MRES SYSTEMS AND SYNTHETIC BIOLOGY 2024-25
Essentials for Physical Scientists
LIFE70038 (S1a)
COURSEWORK

IMPERIAL

# Modelling of Biological Systems: Mathematical Analysis of Reaction Diffusion Systems.

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# Introduction

WE are performing modelling of two biological systems related ODEs. the first one will look at a circular irriversible process **Figure 1**, then at the Dual forward Circuit . and **Figure 3.**. After creating the mathematical modells **Box 1.**, **Box 5.**, we will examine the ODEs using a Machine Learning Pipeline **Figure 4.**.

## Irreversable Circular Reaction

The reaction is depicted in Figure 1, with species B katalysing its own production and therefore B$k_1$ beeing a second order kinetics. Part a will focus on modell building and part b on applying the Machine learning model, which is viewable on google codelab via s1a-coursework-supplemental.ipynb

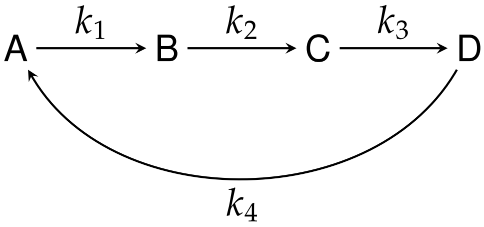


**Figure 1 Irreversable Circular Reaction Pathway.**Created with Tikz

## Dual Feedforward Circuit Analysis.

In task 2 we will first create the reaction circuit , **Figure 3.** and then the kinetics **Box 5.** and **Box 4.** before creating the ODEs in **Box 6.**, **Box 7.** and **Box 8.**. We will then conclude the task by looking at the reaction dynamics for high and low Signal.

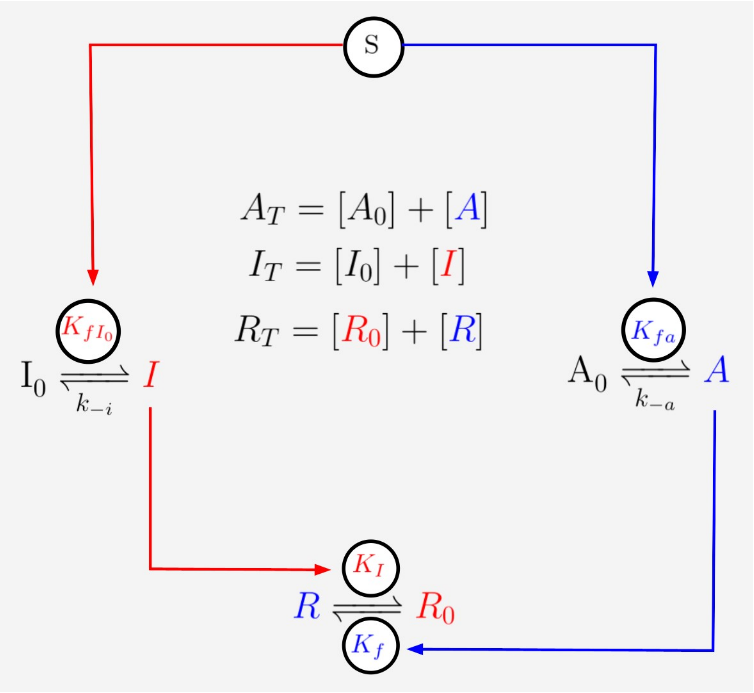


**Figure 2. Reaction Pathway. Dual Feedforward Circuit.** Created with PSTricks

Figure created with Biorender.com with adoption from Storch et al., 2015

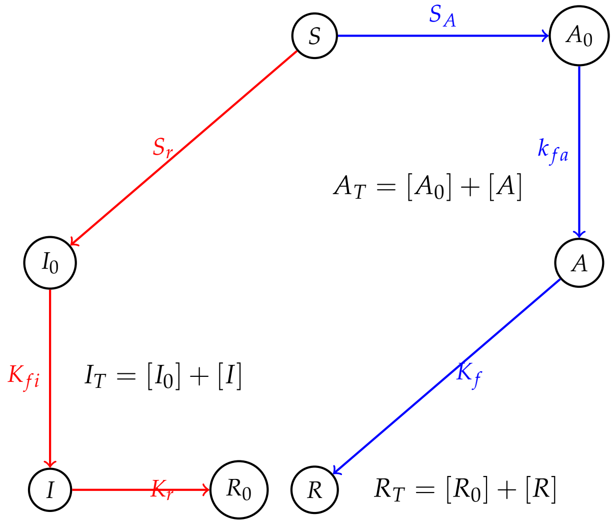
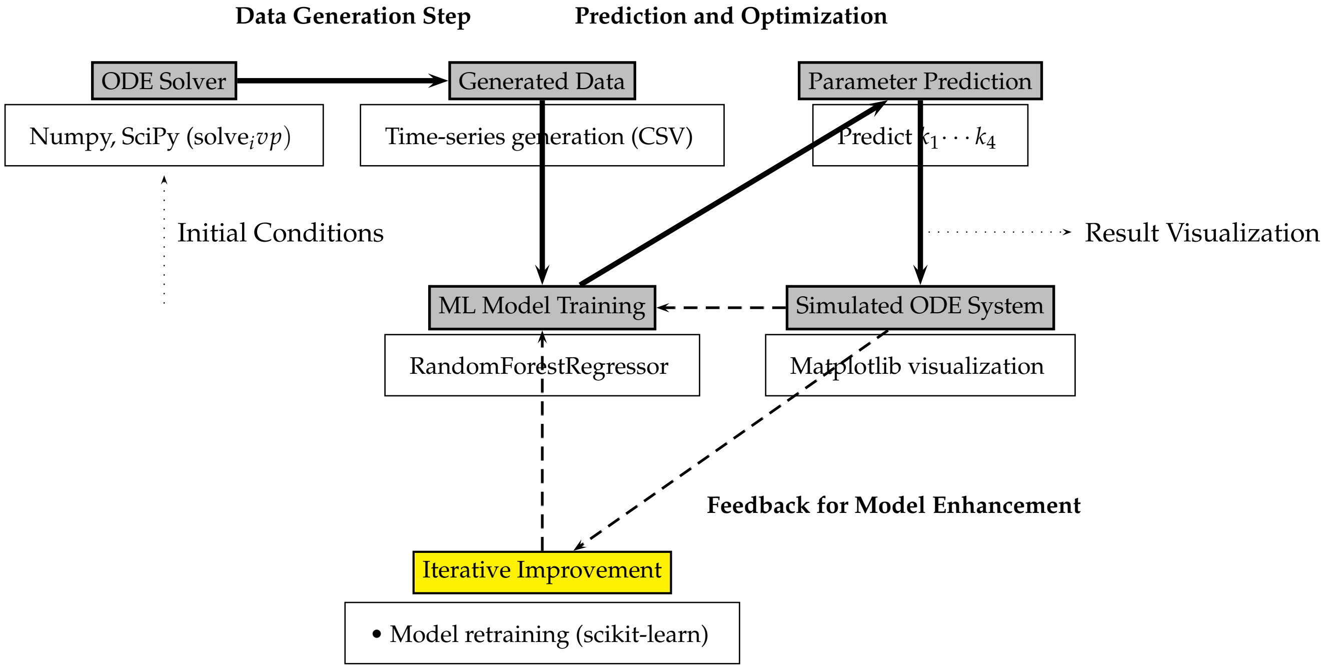


**Figure 3. Dual Feedforward Circuit.** Created with PSTricks.

## Machine Learning Implementation

We have build a trianing pipele on https://colab.research.google.com/drive/1Jj8BLVi6-IXPqv54bta5eGCVJjZAFVoR?usp=sharing to examine the ODEs.



**Legend**. Solid lines represent process flow, dashed lines represent feedback or iterative steps.

**Figure 4. Machine Learning Training Pipeline**. Created with PSTricks

# Results.

## Task 1: Circular Pathway Analysis

The focus in this section is the analysis of the concentrations [A],[B],[C],[D] at equillibrium. We will analyse the obtained solutions of that ODE and examine its physical behaviour through simulations and a machine learning model. We starting by understanding the mathematical model, which describs the stoichiometric system in **Figure 1**. Its derivation is shown in **Box 1.** below.

**Stochiometric Reaction Pathway (Task 1a):**

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} D \quad \text{with feedback} \quad D \xrightarrow{k_4} A$$

**Reaction Kinetic Equations:**

- **Reaction 1:** $A \xrightarrow{k_1} B$ (Second Order Kinetics)

$$\frac{d[A]}{dt} = -k_1[A][B] \quad \text{(Consumption of A, Catalysed by B)}$$
$$\frac{d[B]}{dt} = k_1[A][B] \quad \text{(Creation of B, Catalysed by B)}$$

- **Reaction 2:** $B \xrightarrow{k_2} C$

$$\frac{d[B]}{dt} = -k_2[B] \quad \text{(Consumption of B to form C)}$$
$$\frac{d[C]}{dt} = k_2[B] \quad \text{(Creation of C)}$$

- **Reaction 3:** $C \xrightarrow{k_3} D$

$$\frac{d[C]}{dt} = -k_3[C] \quad \text{(Consumption of C)}$$
$$\frac{d[D]}{dt} = k_3[C] \quad \text{(Creation of D)}$$

- **Feedback Reaction:** $D \xrightarrow{k_4} A$

$$\frac{d[D]}{dt} = -k_4[D] \quad \text{(Consumption of D)}$$
$$\frac{d[A]}{dt} = k_4[D] \quad \text{(Recreation of A)}$$

**Complete ODE System:**

$$\begin{aligned}\frac{d[A]}{dt} &= -k_1[A][B] + k_4[D],\\ \frac{d[B]}{dt} &= k_1[A][B] - k_2[B],\\ \frac{d[C]}{dt} &= k_2[B] - k_3[C],\\ \frac{d[D]}{dt} &= k_3[C] - k_4[D].\end{aligned}$$

**Explanation:**

- Mathematical model for the irreversable cyclic reaction pathway.
- Rate constants: $(k_1, k_2, k_3, k_4)$; Species: $A, B, C, D$.

**Box 1.** Mathematical Model Cyclic Feedback Reaction

Now that we have our mathematical model, we can examine what happens at equilibrium conditions, when the net change in Species consuption vs. creation is 0: $\frac{d}{dt} = 0$.

**Equilibrium State:**

$$\text{Reaction: } A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} D$$
$$\text{Feedback: } \quad D \xrightarrow{k_4} A$$

**ODEs for Steady State:**

$$\begin{aligned}\frac{d[A]}{dt} &= -k_1[A][B] + k_4[D] = 0,\\ \frac{d[B]}{dt} &= k_1[A][B] - k_2[B] = 0,\\ \frac{d[C]}{dt} &= k_2[B] - k_3[C] = 0,\\ \frac{d[D]}{dt} &= k_3[C] - k_4[D] = 0.\end{aligned}$$

**Box 2.** Steadystate Analysis.

With the ODE in **Box 2.** we can solve the equation using Python.

Code Box

**Python solution for ODE (Box 1.):**

```python
from sympy import symbols, Eq, solve
A, B, C, D, k1, k2, k3, k4 = symbols('A B C D k1 k2 k3 k4')

eq1 = Eq(-k1 * A + k4 * D, 0)
eq2 = Eq(k1 * A - k2 * B, 0)
eq3 = Eq(k2 * B - k3 * C, 0)
eq4 = Eq(k3 * C - k4 * D, 0)

solution = solve((eq1, eq2, eq3, eq4), (A, B, C, D))

# Output:
[(A, 0, 0, 0), (k2/k1, D*k4/k2, D*k4/k3, D)]
print(solution)
```

**Snippet 1.** Solution for Equilibrium Concentrations using Python.

Looking at the output of the code above, we can summaries the result of the python code as described in **Box 3.** below.

**Steady-State 1:**

$$A = \text{const.}, B = C = D = 0$$

**Steady-State 2:**

$$A = \frac{k_2}{k_1}, B = [D]\frac{k_4}{k_2}, \quad C = [D]\frac{k_4}{k_3}, D = \text{const.}.$$

**Box 3.** The ODE in **Box 2.** has two solutions. **Solution 1** represents initial state, where only species A is present. **Solution 2** represents the steady state, with species A,B and C depending on D. Species D is independent and can be determined by defining a total concentration amount: $X_0 = [A] + [B] + [C] + [D]$, constraining all species.

## Task 2: Dual Feedforward Circuit Analysis.

For task 2 we also start by formulating the mathematical model.

**Stoichiometric Reactions ()**

**Reaction 1. Enzyme $A$ - Kinetics:**

$$\text{Feedforward Reaction:} \quad S + A_0 \xrightarrow{k_{fa}} S + A$$

$$\text{Reverse Reaction:} \quad A \xrightarrow{k_{-a}} A_0$$

**Reaction 2. Enzyme $I$ - Kinetics:**

$$\text{Feedforward Reaction:} \quad S + I_0 \xrightarrow{k_{fi}} S + I$$

$$\text{Reverse Reaction:} \quad I \xrightarrow{k_{-i}} I_0$$

**Reaction 3. Conversion of $R_0$ to $R$ Catalyzed by $A$ and $I$:**

$$\text{Activation by } A: \quad R_0 \xrightarrow{k_f} R$$

$$\text{Deactivation by } I: \quad R \xrightarrow{k_r} R_0$$

**Total Enzymes:**

$$A_T = [A_0] + [A]$$
$$I_T = [I_0] + [I]$$
$$R_T = [R_0] + [R]$$

**Box 4. Stoichiometry of the Dual Feedforward Cuircuit.**

Now that we understand the reaction process involved in this Cuircuit, we can create the ODEs by looking at the individual Reactions.

**Mathematical Modelling: Dual Feedforward Cuircuit (Figure 3.)**

$$S \to A_0 \xrightarrow{k_{fa}} A[k_{-a}]A_0, \quad S \to I_0 \xrightarrow{k_{fi}} I[k_{-i}]I_0, \quad A + R_0 \xrightarrow{k_f} R, \quad I + R \xrightarrow{k_r} R_0$$

**Reaction Kinetic Equations:**

- **Activation of $A$:**
$$\frac{d[A]}{dt} = k_{fa}[S][A_0] - k_{-a}[A] \quad (\text{Activation of } A_0\text{, Deactivation of } A)$$
- **Activation of $I$:**
$$\frac{d[I]}{dt} = k_{fi}[S][I_0] - k_{-i}[I] \quad (\text{Activation of } I_0\text{, Deactivation of } I)$$
- **Conversion of $R_0$ to $R$ (Catalyzed by $A$):**
$$\frac{d[R]}{dt} = k_f[A][R_0] - k_r[I][R] \quad (\text{Production of } R\text{, Consumption of } R)$$

**Complete ODE System:**

$$\begin{aligned}\frac{d[A]}{dt} &= k_{fa}[S][A_0] - k_{-a}[A],\\ \frac{d[I]}{dt} &= k_{fi}[S][I_0] - k_{-i}[I],\\ \frac{d[R]}{dt} &= k_f[A][R_0] - k_r[I][R].\end{aligned}$$

**Explanation:**

- Reaction dynamics (Activation vs. Deactivation) of Enzyme species $A, A_0, I, I_0, Rand R_0$.
- Rate constants: $k_{fa}, k_{-a}, k_{fi}, k_{-i}, k_f, k_r$

**Box 5. Mathematical Model of the Dual Feedforward Cuircuit.**

We can determine the Equilibrium states by setting the ODEs to zero and obtain the steady states.

**ODEs for Steady State:**

$$\begin{aligned}\frac{d[A]}{dt} &= k_{fa}[S][A_0] - k_{-a}[A] = 0,\\ \frac{d[I]}{dt} &= k_{fi}[S][I_0] - k_{-i}[I] = 0,\\ \frac{d[R]}{dt} &= k_f[A][R_0] - k_r[I][R] = 0.\end{aligned}$$

**Box 6. Steady States** for Dual Feedforward Cuircuit.

We can now solve the ODE using Python again:

Code Box

**Python solution for ODE (Box 6.):**

```
from sympy import symbols, Eq, solve

# Define the symbols
A, I, R, A0, I0, R0, kfa, k_a, kfi, k_i, kf, kr, S = symbols('A I R A0 I0 R0 kfa k_a kfi k_i kf kr S')

# Steady states of A,I,R
eq1 = Eq(kfa * S * A0 - k_a * A, 0)
eq2 = Eq(kfi * S * I0 - k_i * I, 0)
eq3 = Eq(kf * A * R0 - kr * I * R, 0)

# Solve for A, I, and R in terms of known parameters
solution = solve((eq1, eq2, eq3), (A, I, R))

# Print the solution
print(solution)

#Output
#[(A0*S*kfa/k_a, I0*S*kfi/k_i, A0*R0*k_i*kf*kfa/(I0*k_a*kfi*kr))]
```

**Snippet 2.** Solution for Equilibrium Concentrations using Python.
Looking at the output of the code above, we can summaries the result of the python code as described in **Box 3.** below.

Looking at the Python output we find the following equilibria for the species:

**Steady-States:**

$$\text{Steady State for } A: \quad A = \frac{A_0 S k_{fa}}{k_{-a}},$$

$$\text{Steady State for } I: \quad I = \frac{I_0 S k_{fi}}{k_{-i}},$$

$$\text{Steady State for } R: \quad R = \frac{A_0 R_0 k_{-i} k_f k_{fa}}{I_0 k_{-a} k_{fi} k_r}.$$

**Box 7. Steady States for Dual Feedforward Circuit. The steady-state for A** depends on the initial concentration of the inactive form of species A, the signal, and the balance between activation ($k_{fa}$) and deactivation ($k_{-a}$) rates. **The steady-state for I** depends on the signal, the concentration of the initial inactive species I, and the balance between activation ($k_{fi}$) and deactivation ($k_{-i}$) rates. **The steady-state of R** is a complex balance between activating ($k_f, k_{fa}, k_i$) and deactivating ($k_{-a}, k_{fi}, k_r$) rates and initial species concentrations $\left(\frac{A_0}{I_0}\right)$. This dual control is offset by the initial concentration of the inactive form of enzyme R.

Having the Steady-States, we can now perform the substitution and express R in terms the forward and reverse reactions.

Given equillibrium states:

$$A = \frac{A_0 S k_{fa}}{k_{-a}}, \quad I = \frac{I_0 S k_{fi}}{k_{-i}}, \tag{1}$$

Rearrange to obtain $A_0$ and $I_0$ :

$$A_0 = \frac{A k_{-a}}{S k_{fa}}, \quad I_0 = \frac{I k_{-i}}{S k_{fi}}. \tag{2}$$

Substitute in $R$ :

$$R(S) = \frac{\left(\frac{A k_{-a}}{S k_{fa}}\right) R_0 k_{-i} k_f k_{fa}}{\left(\frac{I k_{-i}}{S k_{fi}}\right) k_{-a} k_{fi} k_r}. \tag{3}$$

Simplify

$$R(S) = \frac{A R_0 k_f}{I k_r}. \tag{4}$$

denominator=$R_{forward}$, nominator=$R_{reverse}$

$$R_{\text{forward}}(S) = \frac{A R_0 k_f S}{I k_r}, \tag{5}$$

$$R_{\text{reverse}}(S) = \frac{A R_0 k_f k_{fa} S}{I k_r k_{-a} k_{fi}}. \tag{6}$$

The final steady states for R as a function of S are as follows:

**Steady-States R(S)**

$$R(S) = \frac{AR_0}{I} \cdot \frac{k_f}{k_r}, \tag{1}$$

$$R_{\text{forward}}(S) = \frac{AR_0S}{I} \cdot \frac{k_f}{k_r}, \tag{2}$$

$$R_{\text{reverse}}(S) = \frac{AR_0S}{I} \cdot \frac{k_f k_{fa}}{k_r k_{-a} k_{fi}}. \tag{3}$$

**Box 8. R steady states.** Steady state conditions for R as expression of S. General (1), for the forward (2) and reverse (3) reactions. The forward reaction considers R activating species $(A, R_0)$ and reaction rates $(k_f k_{fa})$. The reverse reaction considers R deactivating species $(I, R)$ and reaction rates $(k_{fi} k_r)$

### Irreversable Circular Reaction Pathway

The high incauracy can be explained by several factors. misalignment of DNA Parts and linker lead to failed assembly. Another possible explanation the fact that the concentration of the DNA fragments varied between the groups, which is plausable since there was no step to verify or check for that concentration. Therefore if the concentrations were indeed too low it would lead to insufficient ligation. This is likely what caused the high inacuracy since the variations between the groups also indicates a fluctuating accuracy. Another likely event could be contamination in the reaction mix leading to DNA ligase not digesting the BsaI site, this is however unlikely since most groups had a positive gel electrophoresis signal. Implementing multiple negative controls testing for diferent assebly steps would also optimise the accuracy. Comparing the results to the control, which had a control step for the DNA fragments purity Storch et al., 2015, (Supplemental Material), we can conclude that considering the absense of essential control steps the BASIC assembly method is highly accurate and fast when looking at the control.

### Condition for Full Activation at High S and Low S

To fully active R under steady state conditions, where S is negligable ($S << R(S)$), the balance between activating species and favorable reation rates must be $(R_0 * A * k_f) > (I * K_r)$, so that $R_{forward}(S) > 1$ and S must enable activation at very low treshholds. To fully active R under steady state conditions, where S is very high, the balance between activating and deactivating reaction rates $\frac{k_f k_{fa}}{k_r k_{-a} k_{fi}}$ will dominate the concentration of Active vs. Inactive species $\frac{AR_0S}{I}$. What will inturn drive the activation rate of R. Therefore to achieve full activation at equillibrium the activating rates are favorable only if $\frac{k_f k_{fa}}{k_r k_{-a} k_{fi}} > 1$.

## Literature

No lterature was consulted, all code was taken from my github: https://wwww.github.com/edunseng Ml Model and simulations can be viewed from: https://colab.research.google.com/drive/1Jj8BLVi6-IXPqv54bta5eGCVJjZAFVoR?usp=sharing