

Slides, videos, links and more:

<https://github.com/physicell-training/ws2023>

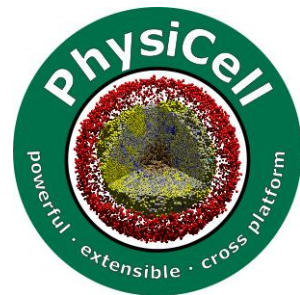
# Advanced Session 4: Intracellular with libRoadrunner (introduction)

Furkan Kurtoglu

 [@PhysiCell](https://twitter.com/PhysiCell)

## PhysiCell Project

August 2023



# What we have learned so far

- PhysiCell folder structure
- PhysiCell model domain
  - Different dts (diffusion\_dt, mechanic\_dt, phenotype\_dt)
- PhysiCell Studio to create config file
- 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

# Agenda:

- First Session (Advanced Session 4)
  - PhysiCell Intracellular Class
  - Kinetic SBML
  - PhysiCell Integration
    - Model Design
    - Results
    - Convergence Tests
  - libRR Add-on
    - Functions
    - Phenotypic Changes

# 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



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# 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



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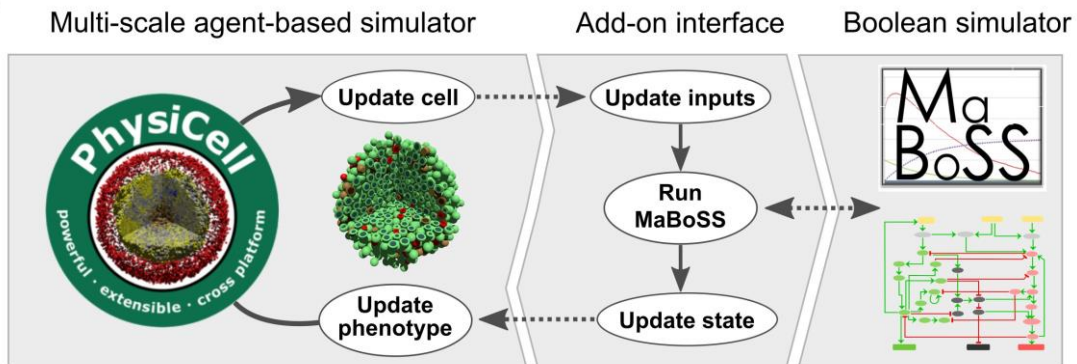
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# PhysiBoSS

- Boolean Network
  - MaBoSS
- SysBioCurie & BSC
  - Vincent, Arnau
- Fully integrated as “add-on” to PhysiCell
- Session 9-10:
  - Please visit [agenda](#) for slides and video

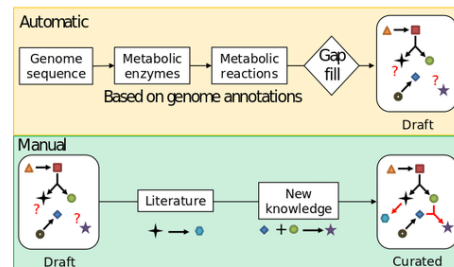




# PhysidFBA

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

## a) Genome-scale metabolic reconstruction



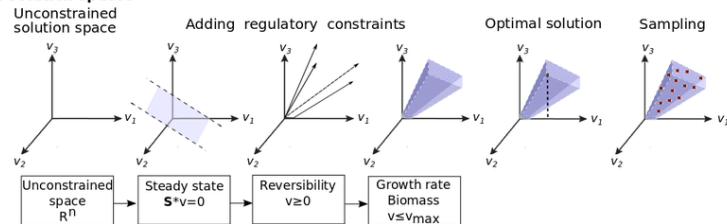
## b) Flux balance analysis

Maximize/minimize an objective function  
 $Z = c_1 v_1 + c_2 v_2 + \dots + c_j v_j$ , such that:

$$\begin{matrix} & \text{Reactions} \\ & R_1 & R_2 & R_3 & R_4 & R_5 \\ \text{Metabolites} & \begin{pmatrix} -1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 & -1 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix} & \otimes & \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{pmatrix} & = & \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} \end{matrix}$$

S-matrix      Flux vector  
 and for every reaction  $i$ :  $lb_i < v_i < ub_i$

## c) Solution spaces



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>  
[...]
```



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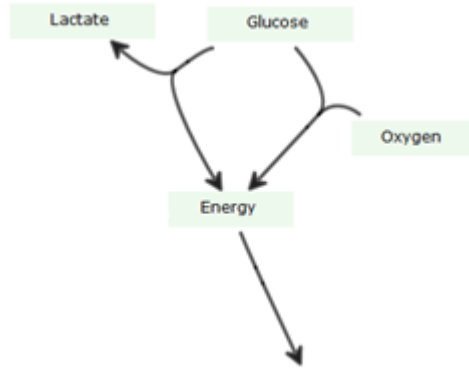
# SBML

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

```
▼ COPASI
  ▼ Model
    ▼ Biochemical
      ▼ Compartments [1]
        Intracellular
      ▼ Species [4]
        Energy
        Glucose
        Lactate
        Oxygen
      ▼ Reactions [3]
        Aerobic
        Anaerobic
        Energy_Usage
      ▼ Global Quantities [3]
        k_aer
        k_ane
        k_usage
```

#	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	

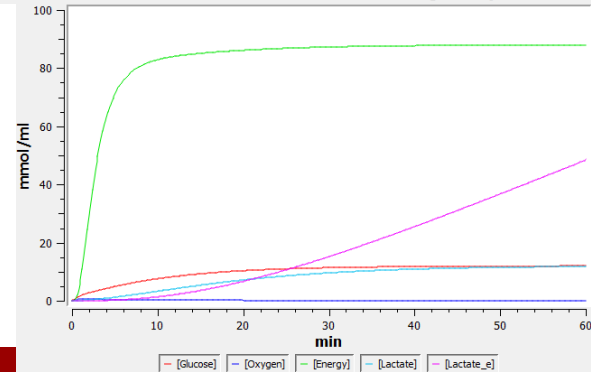
$$\frac{d([Glucose] \cdot V_{Intracellular})}{dt} = -V_{Intracellular} \cdot (k_{aer} \cdot [Glucose] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen]) - V_{Intracellular} \cdot (k_{ane} \cdot [Glucose])$$

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

$$\frac{d([Energy] \cdot V_{Intracellular})}{dt} = +38 \cdot V_{Intracellular} \cdot (k_{aer} \cdot [Glucose] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen] \cdot [Oxygen]) + 2 \cdot V_{Intracellular} \cdot (k_{ane} \cdot [Glucose]) - V_{Intracellular} \cdot (k_{usage} \cdot [Energy])$$

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

**Concentrations, Volumes, and Global Quantity Values**



# SBML

- Generally, SBMLs have more than one compartment
  - Extracellular
  - Intracellular
  - Mitochondria (Sometimes)
  - Nucleus (?)
- And have two or more species for one substrate
  - Glucose[e]  $\rightarrow$  extracellular
  - Glucose[i]  $\rightarrow$  intracellular
- Transfer reaction between compartments
  - Glucose[e]  $\leftrightarrow$  Glucose[i]



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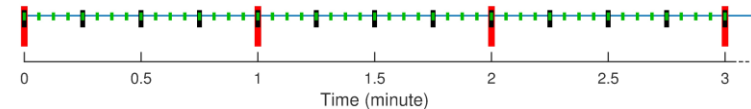
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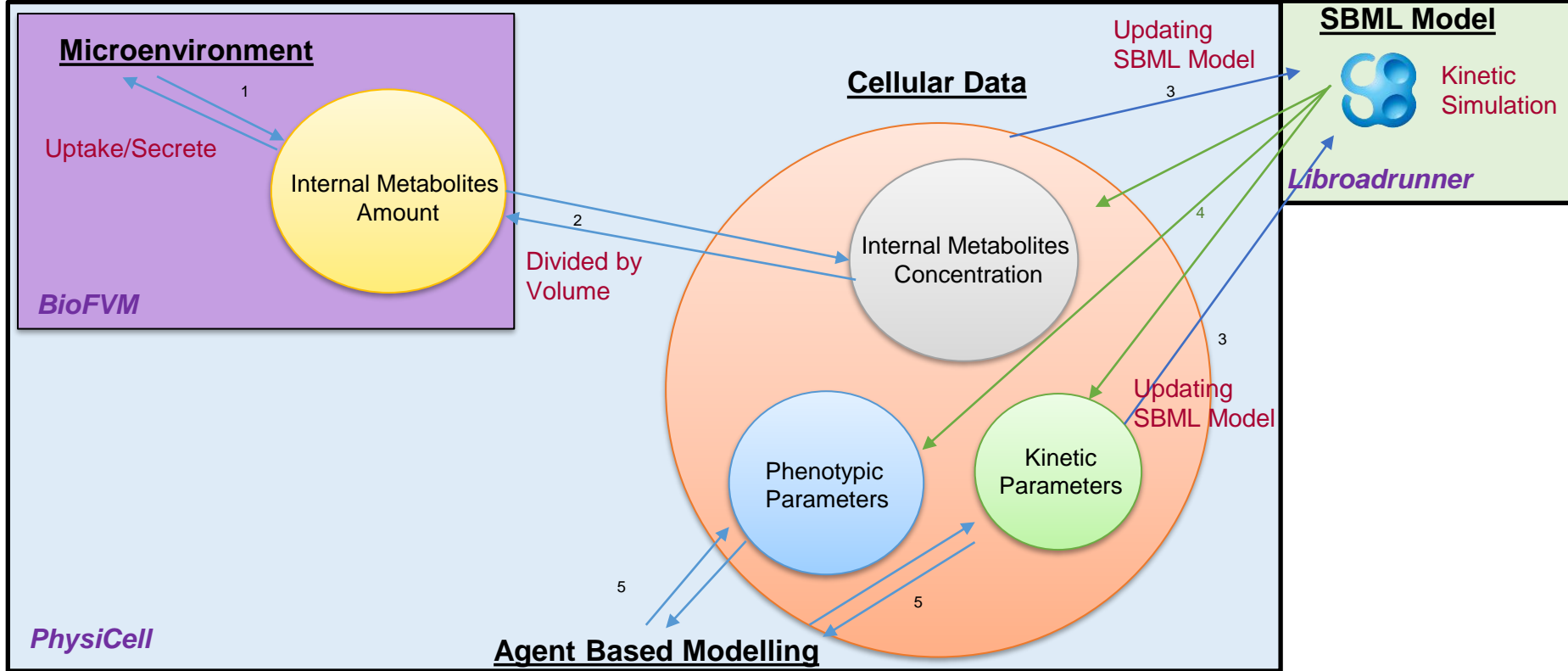
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# Assumptions

- **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)

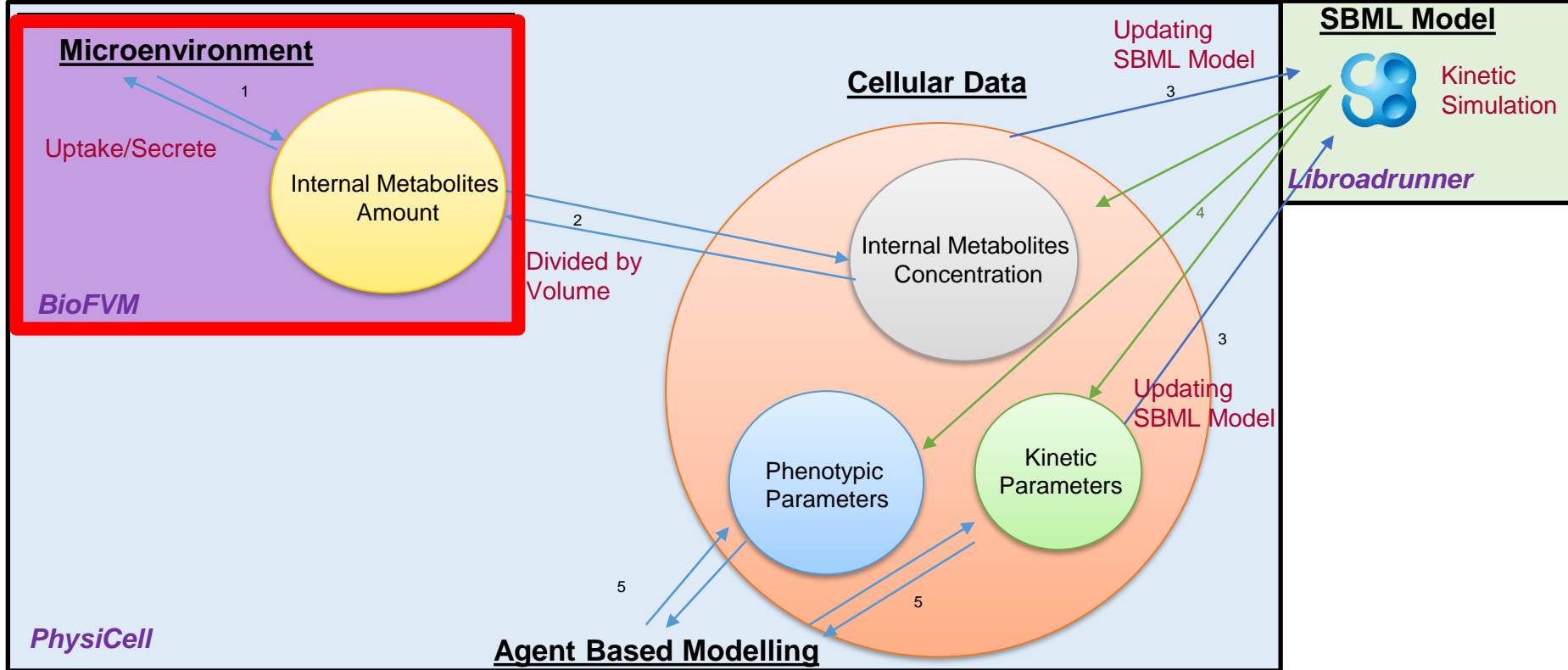


# Integration Design

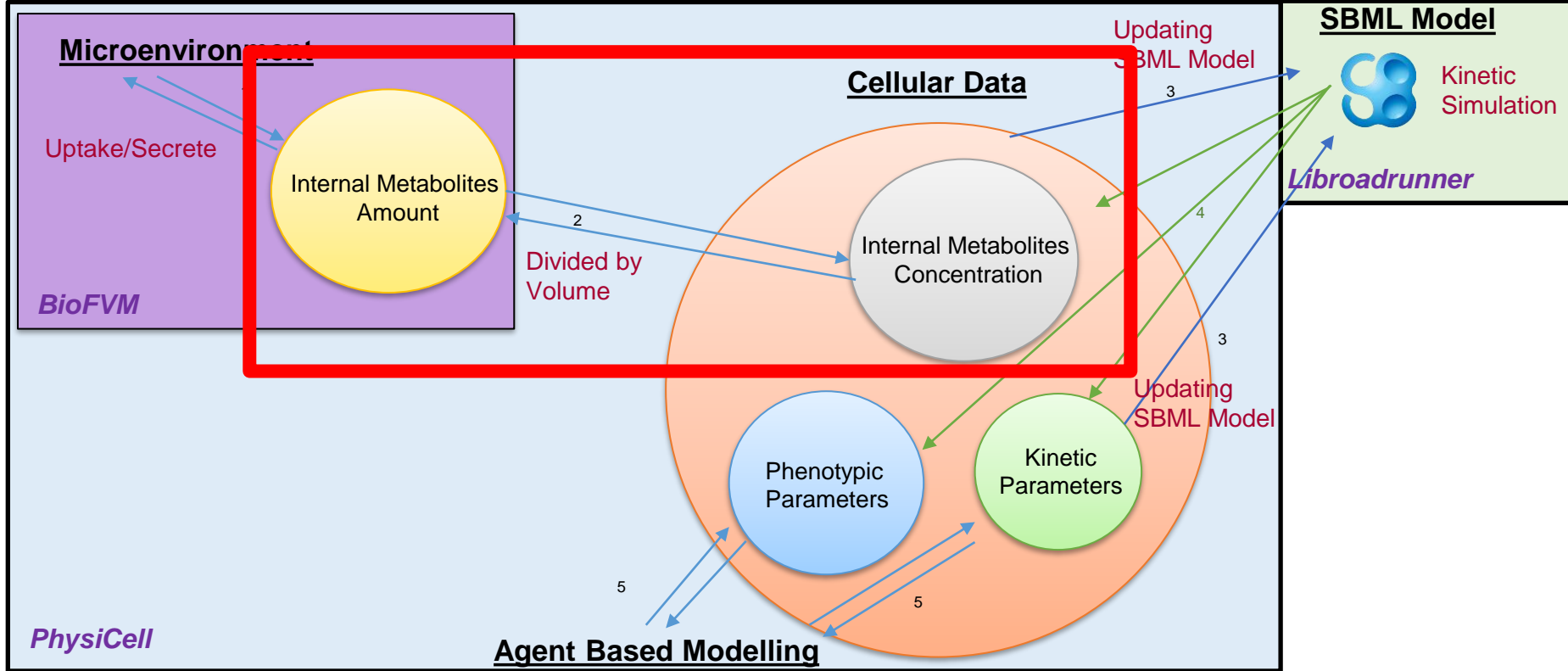




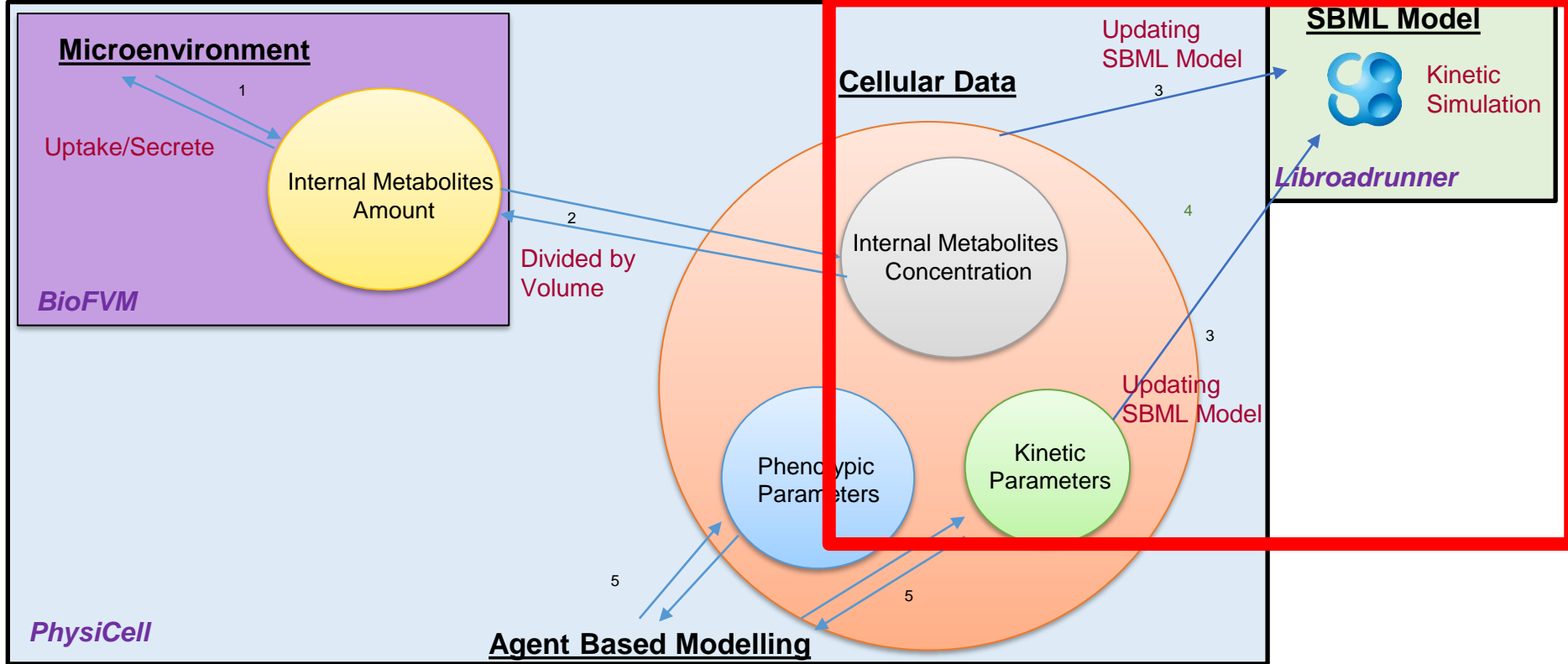
# Integration Design



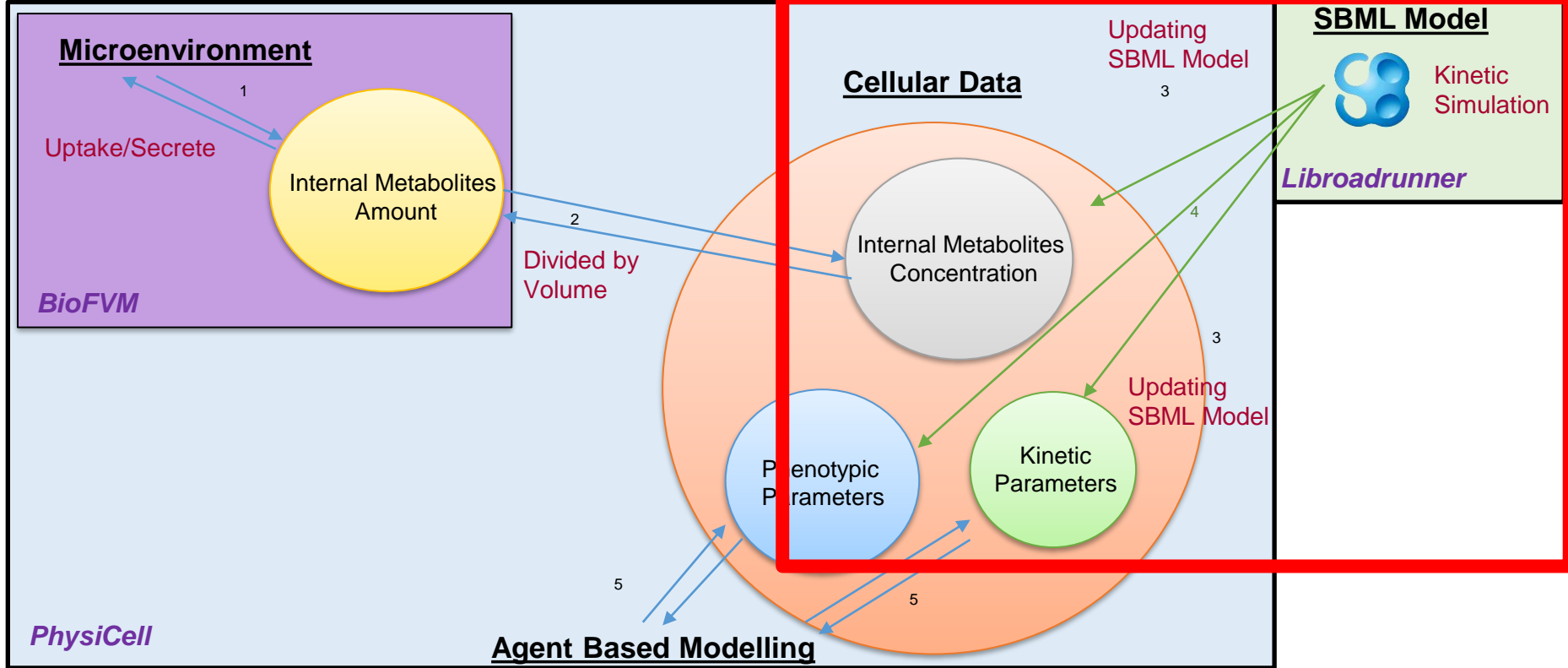
# Integration Design



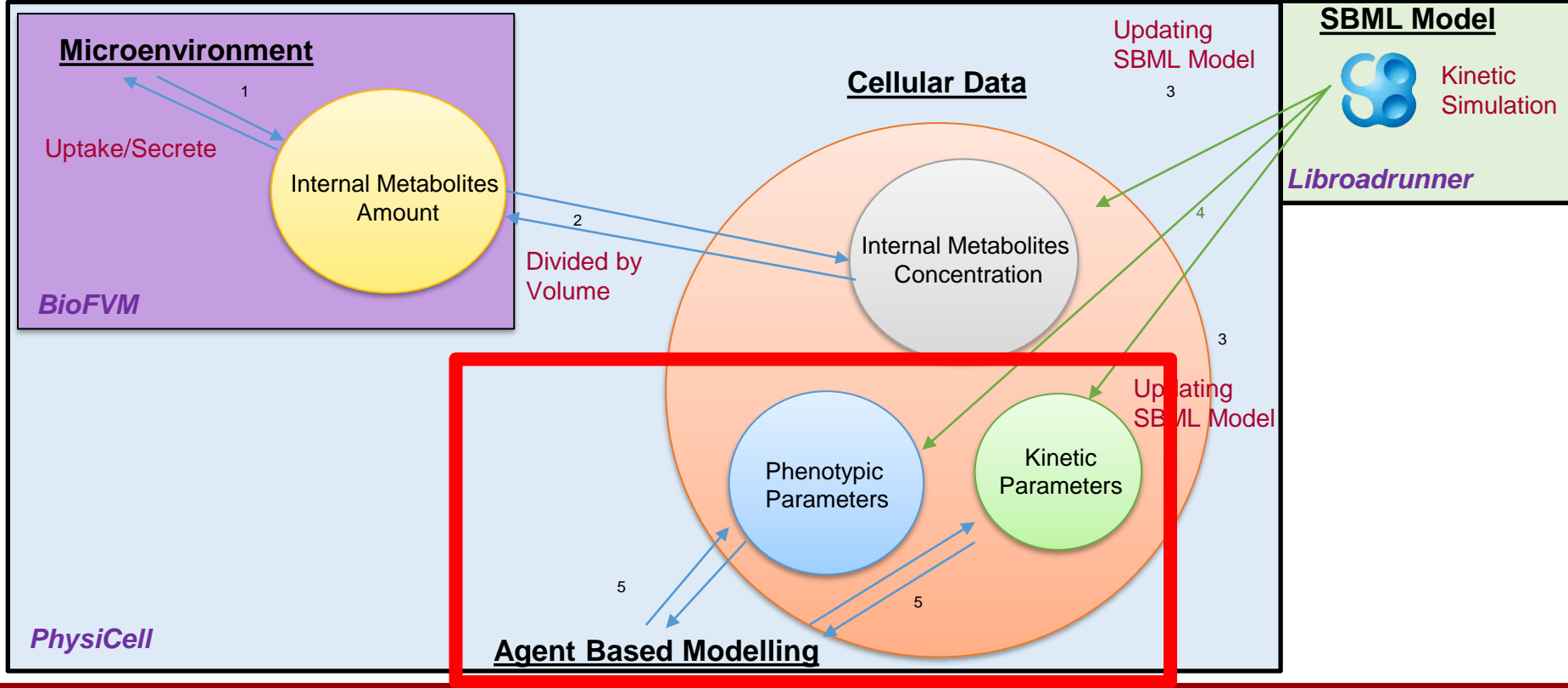
# Integration Design



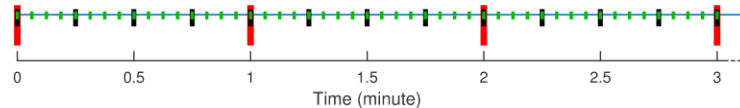
# Integration Design



# 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>
    </phenotype>
  </cell_definition>
</cell_definitions>

<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>
  </secretion>
</volume>
</phenotype>
```



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# 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



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# Some Results



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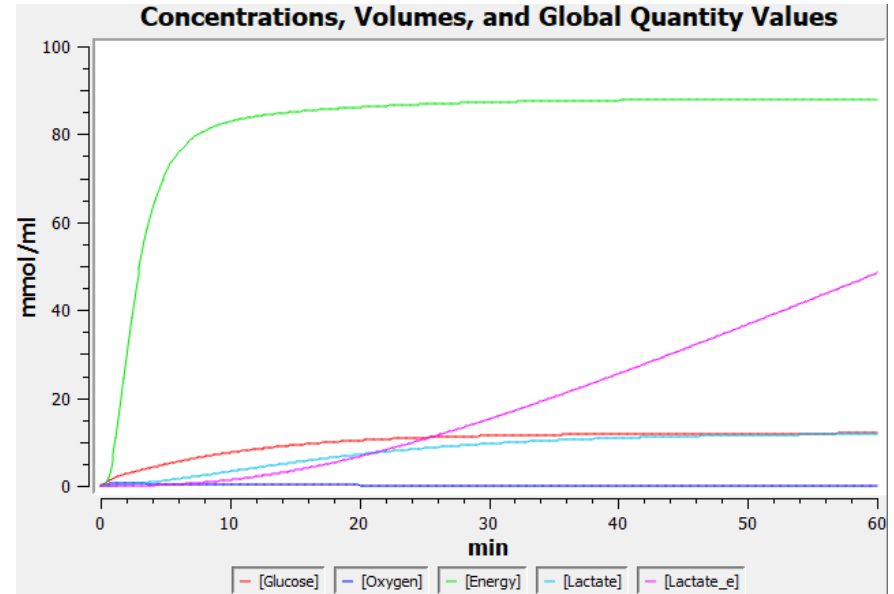
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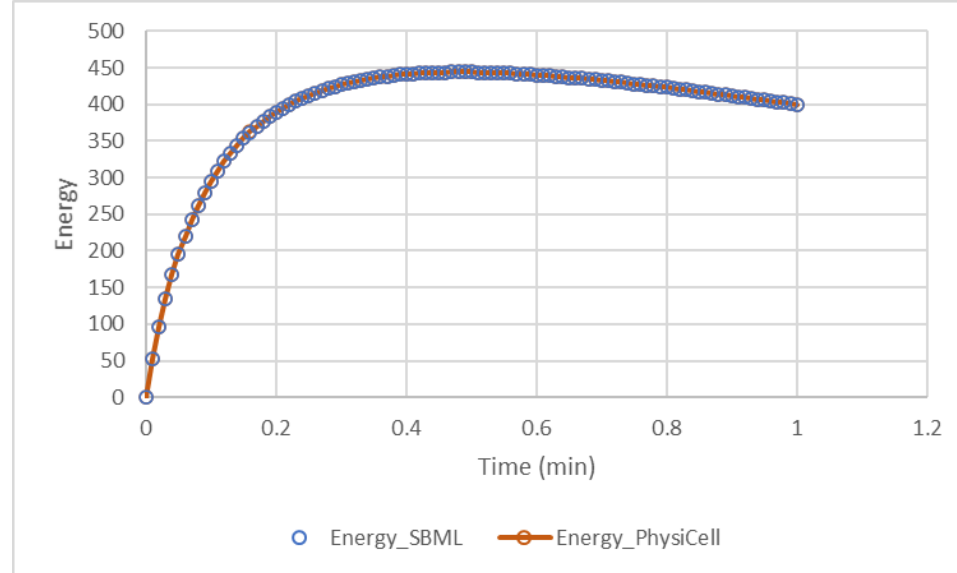
# Model 0 - SBML Model

- 4 Species
  - Oxygen
  - Glucose
  - Lactate
  - Energy
- 3 Internal Reactions
  - ♦ Aerobic reaction
    - »  $\text{Glucose} + \text{Oxygen} \rightarrow \text{Energy}$
  - ♦ Anaerobic reaction
    - »  $\text{Glucose} \rightarrow \text{Energy} + \text{Lactate}$
  - ♦ Energy Usage
    - »  $\text{Energy} \rightarrow$



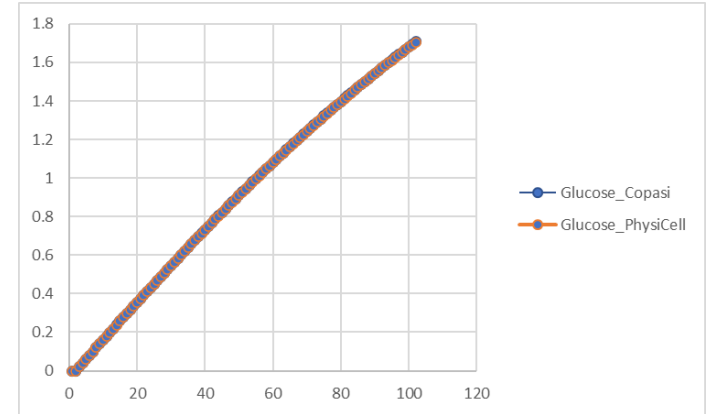
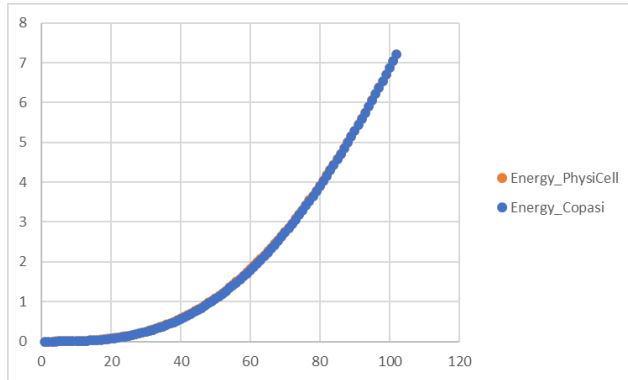
# Model 0

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



# Model 1

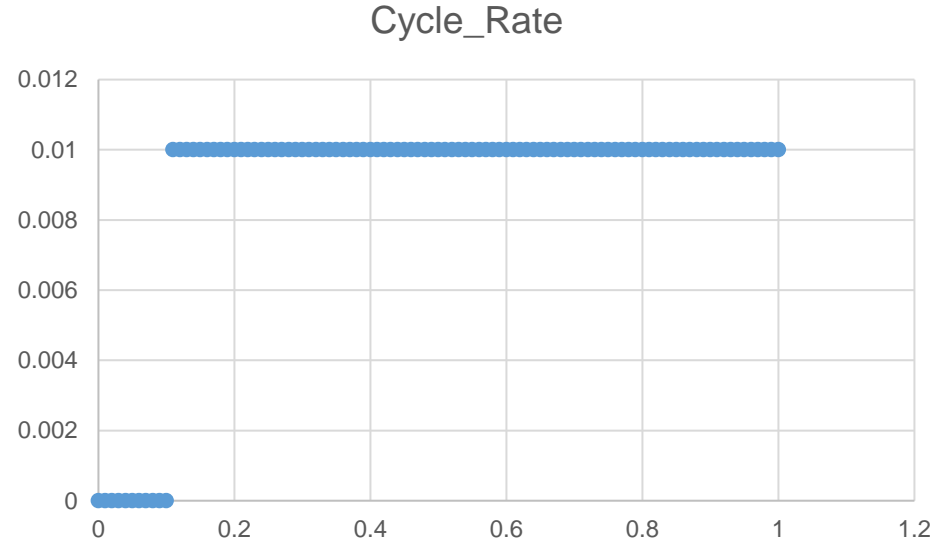
- Uptake glucose, oxygen
- Produces Energy internally.



# 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



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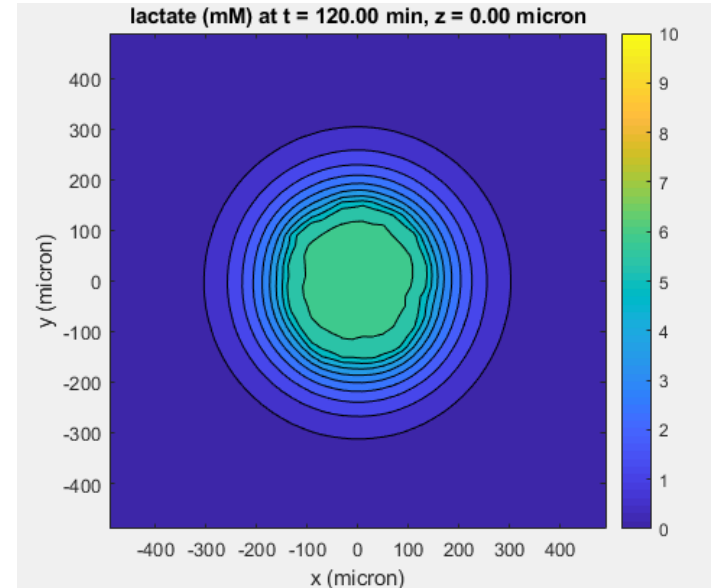
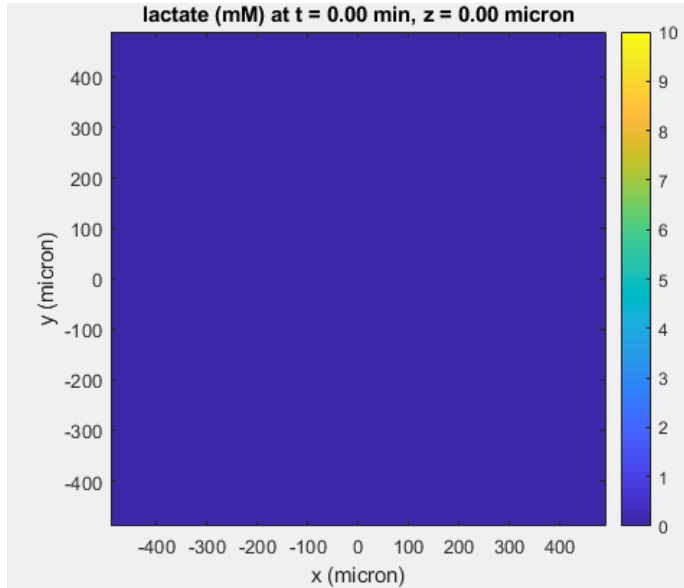
PhysiCell.org

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# 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



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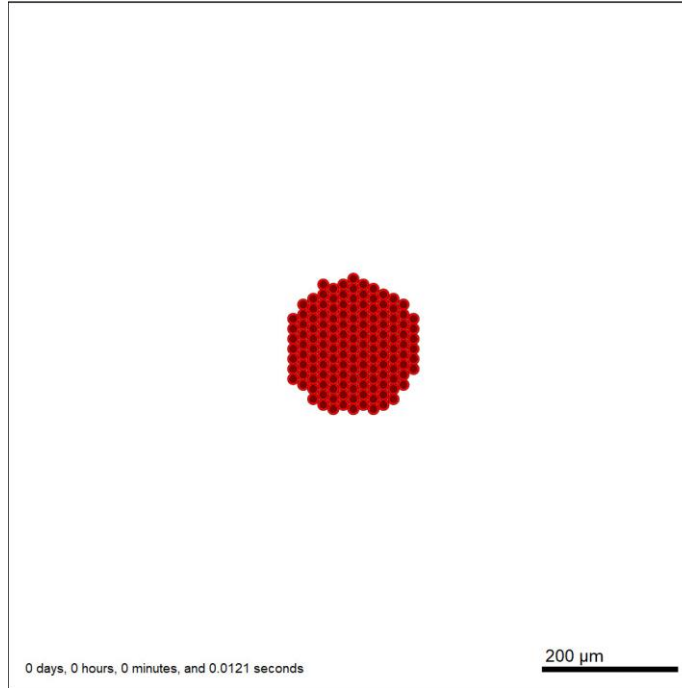
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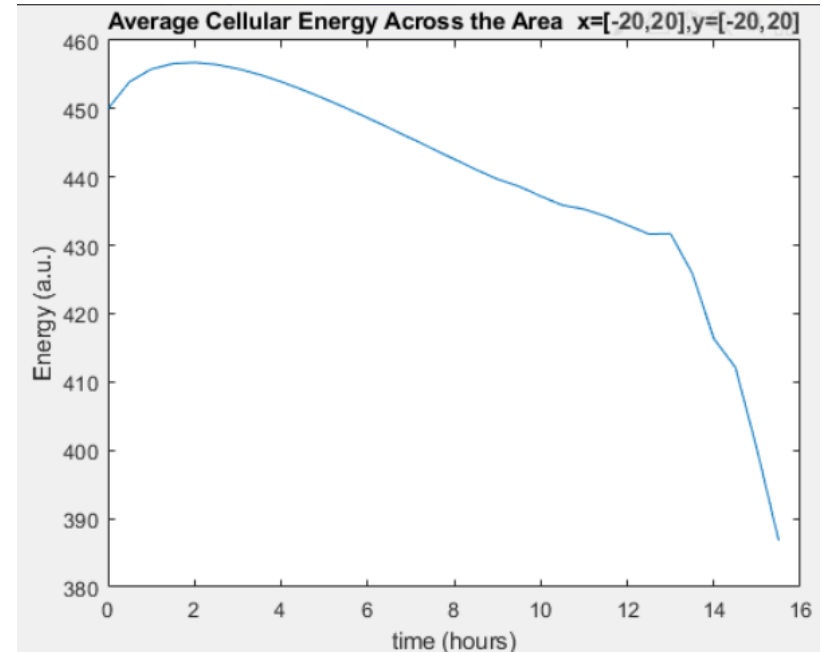
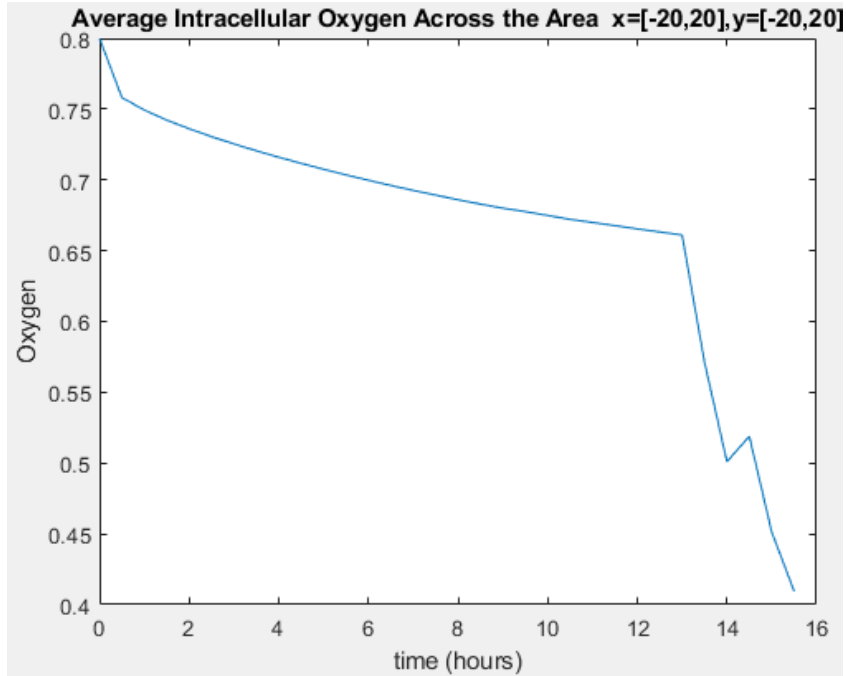
@PhysiCell

Current time: 0 days, 0 hours, and 0.00 minutes,  $z = 0.00 \mu\text{m}$   
144 agents





# Intracellular



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[@PhysiCell](https://twitter.com/PhysiCell)

# 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\_parameter\_value()** = to get value from SBML
  - **set\_parameter\_value()** = 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



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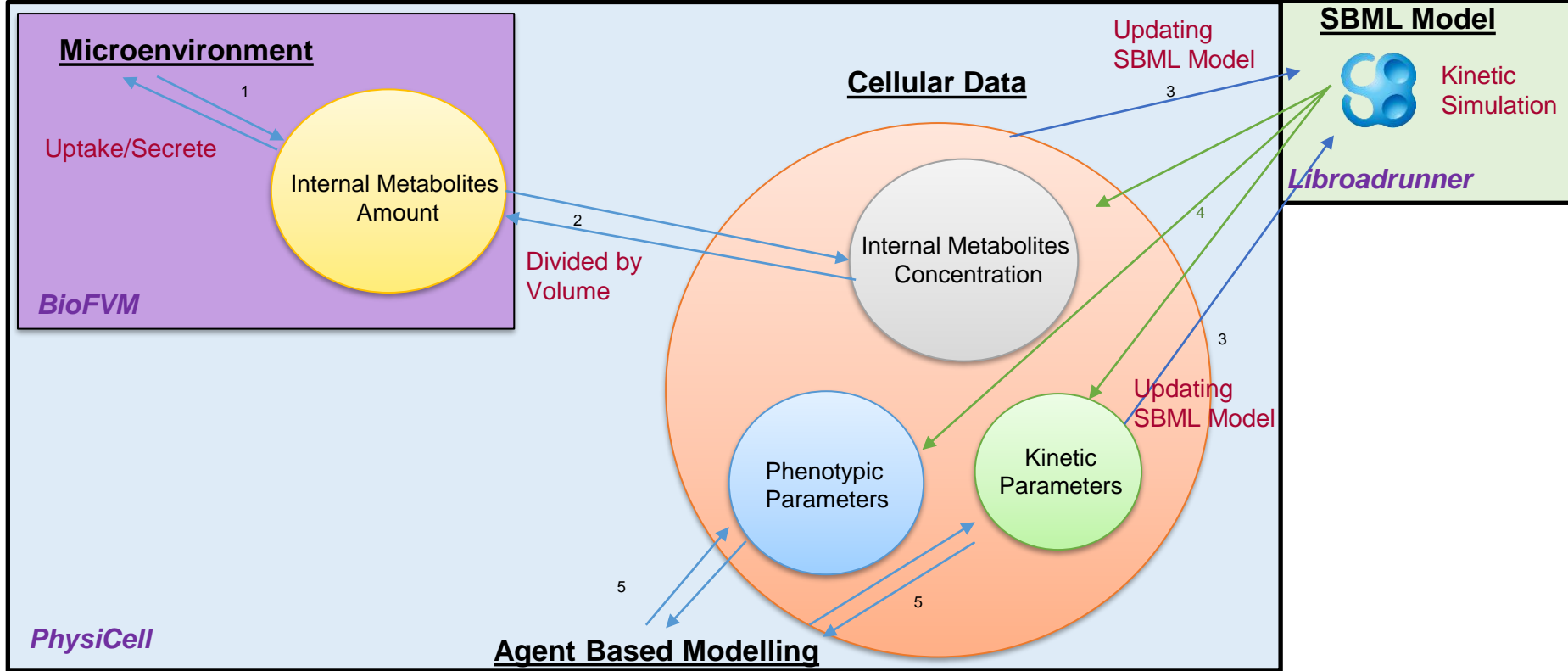
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# Integration Design



# 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

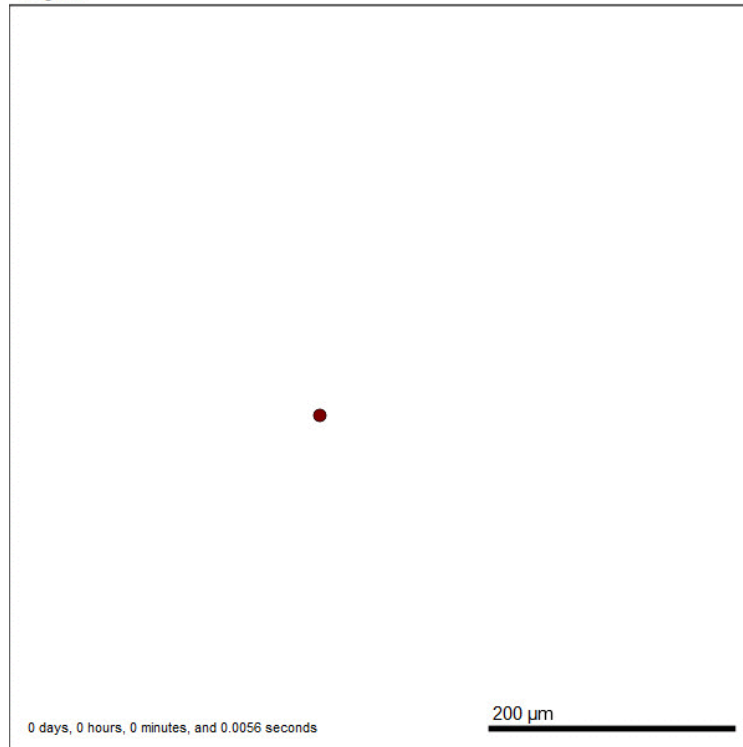
# Migration Speed

## 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>
. . .
```

Current time: 0 days, 0 hours, and 0.00 minutes, z = 0.00  $\mu\text{m}$   
1 agents

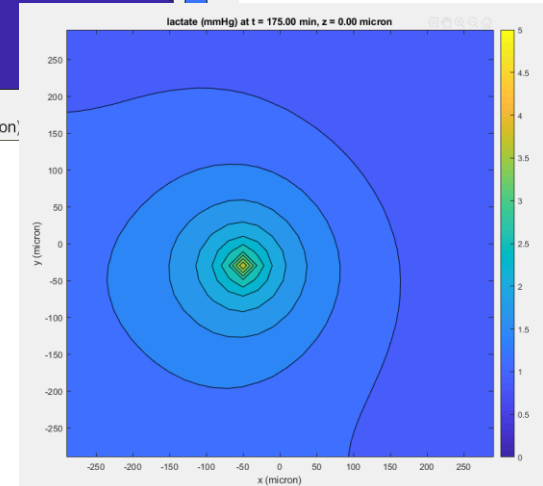
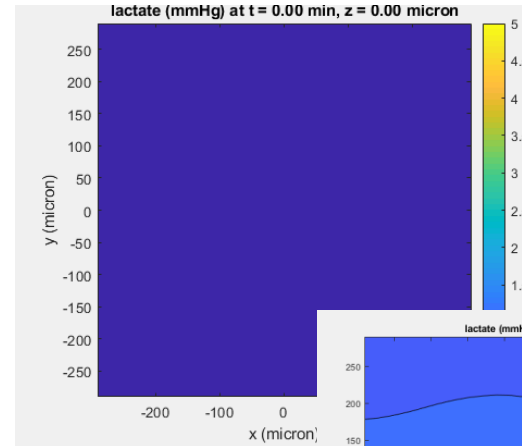


# Lactate Distribution

## 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.

# Funding Acknowledgements



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CANCER  
INSTITUTE



leidos



## PhysiCell Development:

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

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