Input/Output Modeling of Simple Artificial Cell Using Modularized Approach

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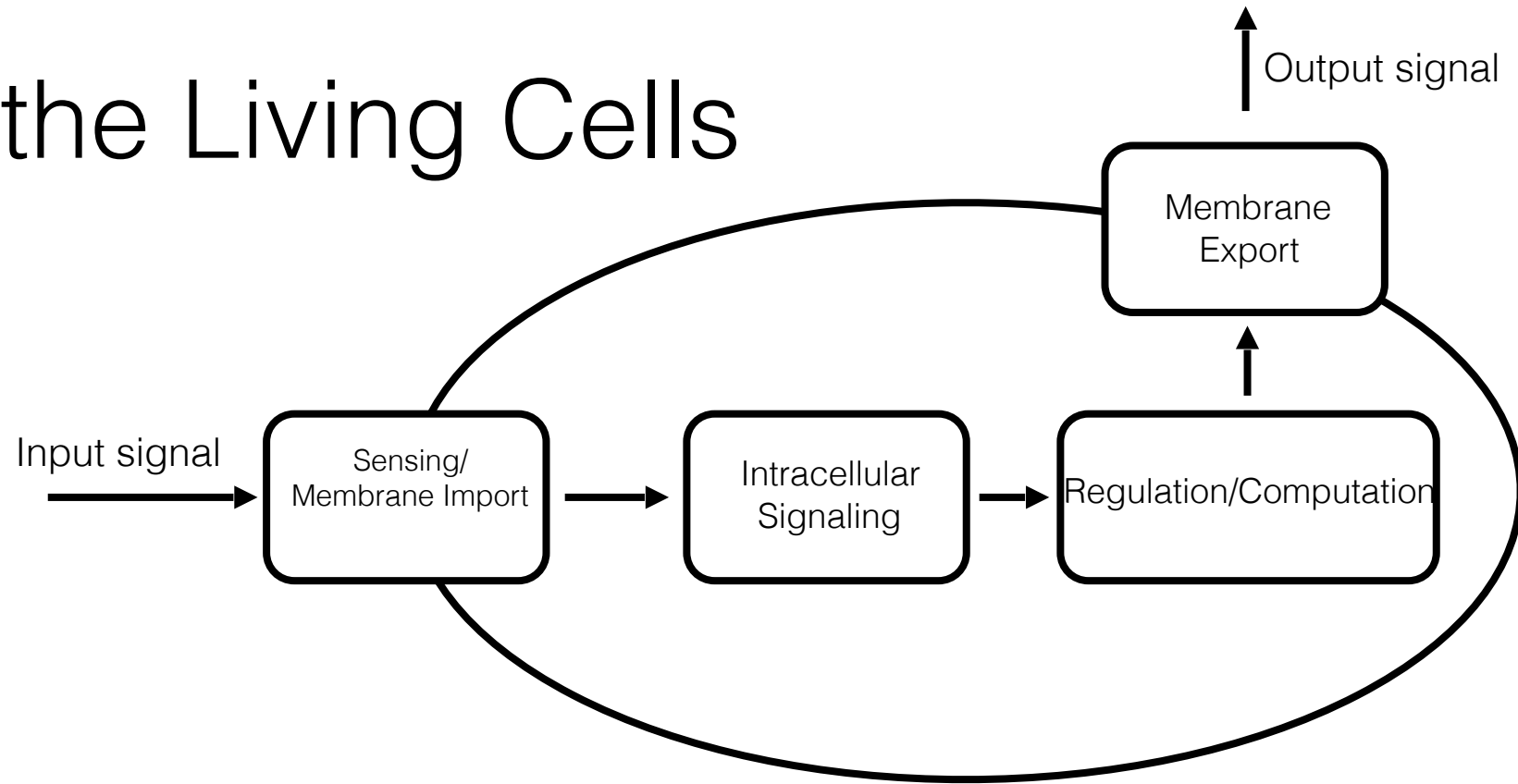
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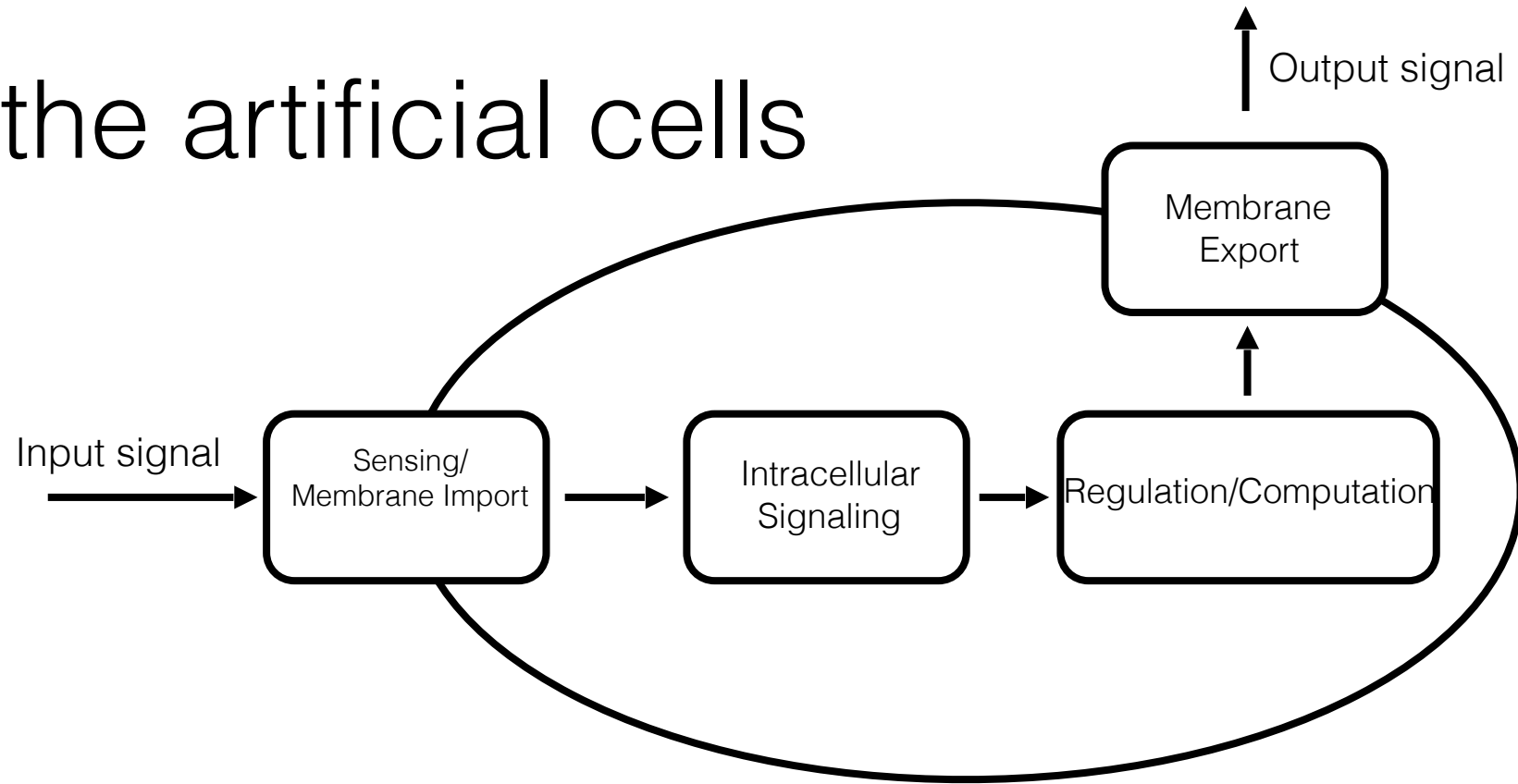26 September 2017

Contents

Properties of the Living Cells

- Self-organization
- Homeostasis
- Reproduction
- Growth
- Metabolism
- Adaptation to the surrounding environment
- Response to the environmental stimuli

Output signal

Membrane Export

Input signal → Sensing/ Membrane Import → Intracellular Signaling → Regulation/Computation

Alberts, B. (2014). *Essential cell biology*. New York: Garland Science.

Properties of the artificial cells

- Self-organization
- Homeostasis
- Reproduction
- Growth
- Metabolism
- Adaptation to surrounding environment
- **Response to environmental stimuli**
- Reproduction

Input signal → Sensing/Membrane Import → Intracellular Signaling → Regulation/Computation → Membrane Export → Output signal

Alberts, B. (2014). *Essential cell biology*. New York: Garland Science.

Modular "Bottom-Up" Approach

- Biomolecular circuits as the subsystems
- Various subsystems:
  - Sensing
  - Transport
  - Signaling
  - Metabolic
  - Regulation/Computation
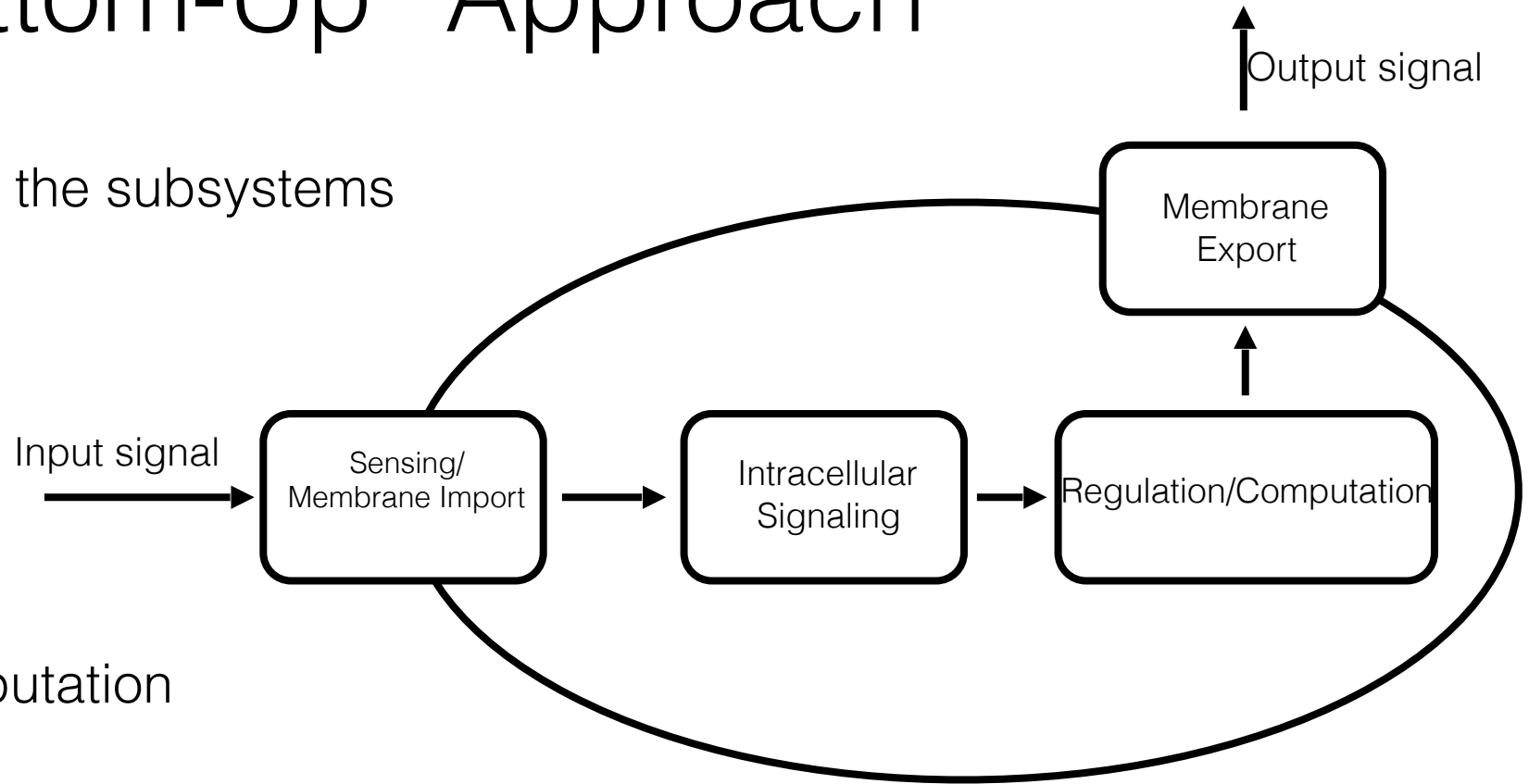
- Synthetic cell: A set of interconnected biomolecular subsystems that perform specific functions

Alberts, B. (2014). *Essential cell biology*. New York: Garland Science.
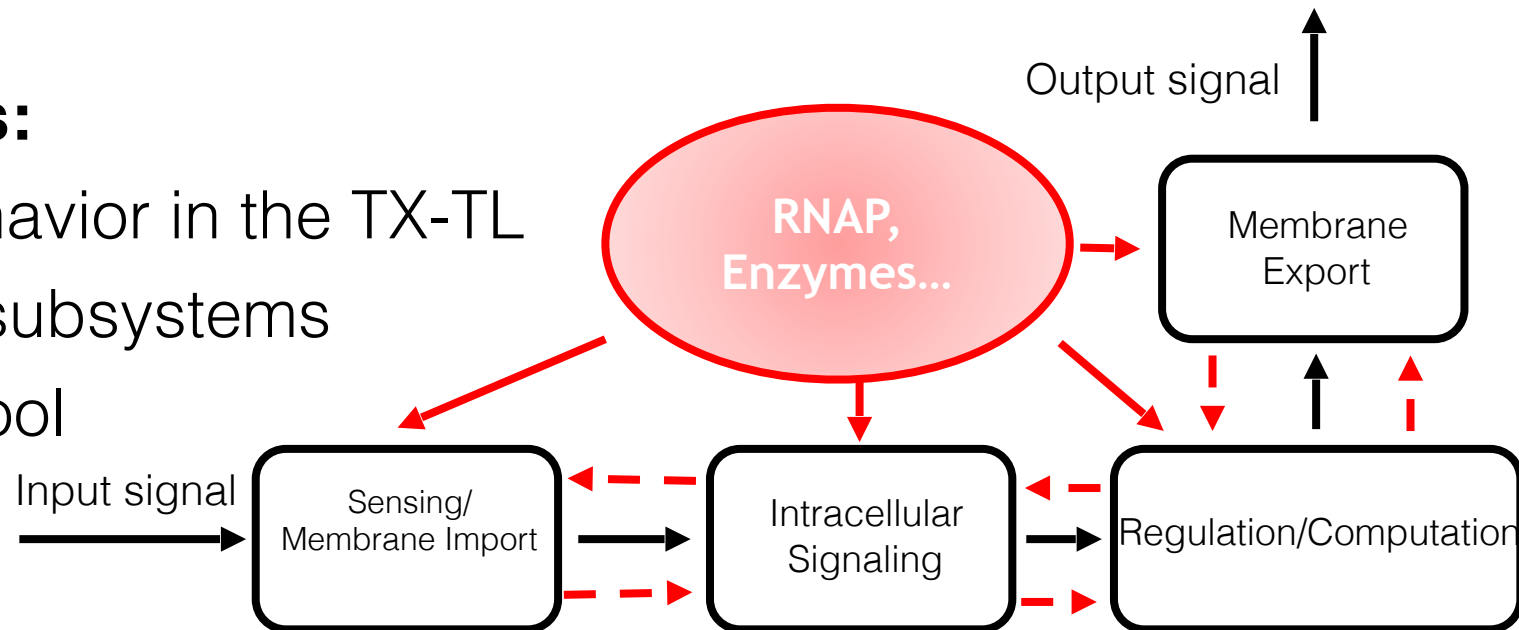
Input/Output Analysis

- **Current Tools:**

    - **Experimental:** *in vitro* cell-free Transcription/Translation machinery

    - **Computational:** MATLAB SimBiology, TX-TL modeling toolbox

- **Computational Challenges:**

- Need to model modular behavior in the TX-TL

- No way to reliably connect subsystems

- Need for simple modeling tool

    for Input/Output modeling

Output signal

RNAP, Enzymes...

Membrane Export

Input signal

Sensing/ Membrane Import

Intracellular Signaling

Regulation/Computation

Del Vecchio, D. and Murray, R. (2015). *Biomolecular feedback systems*. Princeton, NJ [u.a.]: Princeton Univ. Press.

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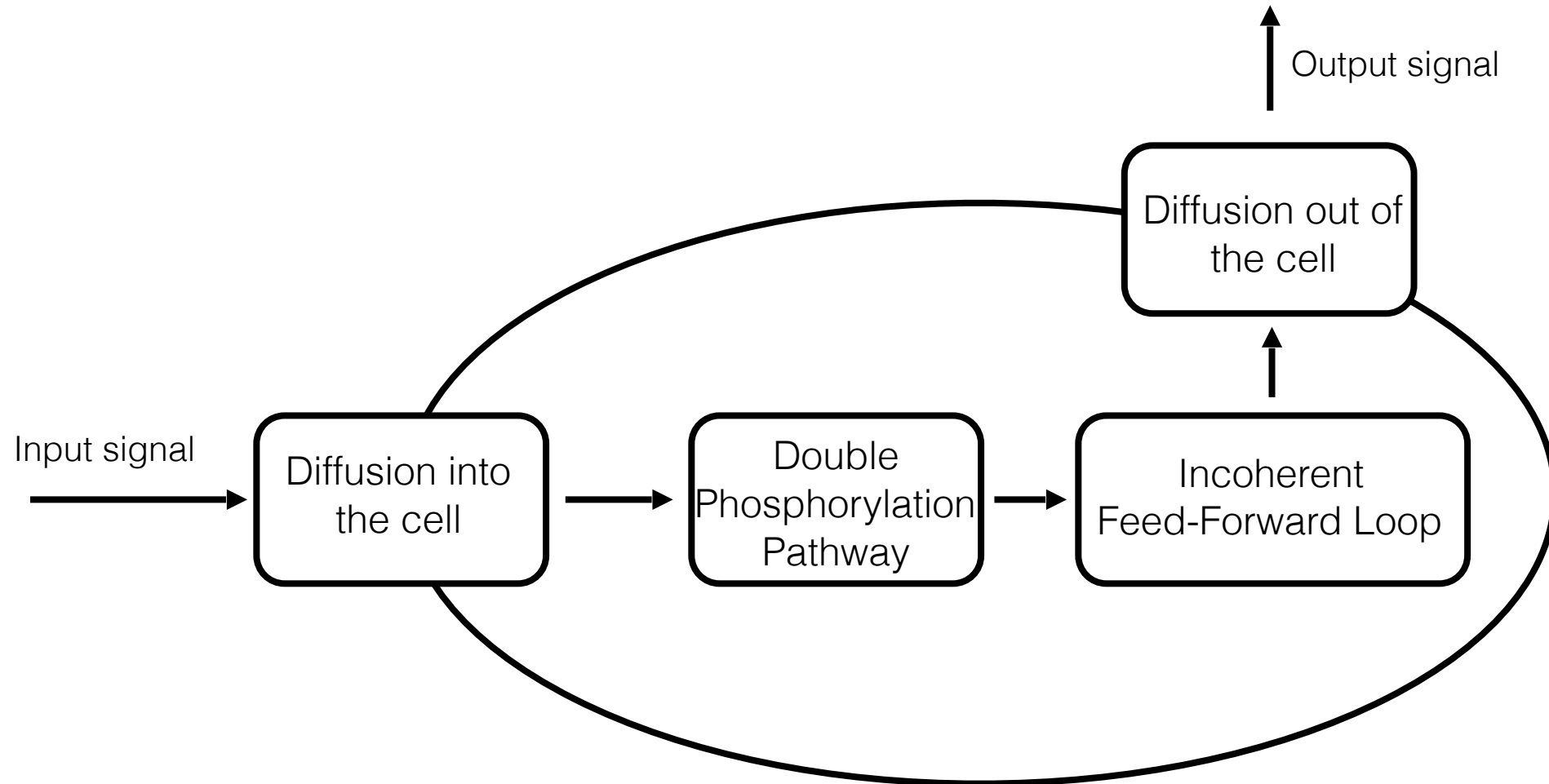
E. Summary of the Experimental Work

F. Conclusion and the Future Plans

BioSIMI

- **BioSIMI** ("**Bio**molecular **S**ubsystems **I**nterconnection **M**odeling **I**nstrument")

- Tool for simple Input/Output modeling of interconnected biomolecular subsystems

- Multiple Inputs/Single Output (MISO) modeling

- User-defined (mainly transport) and TX-TL-defined (standard assembly) subsystems

- Accounts for retroactivity and resource competition

# Modeling of Interconnection of Simple Cellular Subsystems

Output signal

Diffusion out of the cell

Input signal

Diffusion into the cell

Double Phosphorylation Pathway

Incoherent Feed-Forward Loop

BioSIMI (I/O Model of Vesicle)

```matlab
% Create SimBiology model object vesicle
vesicle = BioSIMI_make_vesicle('vesicle')
% Create subsystem with reactions and species in DiffusionIn subsystem
DiffIn = BioSIMI_make_subsystem('DiffusionIn','input','output','DiffIn')
% Add reactions and species in DiffusionIn subsystem to the appropriate
% compartments of vesicle object
BioSIMI_add_subsystem(vesicle,{'int','ext'},DiffIn)
% Create subsystem with reactions and species in Double-Phosphorylation subsystem
DP_Subsystem = BioSIMI_make_subsystem('DP','in','out','DP_Subsystem')
% Add reactions and species in Double-Phosphorylation subystem to the appropriate
% compartments of vesicle object
BioSIMI_add_subsystem(vesicle,'int',DP_Subsystem)
% Connect DiffusionIn subsystem with Double-Phosphorylation subsystem and
% create object of class subsystem that contains connection of both
% subsystems
DiffDP = BioSIMI_connect(vesicle,'int',DiffIn,DP_Subsystem,'DiffDP')
% Create subsystem with reactions and species in IFFL subsystem
IFFL_Subsystem = BioSIMI_make_subsystem('IFFL','in','out','IFFL_Subsystem')
% Add reactions and species in IFFL subystem to the appropriate
% compartments of vesicle object
BioSIMI_add_subsystem(vesicle,'int',IFFL_Subsystem)
%  Connect the combination of DiffusionIn and Double-Phoshporylation subsystem with IFFL
%  subsystem and create object of class subsystem that contains connection
%  of all three subsystems
Diff_DP_IFFL = BioSIMI_connect(vesicle,'int',DiffDP,IFFL_Subsystem,'Diff_DP_IFFL')
% Create subsystem with reactions and species in DiffusionOut subsystem
DiffOut = BioSIMI_make_subsystem('DiffusionOut','input','output','DiffOut')
% Add reactions and species in DiffusionOut subystem to the appropriate
% compartments of vesicle object
BioSIMI_add_subsystem(vesicle,{'int','ext'},DiffOut)
%  Connect the combination of DiffusionIn, Double-Phoshporylation, and IFFL
%  subsystem with Diffusion Out subsystem and create object of class subsystem
%  that contains connection of all four subsystems subsystems
FinalSystem = BioSIMI_connect(vesicle,'int',Diff_DP_IFFL,DiffOut,'FinalSystem')
% Run the simulation of the Final System
SimData = BioSIMI_runsim(FinalSystem,20)
% Plot the Input/Output relationship in the Final System
BioSIMI_plot(FinalSystem,SimData)
```
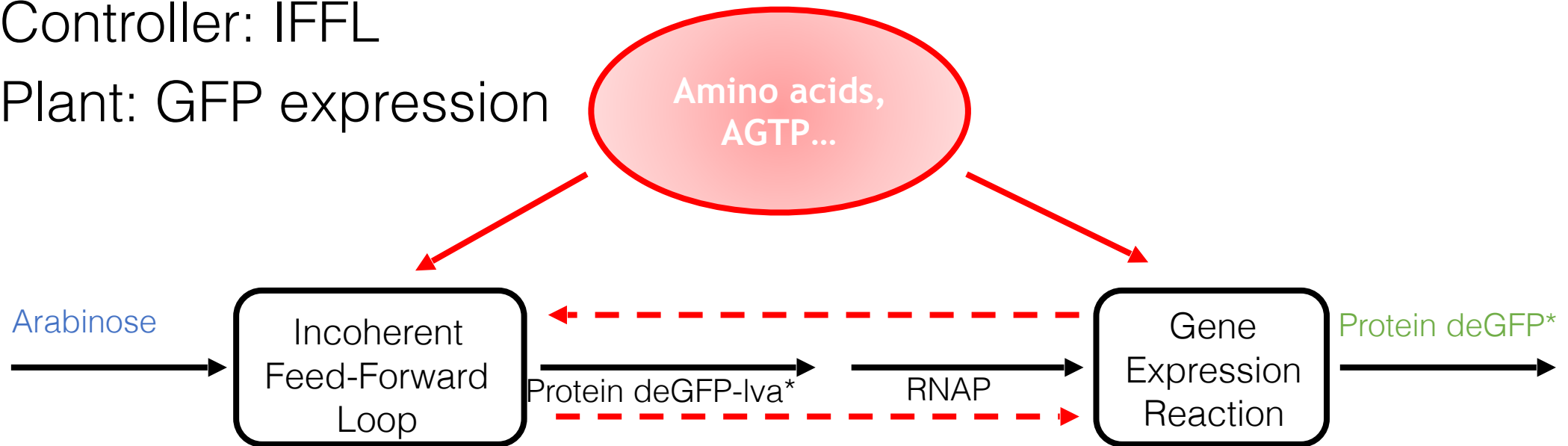

Temporal response of output to input in "FinalSystem" system
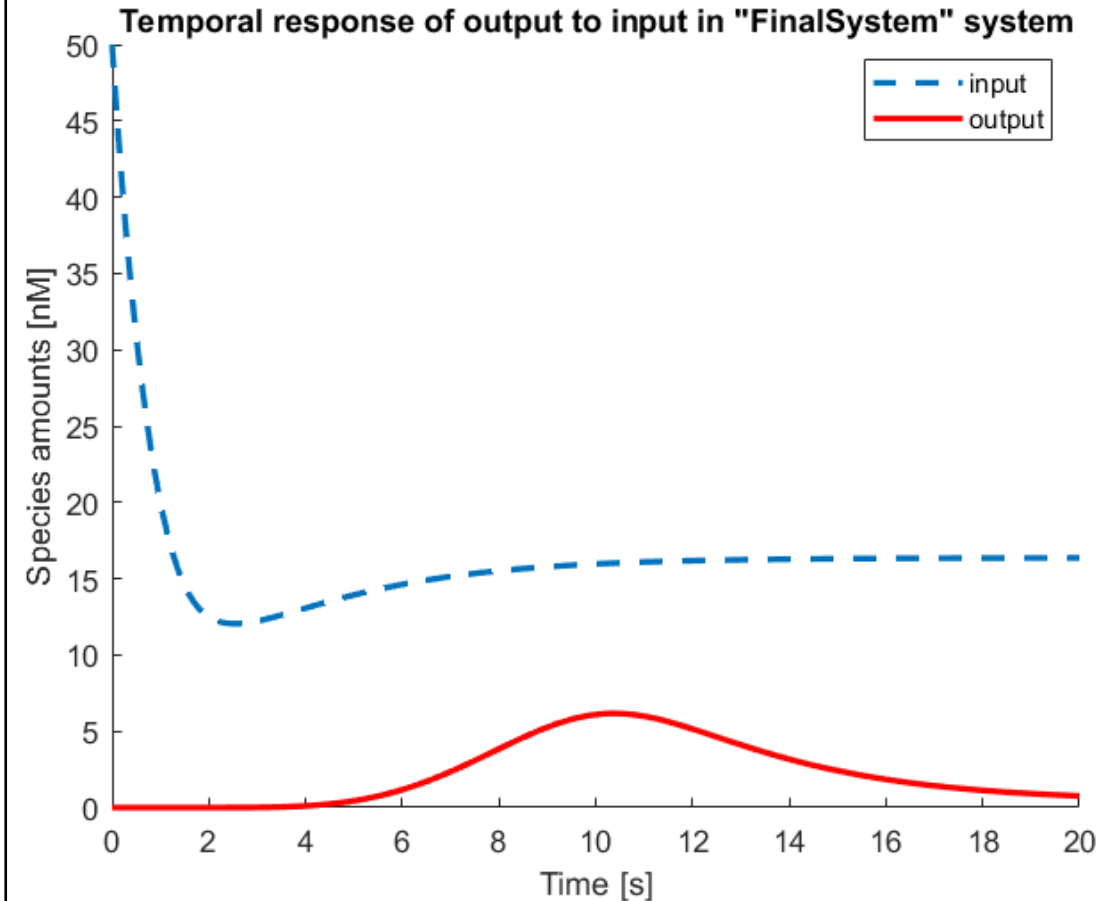
BioSIMI (Multiple Inputs/Single Output)
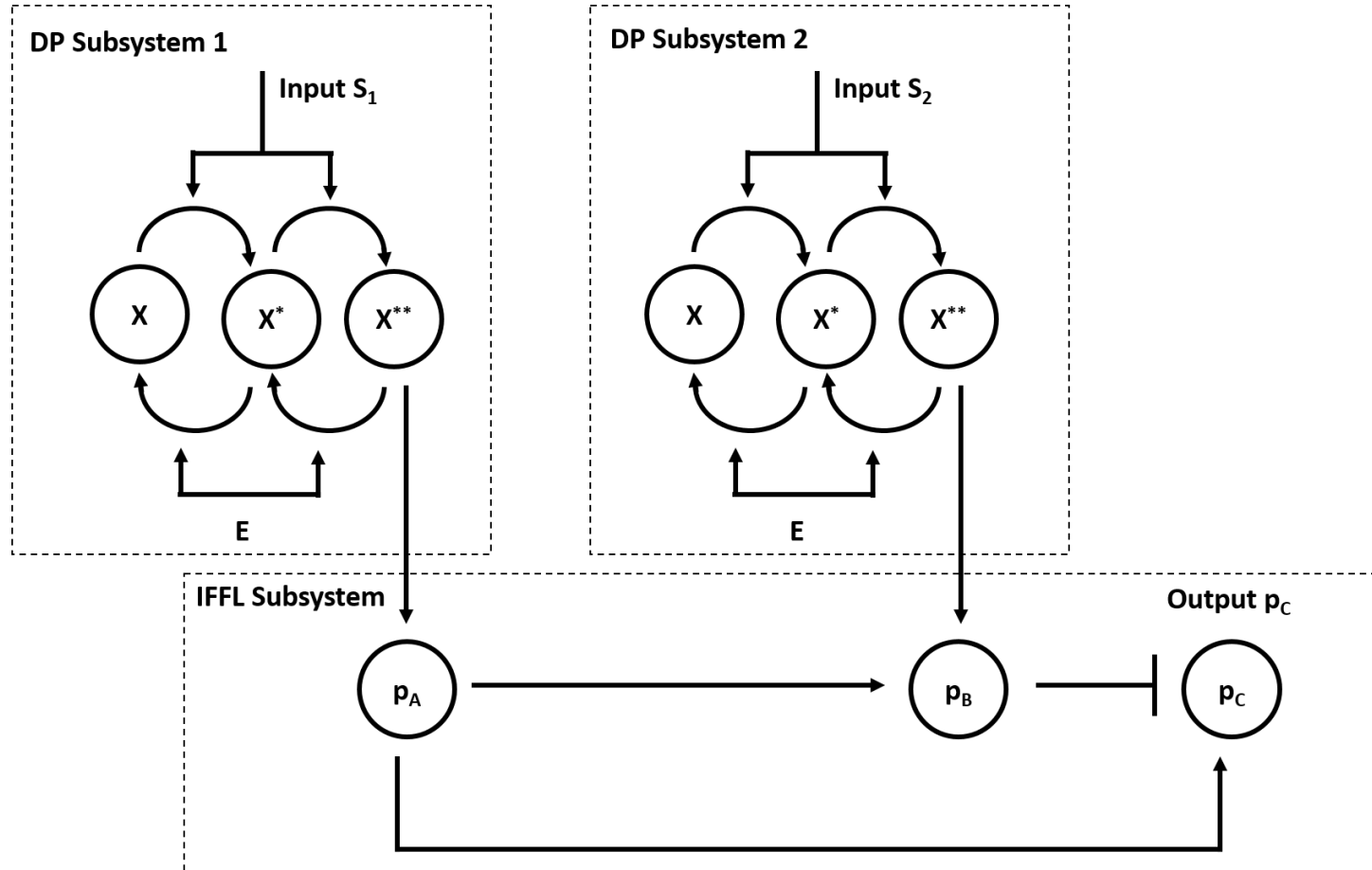
BioSIMI (Multiple Inputs/Single Output)

```matlab
% Create SimBiology model object vesicle
vesicle = BioSIMI_make_vesicle('vesicle');
% Create subsystem with reactions, parameters and species in the first Double-Phosphorylation subsystem
DP_Subsystem1 = BioSIMI_make_subsystem('DP','in','out','DP_Subsystem1');
% Create subsystem with reactions, parameters and species in the second Double-Phosphorylation subsystem
DP_Subsystem2 = BioSIMI_make_subsystem('DP','in','out','DP_Subsystem2');
% Create subsystem with reactions, parameters and species in Incoherent Feed-Forward Loop subsystem
% Choose species activator protein A ('pA') and repressor protein B ('pB')
% as inputs of IFFL subsystem
IFFL_Subsystem = BioSIMI_make_subsystem('IFFL',{'pA','pB'},'out','IFFL_Subsystem');
% Add all subsystems into vesicle's internal compartment
BioSIMI_add_subsystem(vesicle,'int',DP_Subsystem1);
BioSIMI_add_subsystem(vesicle,'int',DP_Subsystem2);
BioSIMI_add_subsystem(vesicle,'int',IFFL_Subsystem);

% Create final system that consists of input subsystems and output
% subsystem
FinalSystem = BioSIMI_connect(vesicle,'int',{DP_Subsystem1,DP_Subsystem2},IFFL_Subsystem,'FinalSystem');
% Run the simulation of the Final System
SimData = BioSIMI_runsim(FinalSystem);
% Plot the Input/Output relationship in the Final System
BioSIMI_plot(FinalSystem,SimData);
```
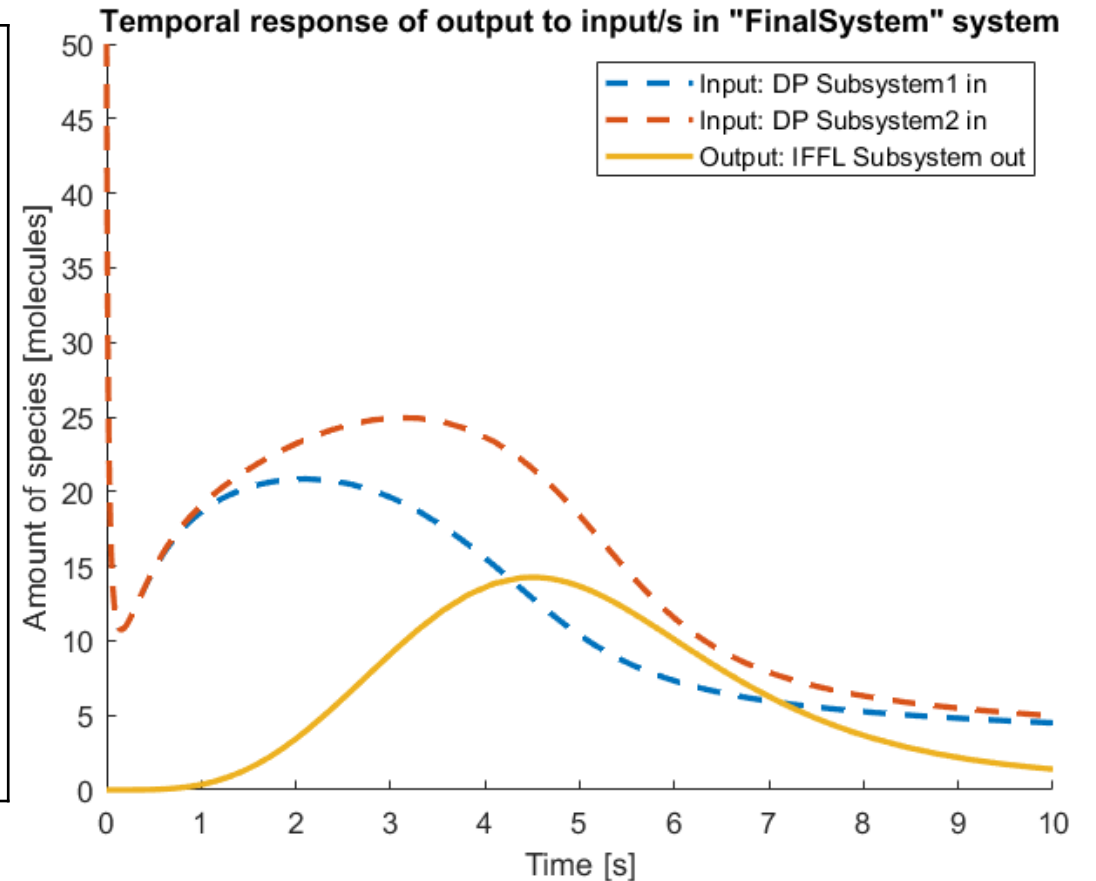

Temporal response of output to input/s in "FinalSystem" system

Contents

Connecting TX-TL Subsystems

- Incoherent Feed-Forward Loop + GFP Expression TX-TL reaction
- An example of the regulatory subsystems interconnection
- Controller: IFFL
- Plant: GFP expression

BioSIMI (Input/Output Modeling of the Connected TX-TL subsystems)

```matlab
%% Create Incoherent Feed-Forward Loop (IFFL)
circuit in TX-TL modeling toolbox
% Set up the standard TXTL tubes
tube1 = txtl_extract('E30VNPRL');
tube2 = txtl_buffer('E30VNPRL');
tube3 =
txtl_newtube('circuit_closed_loop_withClpX');
% Define the DNA strands (defines TX-TL species +
reactions)
txtl_add_dna(tube3, 'p70(50)', 'utr1(20)',
'AraC(600)',0.5*4.5, 'plasmid');
txtl_add_dna(tube3, 'pBAD(50)', 'utr1(20)',
'tetR(600)', 2*4.5, 'plasmid');
txtl_add_dna(tube3,'pBAD_ptet(150)', 'utr1(20)',
'deGFP(1000)-lva(20)',4*4.5, 'plasmid');
txtl_add_dna(tube3,'p70(50)', 'utr1(20)',
'ClpX(600)',0.1*4.5, 'plasmid');
% Add species to the tube 3
txtl_addspecies(tube3, 'arabinose', 10000);
txtl_addspecies(tube3, 'aTc', 1000);
ClpXToAdd = 80; % ClpX are added
% Mix the contents of the individual tubes
% Create Model Object 'IFFL' that can serve as model
object for 'IFFL subsystem'
IFFL = txtl_combine([tube1, tube2, tube3]);

%% Create subsystem for 'IFFL' circuit assembled in
TX-TL above
IFFL_Subsystem =
BioSIMI_make_subsystem(IFFL,'arabinose','protein
deGFP-lva*','IFFL_Subsystem');
```

```matlab
%% Create simple Gene Expression (GenExp) circuit in
TX-TL modeling toolbox
% Set up the standard TXTL tubes
tube1 = txtl_extract('E30VNPRL');
tube2 = txtl_buffer('E30VNPRL');
tube3 = txtl_newtube('gene_expression');
% Define the DNA strands (defines TX-TL species +
reactions)
dna_deGFP = txtl_add_dna(tube3, ...
  'p70(50)', 'utr1(20)', 'deGFP(1000)', ... %
promoter, rbs, gene
  30, ...                    % concentration (nM)
  'plasmid');                % type
% Mix the contents of the individual tubes
% Create Model Object 'GenExp' that can serve as
model object for 'GenExp subsystem'
GenExp = txtl_combine([tube1, tube2, tube3]);

%% Create subsystem for 'GenExp' circuit assembled
in TX-TL above
GenExp_Subsystem =
BioSIMI_make_subsystem(GenExp,'RNAP','protein
deGFP*','GenExp_Subsystem');

%% Create new subsystem 'FinalSystem' that contains
species from connected 'IFFL' and 'GenExp'
subsystems
FinalSystem =
BioSIMI_connect_txtl('FinalSystem','int',IFFL_Subsys
tem,GenExp_Subsystem,'FinalSystem');
% Create new subsystem 'FS' that removes repeated
species and the unused output of 'input subsystem'
% Set up events, parameters, and reactions in the
final subsystem 'FS'
FS = BioSIMI_assemble(FinalSystem,'FS');
% Simulate final subsystem 'FS' with assembled
reactions for (14*60*60) seconds
SimData = BioSIMI_runsim(FS,14*60*60);
% Plot time dependences of input (arabinose) and
output (protein deGFP*) of interconnection of IFFL
and GenExp subsystems
BioSIMI_plot(FS,SimData);
```
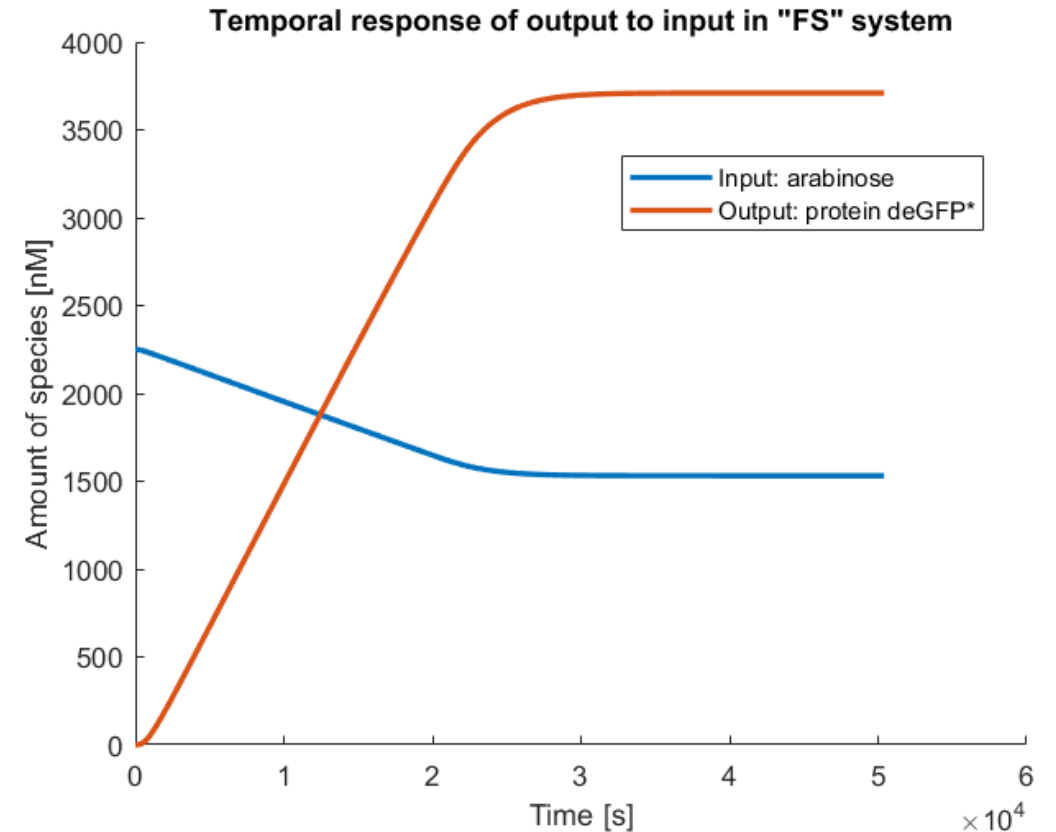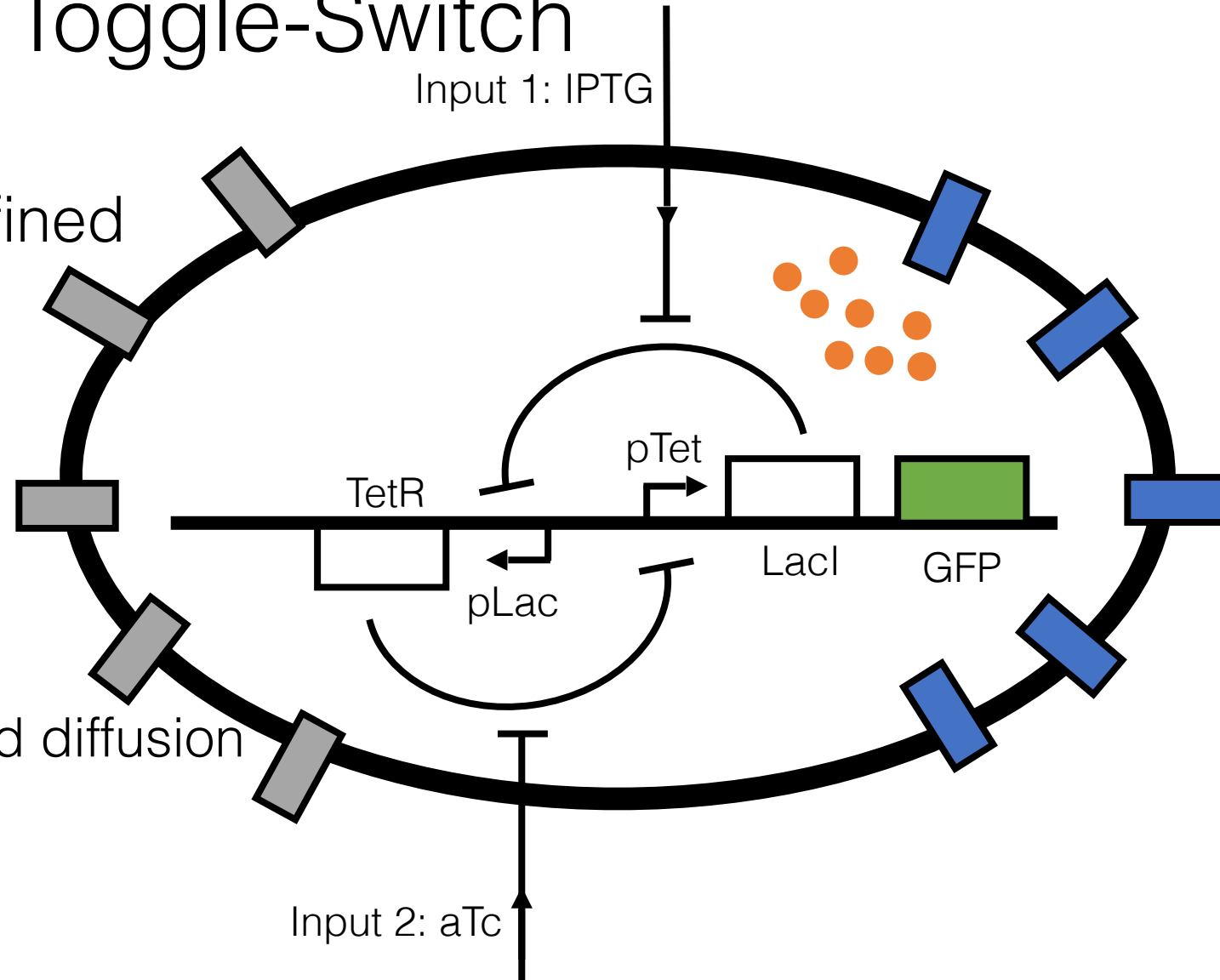

Temporal response of output to input in "FS" system

Legend: Input: arabinose; Output: protein deGFP*

X-axis: Time [s] ×10⁴; Y-axis: Amount of species [nM]

Contents

Input/Output Characteristics of the Liposome-Encapsulated Genetic Toggle-Switch
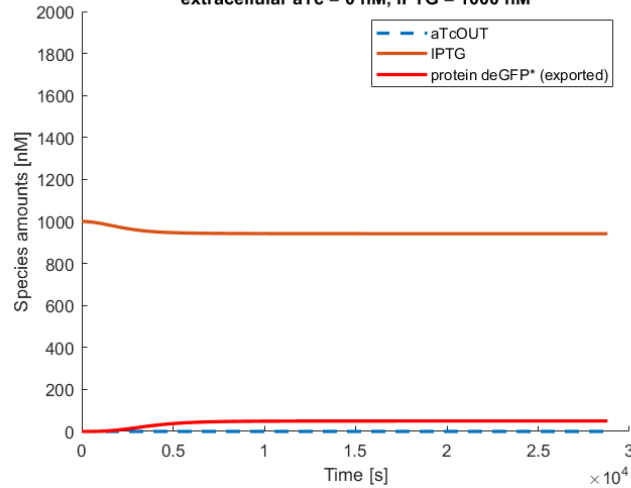
- Interconnection of the user-defined and TX-TL-created subsystems:

  - IPTG initially present ("OFF")
  - Import of aTc through diffusion
  - Sequestration of TetR
  - Increased yield of GFP ("ON")
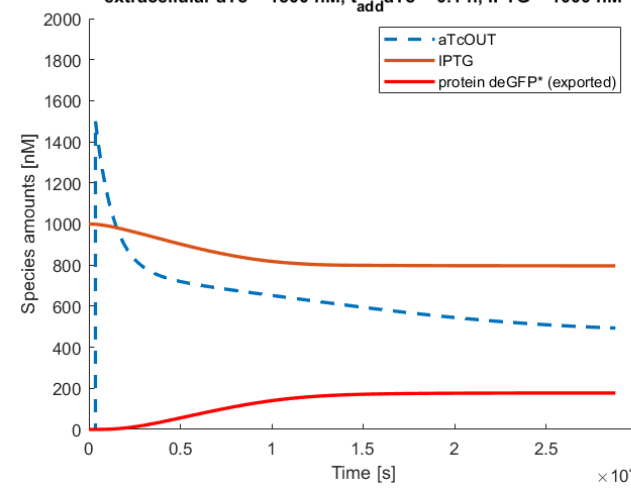 - GFP export by channel-mediated diffusion

Input 1: IPTG

Input 2: aTc

Input/Output Characteristics of the Liposome-Encapsulated Genetic Toggle-Switch

Variation in time of addition of the aTc
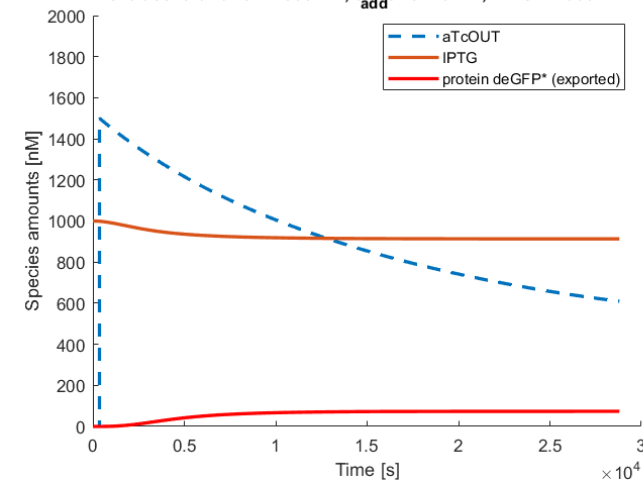
Variation in diffusion rate of the aTc

Contents

Encapsulation of the TX-TL in the Lipid Vesicles

- Liposome vesicles prepared using protocol by Jonsson, Noireaux, Adamala et al. (unpublished)

- Attempt to encapsulate GFP and Luciferase

- Luciferase encapsulation failed, GFP successfully encapsulated

Vesicle with encapsulated GFP
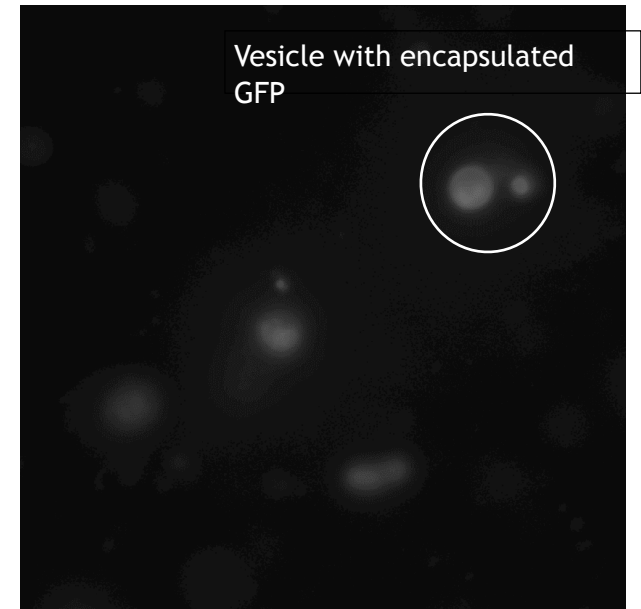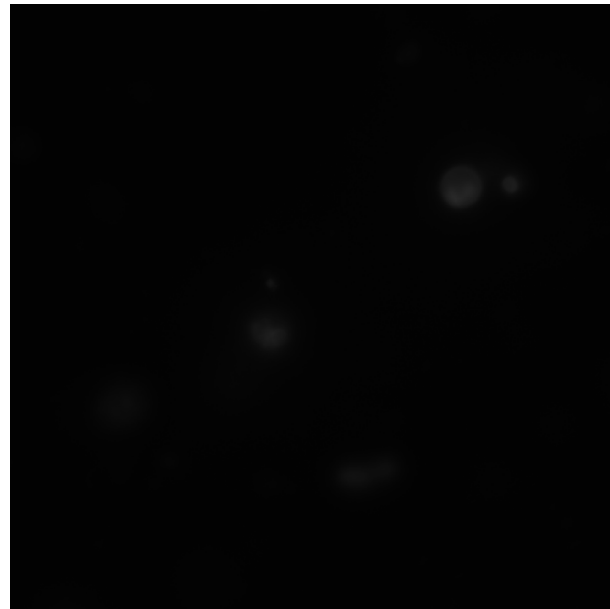
Optimization of the Protocol for the Liposome Preparation

- Variations in the experimental protocol were applied:

    - Decrease in centrifuging speed (20 000g to 5000g)

    - Change in method of the lipid vesicle extraction

      (bottom vs. top)

    - Number of washes by the buffer (1 wash vs. 3 washes)

- Extraction from the bottom of the test tube + multiple washing = no vesicles observed

- For extraction from the bottom, *is single wash optimal*?

Contents

Conclusion

- Preliminary development of the computational framework **BioSIMI** for the modeling of the Input/Output behavior of the biomolecular subsystems interconnection:

  - Development of the several user-defined subsystems

    (transport, signaling,   regulatory)

  - Compatibility with existing TX-TL modeling toolbox

  - Multiple Input/Single Output models

  - Fast, user-friendly modeling of the subsystems

  - Demonstration of several use-cases (multiple subsystems, TX-TL, toggle switch)

- Experimental investigation of liposome formation

  - Contribution to optimization of the experimental protocol

  - Further work required for the robust liposome formation process

Further plans

- Use of the parameter identification to optimize Input/Output characteristics
- Modeling of the sensor-reporter system with genetic toggle switch (orthogonal communication?, concentration sensor?)
- Experimental investigation of the sensor-reporter system?
- Finalization of BioSIMI development (Multiple Inputs-Multiple Outputs?)
- Simulation of the large population of vesicles
- Response to the stochasticity in the input
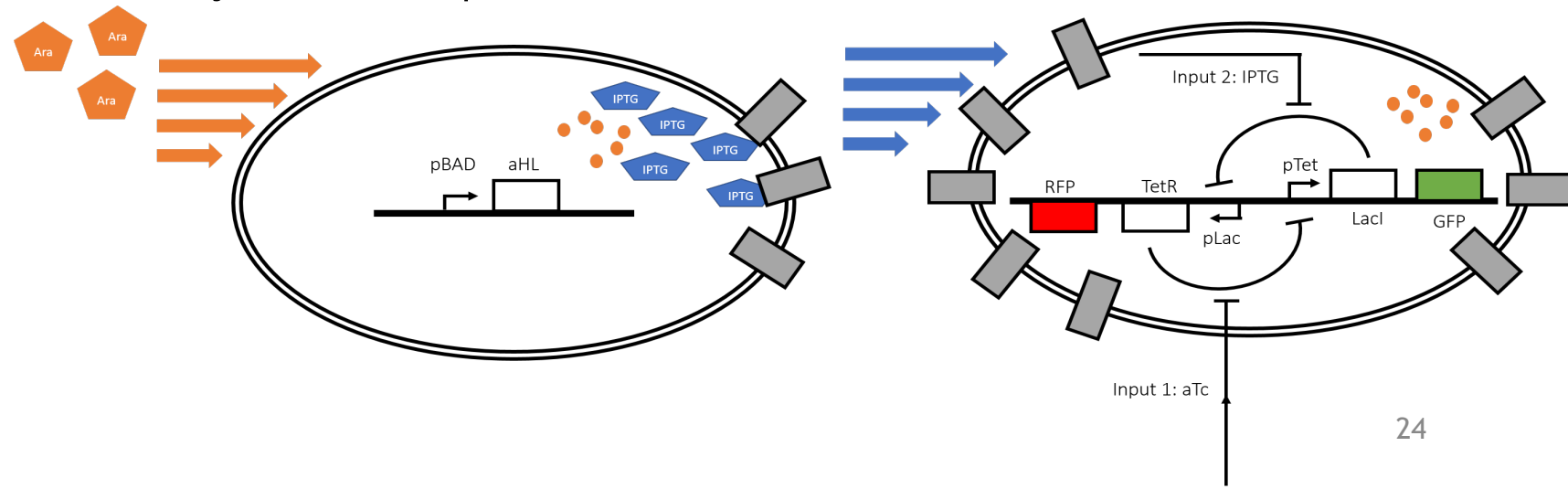
Acknowledgements

- Professor Richard Murray – big-picture insights and challenging suggestions!
- Vipul Singhal – great hands-on and hands-off mentoring and keeping me up!
- Shaobin Guo – patience with my lab efforts and answers to all my lab-related questions!
- Miki Yun – teaching me PCR, TX-TL, proper pipetting and other crucial life skills!

… And thanks to all the members of the Murray Lab!