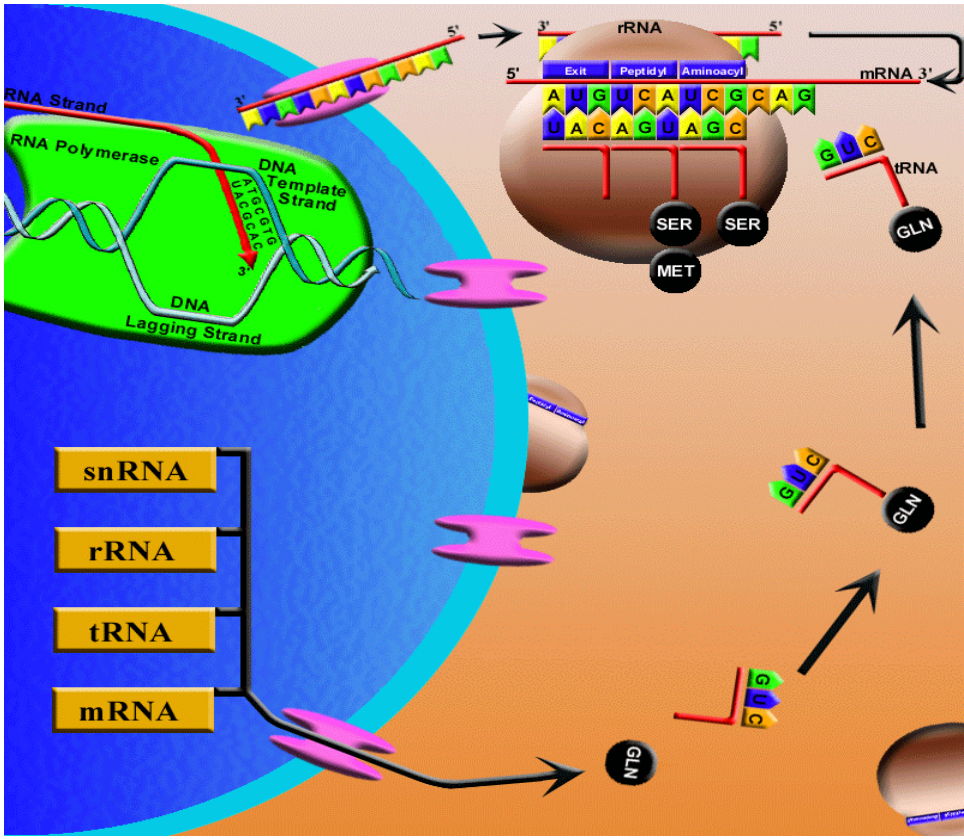

IPRO 302: Synthetic Biology
Engineering a novel organism

Nature's Engineering

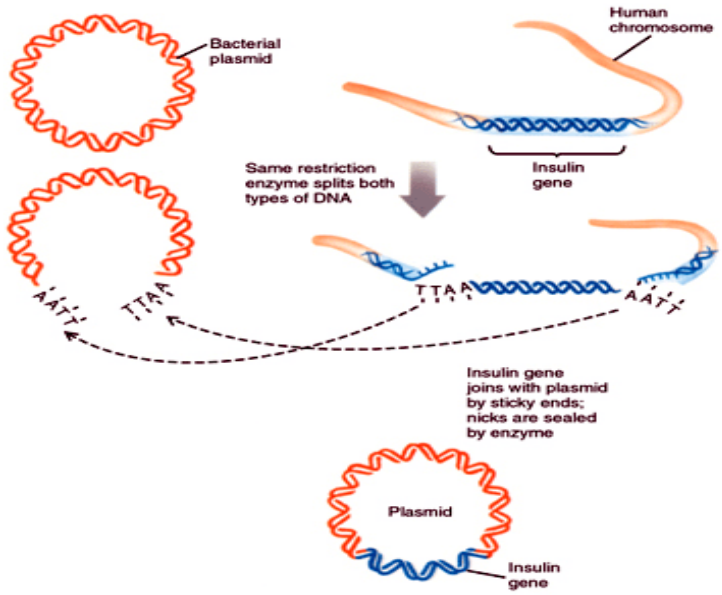
- Living things are made up of biological machines – mostly protein.
- Proteins made according to info stored in DNA
- Each organism has a master plan of DNA
- So, can we make our own blueprints?



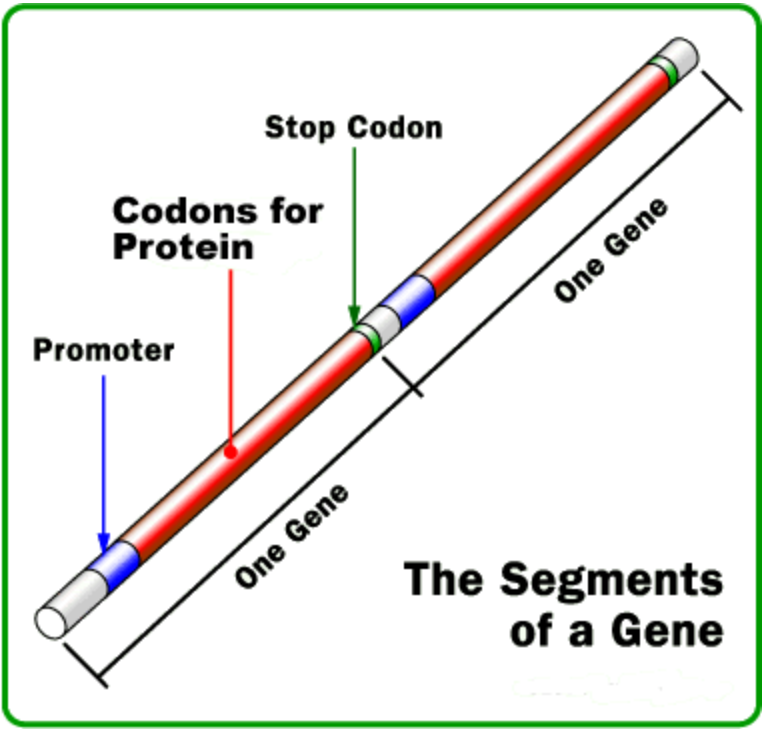
Genetic engineering

Add a DNA piece to a new organism, get a new protein

- 1000 s of examples



Elements of Control

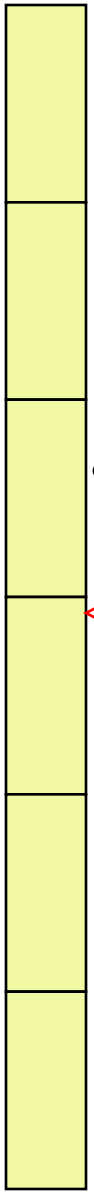


- Not just machines, but the *right* machine in the right place at the *right* time
- **Control**
  - Promoters, terminators, localization signals
  - Syntax and punctuation

Higher Levels of Organization

- More Complex pathways with intercepting parts
  - Omega-3 pigs
  - Landmine sensing plants
  - Golden rice
  - Artemesin

Increasing Complexity



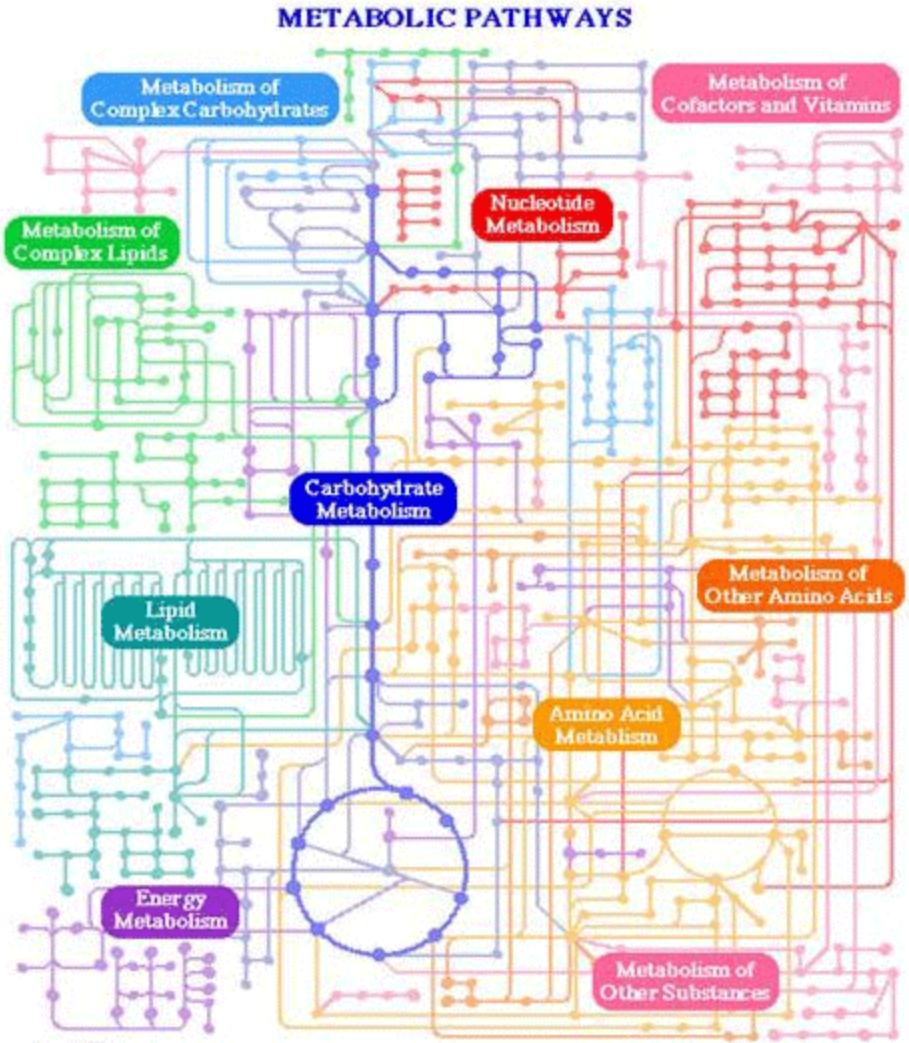
- Single gene = 1
- Gene and control = 2
- Gene and simple sensing system ~5

We are here

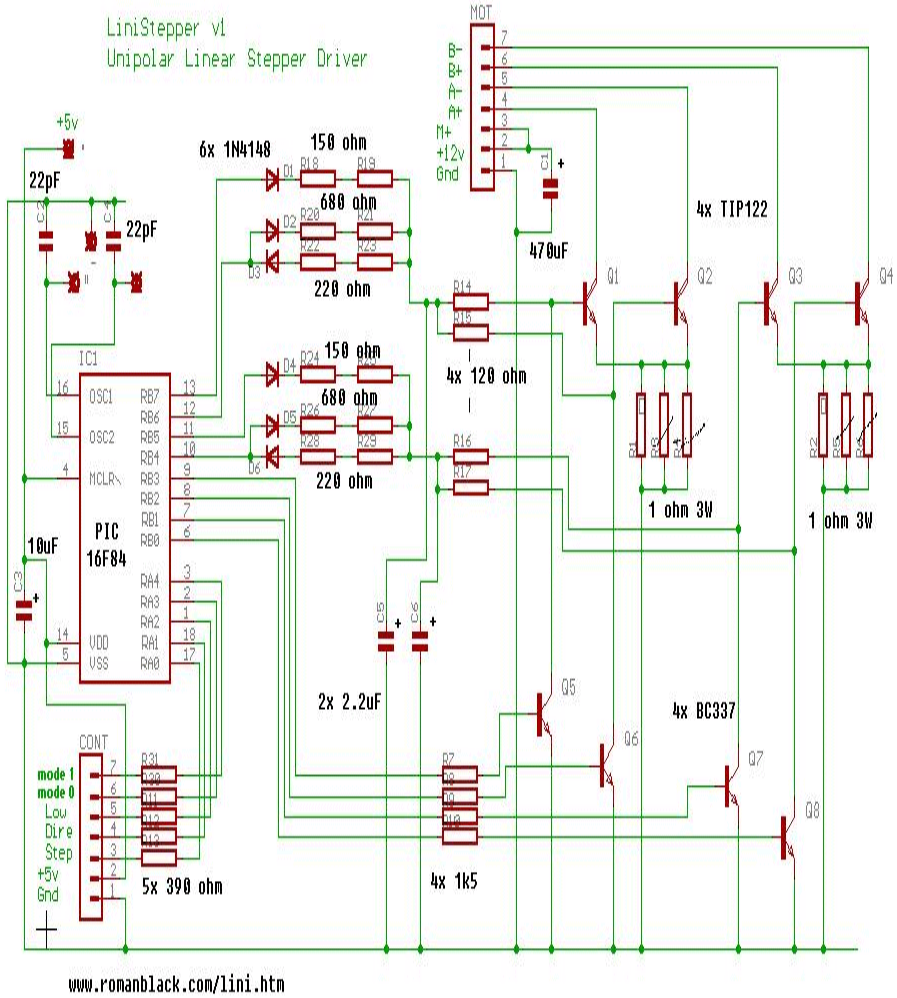
- Viruses ~50 genes
- Bacteria ~5000-30000
- Humans ~30,000, ~100,000 elements.

Genetic circuits

Metabolic pathways



Electronic circuits



Our project

The creation of dynamic system in a bacterium

- Tractable (<50-genes)
- Human readable

Implementation

- Construction through cheap lab methods

Understanding and design

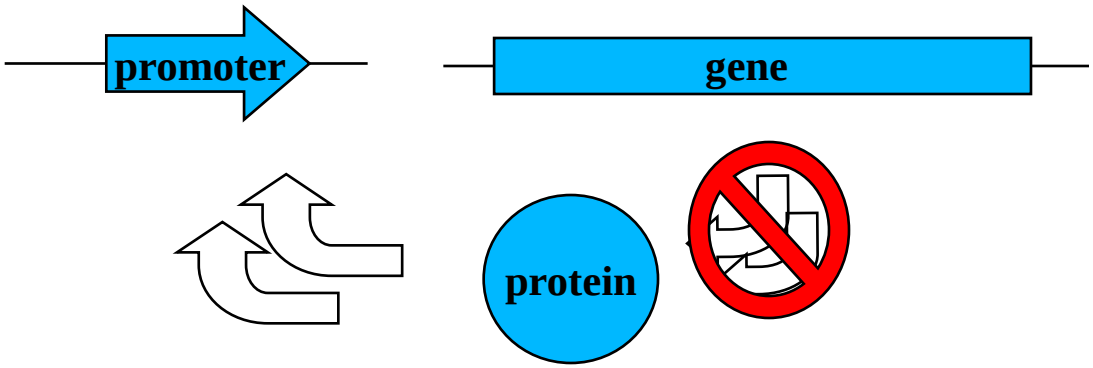
- Simulation through math modeling

Open-ended

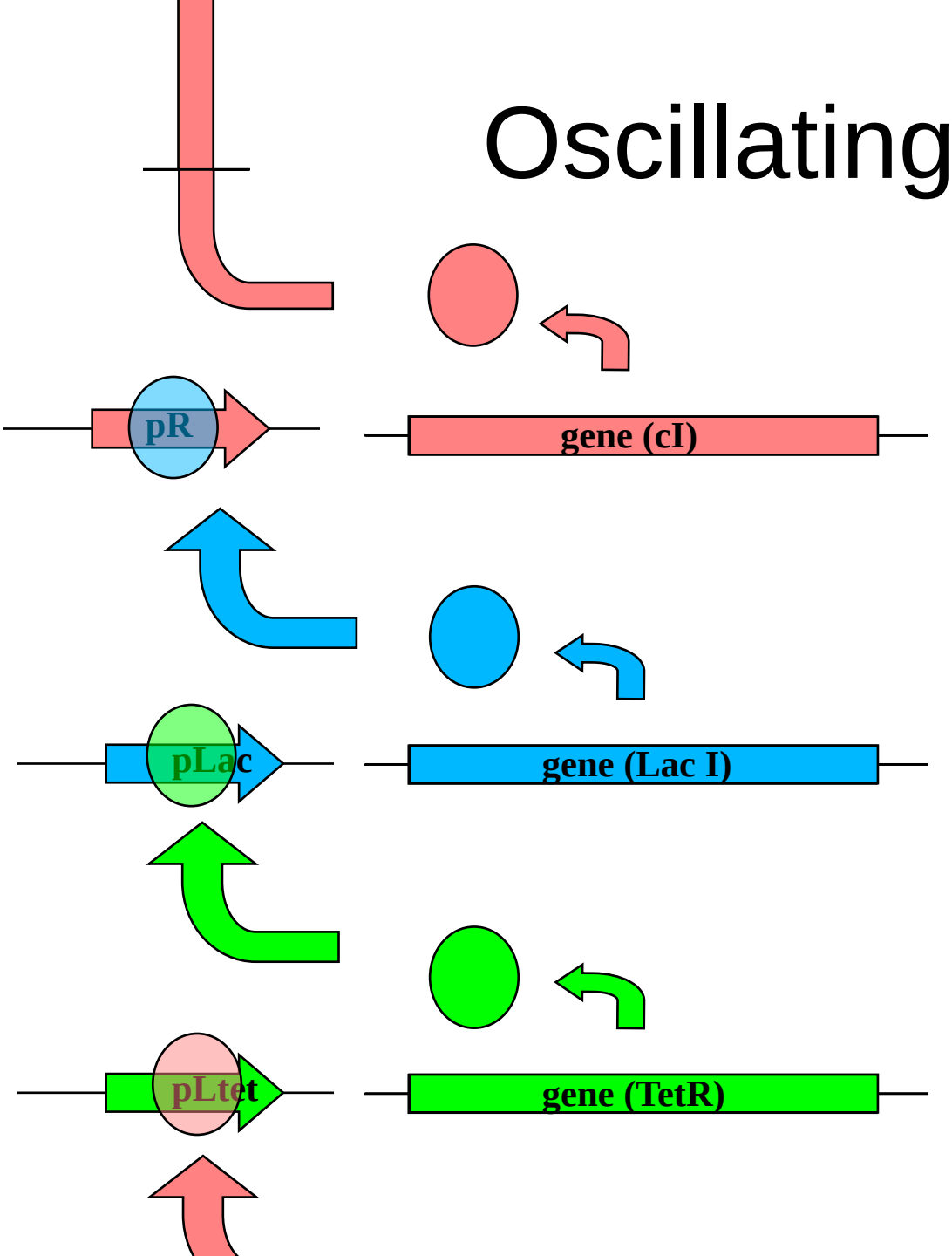
- Synchronization
- More charismatic organisms and designs

Biological System

- Feedback Systems
  - Thermostat
  - Hormone Homeostasis
- Negative Feedback
  - Product Inhibits Production



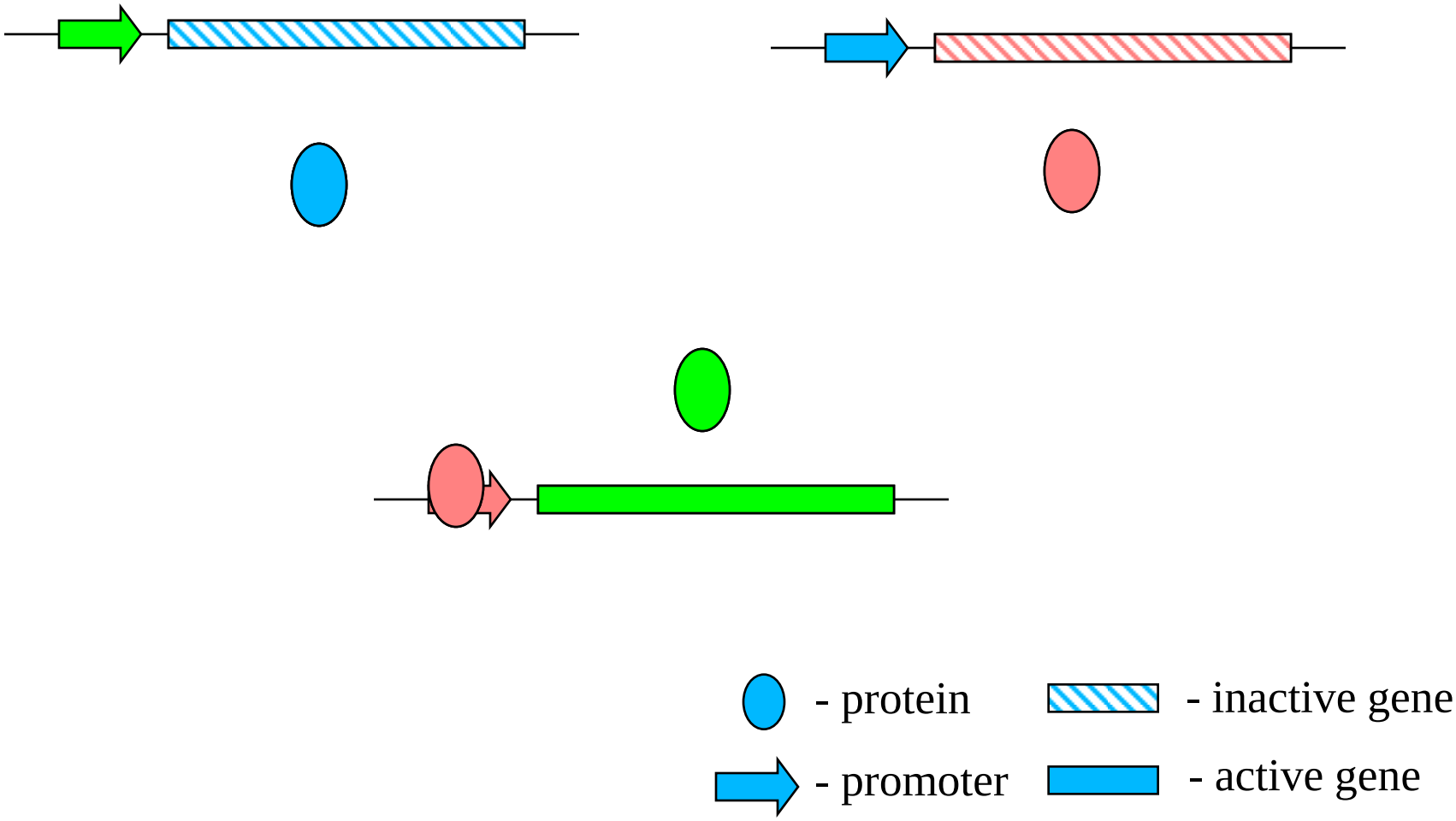
Oscillating System



Three gene system that communicates amongst its parts

From Biology to Engineering

Rearrange gene pieces to obtain an oscillating system – how it all works together?



Obtained Genes from Many Sources

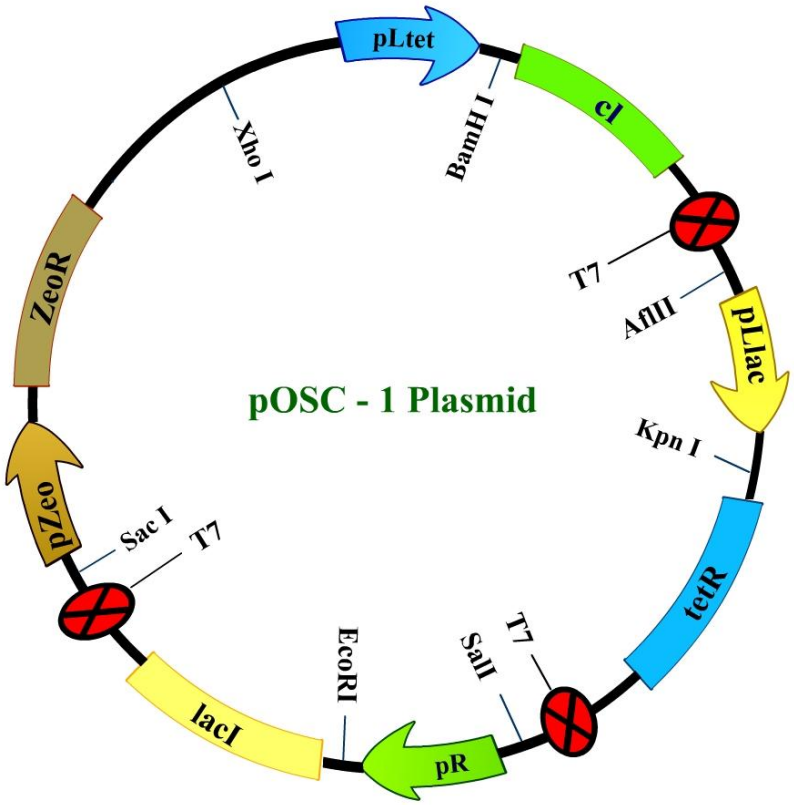
- Jelly Fish
  - XFP
- Bacteria
  - lacI
  - TetR
- Virus/Phage
  - pR – T7
  - cI
- Synthetic
  - pLTet
  - pLlac

Assembly Techniques

- DNA Extraction
  - Remove DNA from Host Cells
- Amplify Target Gene
  - PCR Amplification
- Connect Genes into Plasmids
  - Fusion PCR
- Insert Plasmids into Target Cells
  - Competent Cell Transformations

Oscillator plasmid

- All gene modules are put together in a plasmid
- The system oscillates so that only some genes are active at a certain time



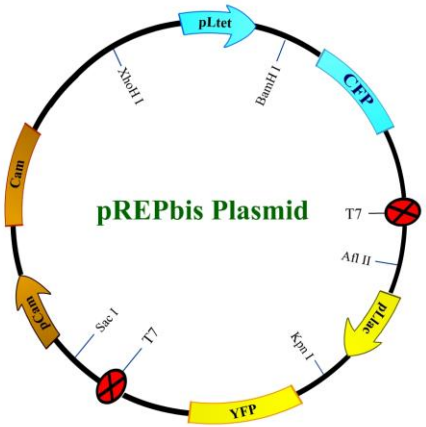
terminator

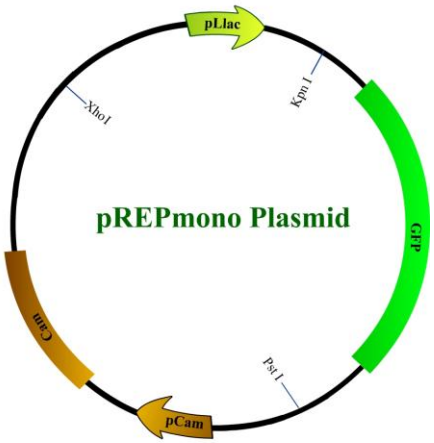
promoter

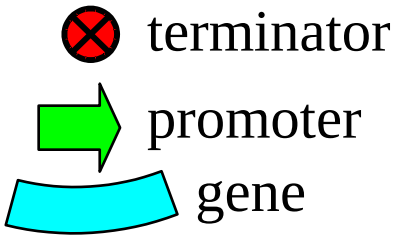
gene

Reporter Plasmid

- Cannot see the Oscillations!!!
- Can See Proteins from Oscillatory Plasmid
  - Duplicate Promoter to Track Active Genes
- Fluorescent Protein Produced Depends on Gene activated in pOSC



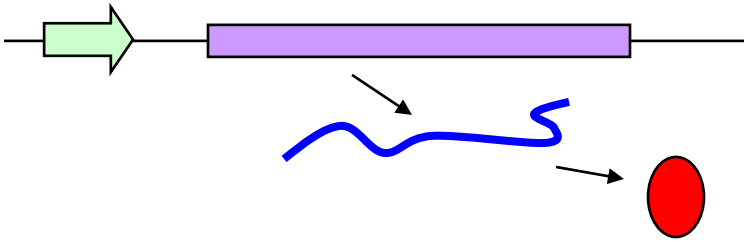




Design Aspects

- In order to have direction in this project, we need:
  - Stochastic arithmetic modeling
  - Imaginative visions for the future

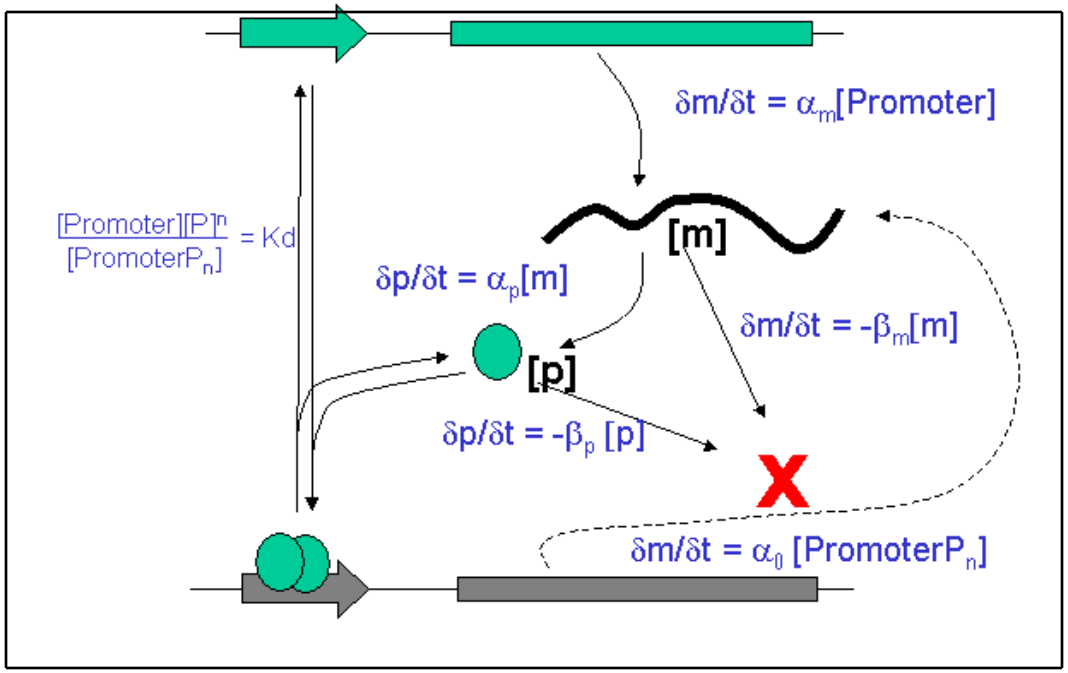
Modeling

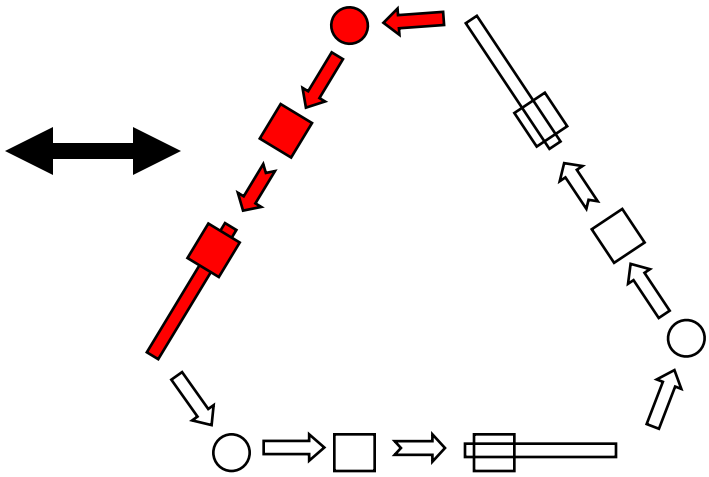


Behavior(t) = Model(α0,b,m,p,k…)

α0 = promotor strength
b = RNA half life
m = amount of mrna
p = amount of protein

Basic system: One branch

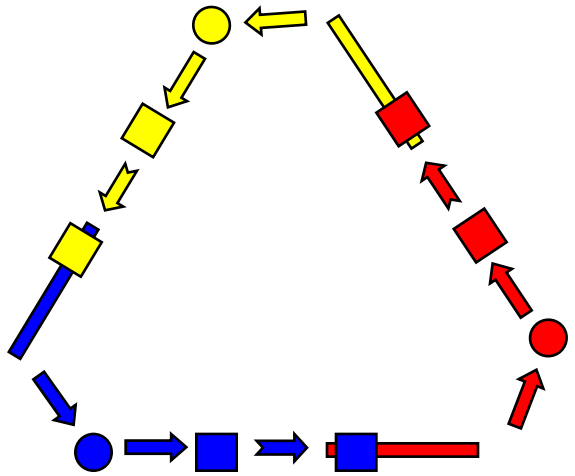




$$\delta m_i/\delta t = \alpha 0_{i+1} + \alpha 1_{i+1}\, p^n_{i+1}/(K^n_{i+1} + p^n_{i+1}) - \beta_m m_i$$

$$\delta p_i/\delta t = \alpha_m m_i - b_p p_i$$

All three



mRNA Equations:

$dm/dt = -\beta_m m + \alpha_1 p^n/(k_d+p^n) + \alpha_0$

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$dm/dt = -\beta_m m + \alpha_1 p^n/(k_d+p^n) + \alpha_0$

Protein Equations:

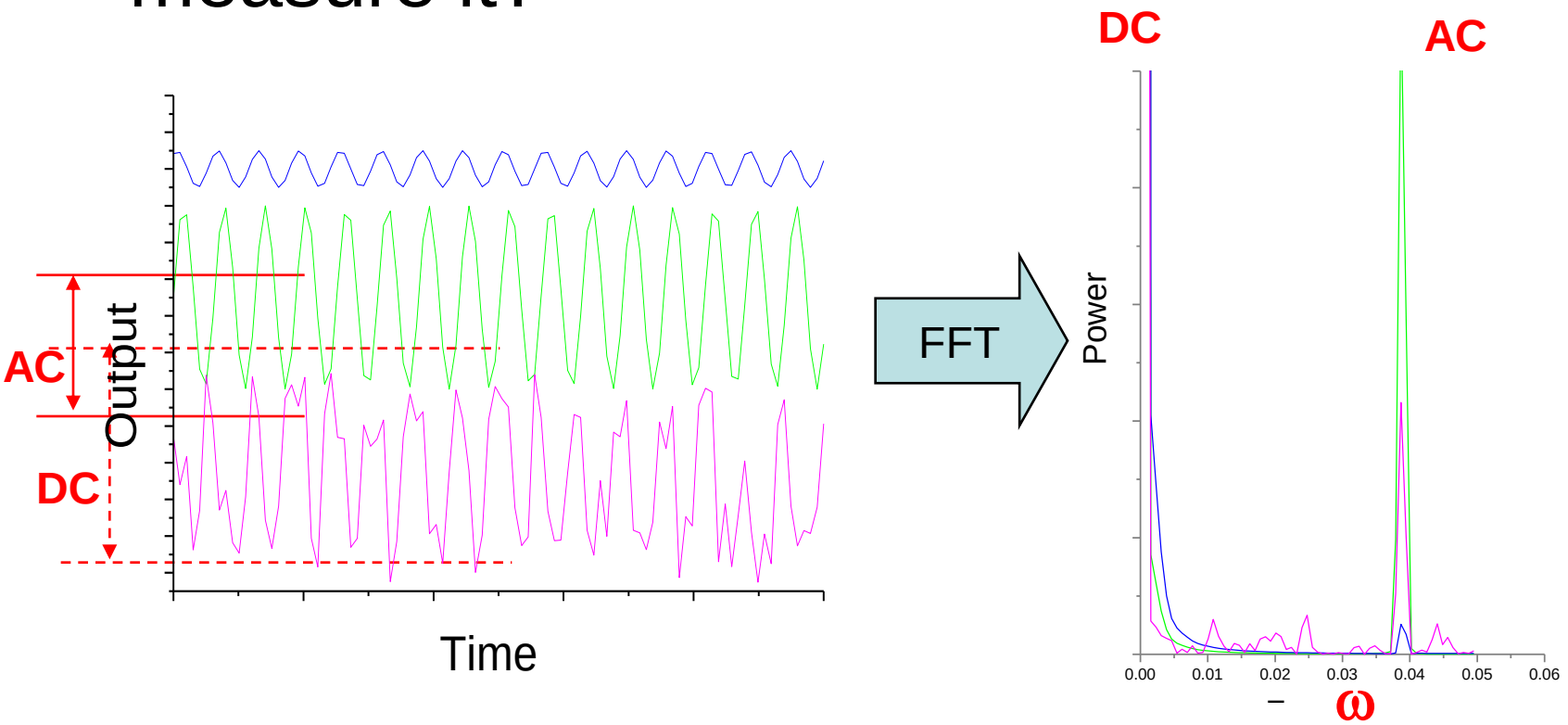
$dp/dt = t_s * m - bp * m$

$dp/dt = t_s * m - bp * m$

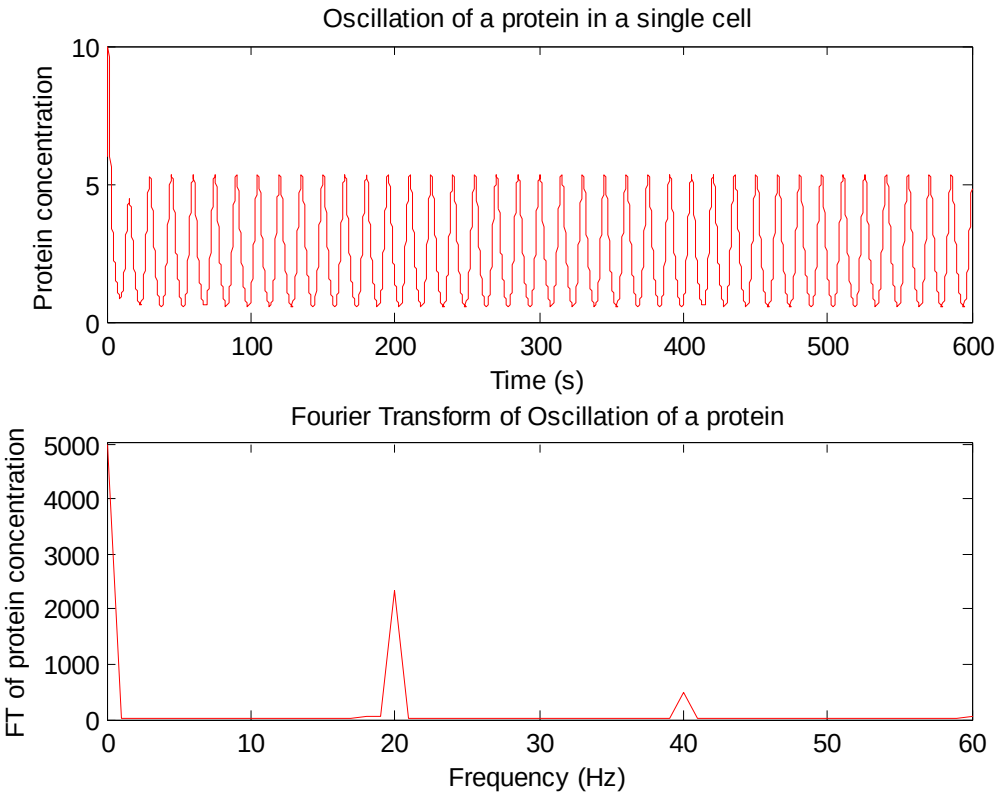
$dp/dt = t_s * m - bp * m$

Analysis

- What do we need to know and how do we measure it?



First simulation:GUI Deterministic



Stochastic Model

- Functional species are limited integer amounts only
  - Poisson distribution

$\lambda_m = -\beta_m m + \alpha_1 p^n/(k_d+p^n) + \alpha_0$

$dp/dt = t_s * m - bp * m$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

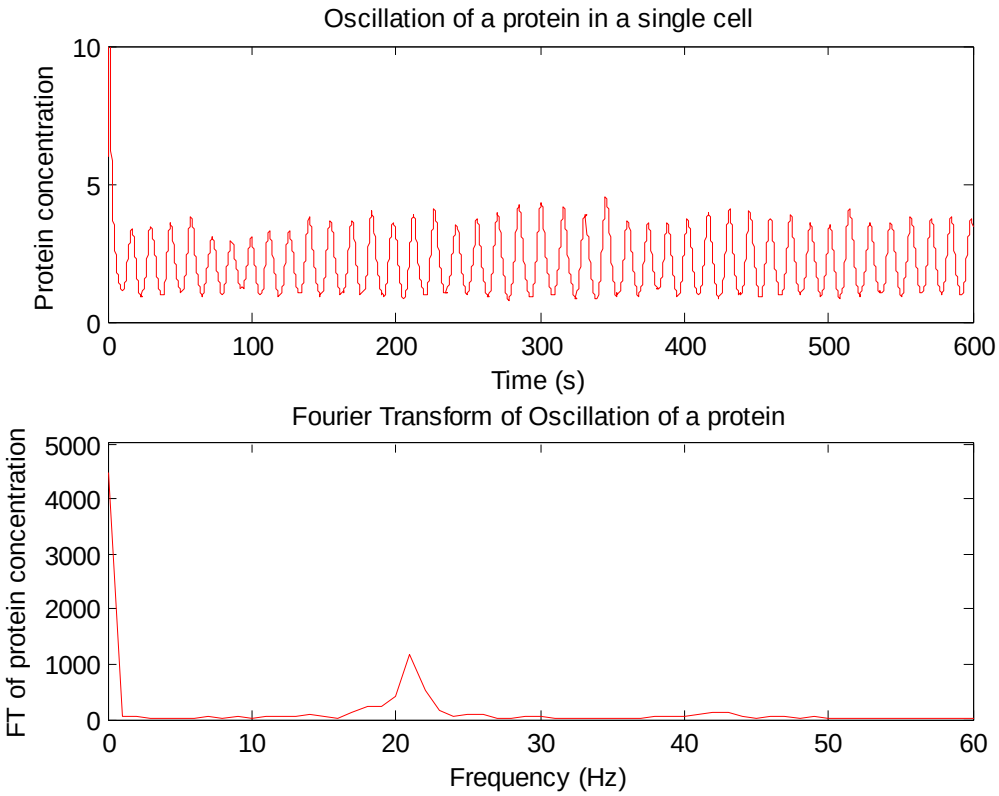
$dm/dt = -\beta_m m + \alpha_1 p^n/(k_d+p^n) + \alpha_0$

$dp/dt = t_s * m - bp * m$

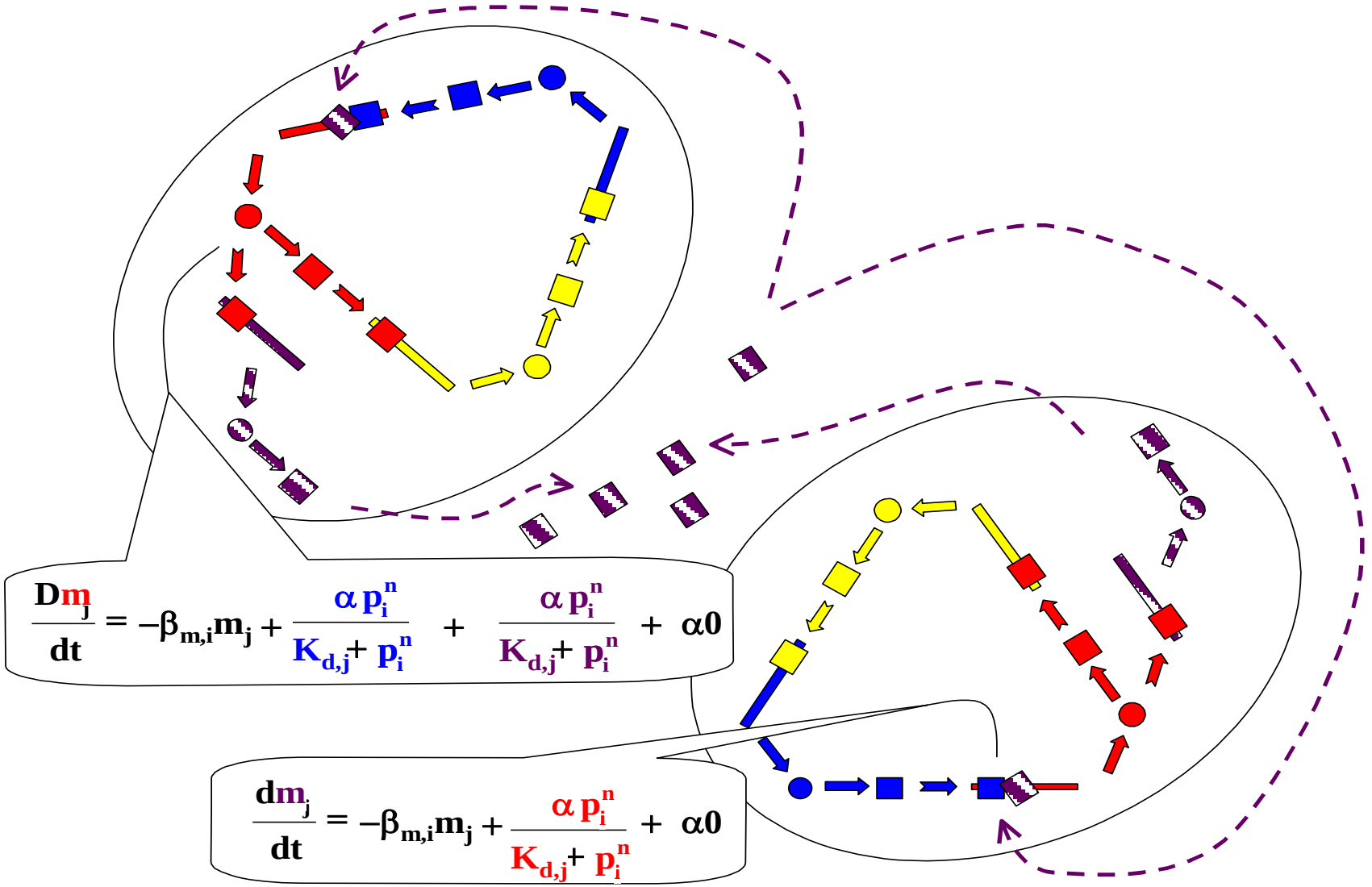
$dm/dt = -\beta_m m + \alpha_1 p^n/(k_d+p^n) + \alpha_0$

$dp/dt = t_s * m - bp * m$

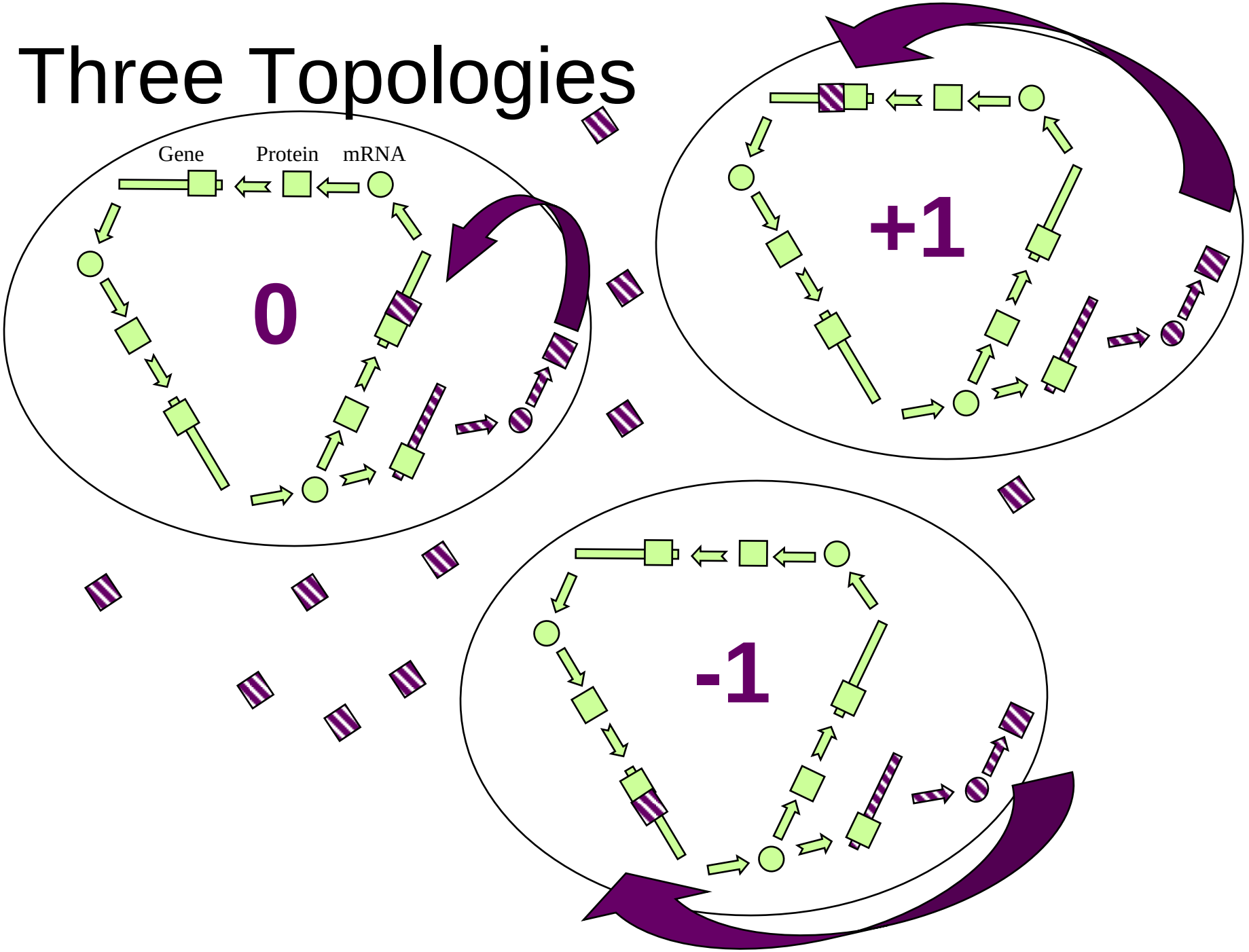
Second Simulation: GUI Stochastic



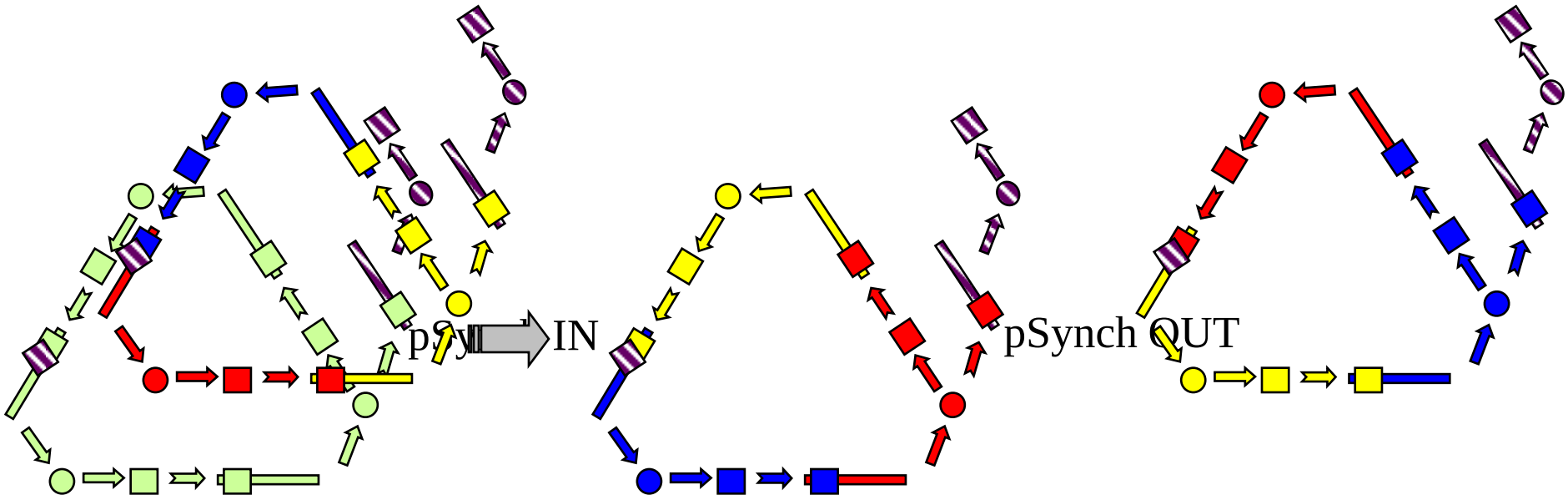
Synchronization



Three Topologies



Three Branches



$$\frac{dm_j}{dt} = -\beta_{m,i} m_j + \frac{\alpha_1}{K_{d,j} + p_i^n} + \alpha_0$$

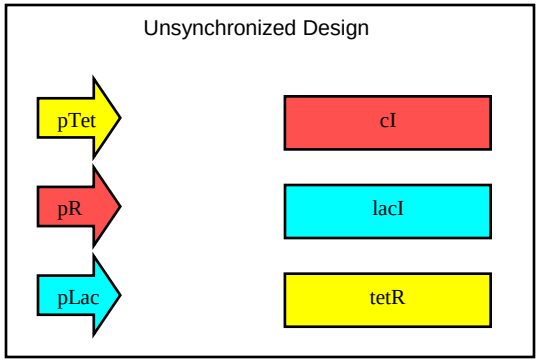
Which one?

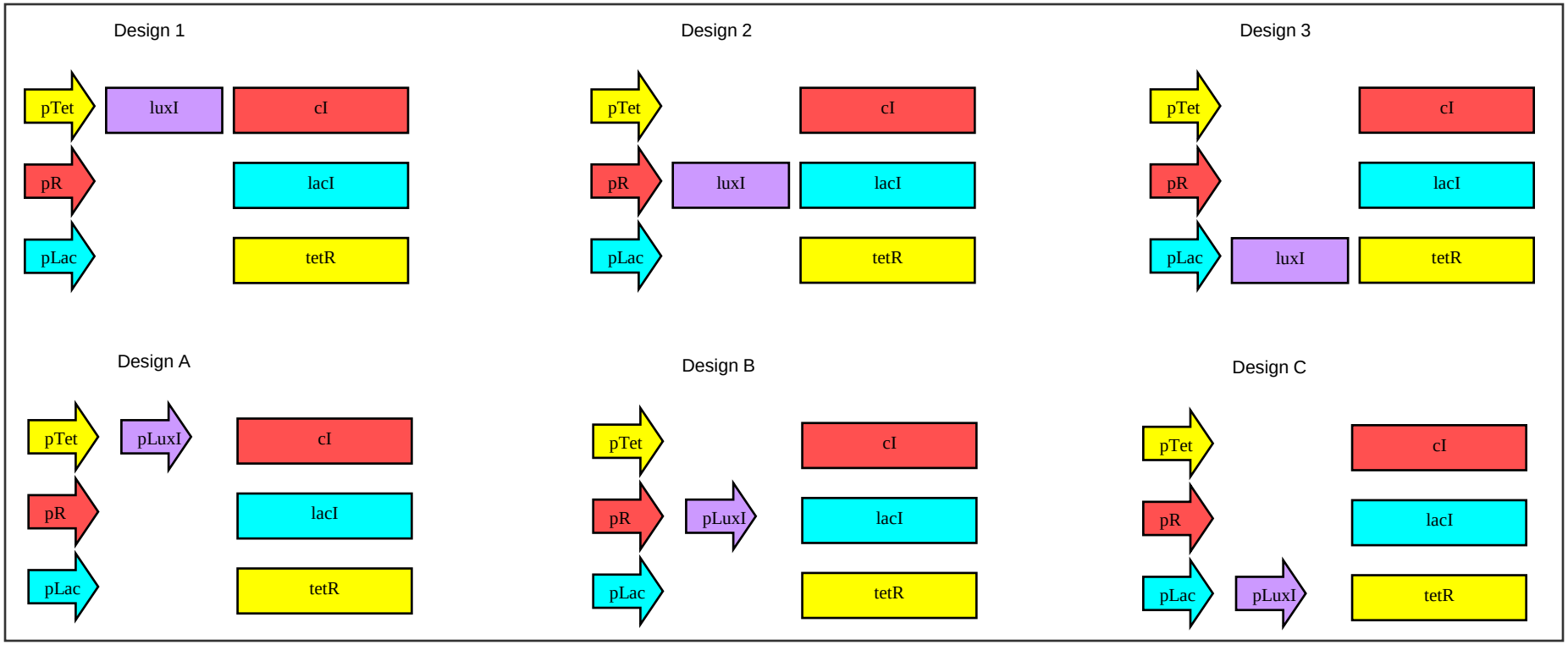
- Strongest α_1?
- Least leaky α_0 ?
- Highest ON/OFF ratio α_1/α_0?
- Kd,s , Kd,out ?
- n_h?

Other factors

- $\beta_{m,s}$
- $\beta_{p,s}$

Synchronization Designs





Modeling

mRNA Equations:

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha_1}{1 + p_k^n} + \alpha_0 + \frac{kS_{int}}{1 + S_{int}}$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dm_j}{dt} = -m_j + \frac{\alpha_1}{1 + p_i^n} + \alpha_0 + \frac{kS_{int}}{1 + S_{int}}$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

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$$\frac{dm_k}{dt} = -m_k + \frac{\alpha_1}{1 + p_j^n} + \alpha_0 + \frac{kS_{int}}{1 + S_{int}}$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

Protein Equations:

$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dp_j}{dt} = -\beta(p_j - m_j)$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dp_k}{dt} = -\beta(p_k - m_k)$$

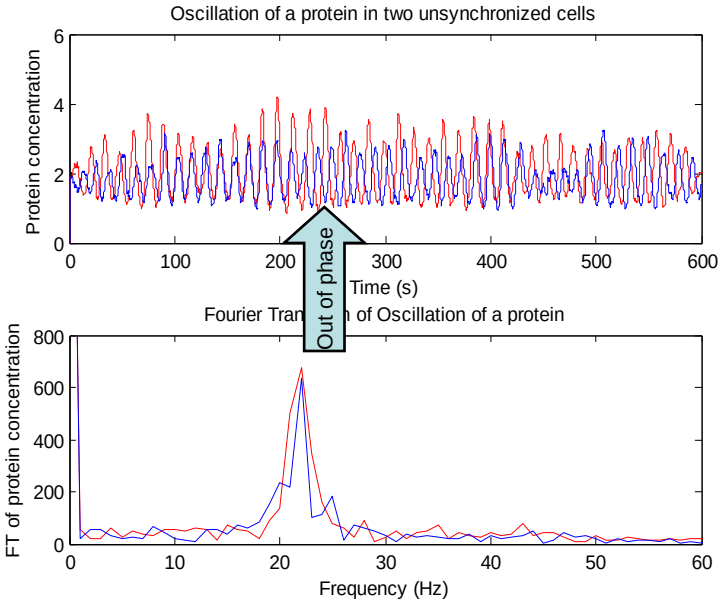
$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

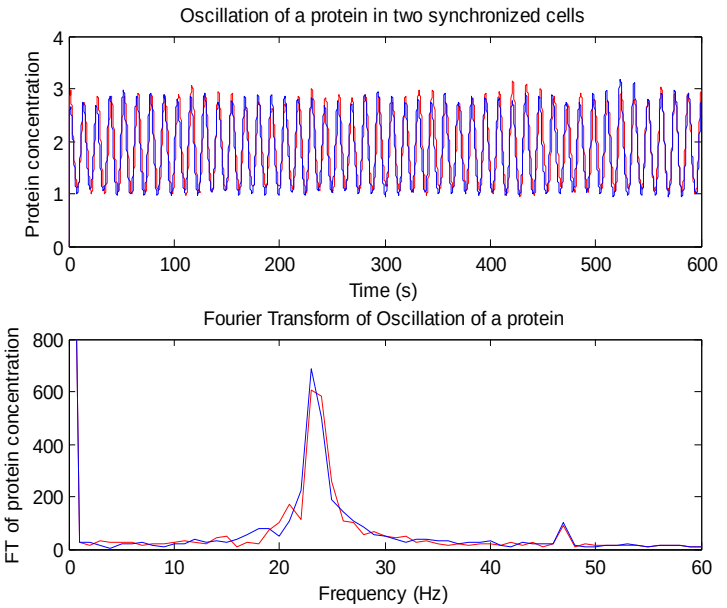
$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dS_{int}}{dt} = -k_{s0}S_{int} + k_{s1}p_{jk} - \eta(S_{int} - S_{ext})$$

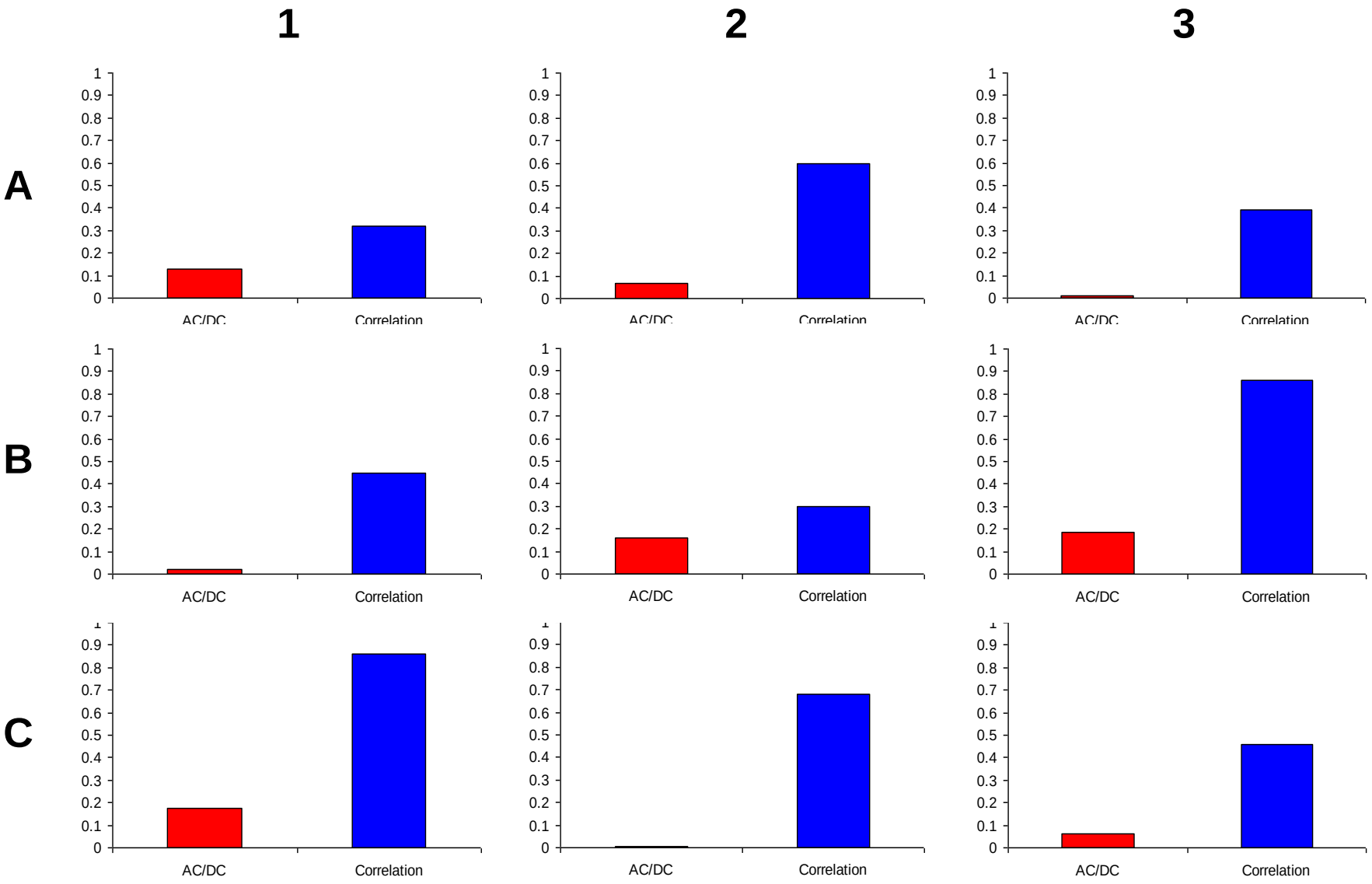
$$\frac{dS_{ext}}{dt} = -k_{se}S_{ext} + \eta\sum_{n=1}^{N}(S_n - S_{ext})$$

Unsynchronized vs. Synchronized

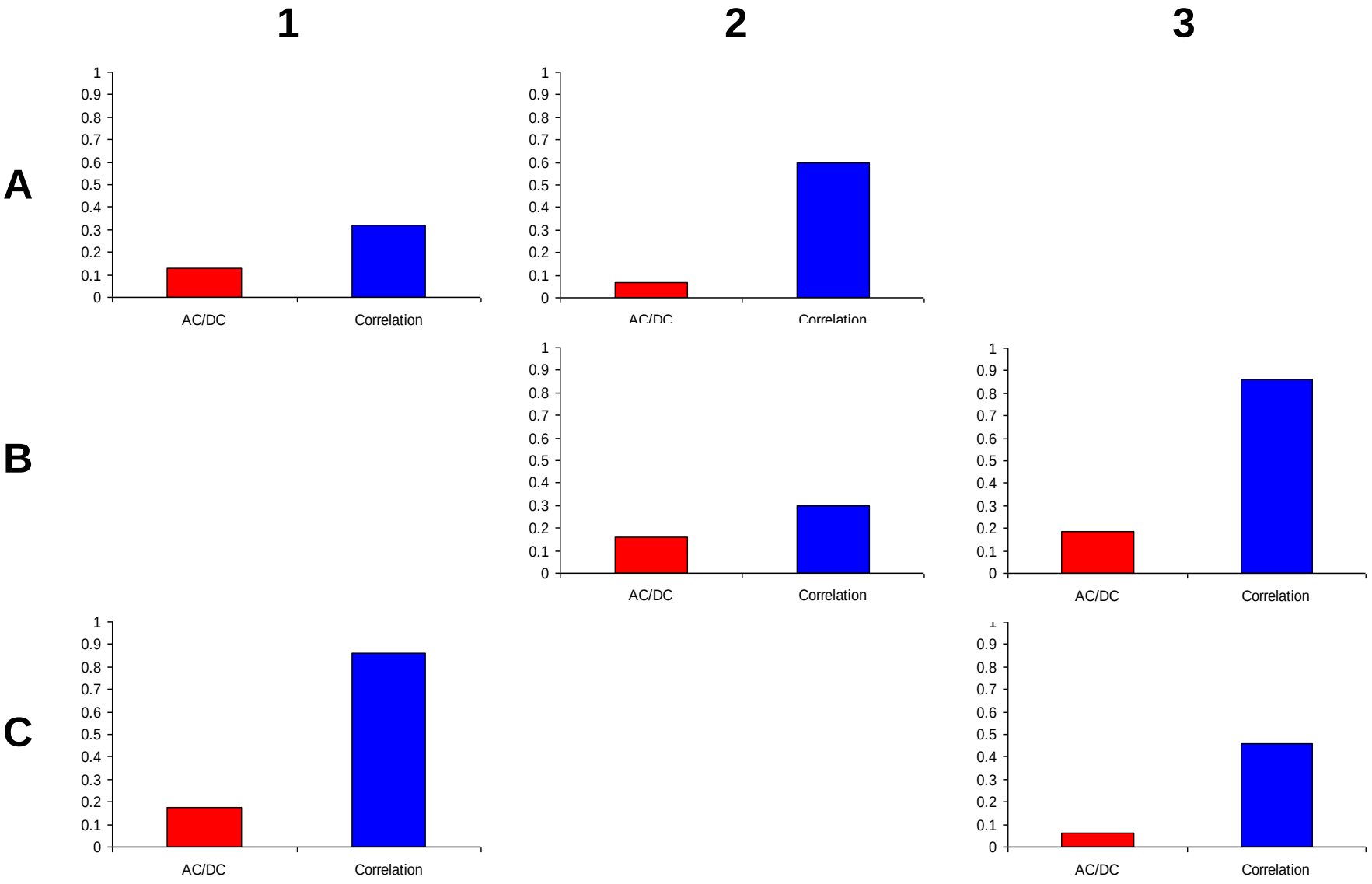




Topology Analysis



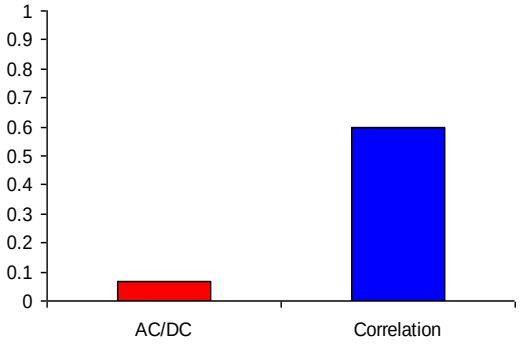
Strong Oscillation



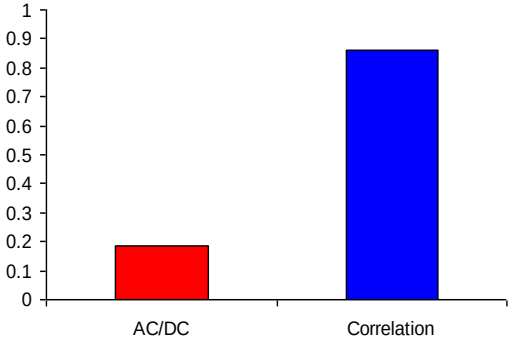
Good Synchronization

1 **2** **3**

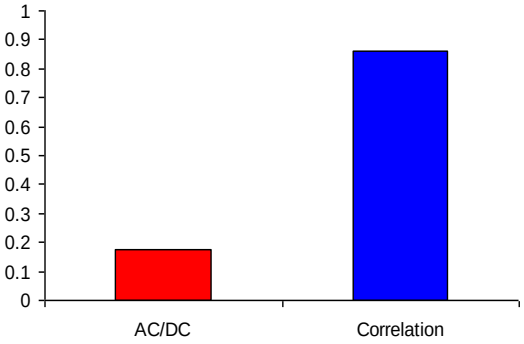
A



B

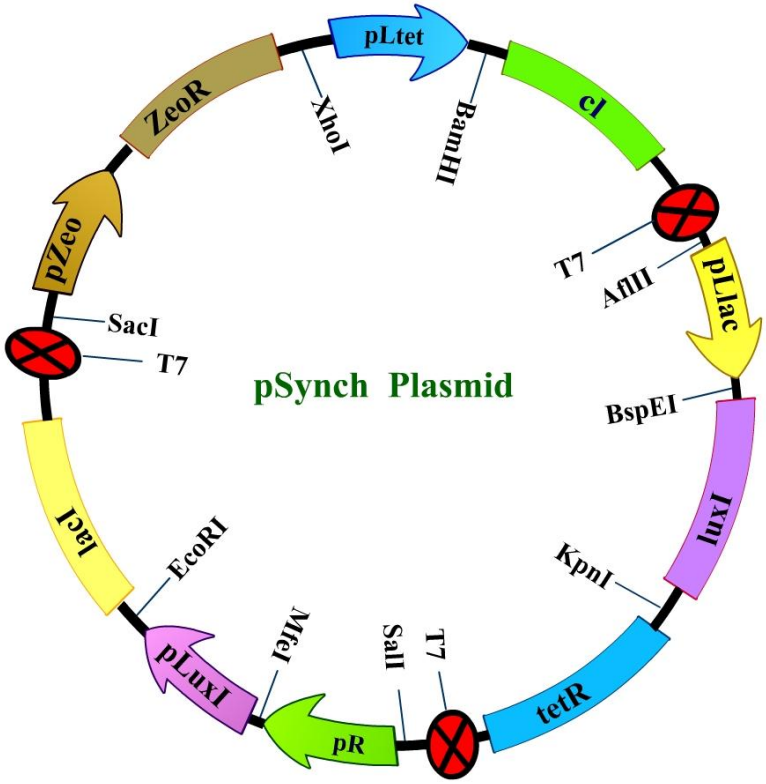


C



New target plasmid: pSynch

- We have the genes and preliminary PCR products
- Need to develop construction blueprint



Development

- Engineering multicellular organisms
  - ✓ Zebrafish
  - ❑ Humans?

–Scientific Issues
  - •Synchronization
  - •Different O/S

–Regulatory Issues

–Ethical Issues

Regulatory & Ethical Issues

- Regulations

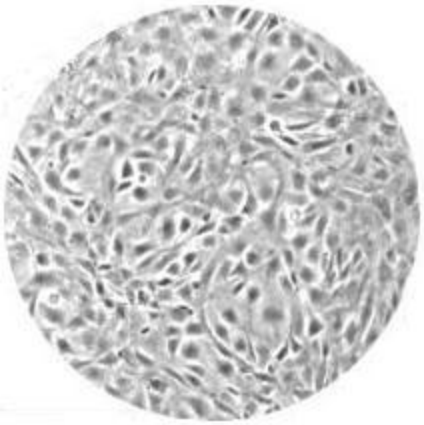
  Bodies like IACUC have final say

  - Justifiable use of animals
  - Minimal pain
  - Purpose

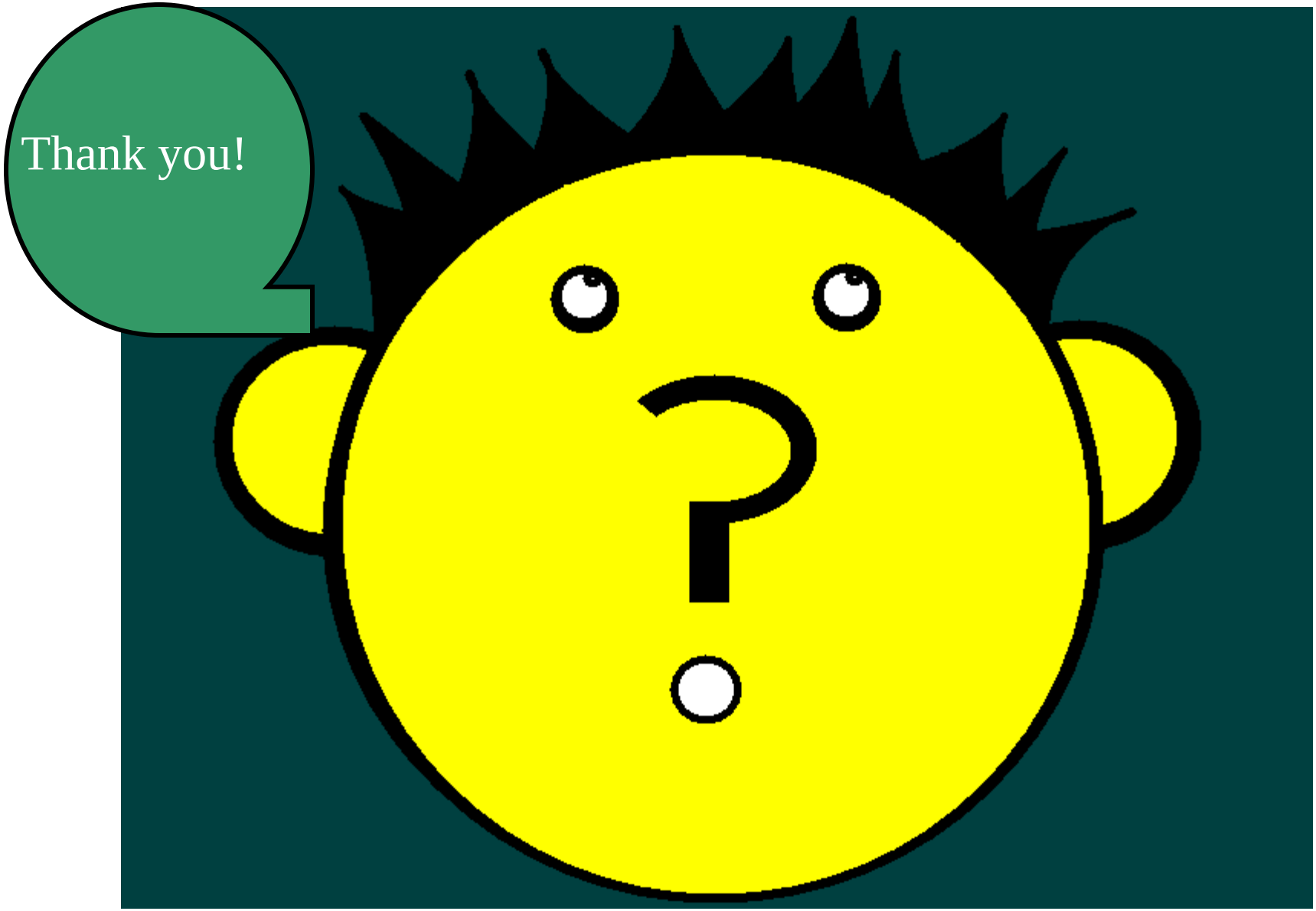
- Ethical Concerns

  Should we be doing this? Why?

  - Understanding complex biomedical systems
  - What is life?



Engineered Zebra Fish Culture Cells


Thank you!