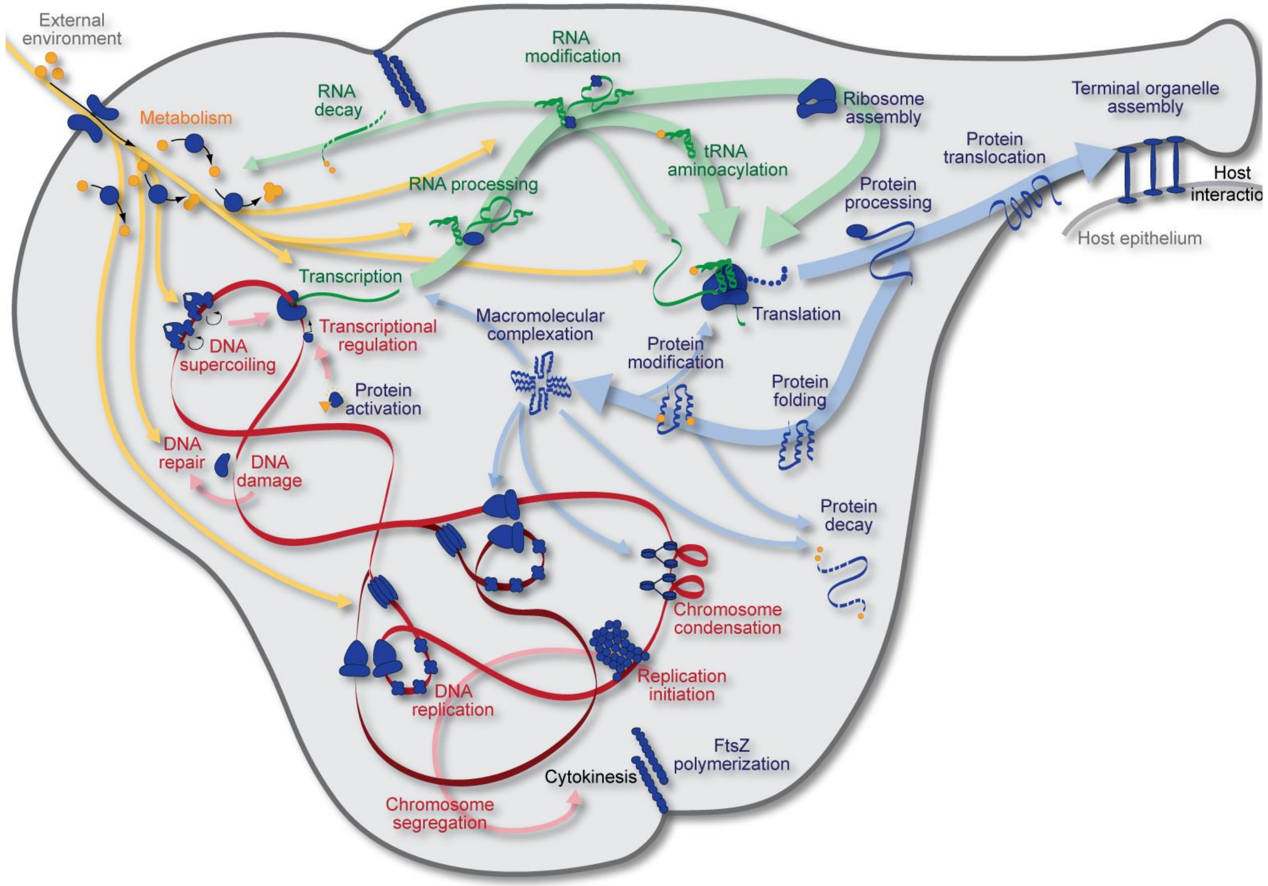
Whole-cell modeling



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Outline

Introduction

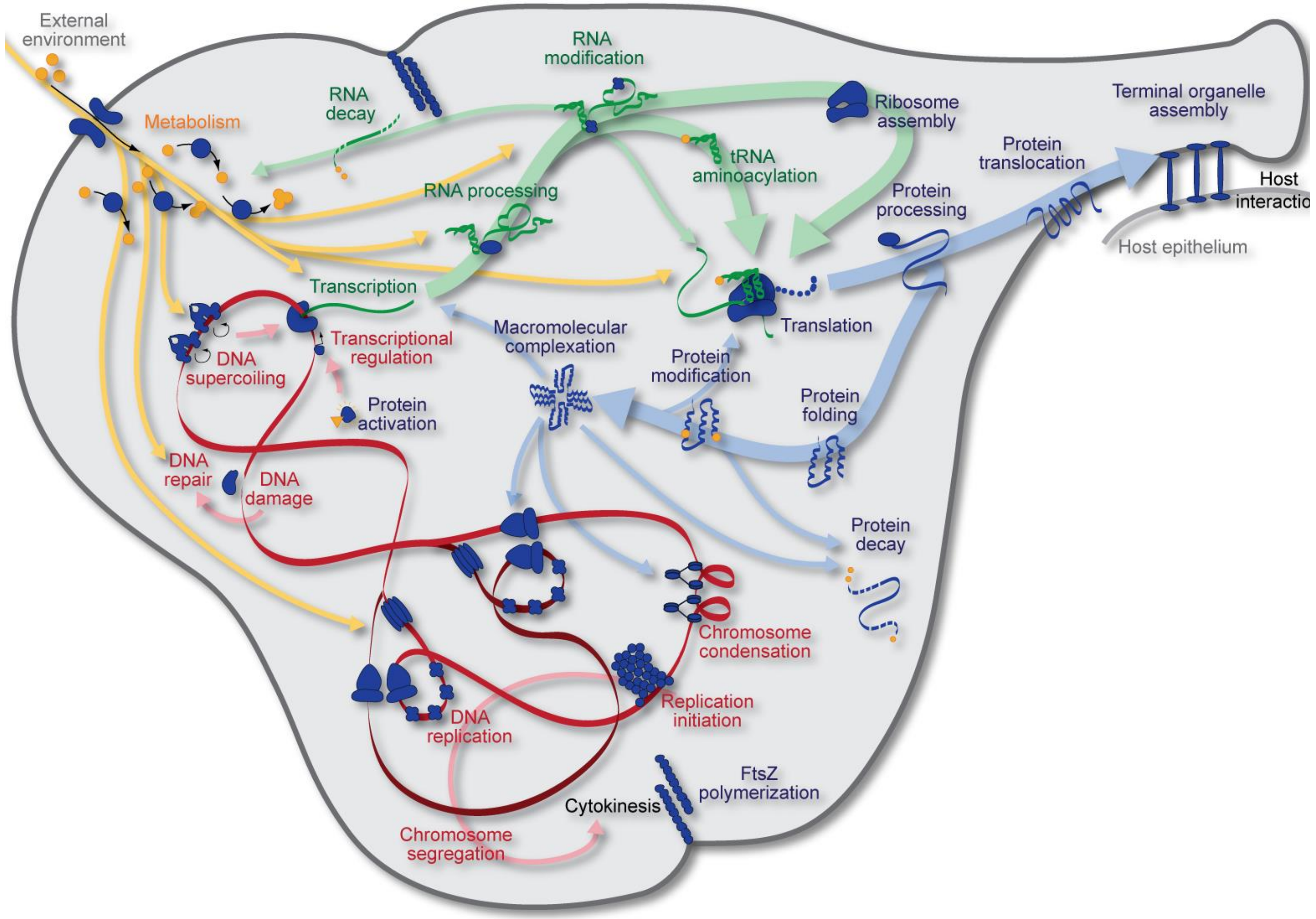
- Goals
- Approaches
- Multi-algorithm simulation
- Parameter estimation, verification, best practices

Exercises

- Model building
- Parameter estimation
- Multi-algorithm simulation
- Submodel simulation
- Model annotation

Introduction

Motivation: Comprehensively understand and manipulate cells



Goals

- Represent multiple pathways with different structures and dynamics
- Represent well- and poorly-characterized pathways
- Integrate heterogeneous data
- Train models from incomplete and noisy data
- Integrate molecular information over many orders of length and time

Approaches

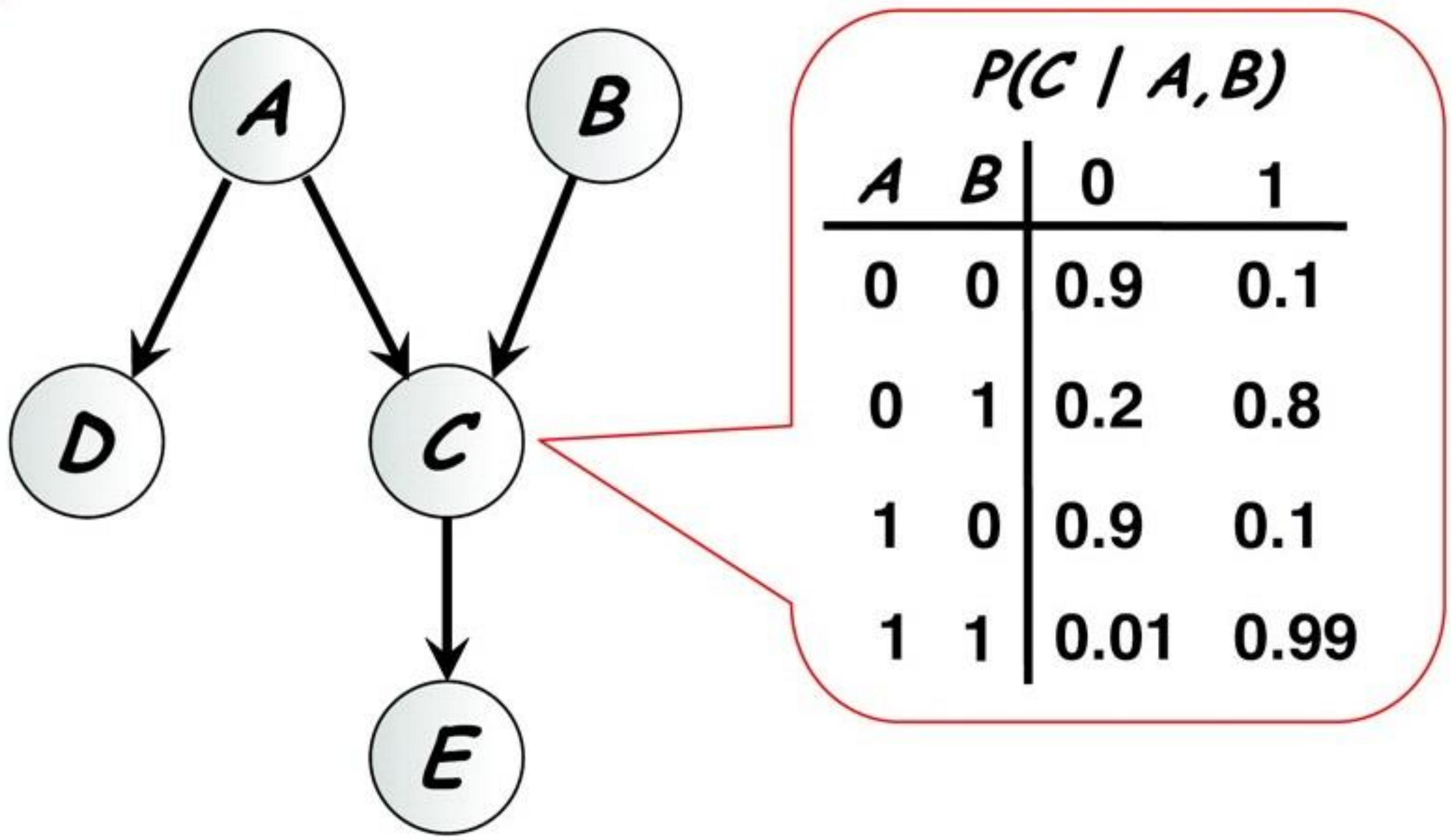
Data-based

- Based on observed phenotypes
- Simpler mathematical formulation
- Easier to construct
- Limited ability to extrapolate beyond training data

Physics-based

- Based on known biochemistry and biophysics
- Complex, non-linear mathematics
- Time-consuming to construct
- Uses universal physical to extrapolate beyond training data

Data-based approaches: Bayesian models

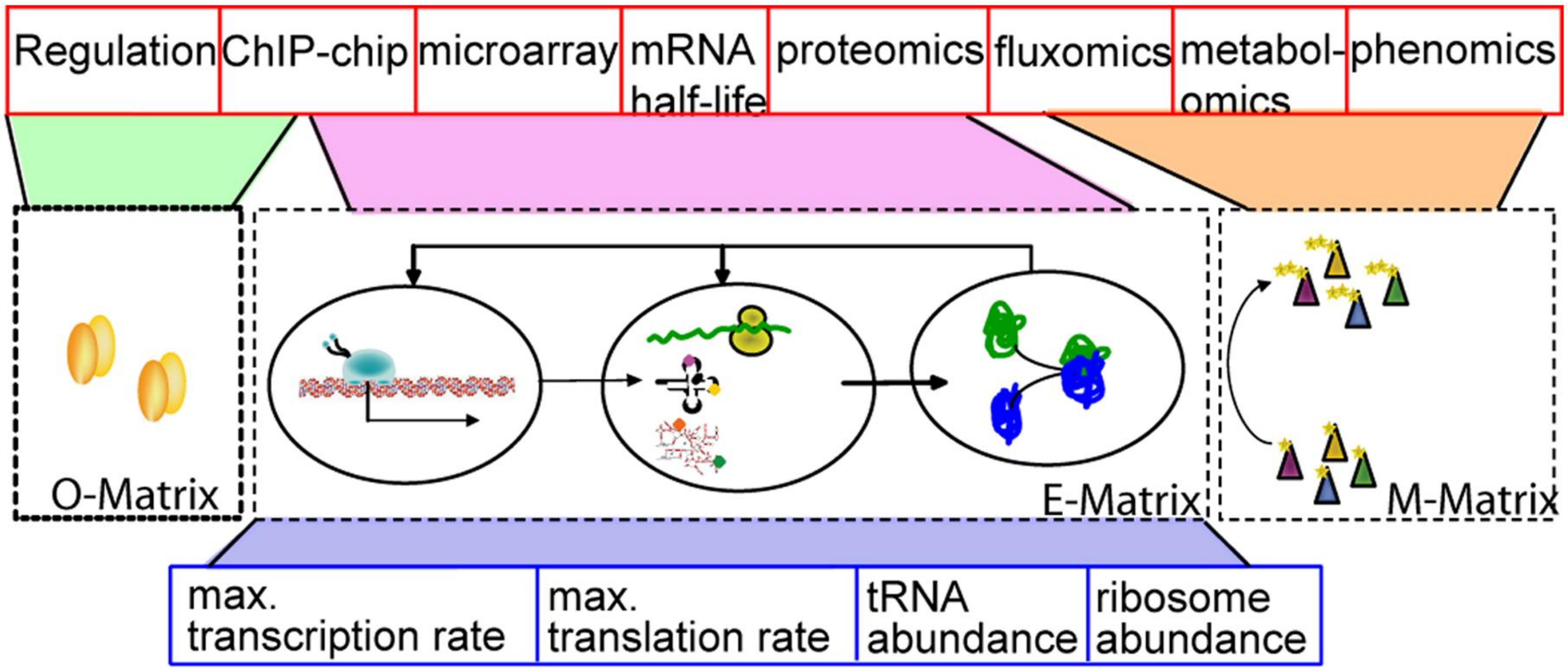


P(C | A,B)

A	B	0	1
0	0	0.9	0.1
0	1	0.2	0.8
1	0	0.9	0.1
1	1	0.01	0.99

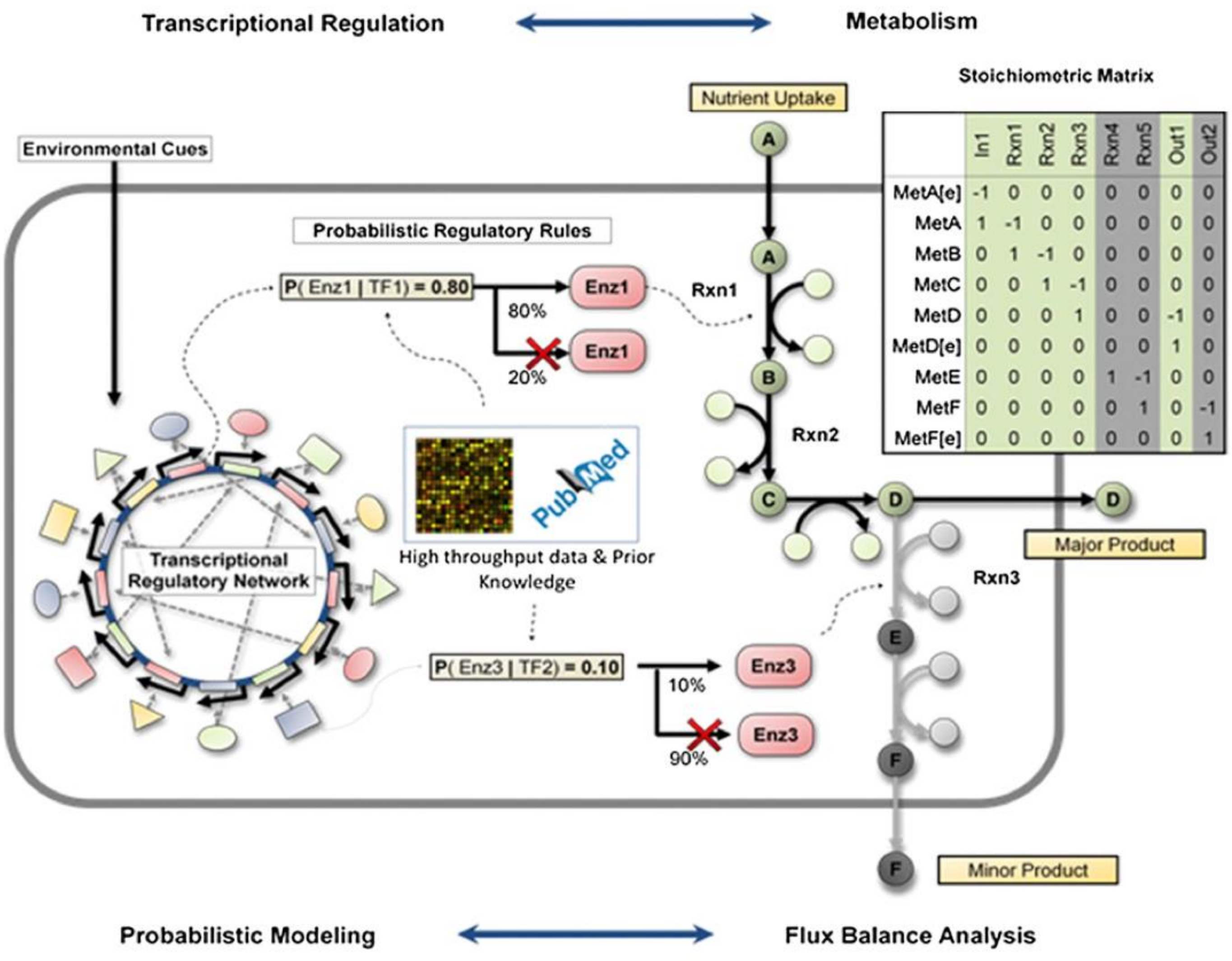
Friedman, 2004

Phenomenological approaches: FBA



Chandrasekaran et al., 2010

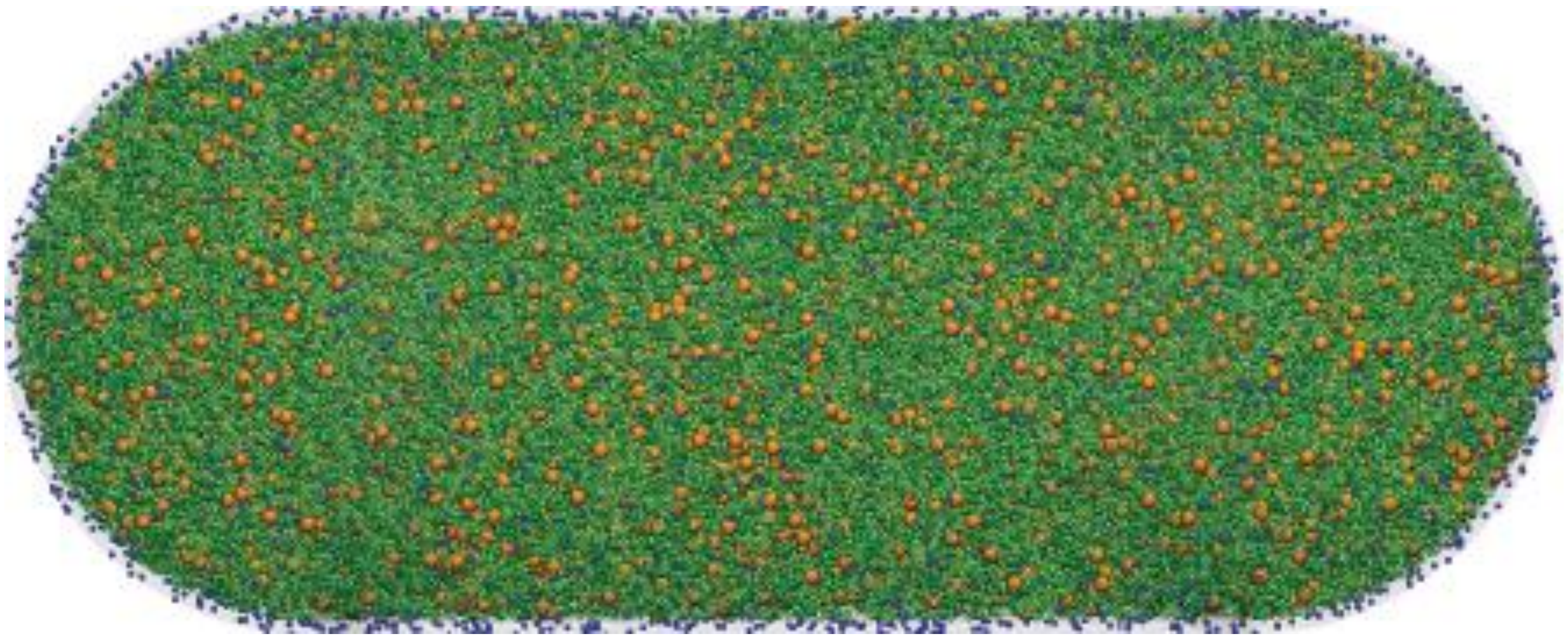
Phenomenological approaches: PROM



	In1	Rxn1	Rxn2	Rxn3	Rxn4	Rxn5	Out1	Out2
MetA[e]	-1	0	0	0	0	0	0	0
MetA	1	-1	0	0	0	0	0	0
MetB	0	1	-1	0	0	0	0	0
MetC	0	0	1	-1	0	0	0	0
MetD	0	0	0	1	0	0	-1	0
MetD[e]	0	0	0	0	0	0	1	0
MetE	0	0	0	0	1	-1	0	0
MetF	0	0	0	0	0	1	0	-1
MetF[e]	0	0	0	0	0	0	0	1

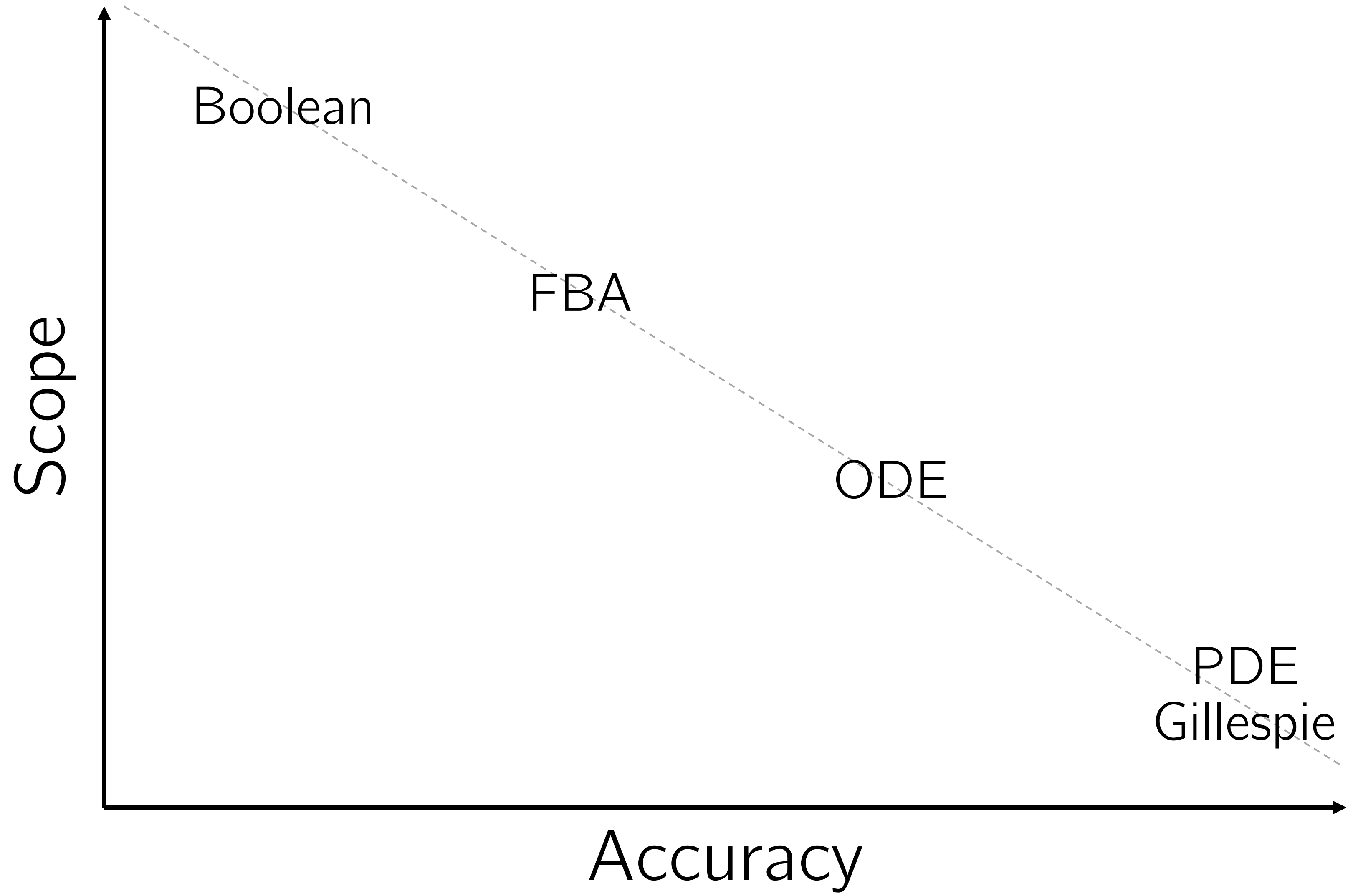
Chandrasekaran et al., 2010

Mechanistic approaches: Coarse-grained MD

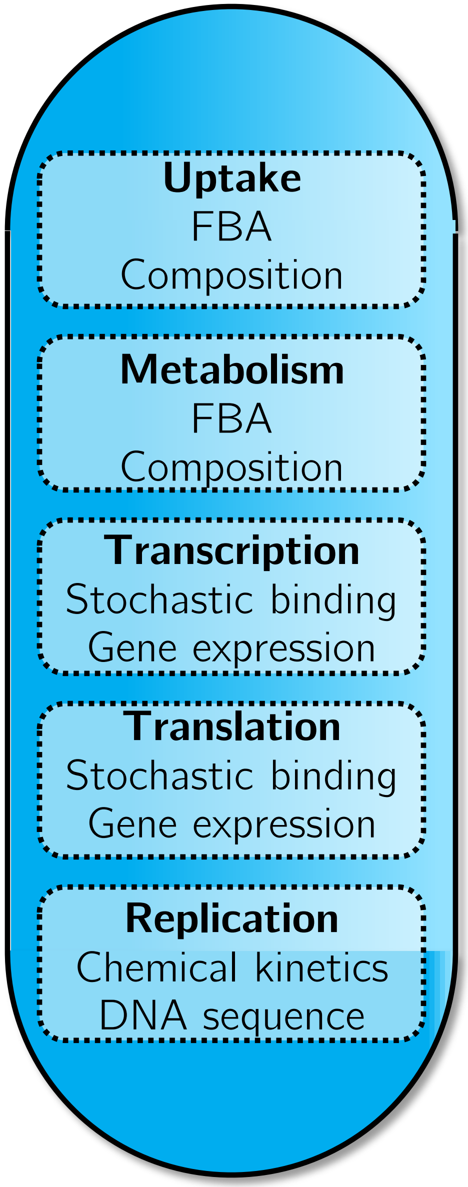


Roberts et al., 2012

Mechanistic modeling formalisms

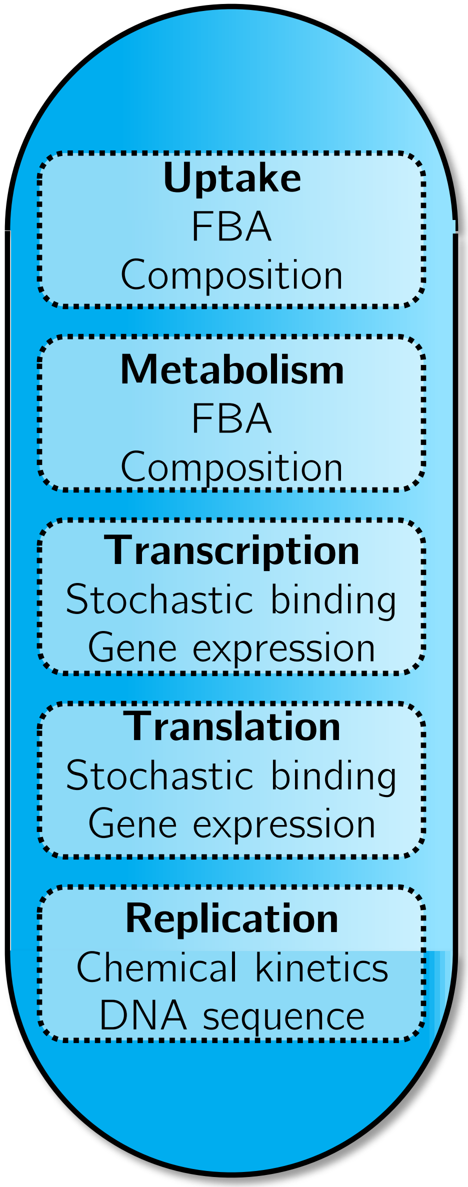


Multi-algorithm modeling



- Models composed of submodels
- Submodels describe individual pathways
- Submodels represented using different math
- Enables representation of well- and poorly-studied pathways

Synonyms



- Model composition
- Integrative modeling
- Hybrid modeling
- Multi-algorithm modeling
- Multi-physics modeling
- Hierarchical modeling

Advantages of multi-algorithm models

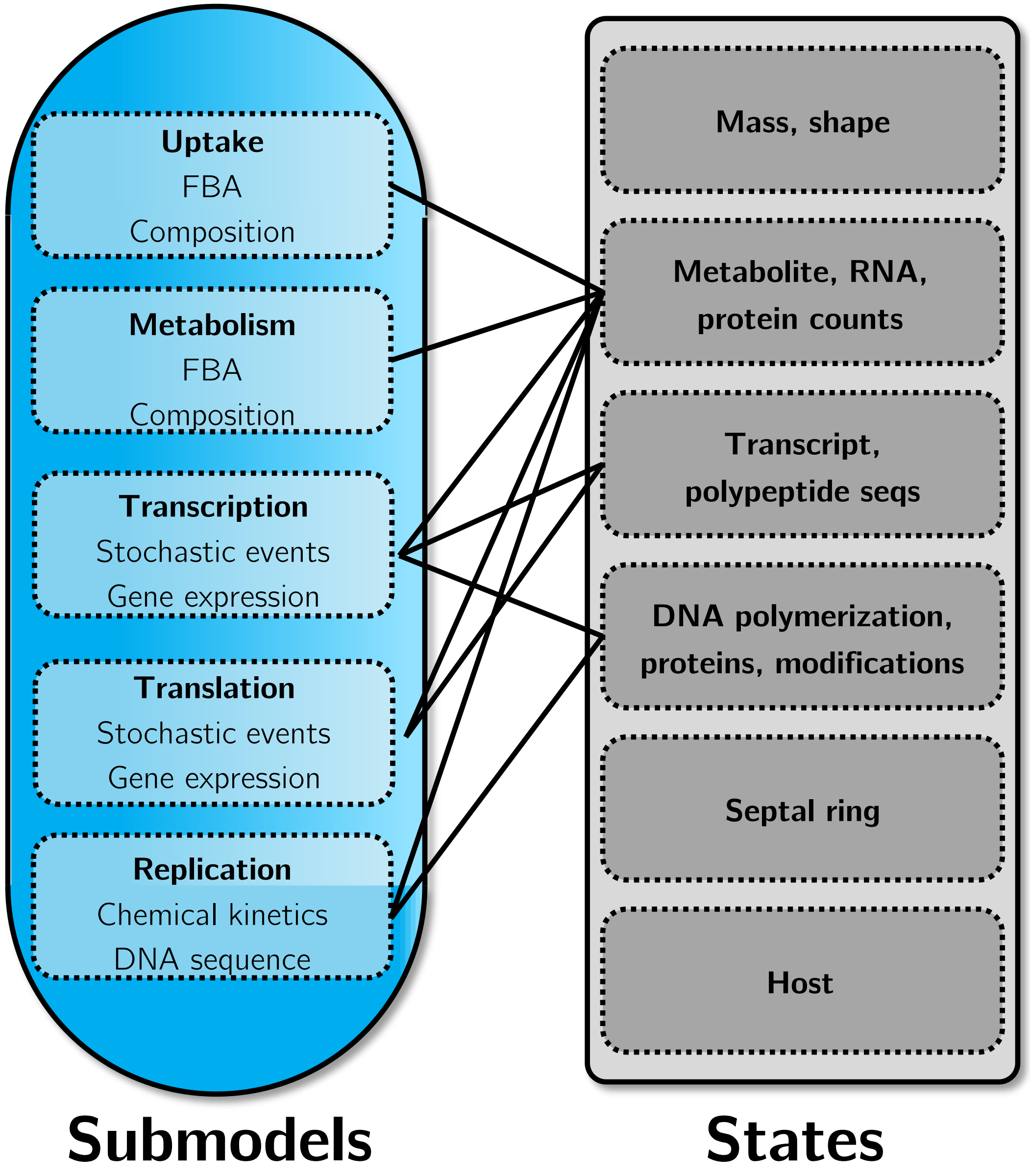
Advantages

- Model composition
  - Encapsulates pathways
  - Enables collaborative model design, estimation, and testing
- Combines coarse- and fine-grained submodels
- Enables thorough representation of knowledge and data
- Avoids unknown parameters
- Simplifies parameter estimation
- Enables more comprehensive and more accurate models

Disadvantages

- Few established methods and software
- Challenging to build, simulate, identify and verify
- Require expert knowledge and intense effort

WC modeling building



1. Curate data
2. Construct submodels
3. Define global state
4. Combine submodels
5. Simulate

Model consistency

- Utilize same species, reaction names
- Resolve conflicting species/reaction representations across submodels
- Calculate RNA and protein sequences from gene sequences
- Calculate species molecular weights from structures
- Calculate transcription, translation, RNA degradation reactions from sequences
- Calculate cell mass, volume from species counts and molecular weights

Reproducibility

Every model element should defined without references to external databases

- Metabolites: InCHI, SMILES
- RNA, protein: sequences
- Reactions: stoichiometry

Cross references should be provided where possible

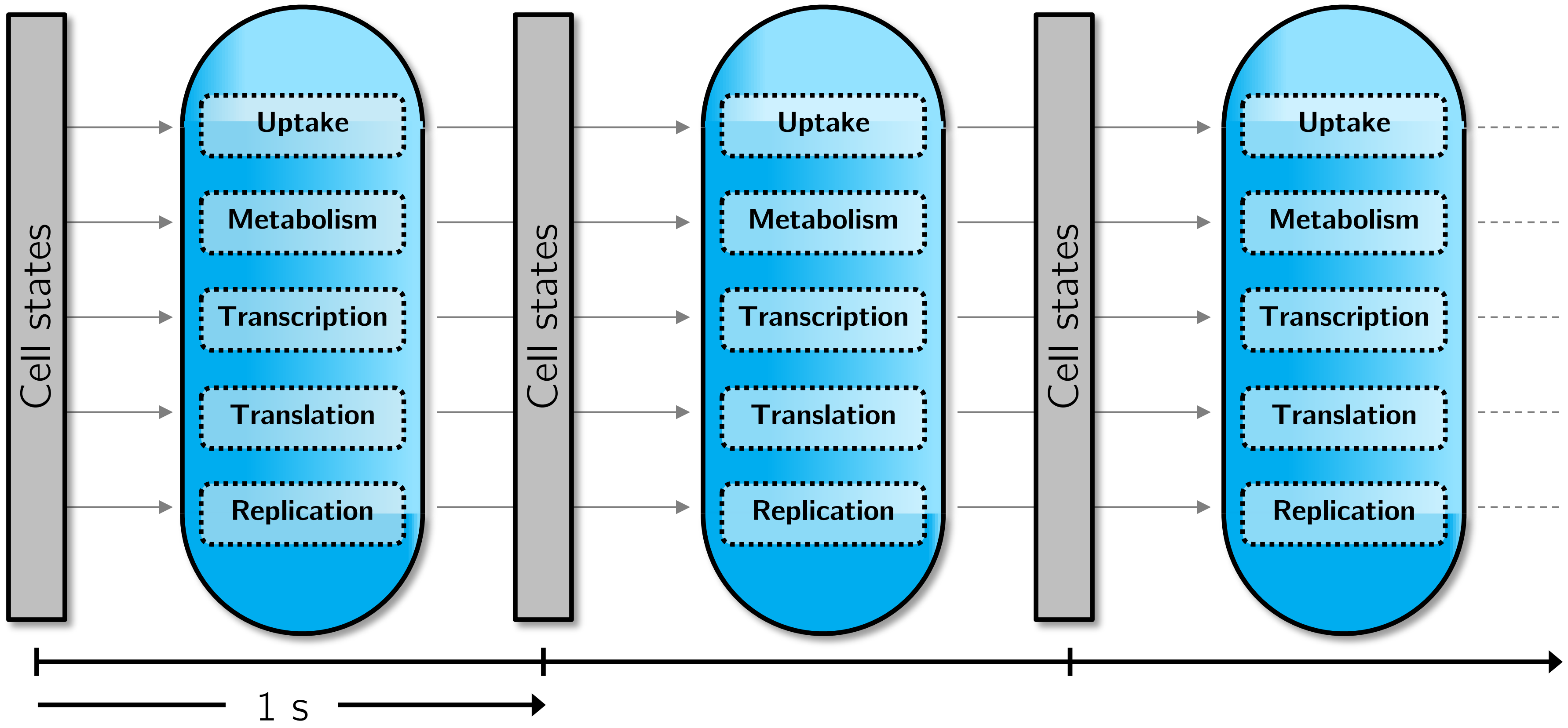
- Metabolites: ChEBI
- RNA: NCBI
- Proteins: UniProt
- Reactions: EC numbers

Every data source and model assumption should be recorded

Multi-algorithm simulation

- Area of active research
- A few algorithms have been developed
- All have limitations
- Tutorial: foundational concepts

Multi-algorithm simulation



Assumption: pathways are independent over short time periods

Concurrent submodel integration

Approximate/continuous/timestep

- Simulate models over short timesteps
- Synchronize models between timesteps
- Low computational cost
- Low numerical accuracy

Exact/discrete

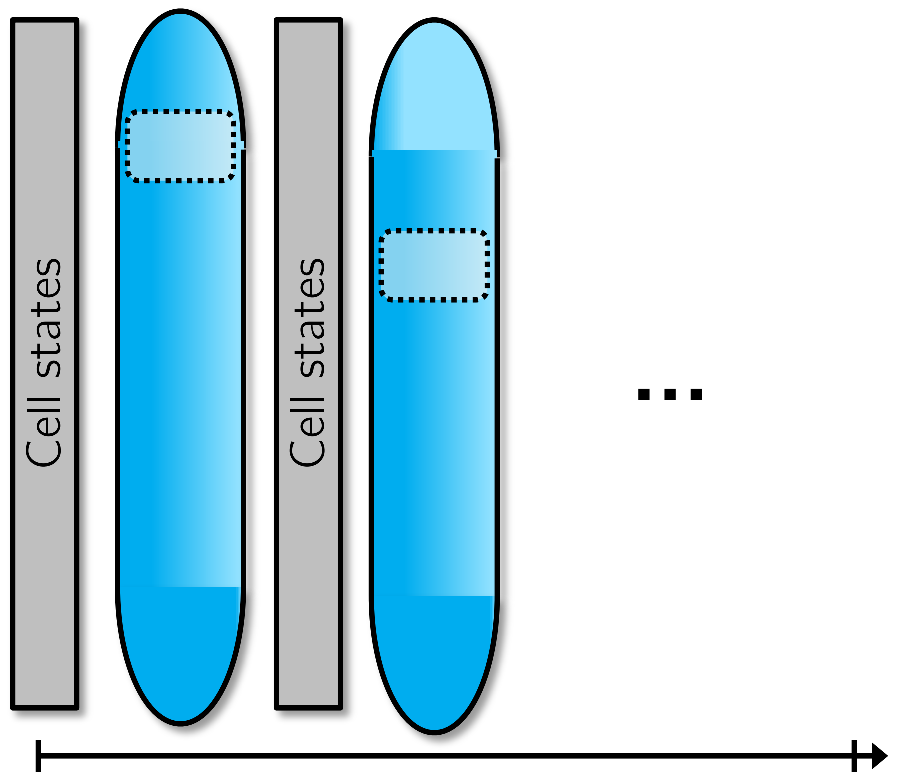
- Resolve order of every individual reaction
- Synchronize submodels after every reaction
- High computational cost
- High numerical accuracy

Approximate simulation

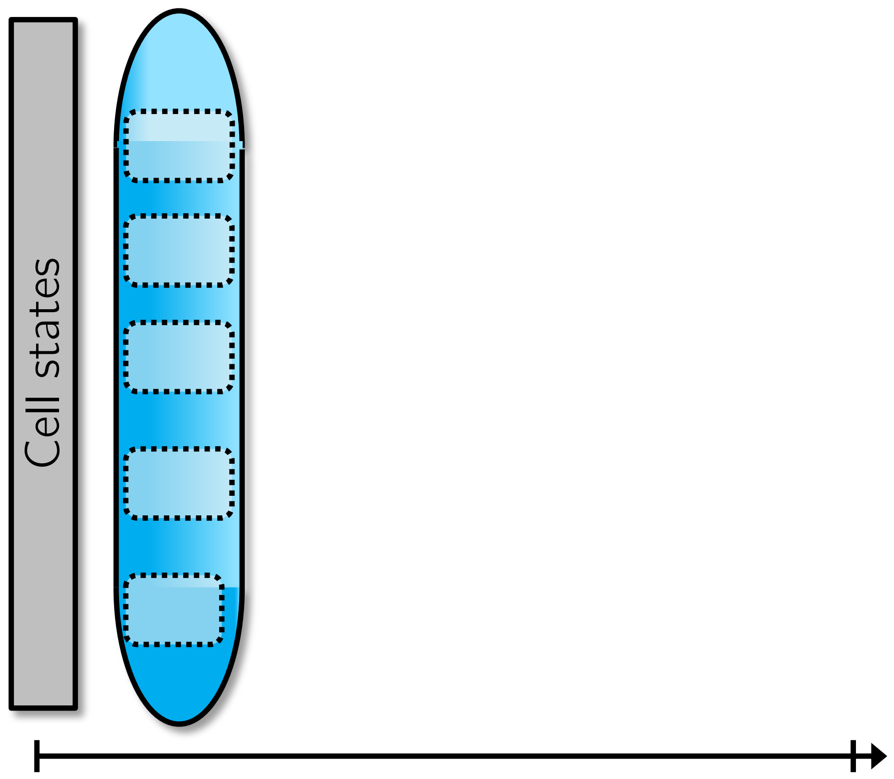
- Divide time into small steps
- Integrate submodels separately
- Update global state
- Reduce timestep until results converge

Submodel execution order

Asynchronous integration



Synchronous integration



Exact simulation

Based on SSA

Where possible, convert submodels to discrete submodels and simulate using SSA

- Simulate ODE with SSA
- Add explicit time to Boolean models
- Merge SSA submodels

Discretely schedule continuous submodel updates

- Treat continuous submodels as a single discrete pseudoreaction whose stoichiometry is time-variant

Exact simulation

- Requires resolution of many events
- Computationally expensive
- Implement using parallel discrete event simulation

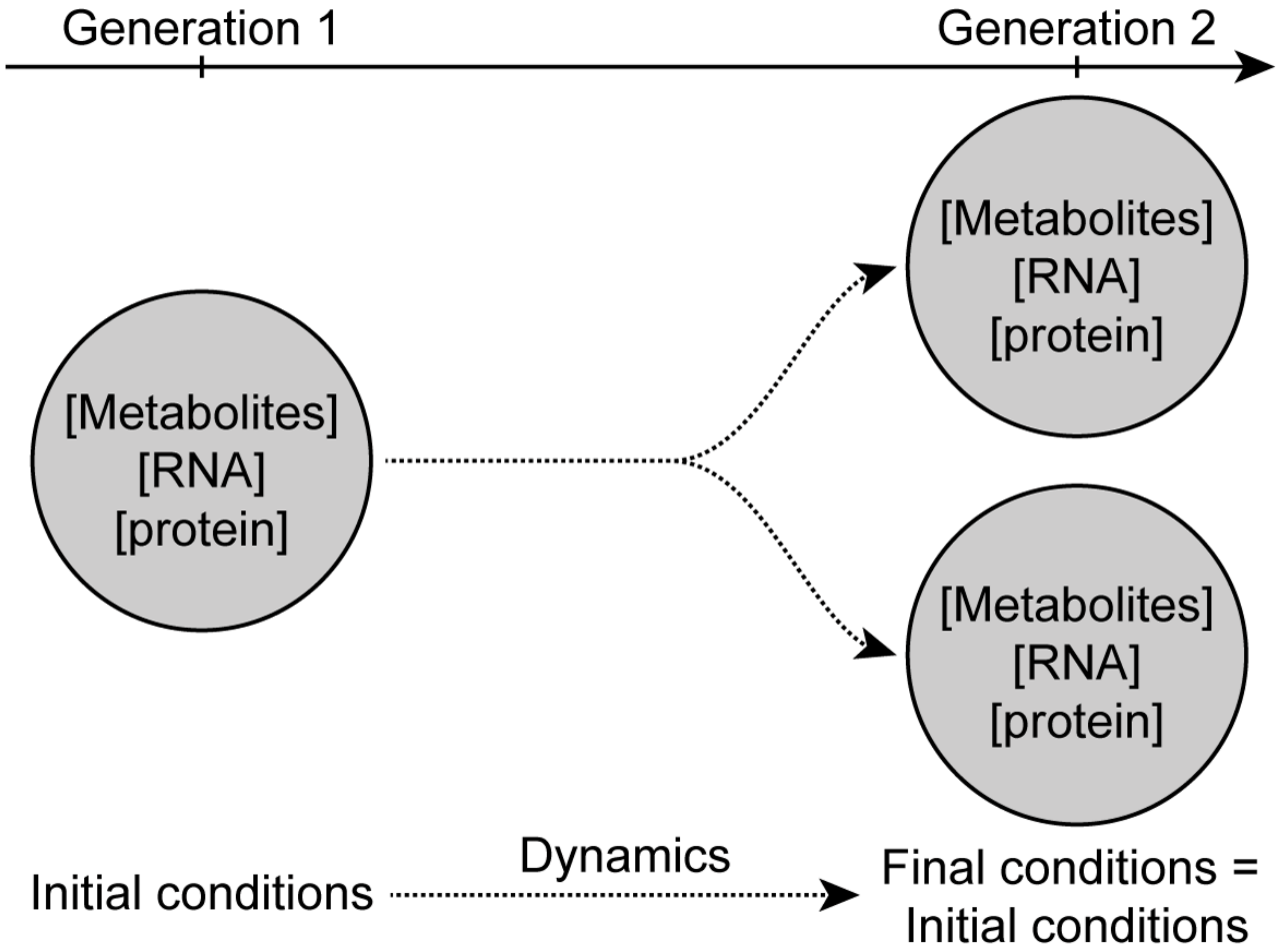
Parameter estimation

Compare model to experimental data

Numerically minimize prediction error

Theory provides additional constraints

Cell theory provides periodic boundary constraint



Cell theory provides periodic boundary constraint

- Phenotype distribution constant over generations
- $\left\langle \text{initial conditions} + \int \text{dynamics}\, dt \right\rangle = \langle \text{initial conditions} \rangle$
- Example:
  - $\left\langle [\text{RNA}]_0 + \int (\text{RNA production} - \text{RNA decay})\, dt \right\rangle = \langle [\text{RNA}]_0 \rangle$
  - $[\text{RNA}](t) = [\text{RNA}]_0 e^{\ln(2)^{t}/_{\tau}}$
  - $\text{RNA production} = \text{k}\, e^{\ln(2)^{t}/_{\tau}}$
  - $\text{RNA decay} = \frac{\ln(2)}{\tau_{1/2}} [\text{RNA}]$
  - $\text{k} = \left(\frac{\ln(2)}{\tau} + \frac{\ln(2)}{\tau_{1/2}} \right) [\text{RNA}]$

Verification

Statically verify model

- E.g. all reactions mass and charge balanced

Dynamically verify submodels

- E.g. protein content doubles over cell cycle duration

Dynamically verify entire model

- E.g. cell divides in observed doubling time

Software engineering

Organization

- Uses methods, objects, and modules to encapsulate data and procedures
- Repository used to track revisions

Style

- Clearly and consistently named variables, methods, classes

Annotation

- Author name, last updated date
- Commented

Testing

- Code is tested formally using unit testing
- Tests evaluated at each revision using continuous integration

Exercises

Exercises

1. **Model building**

   Assemble a small WC model from several data points

2. **Model alignment and parameter estimation**

   Use cell theory to identify parameter values

3. **Multi-algorithm simulation**

   Implement hybrid FBA/SSA simulator

4. **Individual submodel simulation**

   Simulation single submodel

5. **Best practices**

   Annotate model

Physiology

Model reflects typical cell biology

Motivated by *M. pneumoniae*

Submodels

	Algorithm	Reactions	Enzymes
Metabolism	FBA	Several	Several
Transcription	SSA	1 per RNA	RNA polymerase
Translation	SSA	1 per protein	Ribosome
RNA degradation	SSA	1 per RNA	Rnase

Metabolism submodel

Metabolite	Biomass	Produce for other pathways	Recycle from other pathways	Import from media
Glucose				Y
Nucleobases				Y
NMPs	Y		Y	
GDP	Y		Y	
NTPs	Y	Y		
Amino acids	Y	Y		Y
NAD				Y
PPi	Y		Y	
Pi	Y		Y	Y
H_2O	Y	Y	Y	Y
H^+	Y		Y	Y
O_2				Y
CO_2				Y
Internal metabolites				

Non-metabolic submodels

Transcription

a ATP + c CTP + g GTP + u UTP + H_2O → RNA + l PPi + H^+

$l = a + c + g + u$

Translation

a Ala + c Cys + ... + y Tyr + (2 l + 3) GTP + (l + 4) H_2O →
Protein + (2 l + 3) GDP + (2 l + 3) Pi + (2 l + 3) H^+

RNA degradation

RNA + (l – 1) H_2O → a AMP + c CMP + g GMP + u UMP + (l - 1) H^+

Species

Enzymes needed for submodels

- RNA
- Protein

Metabolic reactants and byproducts of all submodels

- Amino acids
- NTPs
- NDPs
- NMPs
- PPi
- Pi
- H2O
- H+

Rate laws

Metabolism

- $v = v_{\max}[\text{Enzyme}]$

Transcription

- $v = v_{\max} \min\limits_{N} \left(\frac{[N\text{TP}]}{K_\text{M} + [N\text{TP}]} \right) [\text{RnaPol}]$
- $K_\text{M} = [N\text{TP}]$

Translation

- $v = v_{\max} \min\limits_{AA} \left(\frac{[AA]}{K_\text{M} + [AA]} \right) [\text{RNA}][\text{Ribosome}]$
- $K_\text{M} = [AA]$

RNA degradation

- $v = v_{\max} \frac{[\text{RNA}]}{K_\text{M} + [\text{RNA}]} [\text{RNase}]$
- $K_\text{M} = [\text{RN}A]$

Files

File	Description
`Model.xlsx`	Template model description and data to build model
`excercise*.py`	Template code for exercises
`model.py`	Reads model from Excel into Python object
`analysis.py`	Plots simulation results
`util.py`	Utility methods

Implementation: Classes

Model represented by `Model` object

- Submodels
  - Species/compartments
  - Reactions
- Compartments
- Species
- Reactions
  - Participants
    - Species
    - Compartment

Implementation: Methods

- `model.getModelFromExcel(<fileName:string>)`
  Reads model from Excel
- `model.Model.calcInitialConditions()`
  Calculates initial conditions
- `model.Submodel.updateLocalState(<model:Model>),`
  `model.Submodel.updateGlobalState(<model:Model>)`
  Updates submodel state from model, updates global state from submodel
- `model.Submodel.calcReactionRates(<reactions:list>,`
  `<speciesConcentrations:dict>)`
  Returns array with rates of every reaction in a submodel
- `model.FbaSubmodel.calcBounds(<timeStep:int>)`
  Returns array with upper and lower bounds for reactions
- `model.Submodel.executeReaction(<speciesCounts:dict>,`
  `<reaction:Reaction>)`
  Updates species counts with stoichiometry of the reaction
- `model.Model.calcMass(), model.Model.calcVolume()`
  Updates cell mass and volume

Implementation: Cell state

- `model.Model.speciesCounts`
  Represents species copy numbers as `numpy` array
  - Rows represent species
  - Columns represent compartments
- `model.Submodel.speciesCounts`
  Represents species copy numbers as `dict`
- `model.Model.getSpeciesCountsDict()`,
  `model.Model.setSpeciesCountsDict(<counts:dict>)`
  Gets, sets `dict` of species counts
- `model.Model.getSpeciesConcentrations()`,
  `model.Submodel.getSpeciesConcentrations()`
  Gets species concentrations
- `model.Model.mass`, `model.Model.volume`,
  `model.Model.extracellularvolume`, `model.Submodel.volume`,
  `model.Submodel.extracellularVolume`
  Cell mass, cell volume, extracellular volume
- `model.FbaSubmodel.growth`,
  `model.FbaSubmodel.reactionFluxes`
  Growth rate and reaction fluxes

Implementation: Methods

- `model.Model.getComponentById(<id:string>)`
  Returns model component with id
- `model.Reaction.getStoichiometryString()`
  Returns string representation of reaction

-
  ```
  analysis.plot(
      <model:Model>,
      <time:numpy.ndarray>,
      <volume:numpy.ndarray>,
      <speciesCounts:numpy.ndarray>,
      <selectedSpeciesCompartments: list of ids e.g. "ATP[c]">,
      <units:str e.g. "mM">,
      <fileName:str>)
  ```
  Plots simulation results
- `numpy.random.seed(<seed:int>)`
  Seeds PRNG

Exercise 1: Building models from data

- Learn how to build WC models from data
- Build list of species, reactions from metabolic reconstruction
- Use transcription, translation, RNA degradation templates to create individual reactions
- Enumerate initial conditions from RNA and metabolite copy numbers/concentrations

Exercise 2: Aligning submodels

- Learn how to build internally consistent models by
  - Calculating transcription, translation, RNA degradation rate parameters
  - Calculating metabolism output pseudoreaction (FBA objective)
- Use cell theory to calculate rate constants
- Sum net effects of non-metabolic submodels and cell composition to calculate metabolism output (FBA objective)
  - $\text{Production}_i = [\text{Metabolite}]_i + \sum_j S_{ij} \int_0^{\tau} v_j \, dt$

Rate parameters

Metabolism

- Curated from literature
- $v = v_{\mathrm{max}}[\mathrm{Enzyme}]$

Transcription

- Calculated from RNA copy number, half-life, and cell cycle length
- $v = \mathrm{degradation} + \mathrm{dilution} = \frac{\ln(2)}{\tau_{\mathrm{RNA}}}[\mathrm{RNA}] + \frac{\ln(2)}{\tau_{\mathrm{cell}}}[\mathrm{RNA}] = v_{\mathrm{max}} \min_{N} \left(\frac{[N\mathrm{TP}]}{K_{\mathrm{M}} + [N\mathrm{TP}]}\right)[\mathrm{RnaPol}]$
- $K_{\mathrm{M}} = [N\mathrm{TP}]$

Translation

- Calculated from amino acids, RNA, and ribosome concentrations
- $v = \mathrm{dilution} = \frac{\ln(2)}{\tau_{\mathrm{cell}}}[\mathrm{Protein}] = v_{\mathrm{max}} \min_{AA} \left(\frac{[AA]}{K_{\mathrm{M}} + [AA]}\right)[\mathrm{RNA}][\mathrm{Ribosome}]$
- $K_{\mathrm{M}} = [AA]$

RNA degradation

- Characterized by typical RNA half-life
- $v = v_{\mathrm{max}} \frac{[\mathrm{RNA}]}{K_{\mathrm{M}} + [\mathrm{RNA}]}[\mathrm{RNase}] = \frac{\ln(2)}{\tau_{\mathrm{RNA}}}[\mathrm{RNA}]$
- $K_{\mathrm{M}} = [\mathrm{RN}A]$

Rate parameters

Submodel	Vmax	Km (mM)
Metabolism	Given	N/A
Transcription	2.33×10^{-4} s^{-1}	1.00
Translation	2.66×10^{2} M^{-1} s^{-1}	5.00
RNA degradation	2.31×10^{-4} s^{-1}	1.81×10^{-4}

Metabolism production

Metabolite	Molecules/cell	Metabolite	Molecules/cell
ALA	3.42×10^4	PHE	2.39×10^4
ARG	4.09×10^4	PRO	3.44×10^4
ASN	2.32×10^4	SER	4.45×10^4
ASP	2.43×10^4	THR	3.62×10^4
ATP	1.10×10^6	TRP	2.22×10^4
CTP	1.16×10^6	TYR	2.20×10^4
CYS	2.23×10^4	UTP	1.13×10^6
GLN	2.47×10^4	VAL	3.38×10^4
GLU	2.27×10^4	AMP	-1.04×10^6
GLY	3.12×10^4	CMP	-1.10×10^6
GTP	1.73×10^6	GDP	-5.81×10^5
H2O	1.52×10^9	GMP	-1.05×10^6
HIS	2.16×10^4	H	-4.97×10^6
ILE	2.93×10^4	PI	-4.71×10^5
LEU	4.12×10^4	PPI	-4.38×10^6
LYS	2.20×10^4	UMP	-1.07×10^6
MET	2.14×10^4		

Exercise 3: Multi-algorithm simulation

Goal

- Learn how to simulate multi-algorithm models by
  - Implementing a simulator for FBA and SSA

Approach

- Combine non-metabolic submodels into a single submodel
- Synchronize state between FBA and SSA models
- Use SSA and FBA and simulate the combined model

Psuedocode

```
Initialize state
for t = 0; t < tMax; t+= dt
    Simulate SSA submodels for dt
        t2=t
        Calculate SSA reaction rates
        Calculate time to next SSA reaction
            dt2 = exponential(sum(rates))
        Calculate next SSA reaction:
            multinomial(rates)
        Update time: t2 = t2 + dt2
        Update state
    Simulate FBA submodel
        Calculate growth rate
        Update state
```

Exercise 4: Testing submodels

Goal

- Learn how to test individual submodels so that submodels can be developed separately by
  - Testing metabolism submodel in a meaningful way <u>without</u> simulating the other submodels

Approach

- Simulate metabolism submodel coupled with other reduced or “mocked” versions of the other submodels
- The other submodels can be mocked by replacing them with a single pseudoreaction with represents their net effect

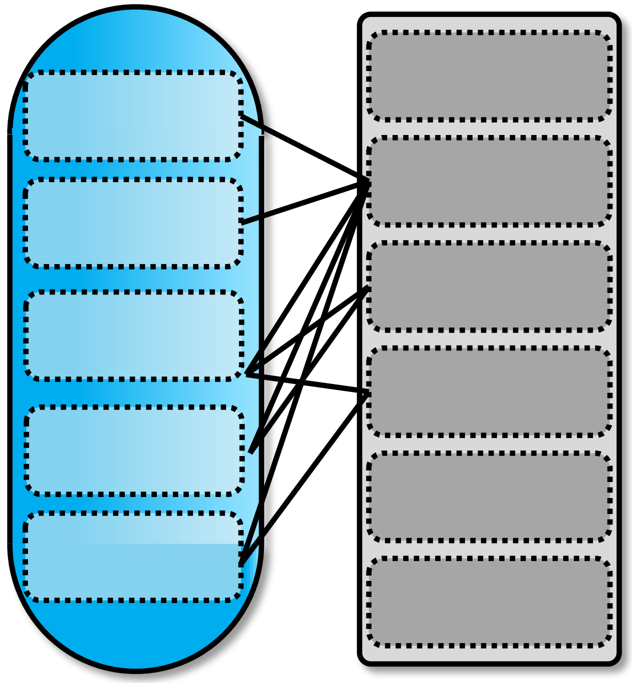
Pseudocode

```
Calculate net transcription, translation, RNA
degradation reactions
Initialize state
t = 0
while t < tMax
    Calculate growth and fluxes
    Update state
    Update sate by growth * net transcription,
    translation, RNA degradation reactions
    Update time: t=t+dt
```

Exercise 5: Best practices

- Learn and reinforce best WC modeling practices by
  - Annotating model with cross references
  - Calculating chemical formulae, molecular weights from structures
  - Testing model
- Use ChEBI, Enzyme, NCBI, and UniProt to annotate species and reactions
- Use ChEBI to annotate metabolite structures
- Use mcule to calculate chemical formulae, molecular weights, charges

Summary



Definition	Represent well- and poorly-characterized pathways
Motivation	Represent well- and poorly-characterized pathways
Advantages	Enables comprehensive models
Disadvantages	Complex, few methods and tools
Construction	Map submodels onto common state
Simulation	Concurrently integrating submodels
Outlook	Research needed to develop simulation algorithms

Feedback

We're very interested in improving the course

Please complete tutorial survey