Application of Pharmacokinetic/Pharmacodynamic Concepts to Modelling in Gene Therapy

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Summary

- Gene therapy
  - Definition/Objectives
  - Concept of drug
  - Data available
- Example

Gene Therapy

- Definition/Objectives
  - The delivery of a gene or genetic information into cells for the purpose of achieving a therapeutic effect

- Similarities/Differences with respect to traditional therapy

Concept of Drug

Traditional Therapy

- Formulation
- Pro-drugs
- Active molecule(s)

Gene Therapy

- Formulation
- Promoter
  - DNA sequence located upstream of a gene, and which function is the regulation of this gene expression
- Genes

Data available

Traditional Therapy

- Dose
- Route
- Measure of exposure
- Response
  - Transient response

Gene Therapy

- Dose
- Route
- -
- Response
  - Maintained response

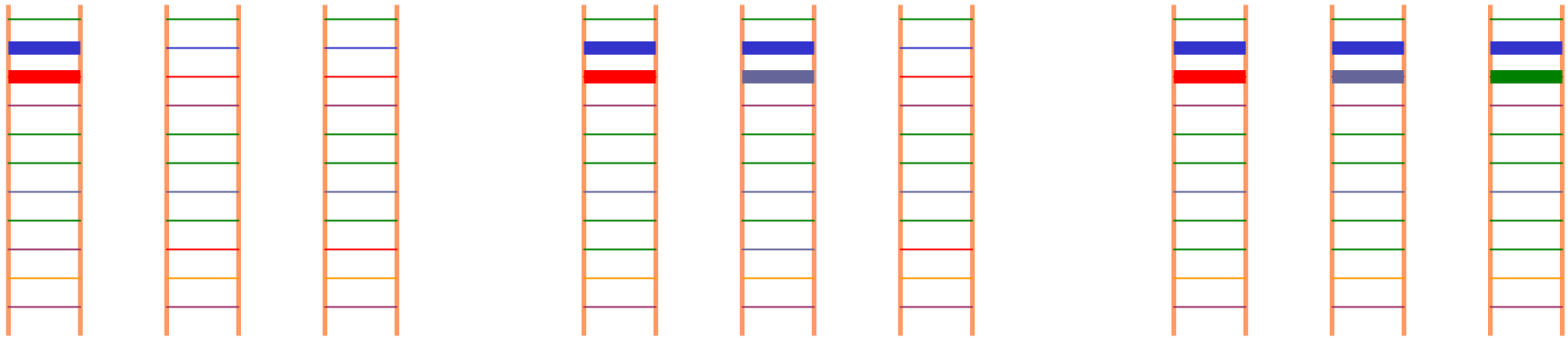
- Novel (but upcoming) field in Gene therapy
  - 8th annual meeting of the American Society of Gene Therapy; 1-5 June; S. Luis (MO). USA
- Even in the PK/PD area
  - Few studies have explored/modelled the time course of gene expression (Jusko's group)
    - The drug was a drug, not a gene

Example (pre-clinical experiment)

- Background
  - Treatment of hepatitis B requires over six months administration with interferon α (IFN-α)
- Aim (general)
  - To promote the expression of INF-α over a long period
- Difficulties/opportunities
  - To find a promoter (PR) insensitive to the INF-α already expressed
- Aim (specific)
  - To explore the effect INF-α at the PR level using **luciferase (Luc) activity** as an indirect measure of PR functionality

Study design

Group I **Group II** **Group III**



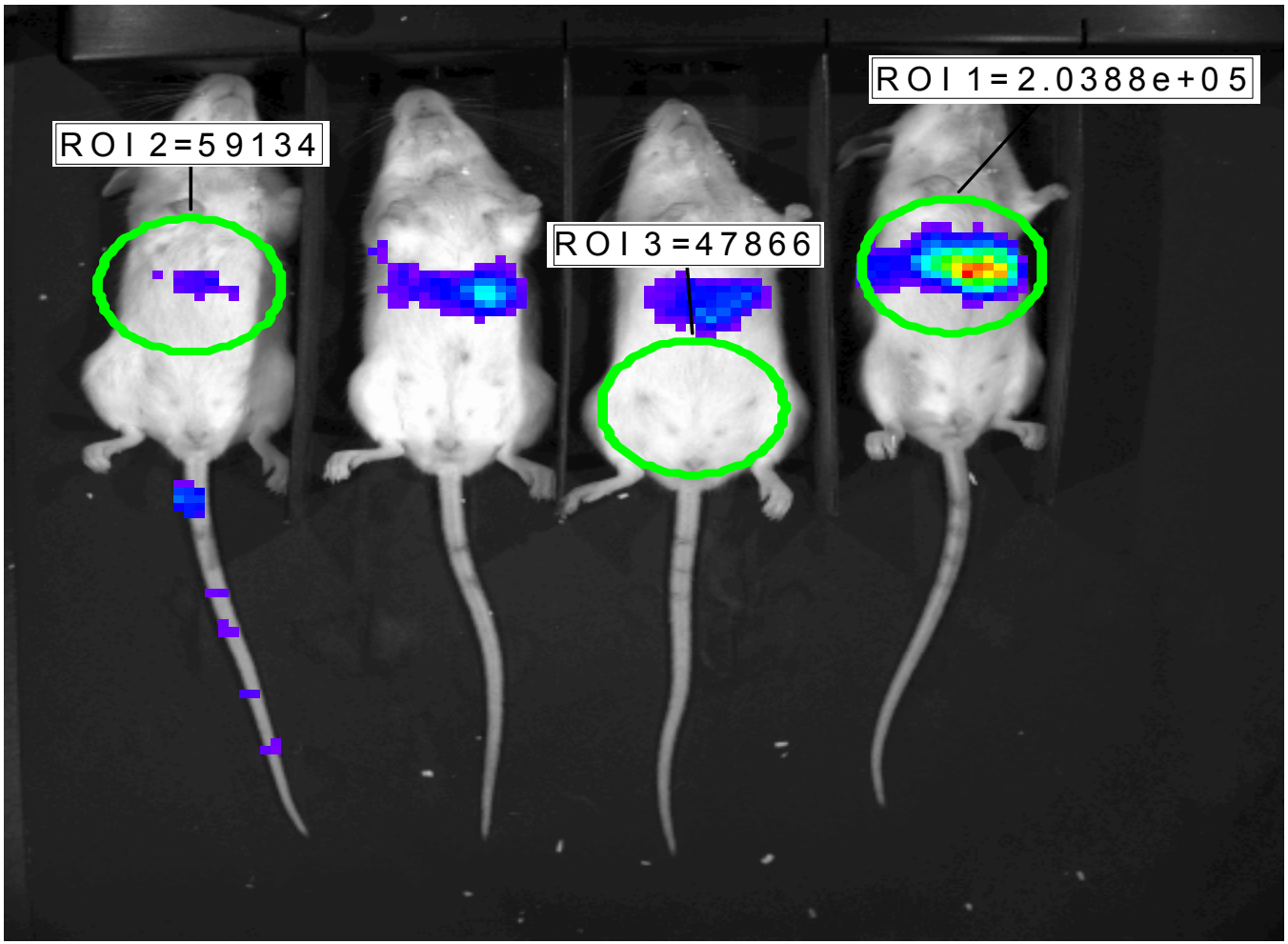
Promoter (elongation factor 1α, EF1α)
Gene expressing Luc
Gene expressing IFN-α
Gene expressing β-galactosidase (lacZ)

- Same “naked” DNA load (10 μg/kg)
- Production of DNA in E.coli
- Purification in absence of endotoxins

Plasmid “drug” Administration

- Hydrodynamic injection
  - Tail vein
  - Rapid injection (7 sec)
  - Large volume of plasmid DNA solution (1.8 ml/20 gr)
- Consequences
  - Bad
    - Transient irregularity of heart function, sharp increase in venous pressure
  - Good
    - Enhancement of membrane permeability of the hepatocytes

Data adquisition



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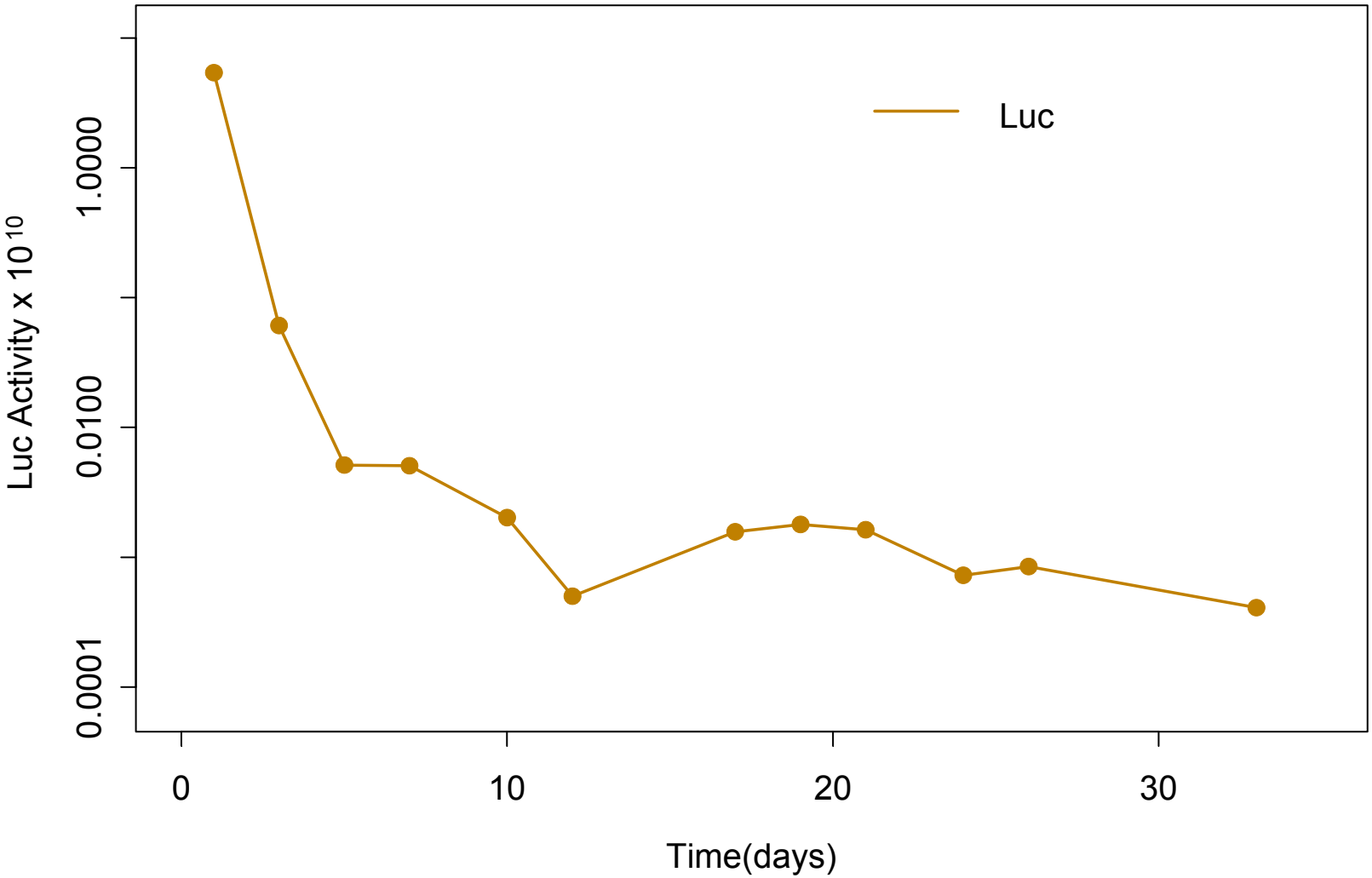
Raw data



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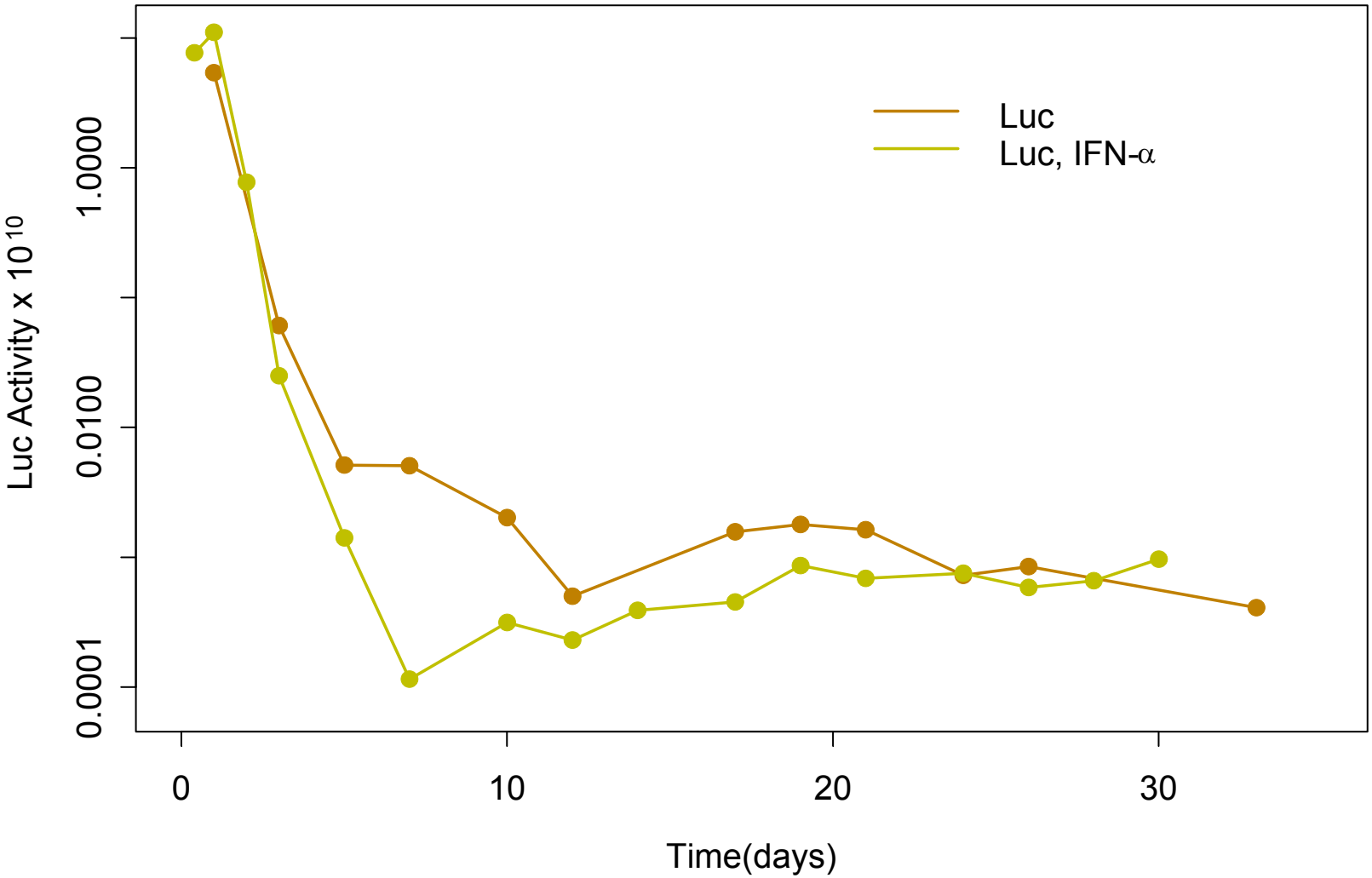
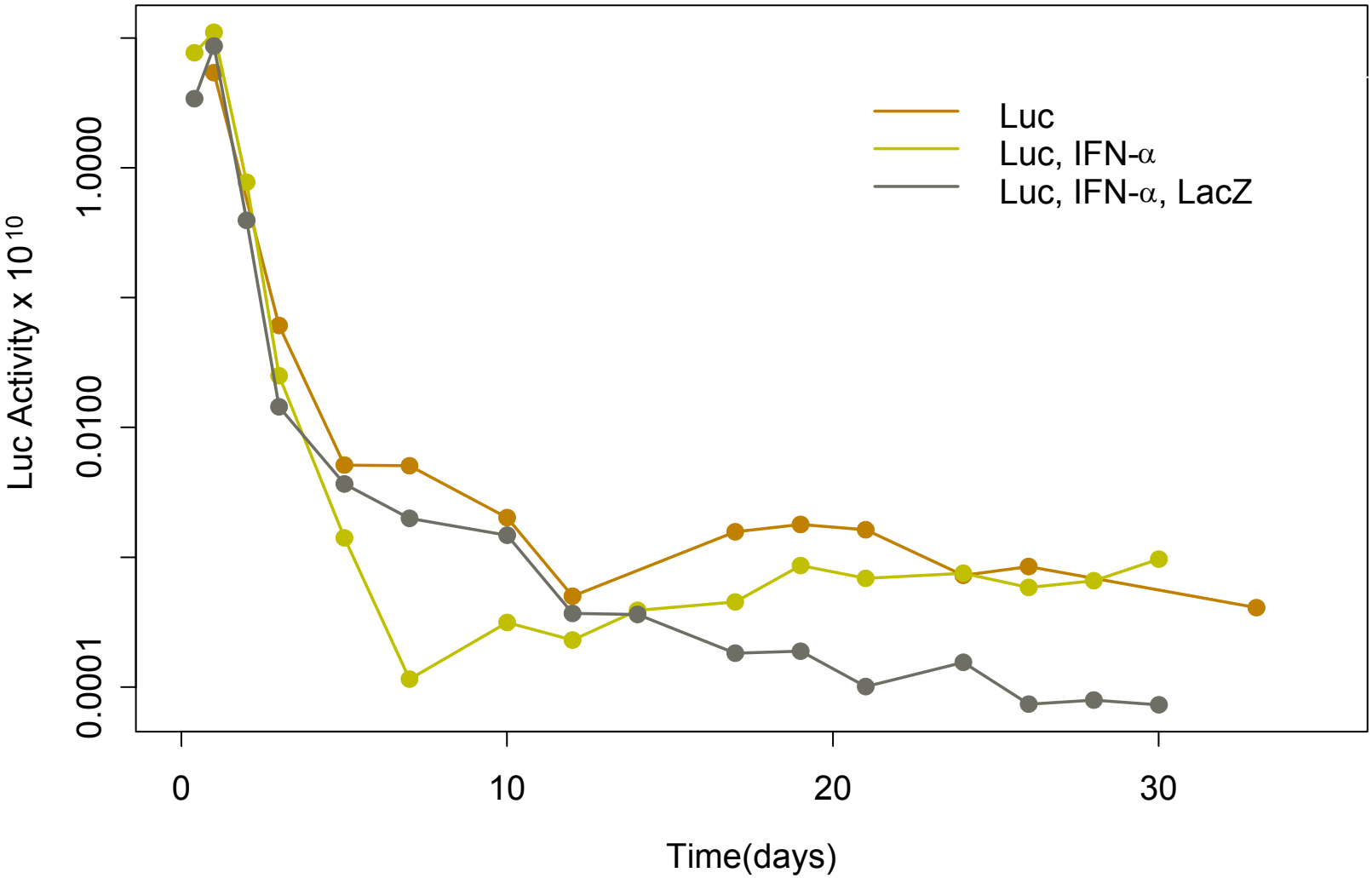
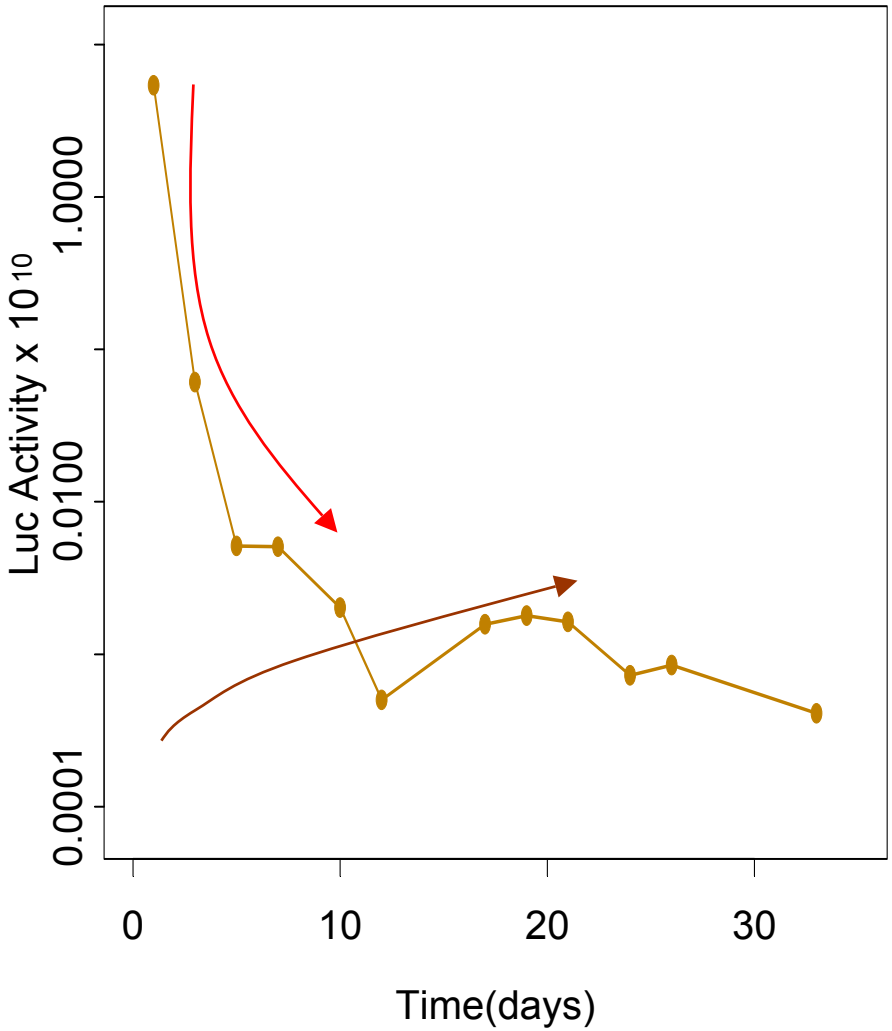

Luc
Luc, IFN-α
Luc Activity x 10^10
1.0000
0.0100
0.0001
0
10
20
30
Time(days)

Raw data


Luc
Luc, IFN-α
Luc, IFN-α, LacZ
Luc Activity x 10^10
1.0000
0.0100
0.0001
0
10
20
30
Time(days)

Data Interpretation



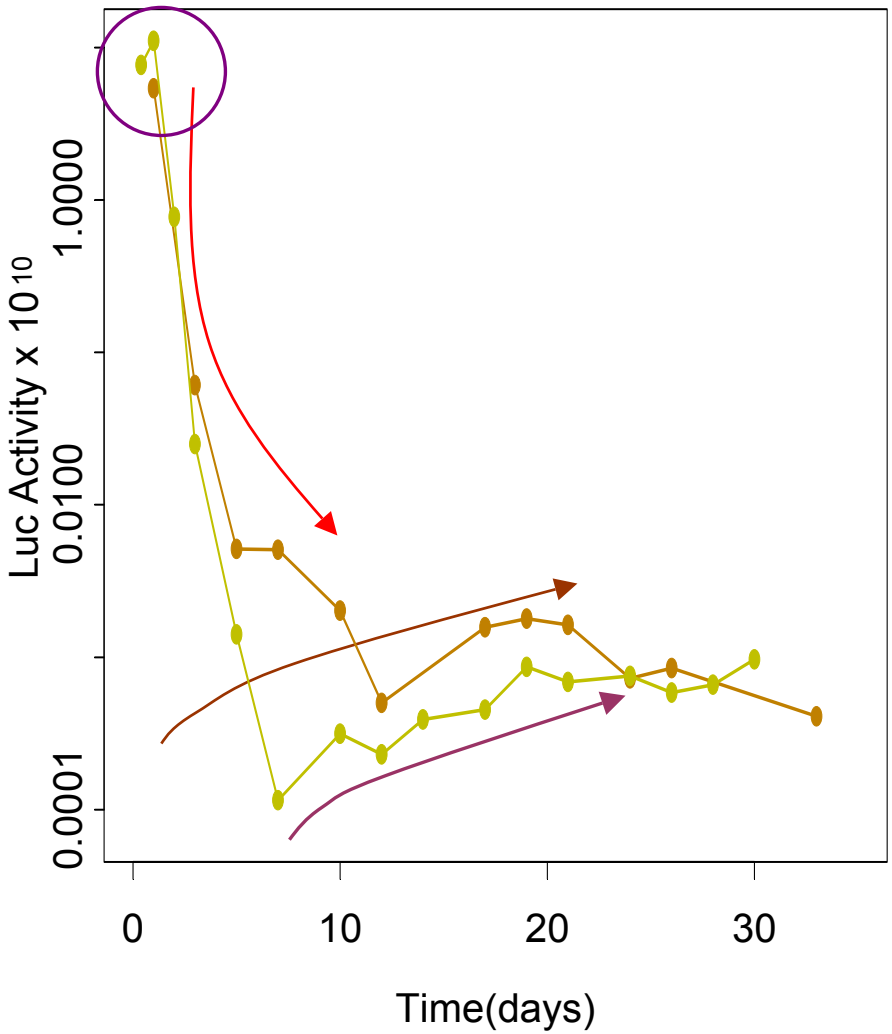
PK/PD modeller

Initial burst (fast expression)

Rapid turn-over

Slower expression

Data Interpretation



PK/PD modeller

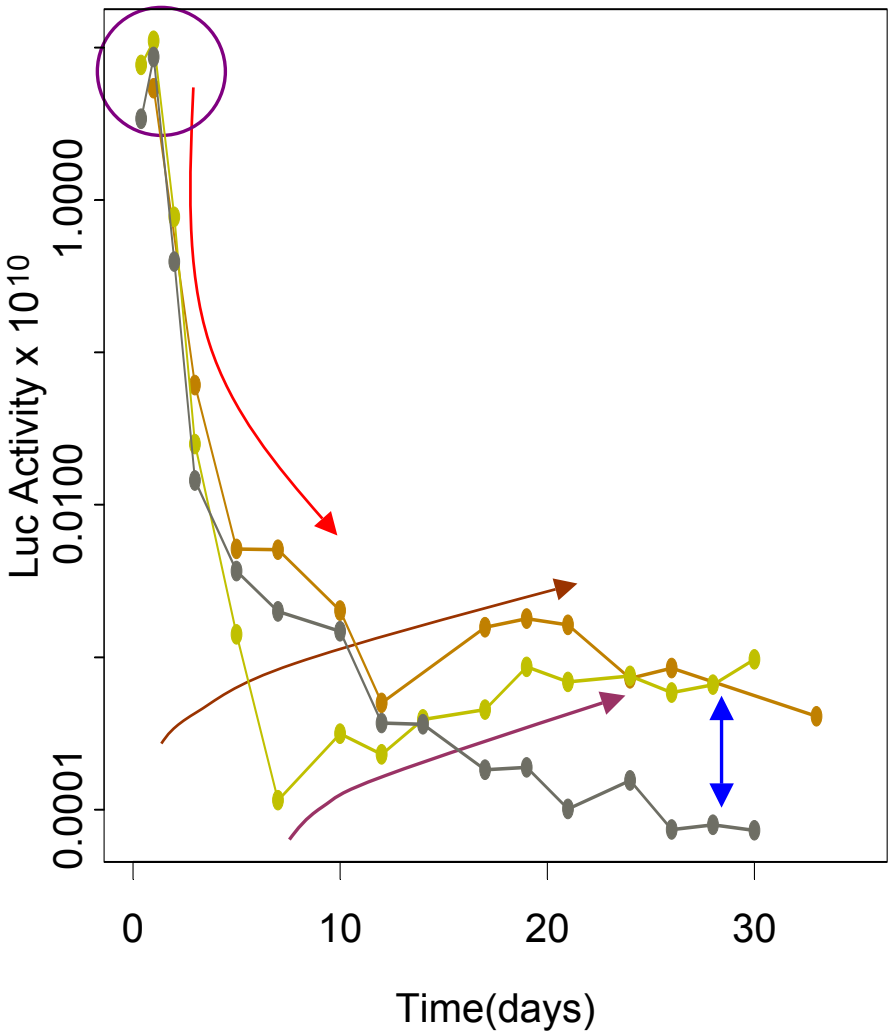
Initial burst (fast expression)

- Rapid turn-over
- No effect of IFN-α

Slower expression

- Reversibly antagonised by INF-α

Data Interpretation



PK/PD modeller

Initial burst (fast expression)

Rapid turn-over

No effect of IFN-α ,and LacZ

Slower expression

Reversibly antagonised by INF-α

Irreversibly antagonised by LacZ

Data Interpretation

PK/PD modeller

Initial burst (fast expression)

Rapid turn-over

No effect of IFN-α ,and LacZ

Slower expression

Reversibly antagonised by INF-α

Irreversibly antagonised by LacZ

Gene therapy scientist

Free integrated DNA expresses Luc rapidly

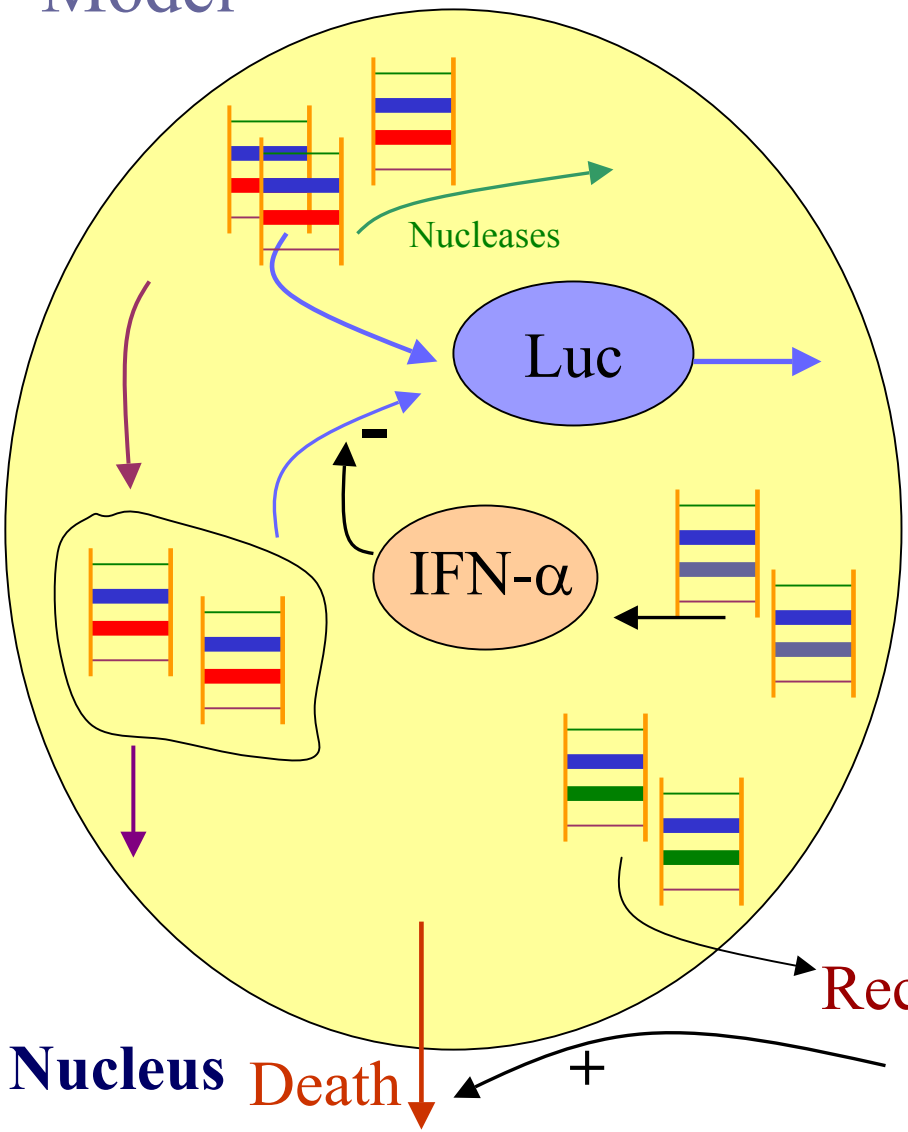
- Quickly eliminated by nucleases

Fraction of the integrated DNA binds to structures

- Insensitive to nucleases
- Expression of IFN-α inhibits promoter activity
- Expression of LACz induces cellular response

Data Interpretation

Model



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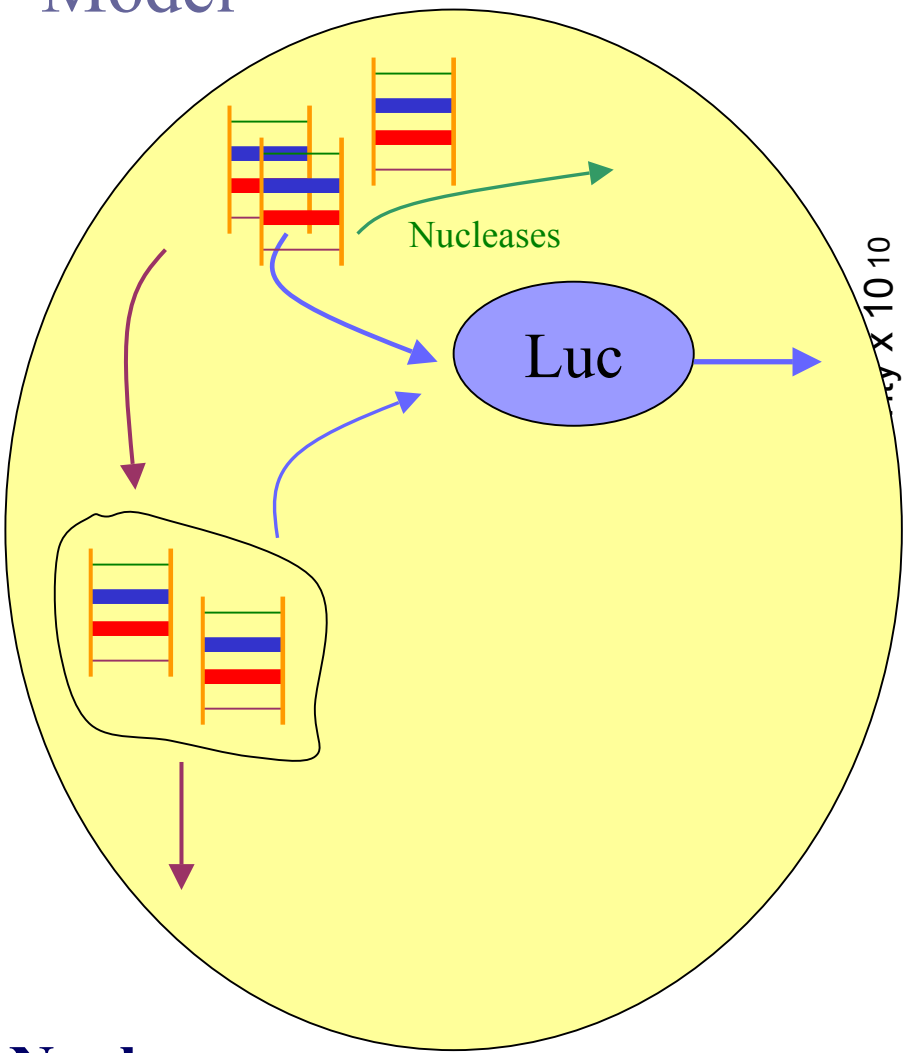
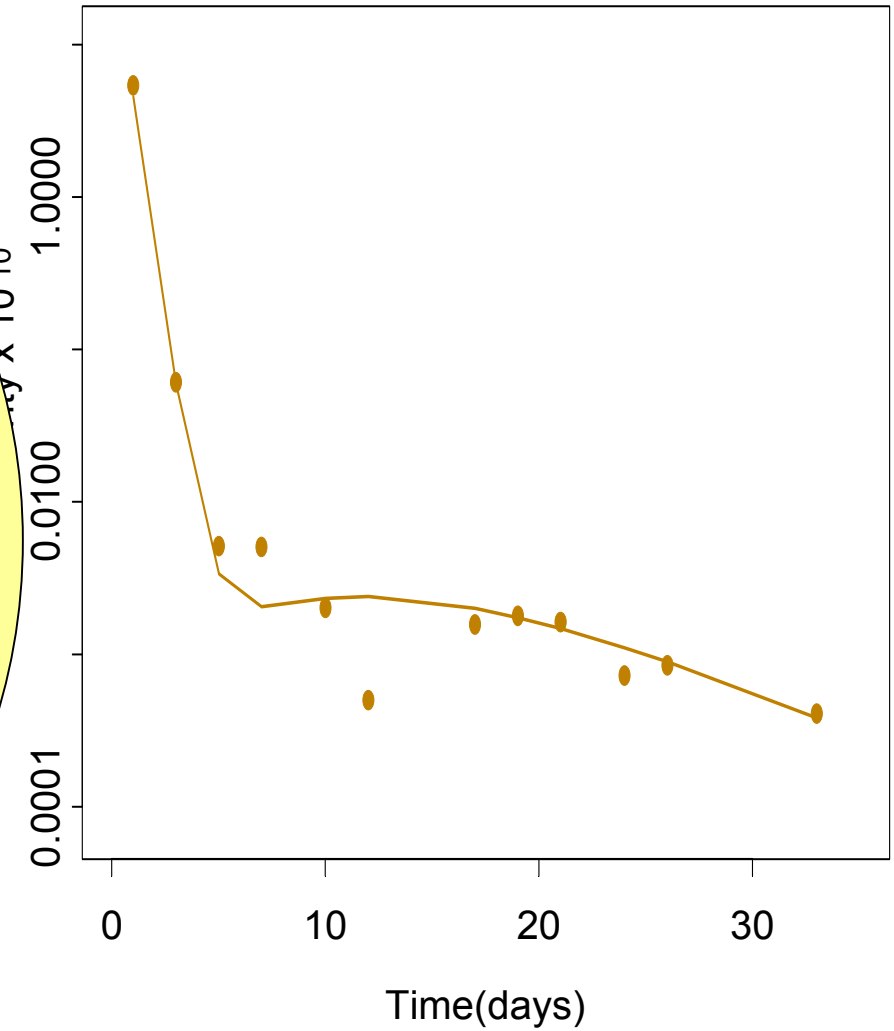
Results

Model

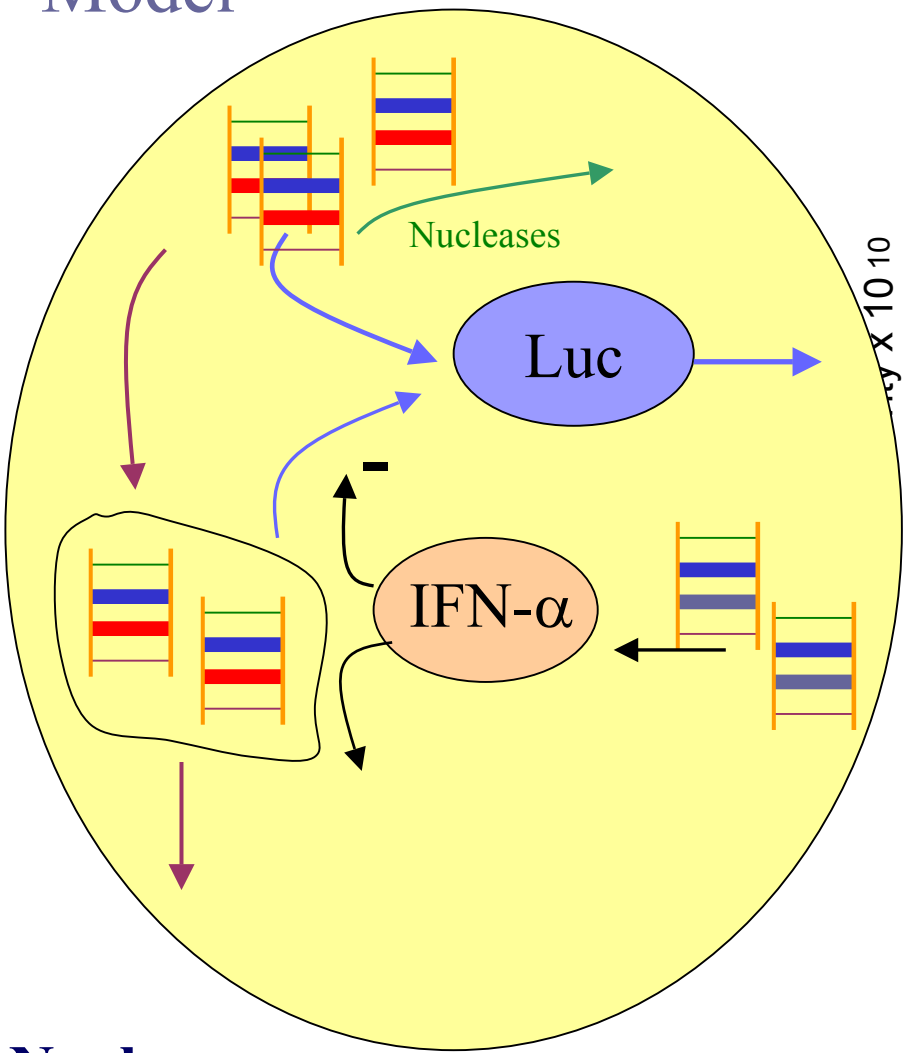


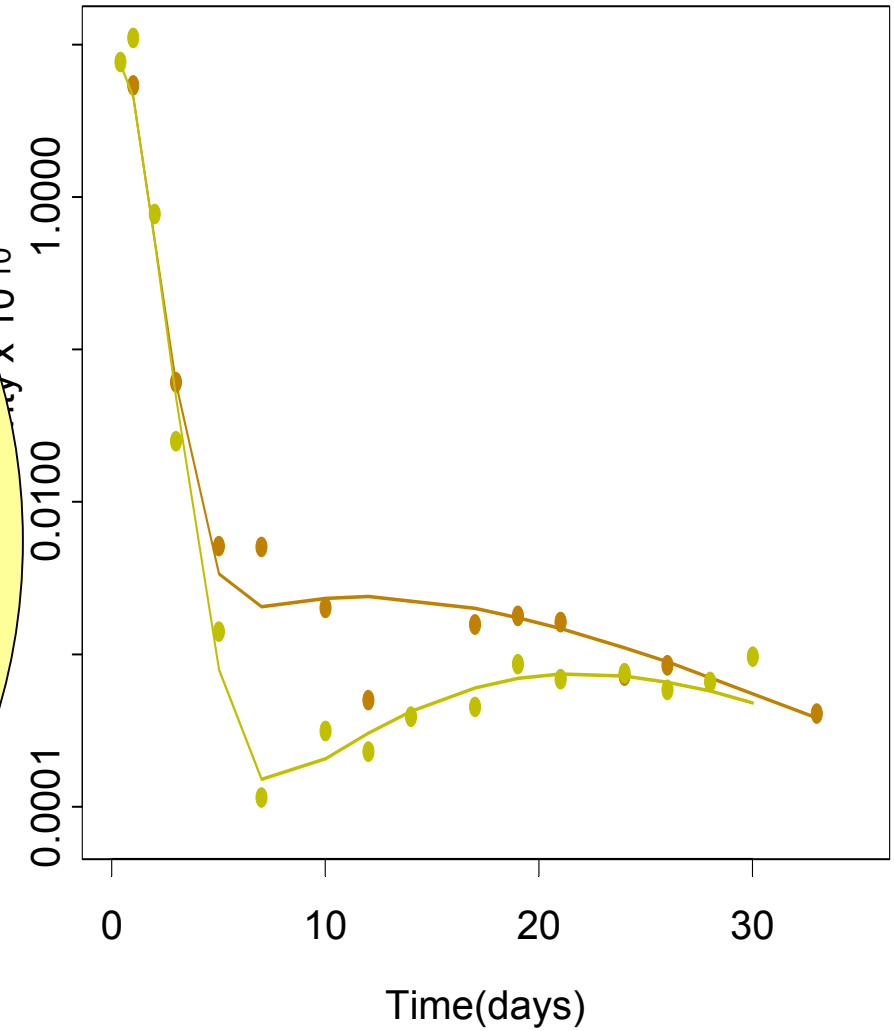
Nucleus



Results

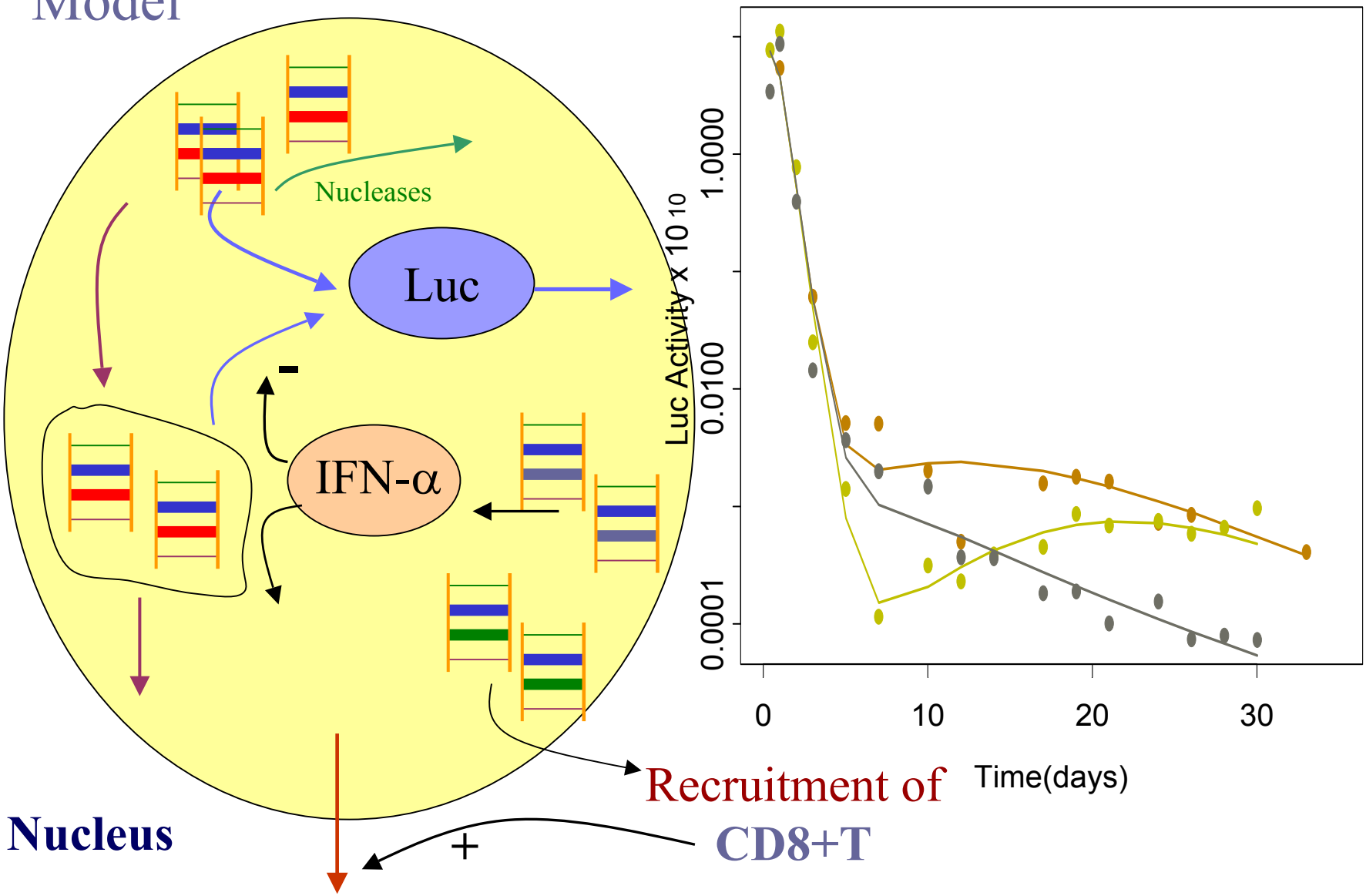
Model





Results

Model



Results

- Modelling allowed to quantitatively characterise
  - Nucleases degradation activity
  - Dynamics of gene expression
  - Time course of IFN-α expression and the magnitude of the competitive interaction
  - Time course of the LacZ induced cellular response and its immunogenic capacity

Final Considerations

- Same applications
  - Discrimination between system, “drug”, and design parameters
- Feed-back
  - Big amount of in vitro and in vivo information
  - Model-based oriented study designs
- Dynamic system analysis