config Basics
  - Microenvironment
  - Cells
- Create SBML on Copasi
- Integrate SBML to PhysiCell model

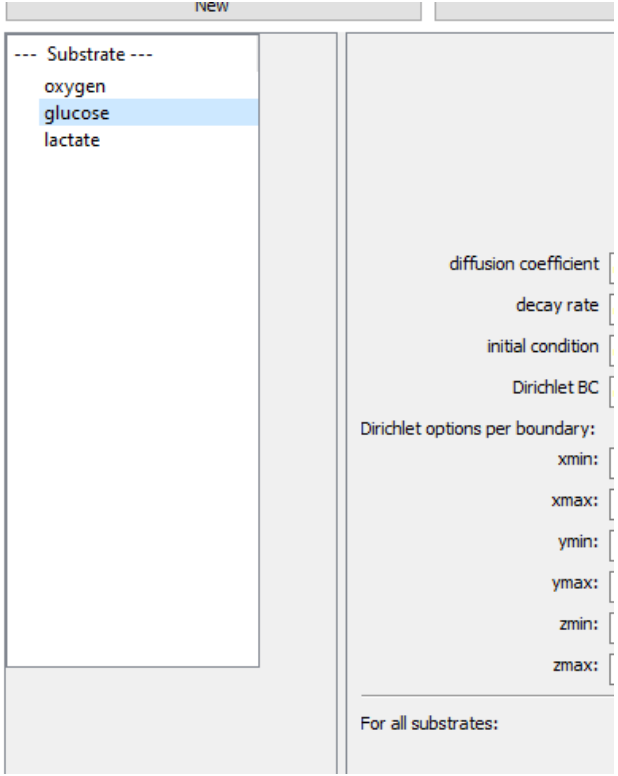
Config Basics

- Domain size
  - X=[-500,500]
  - Y=[-500,500]
  - Z=[-10,10]
  - dx,dy,dz = 20
  - Use_2D = true
- Max-time = 1440 min
- Thread = your choice
- Save data :
  SVG = 30 min, Full = 30 min



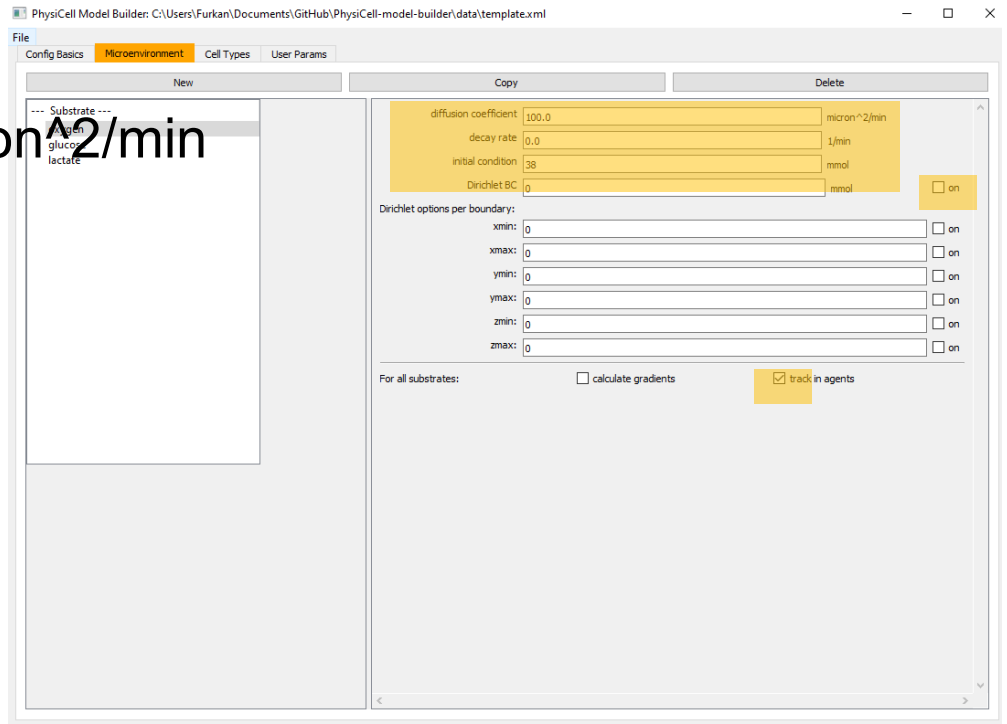
Microenvironment

- Microenvironment Tab
- Let's add "oxygen", "glucose", "lactate"



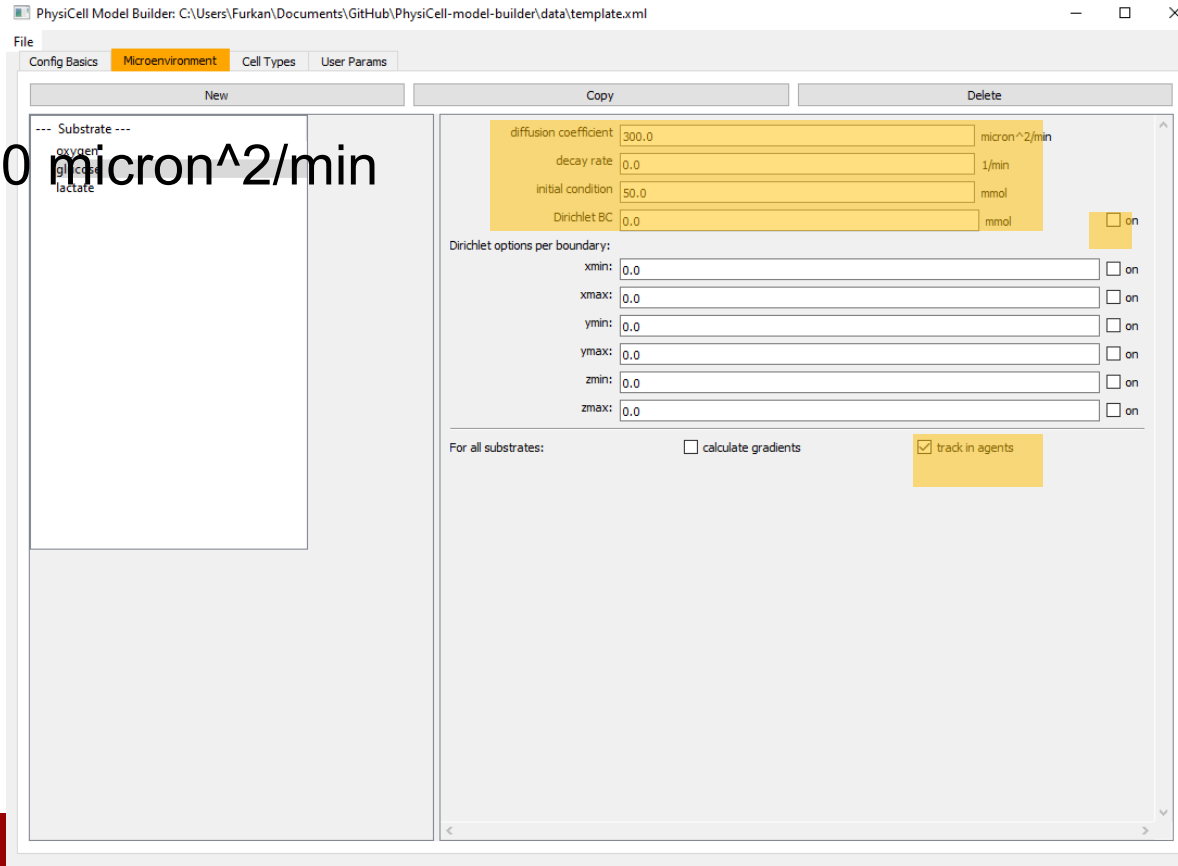
Microenvironment

- Oxygen
- Diffusion Coefficient = 100.0 micron^2/min
- Decay Rate = 0.0 1/min
- Initial condition = 38.0 mmHg
- Dirichlet = OFF
- Track in agents = ON



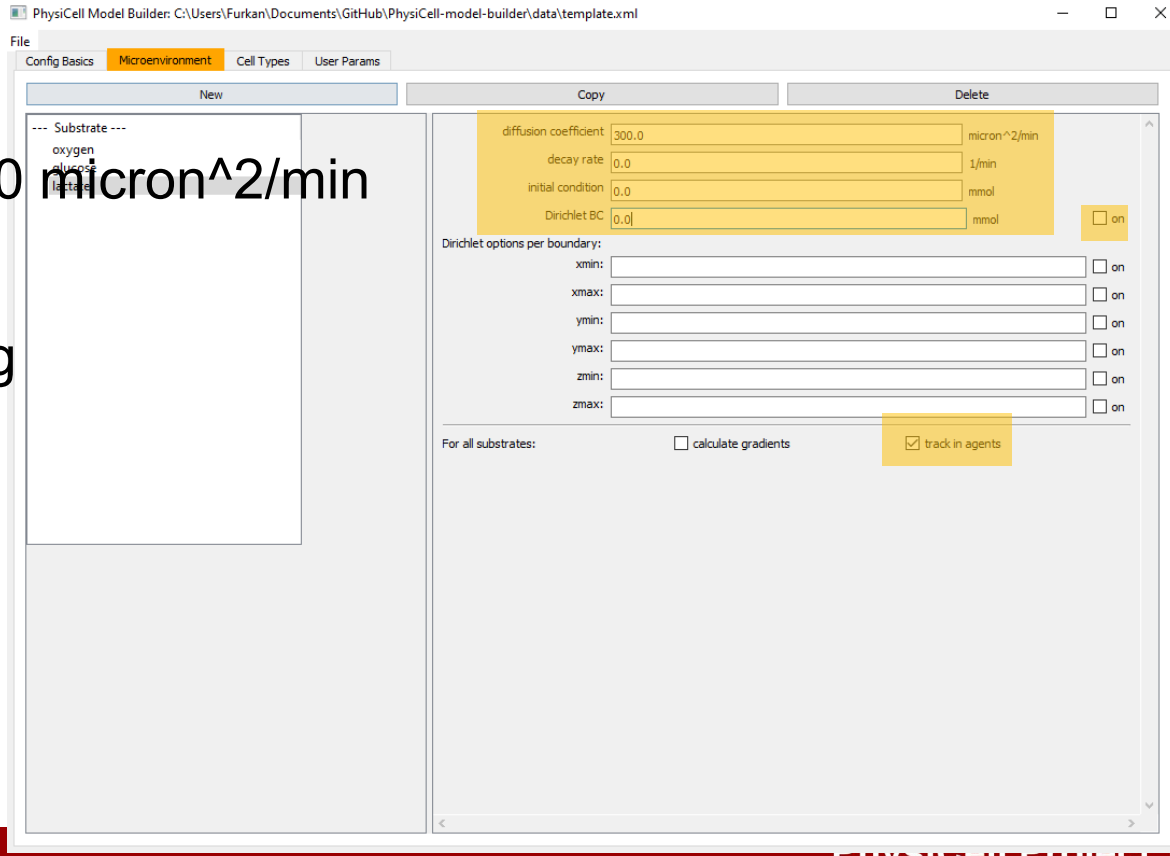
Microenvironment

- Glucose
- Diffusion Coefficient = 300.0 micron^2/min
- Decay Rate = 0.0 1/min
- Initial condition = 50.0 a.u
- Dirichlet = OFF
- Track in agents = ON



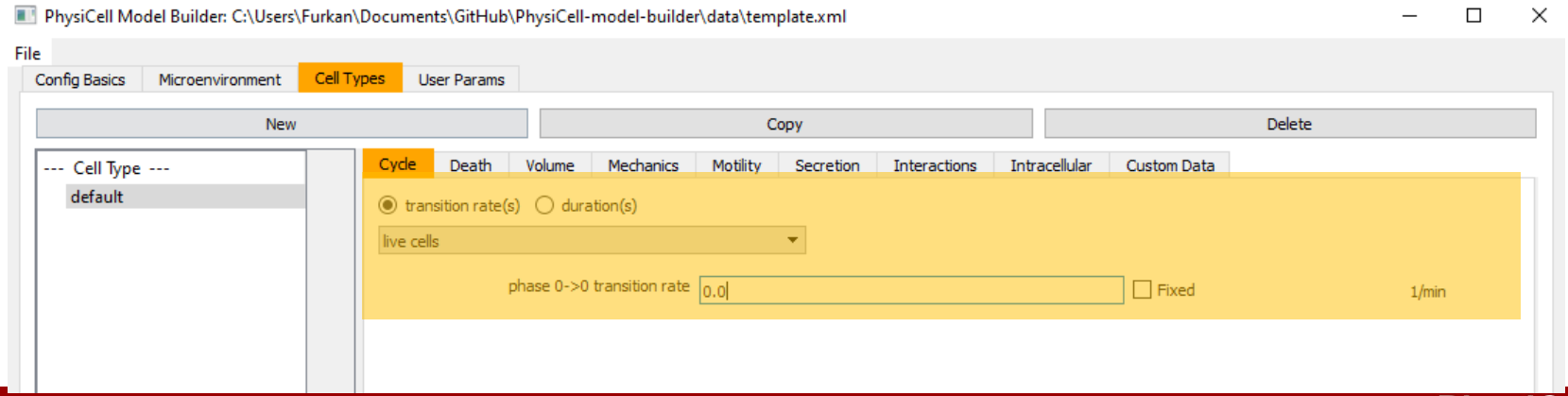
Microenvironment

- Lactate
- Diffusion Coefficient = 300.0 micron^2/min
- Decay Rate = 0.0 1/min
- Initial condition = 0.0 mmHg
- Dirichlet = OFF
- Track in agents = ON



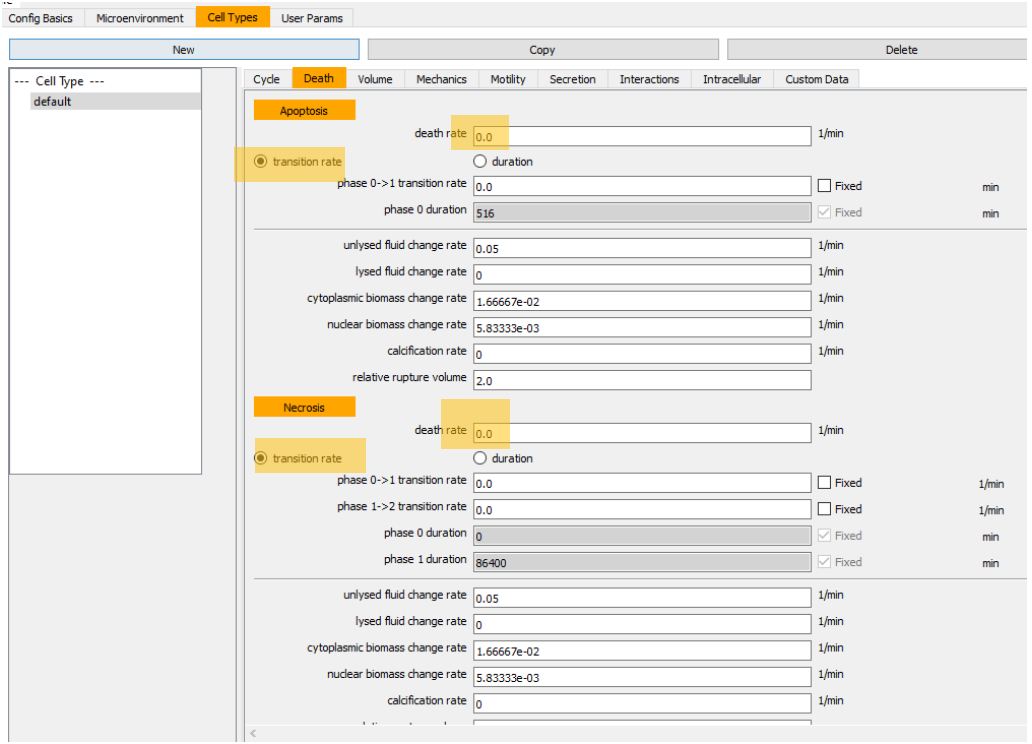
Cell Type

- Only one type of cell in the name of “default”
- Cycle
  - Live Cells
  - Transition rate(s)
  - 0.0



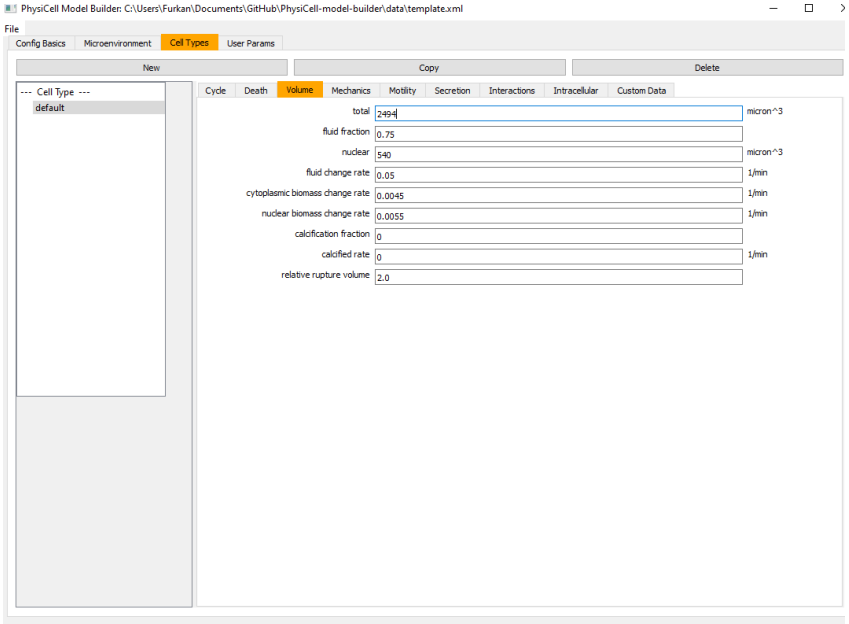
Cell Type

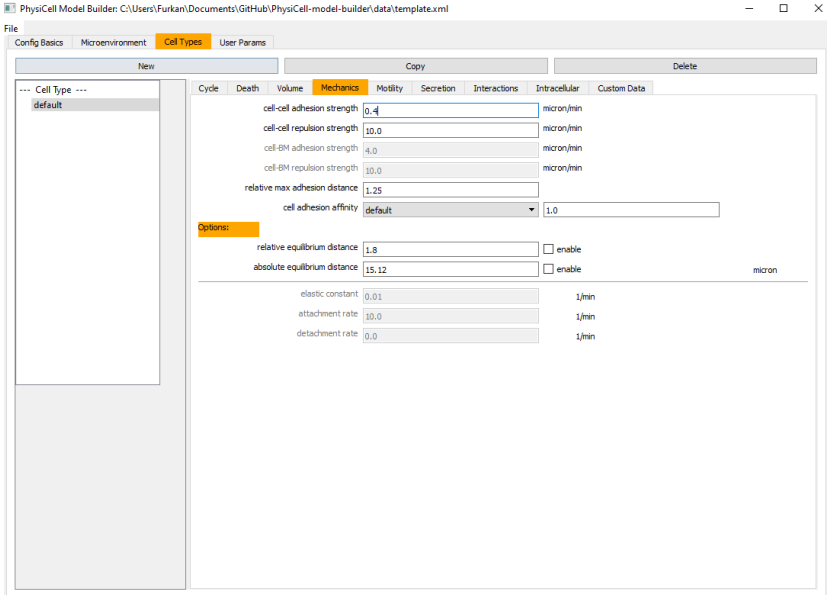
- No Death



Cell Type

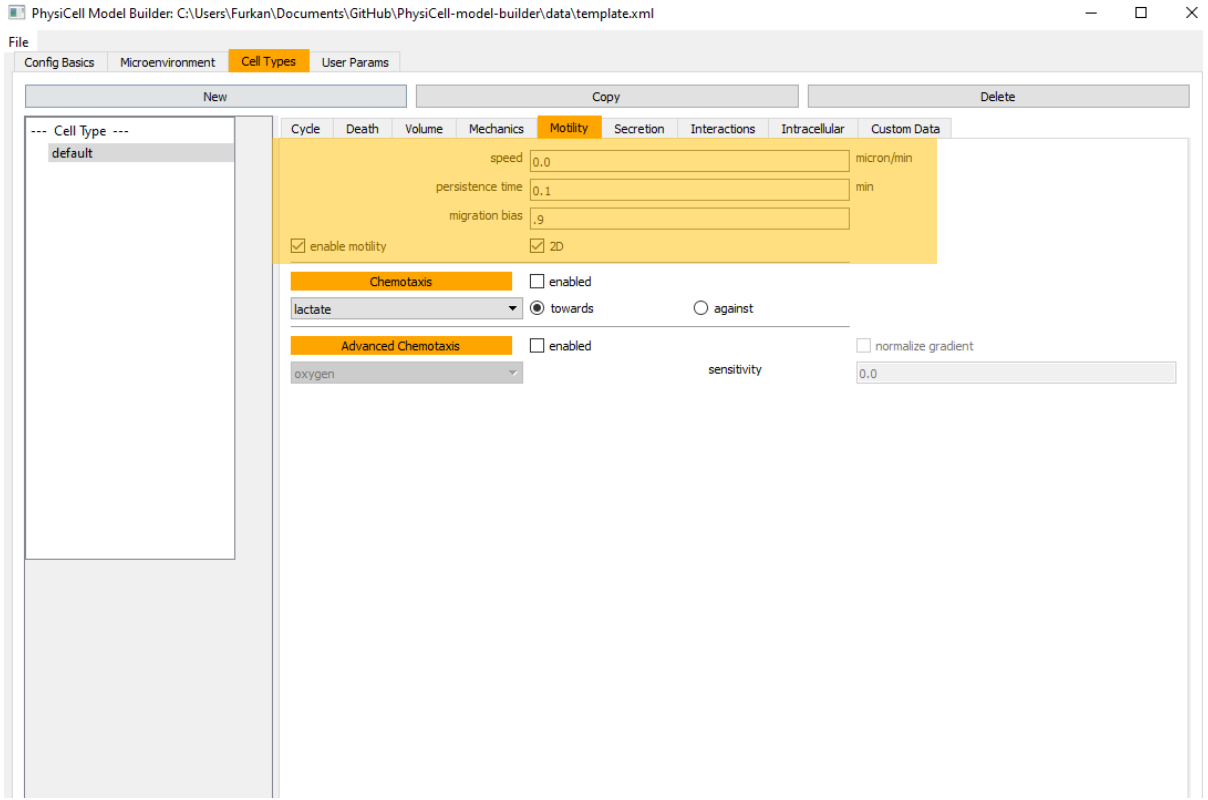
- No change in the Volume and Mechanics Tab





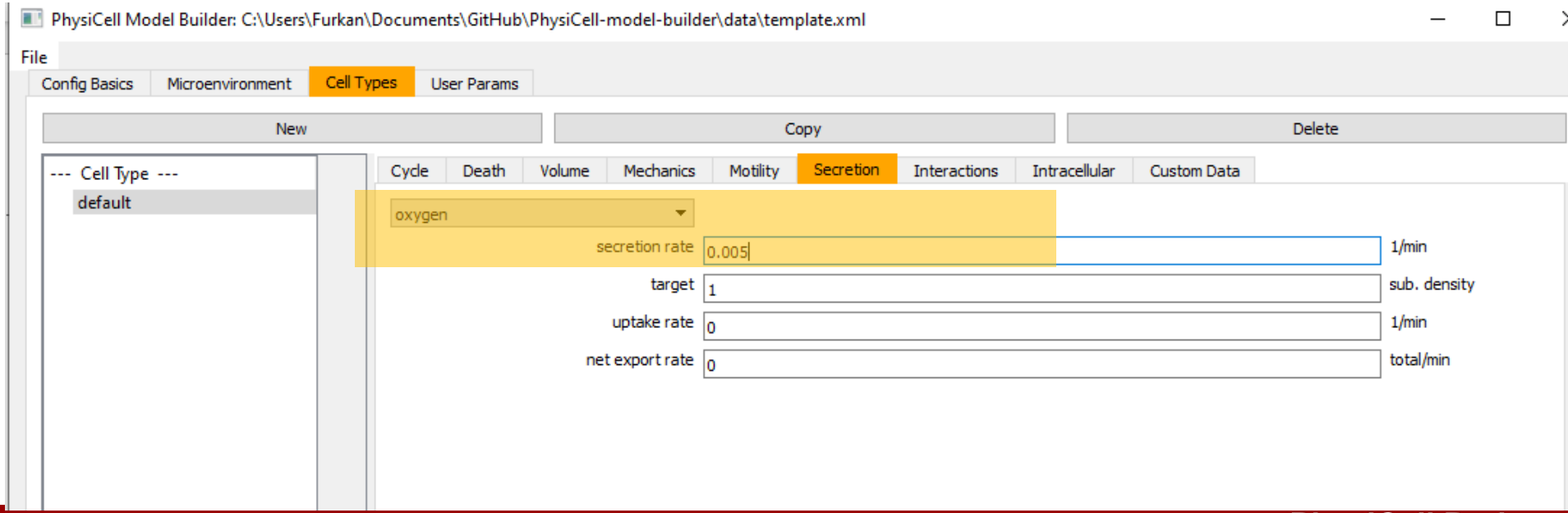
Cell Type

- Motility
- Speed = 0.0
- Persistence Time = 0.1
- Migration Bias = 0.9
- Enable-motility
- 2D = Yes



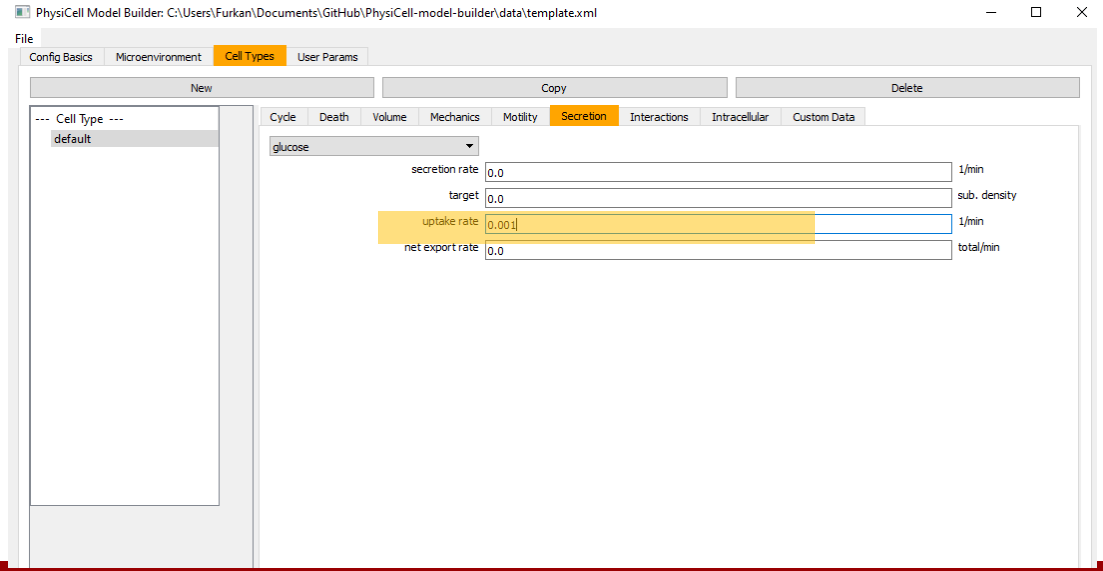
Cell Type : Secretion

- Oxygen Tab
- Uptake rate = 0.005



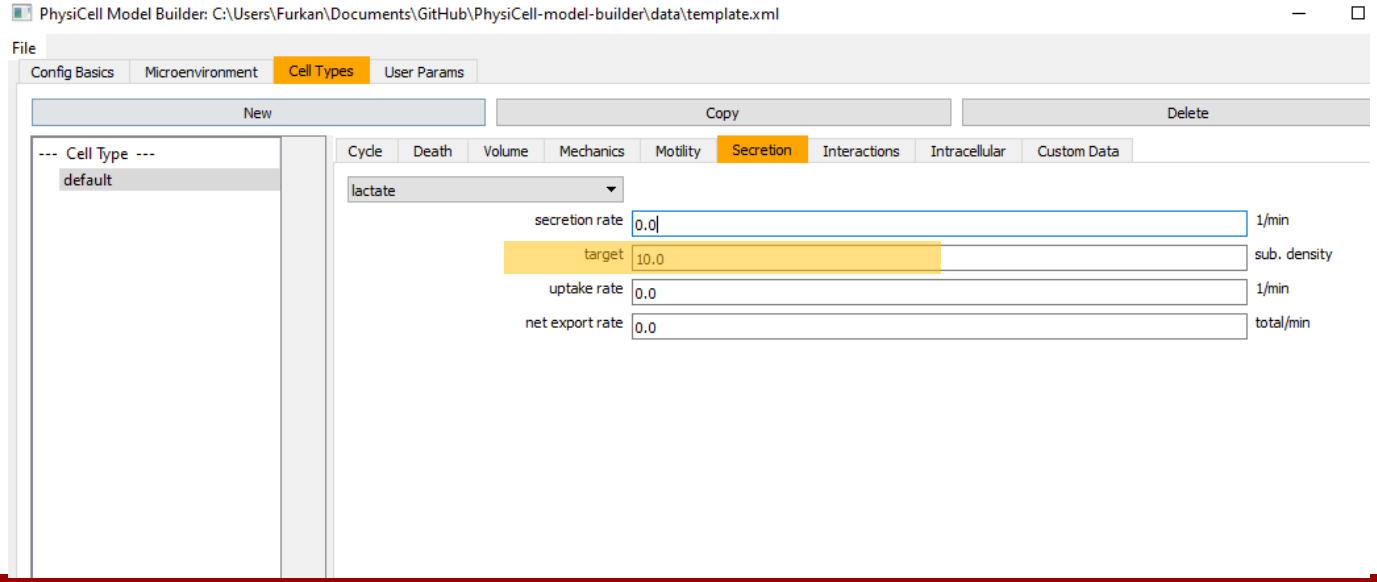
Cell Type : Secretion

- Glucose Tab
- Uptake rate = 0.001



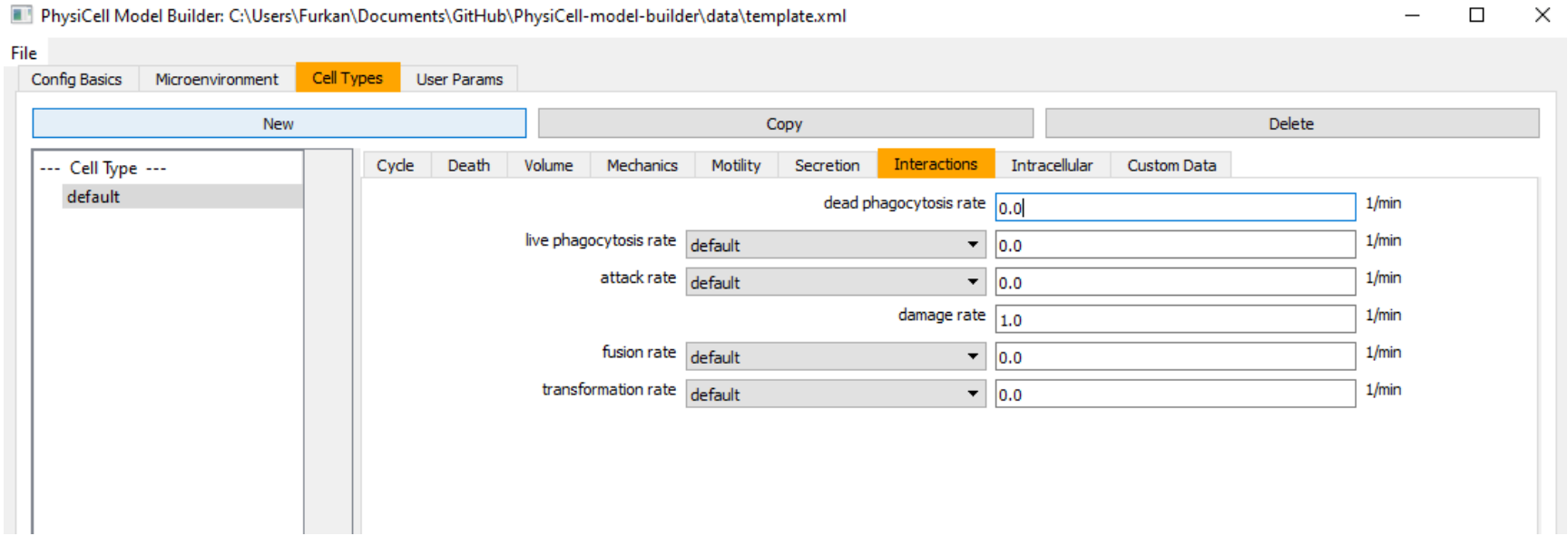
Cell Type : Secretion

- Lactate Tab
- Secretion Target = 10.0



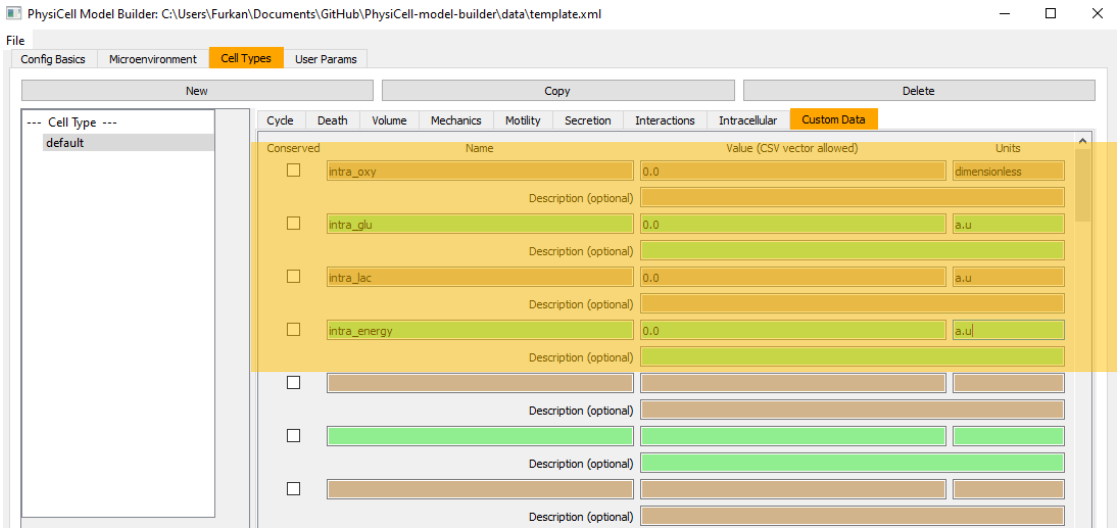
Cell Type

- No Interactions



Cell Custom Data

- We need to create intracellular data to save the data
- intra_oxy = 0.0
- intra_glu = 0.0
- intra_lac = 0.0
- intra_energy = 0.0



User Params

- i
- i
- i
- i

PhysiCell Model Builder: C:\Users\Furkan\Documents\GitHub\PhysiCell-model-builder\data\template.xml

File

Config Basics | Microenvironment | Cell Types | User Params

Append 10 more rows | Clear selected rows

	Name	Type	Value	Units
☐	initial_internal_oxygen	double	0.8	mmHg
	Description:			
☐	initial_internal_glucose	double	15	a.u
	Description:	initial number of cells (for each cell type)		
☐	initial_internal_lactate	double	0.0	a.u
	Description:			
☐	initial_energy	double	450	a.u
	Description:			
☐		double		
	Description:			
☐		double		
	Description:			
☐		double		
	Description:			
☐		double		

Save

- Let’s check is it right

Let’s Simulate

- But First
- Let’s get rid of the all-intracellular related part
  - In the custom module
  - Browse through the setup tissue() function
  - Remove the inner code of the for loop
    - Starting with Line #166 to #183 (except assigning)
  - Remove the inner part of update_intracellular() function
    - Starting with Line #192 to #263
  - That’s all

  - CHECKPOINT - 1

Funding Acknowledgements

PhysiCell Development:

- Breast Cancer Research Foundation
- Jayne Koskinas Ted Giovanis Foundation for Health and Policy
- National Cancer Institute (U01CA232137)
- National Science Foundation (1720625, 1818187)

Training Materials:

- Administrative supplement to NCI U01CA232137 (Year 4)

Other Funding:

- NCI / DOE / Frederick National Lab for Cancer Research (21X126F)
- DOD / Defense Threat Reduction Agency (HDTRA12110015)
- NIH Common Fund (3OT2OD026671-01S4)