Models that include mechanisms or variables artificially induced in a biological experiment

On differences between experimental and real-life models

Models that describe behavior of a natural biological system

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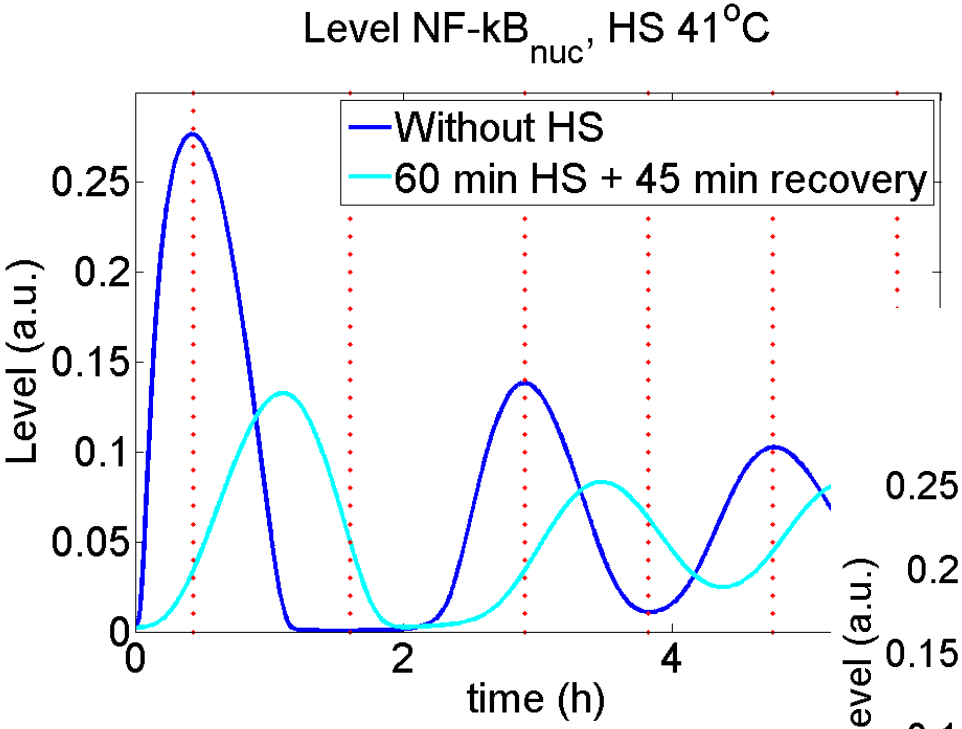
Modeling of intracellular processes

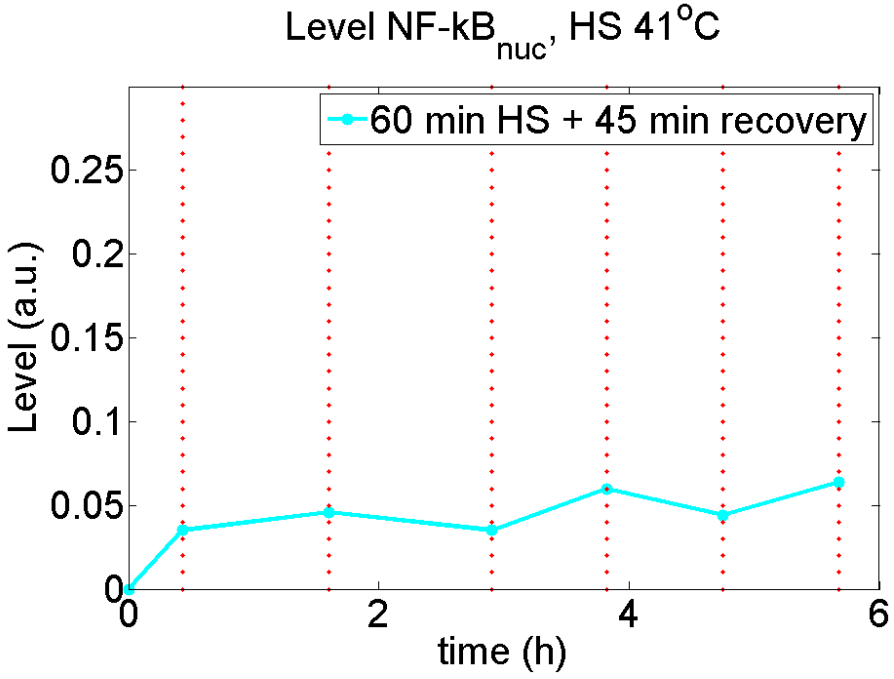
- Goals:
  - To understand control mechanisms
  - Once the mechanisms are known, to control dynamics of cell poplations/tissues

Modeling of intracellular processes

- General procedure:
  - Preliminary biological experiments
  - Model development and parameter estimation
  - Model analysis (properties, robustness/sensitivity, perturbations) – new findings/conclusions
  - Experimental confirmation of the above
- But
  - Different cells respond differently to the same type of stimulus
  - The dynamics may change qualitatively when the same pathway is activated by various stimuli
  - Experiental procedures may in some cases disturb the picture

Problem 1 – choice of time points for measurements





Partial conclusion:

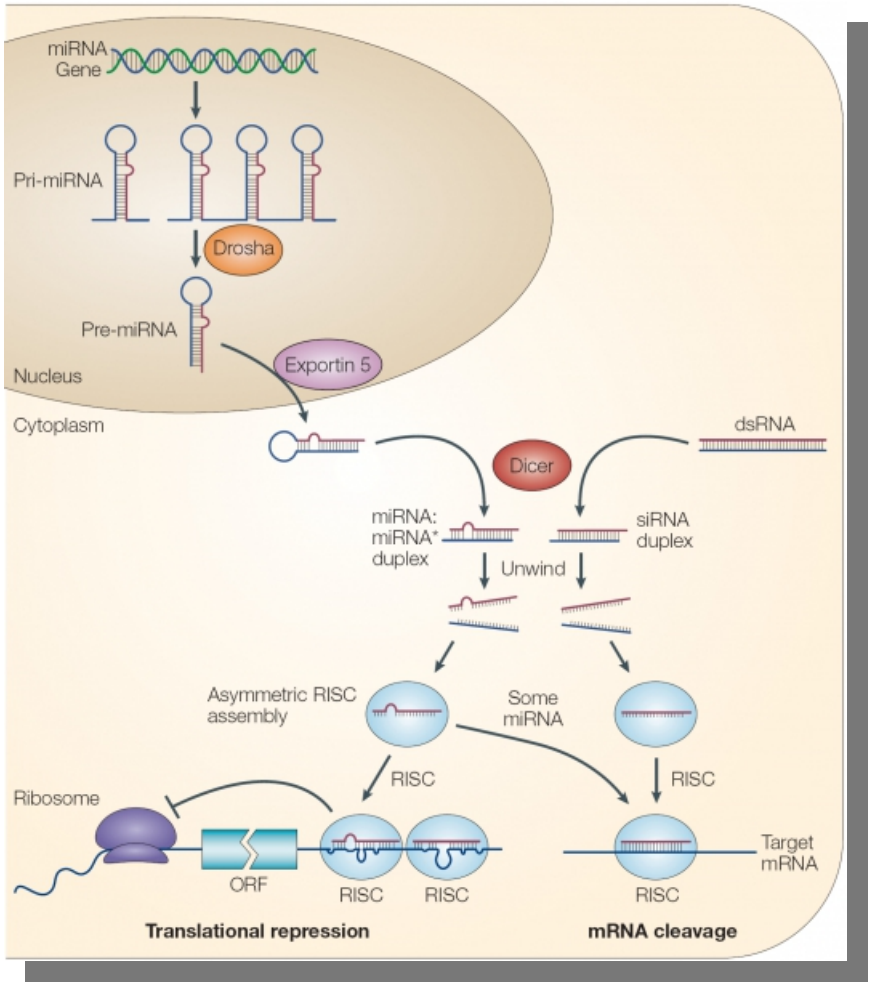
- preliminary modeling part of research may be quite useful

Problem 2 – direct effects of experimental procedures

Transfection experiments

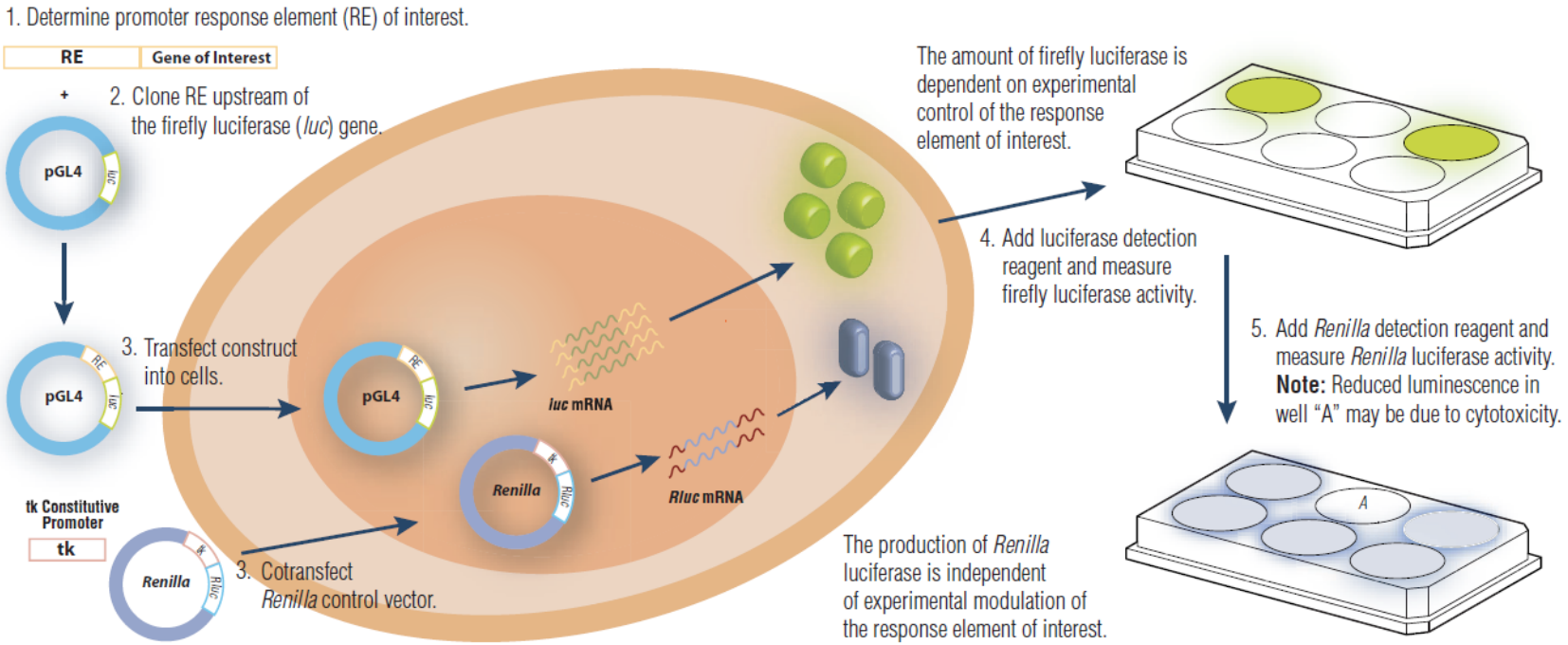
- Consist in introduction of so called reporter plasmids into cells
- Have been shown to heavily influence experimental results
  - no follow-up research results have been published so far (?)
  - Nevertheless, are quite widely used in investigation of control mechanisms governing cell responses to external stimuli

miRNA function and mechanism of action



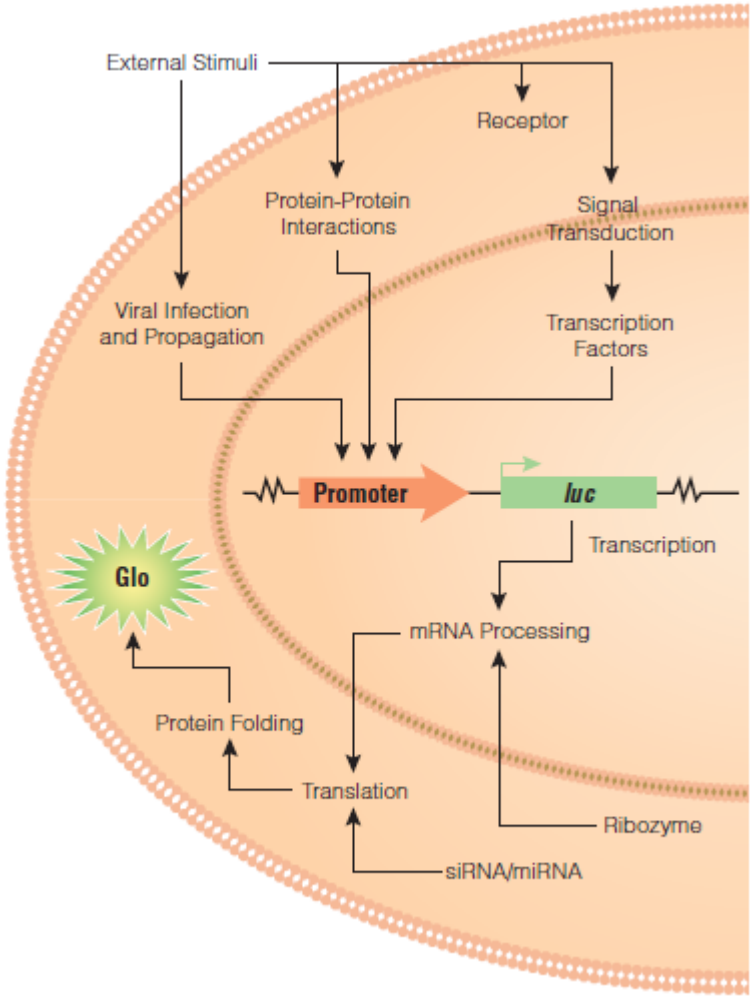
He, L.,Hannon,G.J., MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004;5(7):522-31.PMID: 15211354

Experimental procedure



S. T.M. Allard, K. Kopish, Promega Corporation, Luciferase Reporter Assays: Powerful, Adaptable Tools For Cell Biology Research, CELL NOTES; 2008; 21; 23-26

Experimental procedure



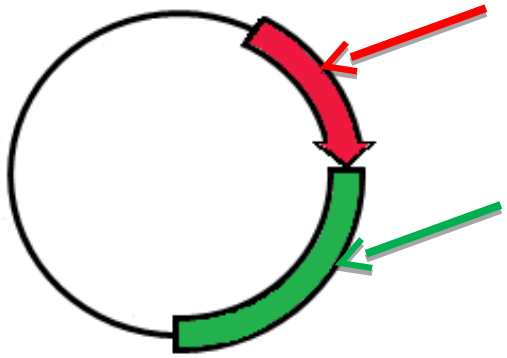
S. T.M. Allard, K. Kopish, Promega Corporation, Luciferase Reporter Assays: Powerful, Adaptable Tools For Cell Biology Research, CELL NOTES; 2008; 21; 23-26

Motivation

- In many cases experimental research on miRNA is based on plasmid transfection
- It has been shown that transfection experiments (in other areas) may result in misleading conclusions
- Mathematical models of miRNA activity do not take into account the nature of experiments whose results the model output should reflect
- Transfection efficiency is not high – what are the implications?

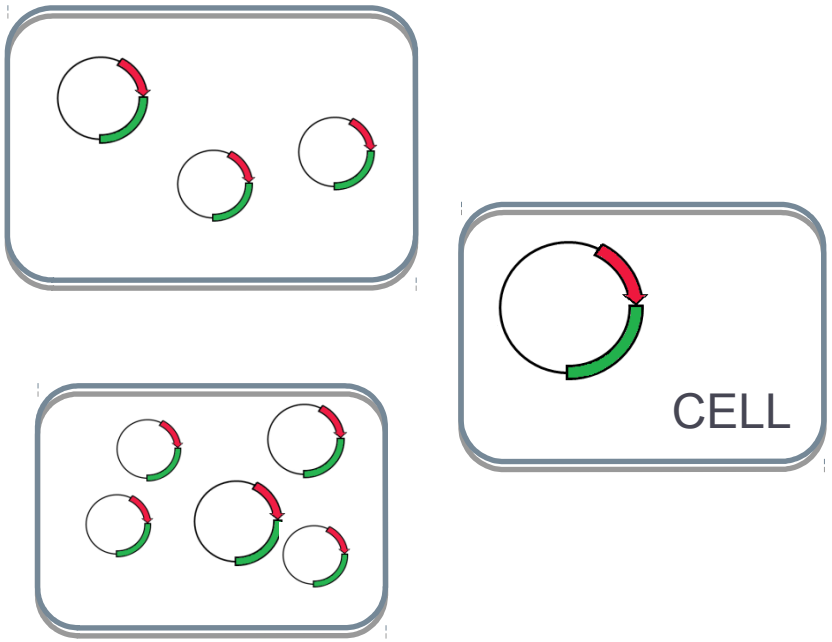
Experimental procedure

- Plasmids used in our experiments:
  - contain specific Response Elements (Binding Sites – BS), which are the same as in the target gene of interest
  - code for mRNA which is fully complementary to the miRNA of interest



Experimental procedure

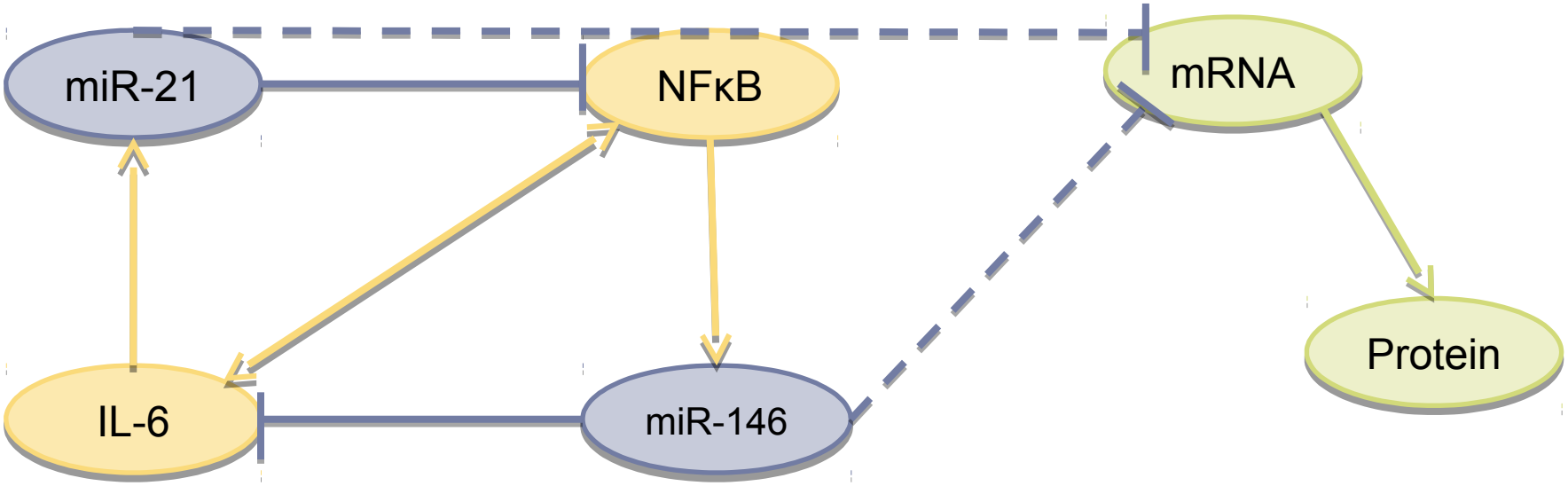
- Cells in a population are transfected with variable number of plasmid copies



Questions to be answered

1. What are the direct (unintended) effects of plasmid introduction on intracellular processes?
2. What are the differences among cells, with respect to the number of plasmids that actually entered them?
3. Can we base the analysis on average results (as given, e.g. by RT-PCR), without taking into account heterogeneity of cell populations?
4. Can we draw conclusions about wild type cells behavior basing on experimental results from transfected cells?

A simple model



Xu Xue, Wang Xi, Hu Wenzhong, A modeled dynamic regulatory network of NF-kappaB and IL-6 mediated by miRNA, 2013

Models to be compared

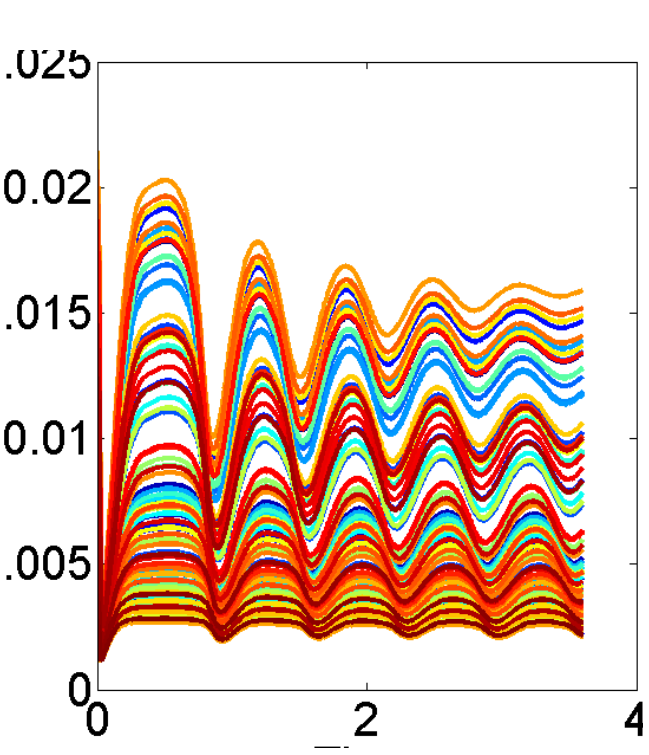
Models describing the dynamics of a signaling pathway under consideration

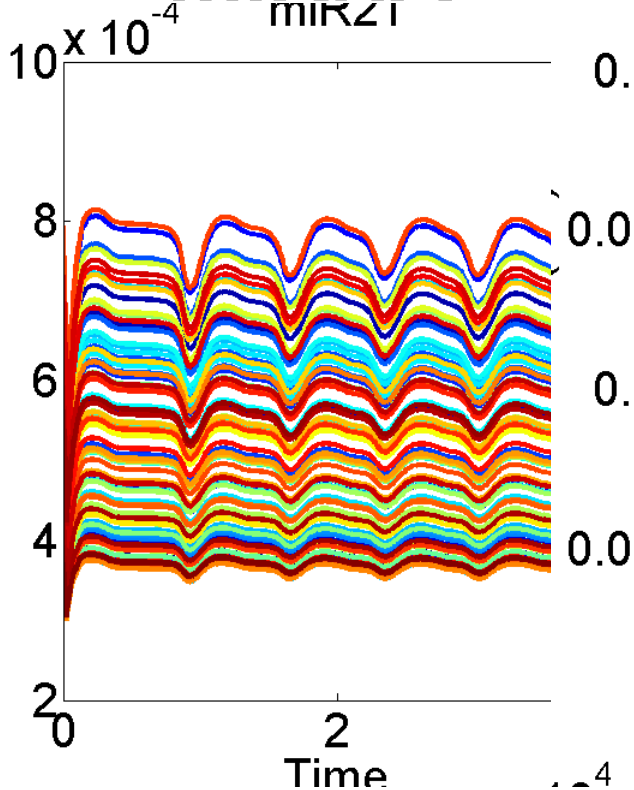
- Describing only the variables and processes that are subject of the analysis (labeled ***„predicted”*** in subsequent plots)
- With additional terms and variables that correspond to molecules introduced in the transfection experiment and their actions
    - After fitting model parameters to match simulation and experimental data, the plasmid concentration is set to 0 (labeled ***„real”*** in subsequent plots)

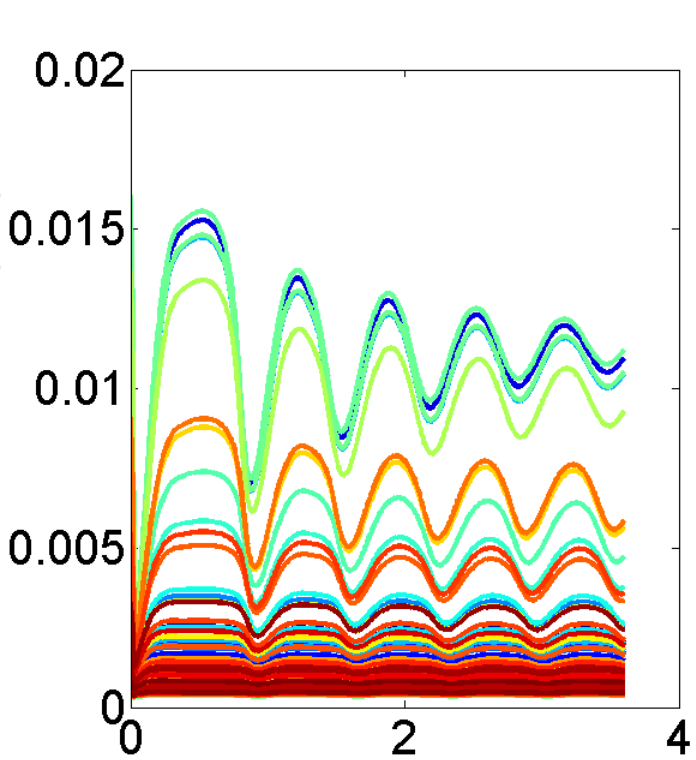
Computational procedure

- Randomize transfection efficiency
  - Generate random numbers that represent numbers of plasmids that got into each cell
    - High transfection efficiency
    - Low transfection efficiency
    - Variable transfection efficiency
- For each cell simulate its behavior

miRNA





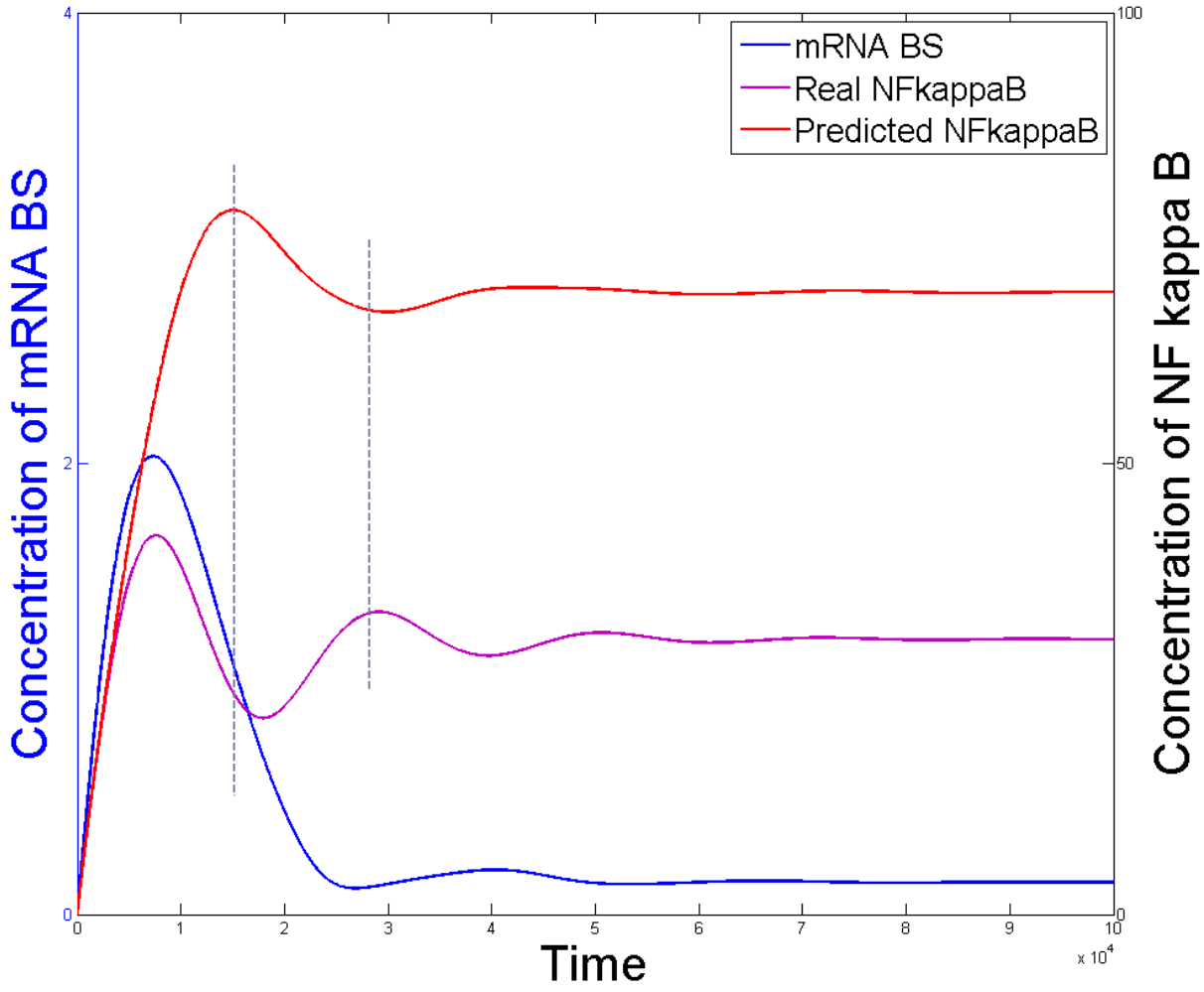


Low transfection efficiency

High transfection efficiency

Variable transfection efficiency

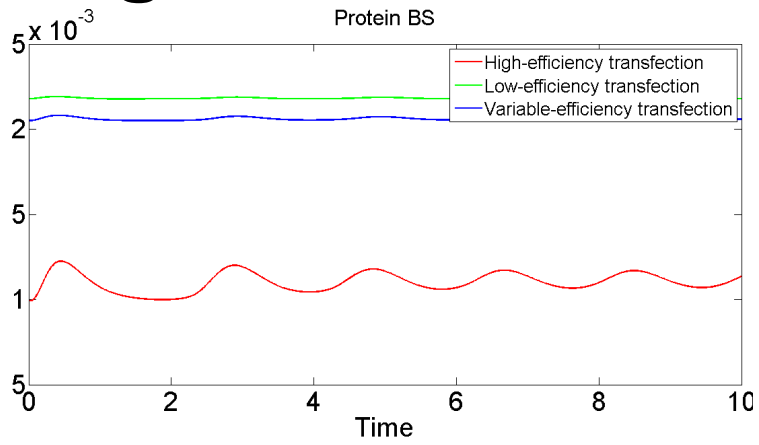
mRNA levels


mRNA BS
Real NFkappaB
Predicted NFkappaB
Concentration of mRNA BS
Concentration of NF kappa B
Time
x 10^4
0
1
2
3
4
5
6
7
8
9
10
0
2
4
50
100

What are we looking at?

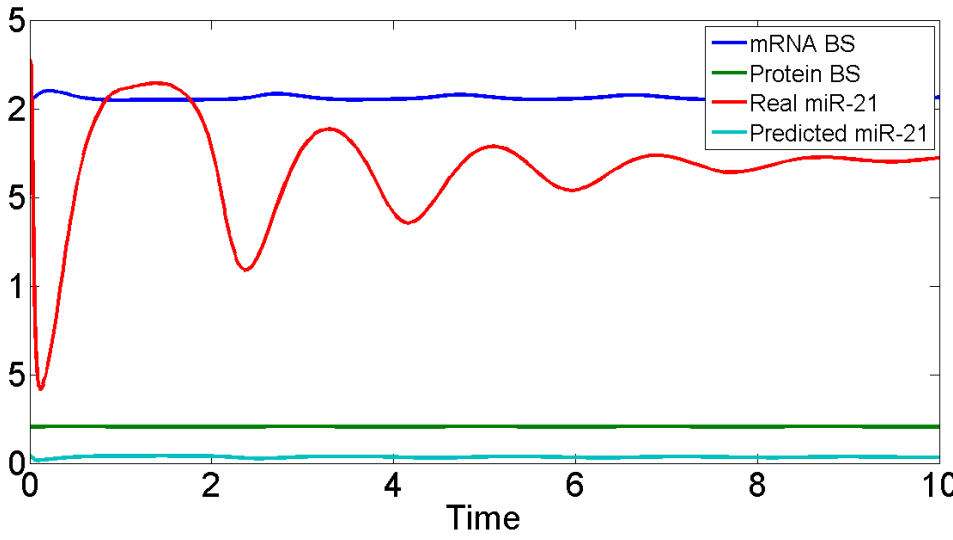
Data available:

- Protein coded by plasmid (waiting for it)
- Data from expriments without plasmid



The goal is to investigate

- miRNA control actions exerted over NFkB pathway
- changes in specific miRNA concentration measured indirectly with the transfected reporter system



Conclusions

- Caution required when drawing conclusions from plasmid-based experiments
- Models whose dynamics is fitted to results of experiments performed with transfected cells should include additional terms and variables that correspond to molecules introduced in the transfection experiment
- Transfection efficiency influence qualitative system behavior

Acknowledgements

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