Zewail City of Science and Technology

BMS 473 - Foundations of Computational & Sys Biol

Paper:

De novo design of protein logic gates

Undersupervison:

Dr. Mohammad ElGamacy

Mr. Ahmed Hesham

By:

Alaa Mohamed 202101529

Hayam Waleed 202100281

Hanin Gamal 202101061

Nader Alfred 201-901-347

Systems Biology Project Report

1. Summary

Regulating protein function post transcriptionally is a significant challenge in synthetic biology. Moreover, such protein-protein interactions are crucial for cellular decision-making and synthetic biology, though most efforts have focused on DNA, transcription, or RNA-based control. Current protein-based circuits are limited by a small pool of building blocks, restricting scalability of their usage. Hence, in this paper [1], Baker et al. developed biological representations of cooperatively-inducible-protein-heterodimer (CIPHR) logic gates (AND, OR, NAND, NOR, XNOR, NOT) to further expand or scale this kind of protein modifications. The input-output tables of these gates are illustrated in figure 1. Heterdimeric complexes were designed with high affinity, opening the door for different combinations with a spectrum of affinities that can achieve the goal of the gates. This is better visualized in figure 2.

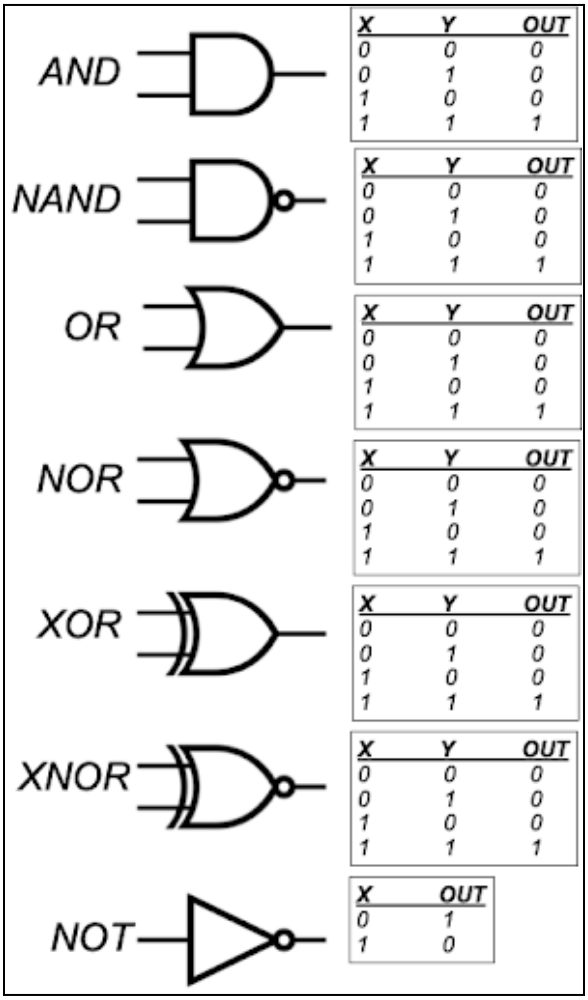


Figure (1): The input-output tables of the CIPHR gates.

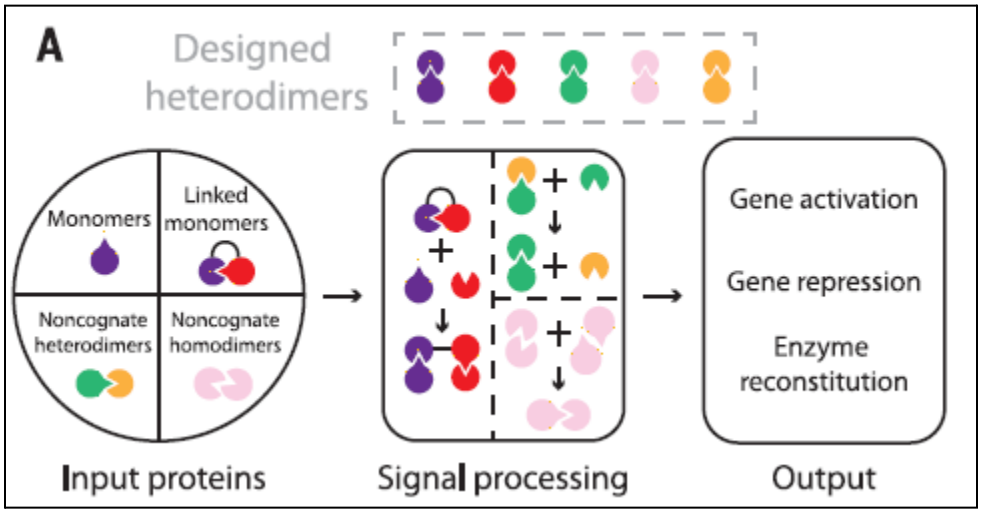


Figure (2): CIPHR gates built from heterodimers with monomers or covalently connected monomers as inputs

For a correct design, building blocks require orthogonality (independent pairs), modularity (similar structures), tunable binding specificities, and cooperativity (insensitivity to stoichiometric imbalances). In this regard, hydrogen bond network-mediated heterodimers in addition to shared topological design satisfy these requirements. Cooperative monomer fusions are engineered to achieve selective gate activation by concealing interaction surfaces, leading to genetic expression / repression. Experiments optimized linker lengths for stability and folding of the dimers, with the lengths of 6-12 residues were found to behave best. Native mass spectrometry was used to assure cooperativity, so the gates complexes showed high cooperativity and insensitivity to stoichiometric imbalances. The validation of these newly designed systems is further explained in the results section. These gates control protein associations (e.g., split enzymes, transcriptional machinery) in various systems, and they were validated in yeast and human T cells, in which TIM3 gene expression, is regulated. This approach allows extensions to more complex logic gates like three-input OR, AND, and disjunctive normal form gates and enables advanced control over biological functions through flexible design methods.

2. Methods

2.1 Creating functions for visualization:

a) "solve_visualize" function: this functions takes the reaction name, initial conditions, and rates and gives the solution solved by odeint and plotted in a graph. It utilizes the "get_y_names" function to extract the names of the species.

b) "Get_y_names" function: this function takes the model and outputs the y components of it, to be used in the "solve_visualize" function.

c) "simulate_condition function": this function is used to plot the bar plot for the 3-in gates, representing the final concentrations of the outputs for each reaction. It takes the initial conditions, the reaction function, the parameters for the reaction, and time. It solves the reactions with Odeint, retrieving the return value from the specified reaction.

2.2 Creating differential models of the CIPHR gates:

This section is intentionally embedded in the results section to better visualize each model according to its equations.

3. Results

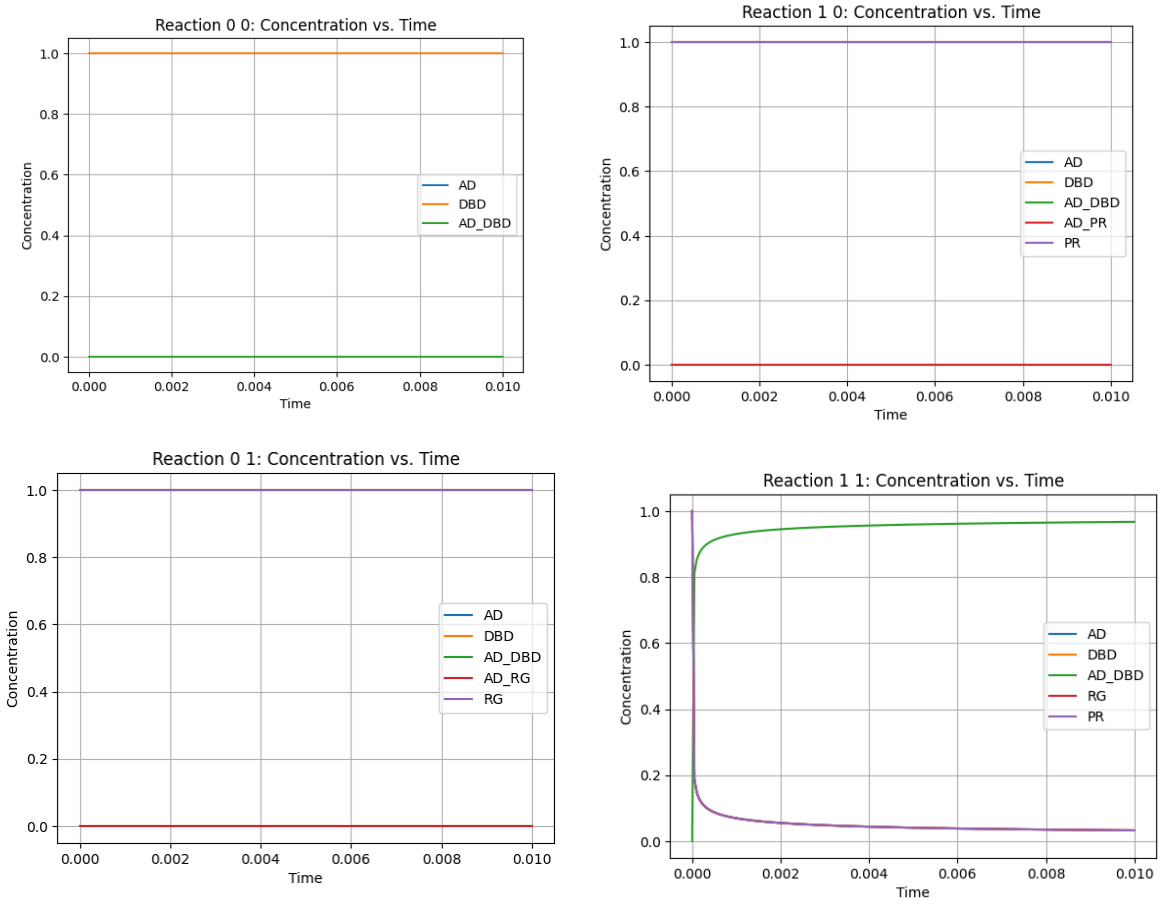
3.1. 2-in AND Gate

In the case of AND gate, the desired input is not active unless the two are present, 1’7 represented in the plot as PR and 6’7 represented in the plot as RG and

The chemical reaction:

AD+DBD ⇌ ADDBD for the case (0 0)
AD+DBD ⇌ ADDBD
AD+P-R' ⇌ ADP-R' (1 0)
AD + DBD ⇌ ADDBD (0 1)
DBD + R-G' ⇌ DBDR-G'
AD + DBD + R-G'+P-R' ⇌ AD_PRRG_DBD(1 10

Outputs:



Figure(3): AND gate reactions species concentration over time

3.2. 2-in OR Gate

The gate was designed by 1-6 fusion to the AD and 7′ to the DBD. The expression of any of these linkers activates the association between AD and DBD and so it affects the growth**.**

Rate equations:

AD + DBD ⇌ AD_DBD (0,0)

AD + DBD + A ⇌ AD-I1-DBD (1,0)

AD + DBD + B ⇌ AD-I2-DBD (0,1)

AD + DBD + A + B ⇌ DBD-L-AD-L-DBD (1,1)

A=1'-7 B=6'-7

Output:

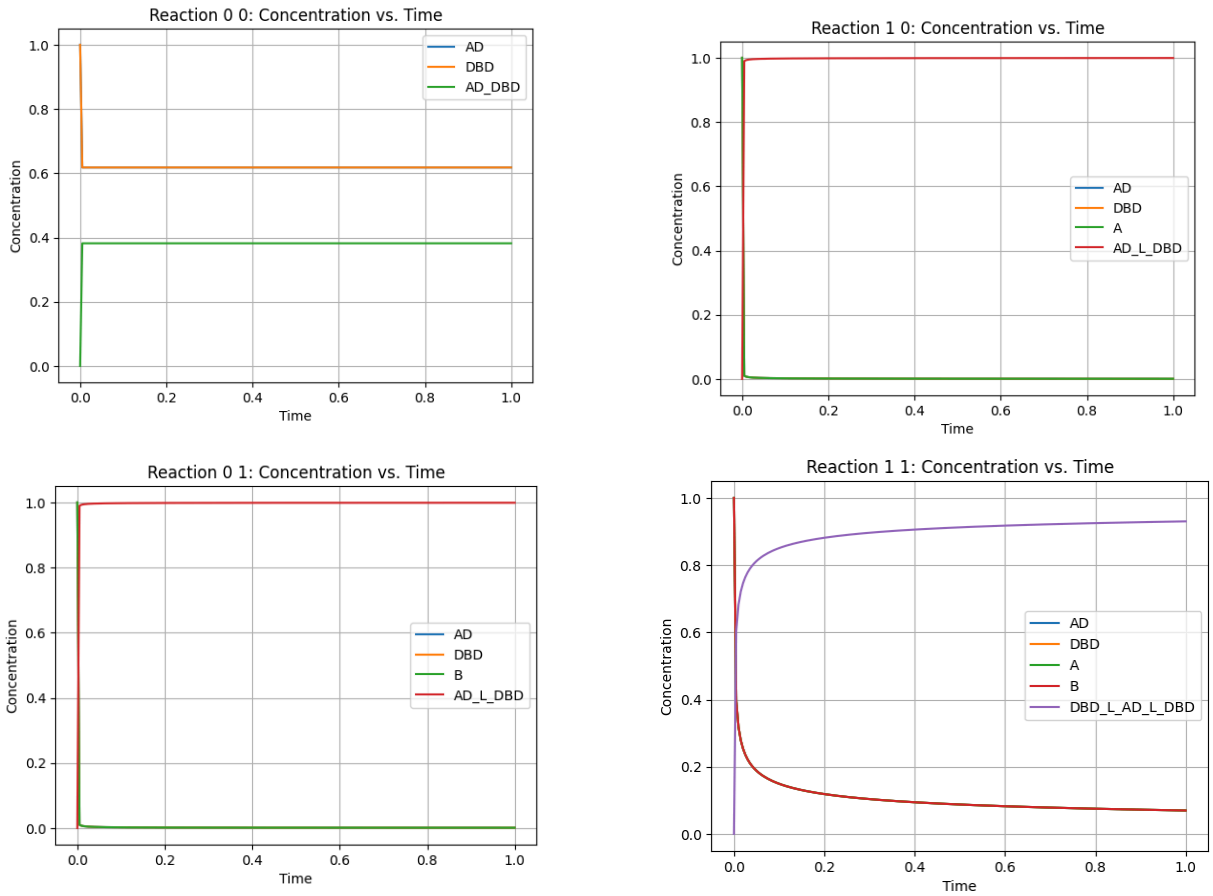


Figure (4): The figure represents the four cases of the 2 input OR gate and shows the relationship between time and concentration.

3.3. 2-in NOT Gate

In the NOT gate the AD_DBD complex is fused with a homodimer 2I where the affinity of forming ADI or DBDI is higher than the affinity for AD_DBD. Therefore in the presence of the homodimer the output formation is ADI and DBDI as shown in the following chemical reaction.

The Chemical Reaction:

AD_DBD + 2I ⇌ ADI + DBDI

Assuming that the k forward is 1e3 and k reverse 1e-3.

Output:

After solving the model numerically using Odeint, the graph showing the behaviour of the system was plotted as shown in figure (5).

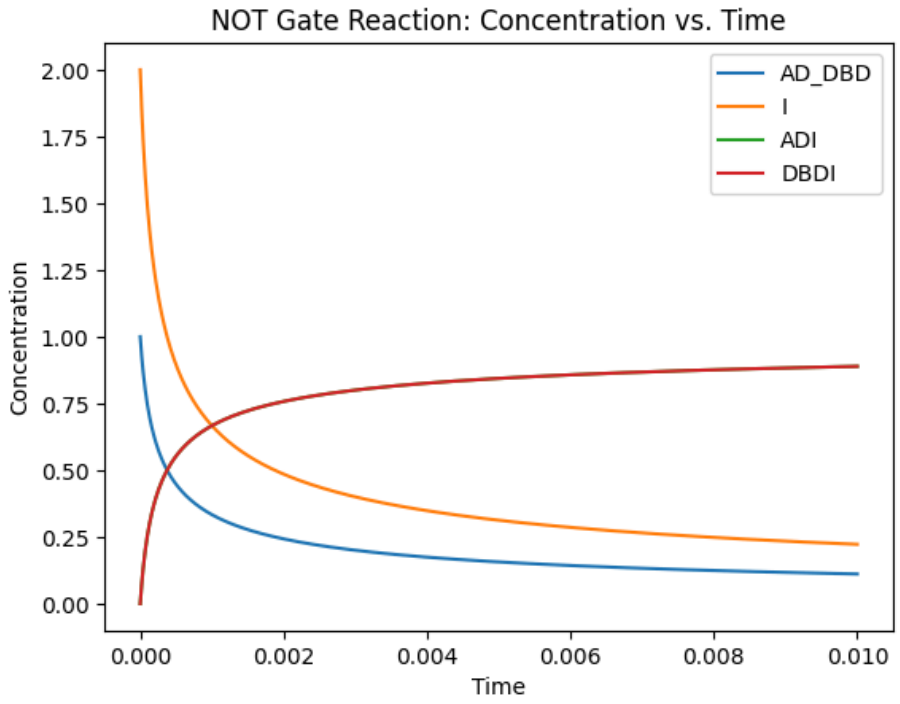


Figure (5): The dynamics of the system on NOT gate

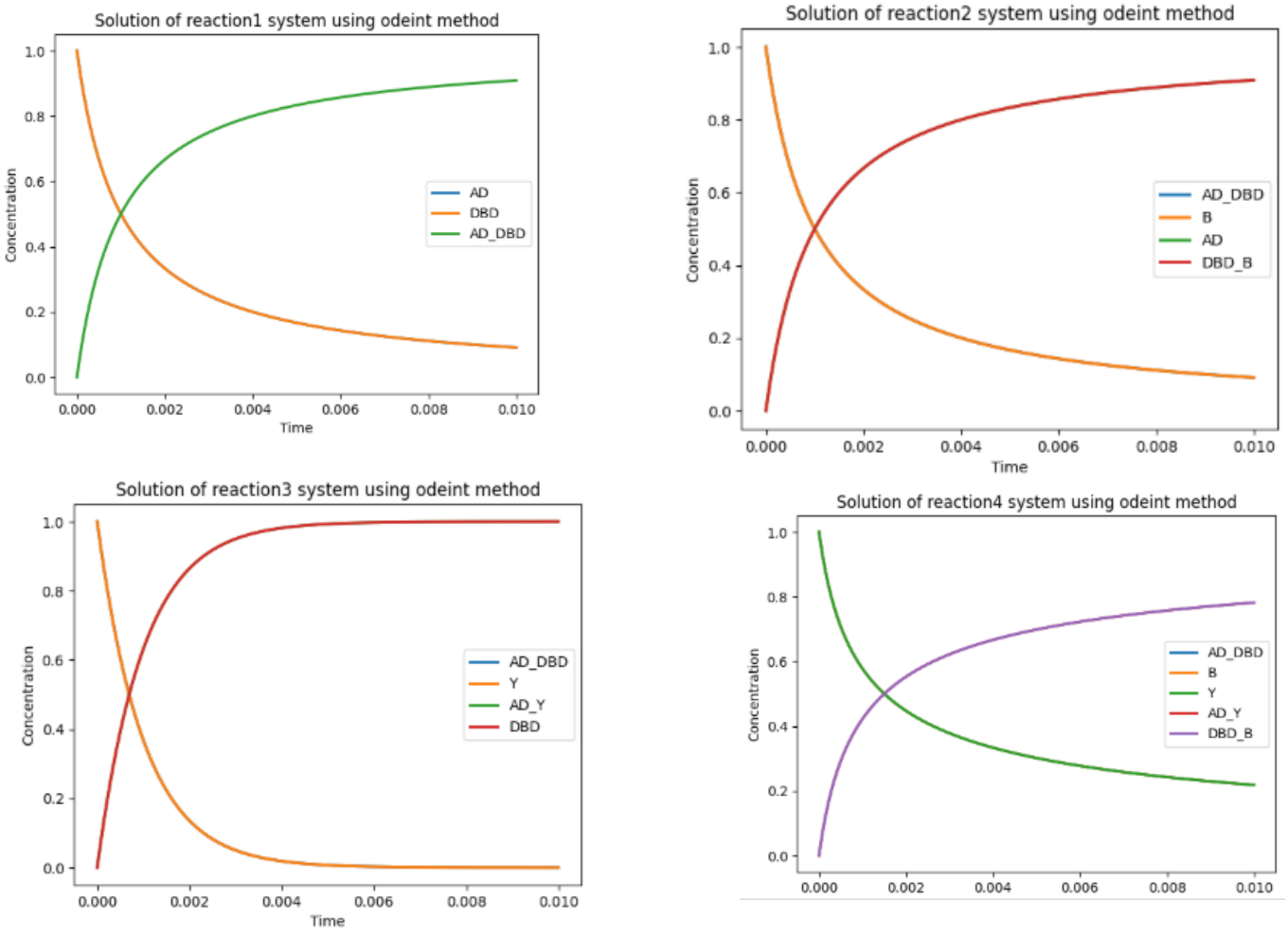
3.4. 2-in NOR Gate

For the NOR gate, the complex AD_DBD is considered as an input. A homolog structure for AD is I1 (B: blue) and another homolog structure for DBD is I2 (Y: Yellow). The base case (0, 0) is when there is no inputs other than the AD_DBD complex itself. The (1, 0) case is when I1 exists as an input with AD_DBD, the (0, 1) case is when I2 exists as an input with AD_DBD, and for (1, 1) the I1, I2, AD_DBD. Giving the affinity for DBD_B > AD_Y > AD_DBD.

The Chemical Reactions:

1) AD + DBD ⇌ AD_DBD
2) AD_DBD + B ⇌ AD + DBD_B
3) AD_DBD + Y ⇌ AD_Y + DBD
4) AD_DBD + B + Y ⇌ AD_Y + DBD_B

Output:



Figure(6): NORgate reactions species concentration over time

3.5. 2-in XNOR Gate

For the XNOR gate, the complex AD_DBD is considered as an input. A homolog structure for AD is I1 and another homolog structure for DBD is I2. The base case (0, 0) is when there is no I1 OR I2 as inputs, the (1, 0) case is when I1 exists as an input with AD_DBD, the (0, 1) case is when I2 exists as an input with AD_DBD, and for (1, 1) the I1, I2, AD_DBD. Giving the affinity for I1_I2 > ADI2, DBDI2 > AD_DBD.

The Chemical Reactions:

5) AD_DBD ⇌ AD_DBD
6) AD_DBD + I1 ⇌ AD + DBD_I1
7) AD_DBD + I2 ⇌ AD_I2 + DBD
8) AD_DBD + I1 + I2 ⇌ AD_DBD + I1_I2

Output:

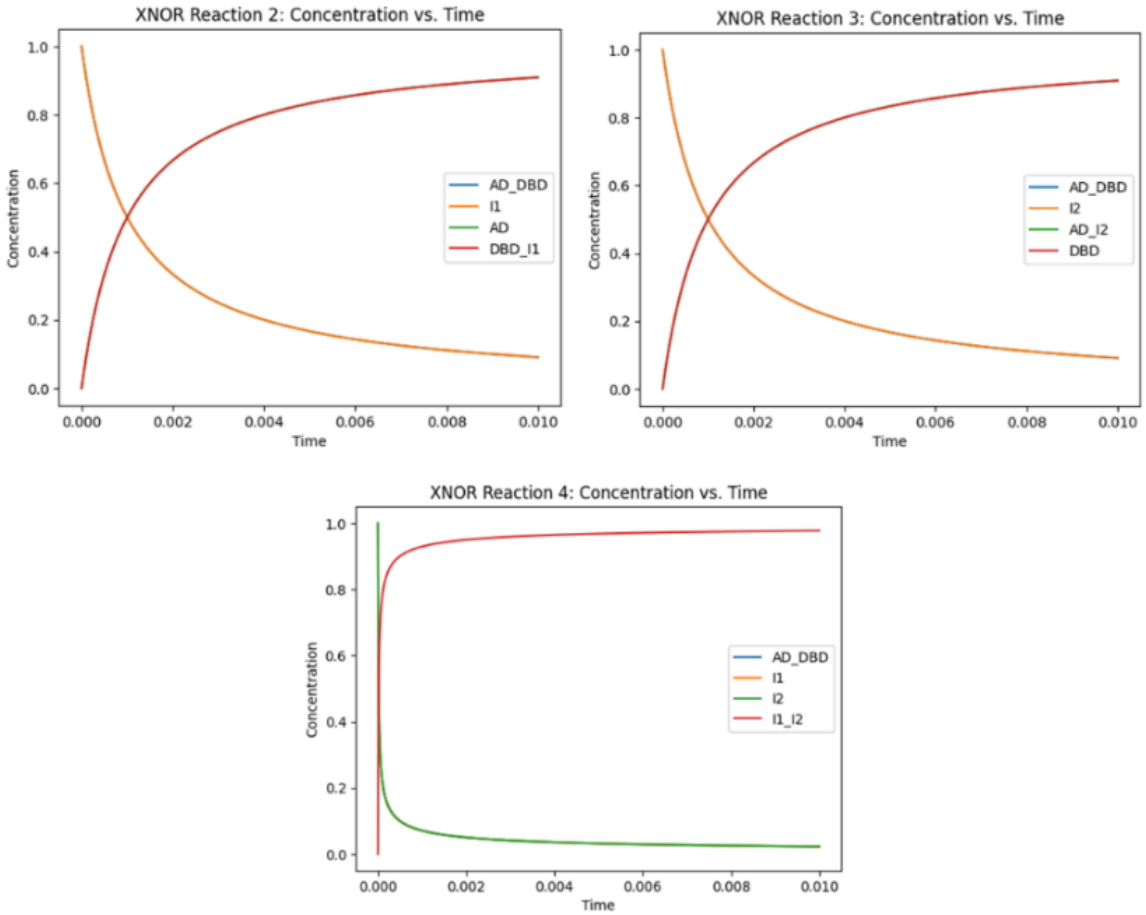


Figure (7): The dynamics of the system for the XNOR gate for reactions 2, 3, and 4.

3.6. 2-in NAND Gate

Except when both inputs are 1, NAND gates produce the desired output. When both are present, the affinity of the two competitive protein binders 1 10 (G B) is higher than that of the AD-DBD complex. Based on that when the 1 and 10 are present, the reaction of ADG and DBDB formation is more favorable.

The chemical reaction:

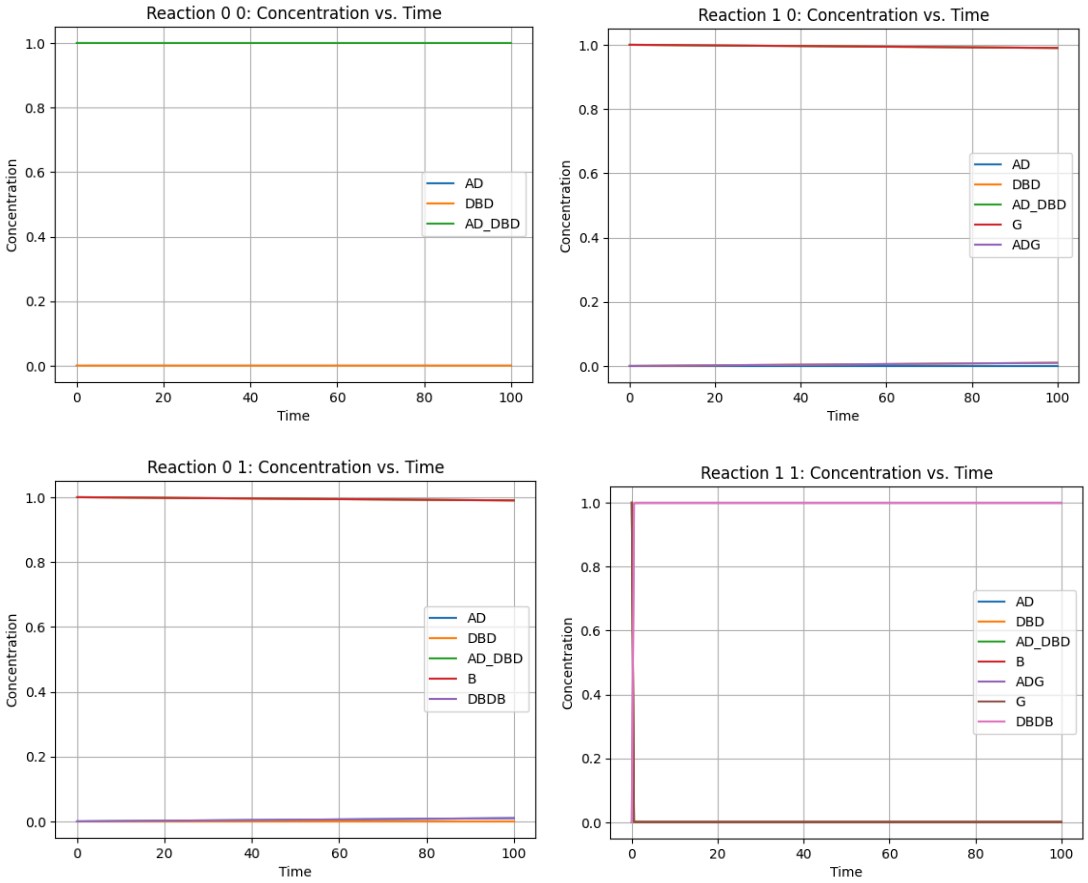
ADDBD ⇌ AD + DBD (0 0)

ADDBD ⇌ AD + DBD (1 0)
AD + G ⇌ ADG

ADDBD ⇌ AD + DBD (0 1)
DBD + B ⇌ DBDB

ADDBD = AD + DBD (1 1)
AD + G ⇌ ADG
DBD + B ⇌ DBDB

Outputs:



Figure(8): NAND gate reactions species concentration over time

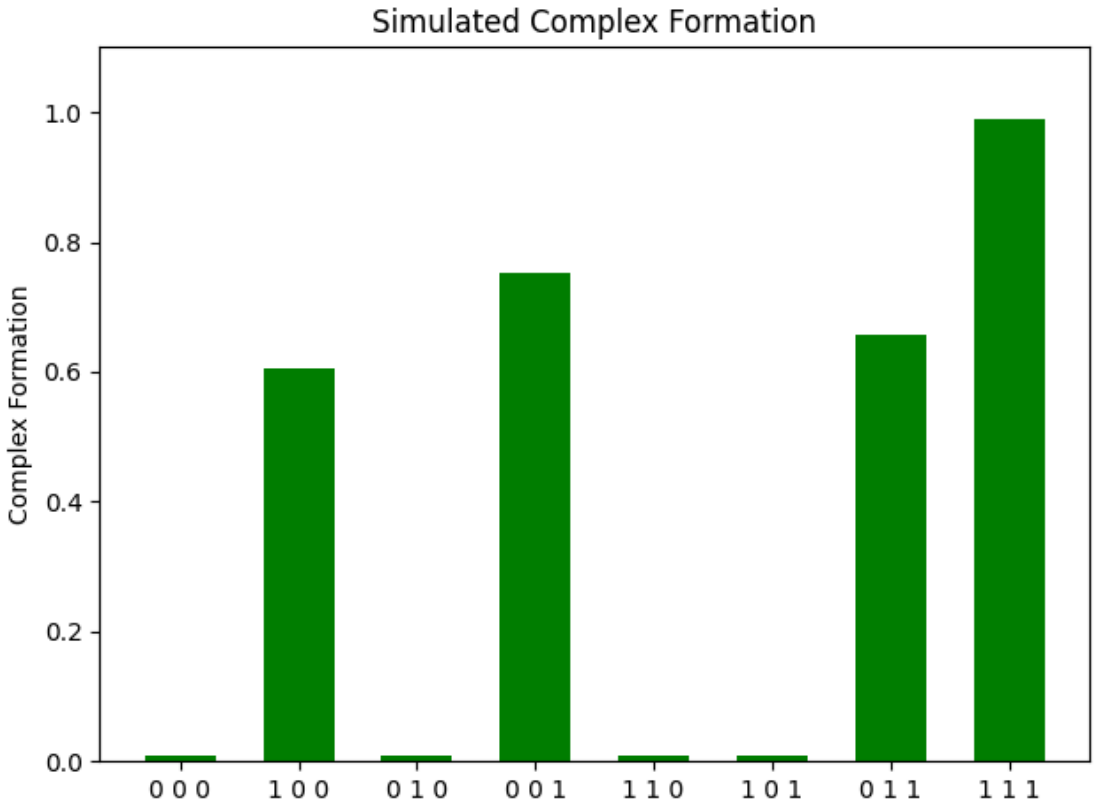
3.7. 3-in AND

And gates are not working unless all the outputs are present, but in the case of the 3 in AND protein logic gates the case is not that restricted because the affinities of some other combinations in this case may encourage the product formation. However, the most favorable reaction is when all the input dimers are present (1'4(GY), 4'3(YR), 3 2' (RP)),

Reactions:

AD+DBD ⇌ ADDBD - - -
AD+DBD+GY ⇌ AD_GY_DBD + - -
AD+DBD+YR ⇌ AD_GY_DBD - + -
AD+DBD+ RP ⇌ AD_RP_DBD - - +
AD+DBD+ YR+ GY ⇌ AD_RPGY_DBD + + -
AD+DBD+ RP+ GY ⇌ AD_RPGY_DBD + - +
AD+DBD+ RP+ YR ⇌ AD_RPYR_DBD - + +
AD+DBD+ RP+ GY + YR ⇌ AD_RPGY_DBD + + +

Output



Figure(9): Bar plot representation of the time 100 AD-DBD complex concentration for the 8 possibilities of the AND gate

3.8. 3-in OR:

To test the 3 inputs inside the cell, A 3 OR gate was designed by fusing 1′-6-7 to AD and 11′ to DBD. The three inputs were (11-1, 11-6′, or 11-7′) and any of them could associate AD to DBD via 1′, 6, or 7. The results were confirmed by measuring the Y2H.

Chemical equations:

AD + DBD ⇌ AD_DBD (-,-,-)

AD + DBD + A ⇌ AD_L_DBD (+,-,-)

AD + DBD + B ⇌ AD_L_DBD (-,+,-)

AD + DBD + C ⇌ AD_L_DBD (-,-,+)

AD + DBD + A+B ⇌ AD_L_DBD (+,+,-)

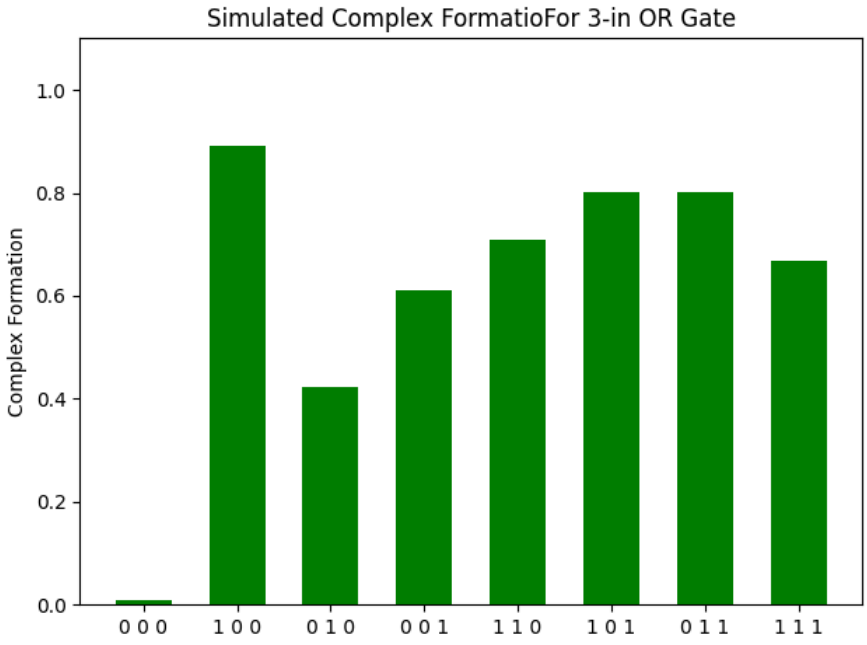
AD + DBD + A + C ⇌ AD_L_DBD (+,-,+)

AD + DBD + B + C ⇌ AD_L_DBD (-,+,+)

AD + DBD + A+B + C ⇌ AD_L_DBD (+,+,+)

A=11-1' B=11-6' C=11-7'

Output:



Figure(10): Bar plot representation of the time 100 AD-DBD complex concentration for the 8 possibilities of the 3-OR gate

3.9. 3-in DNF

The DNF gate is a combination of AND and OR gates assuming the names of the inputs A and B for the AND gate, and D is the second input for the OR gate. Based on the literature[2], the ranges for k forward and k reverse for protein-protein interaction. Consider the representation of the growth for each scenario an indication for the higher and lower bindings of AD_DBD with the used linkers. Therefore, this could be represented by controlling the k-forward values for each reaction by multiplication with a factor for representing the different effects in each case.

The chemical equations:

AD + DBD ⇌ AD_DBD (-,-,-)

AD + DBD + A ⇌ AD_A_DBD (+,-,-)

AD + DBD + B ⇌ AD_B_DBD (-,+,-)

AD + DBD + D ⇌ AD_D_DBD (-,-,+)

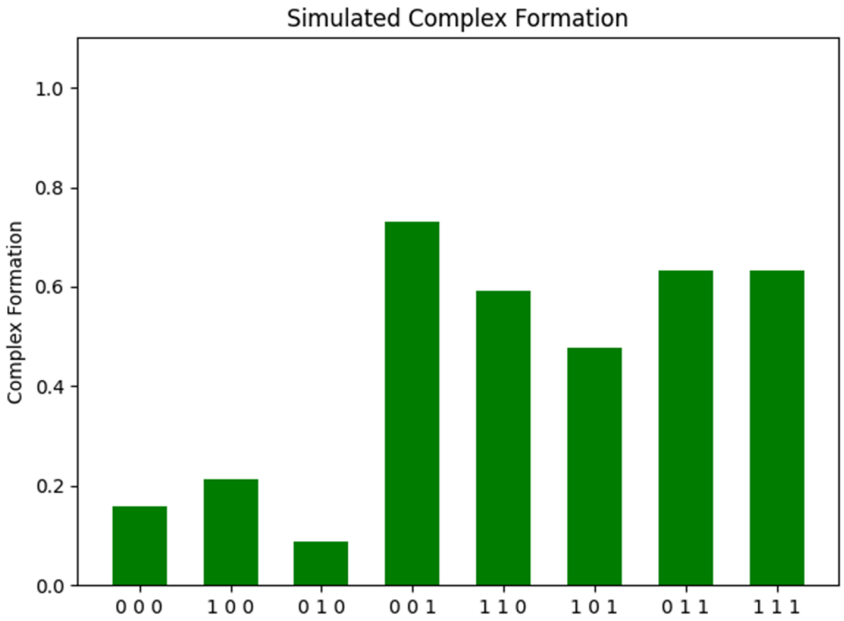
AD + DBD + A_B ⇌ AD_A_B_DBD (+,+,-)

AD + DBD + A + D ⇌ AD_A_D_DBD (+,-,+)

AD + DBD + B + D ⇌ AD_B_D_DBD (-,+,+)

AD + DBD + A_B + D ⇌ AD_A_B_D_DBD (+,+,+)

The Output:



Figure(11): Bar plot representation of the time 100 AD-DBD complex concentration for the 8 possibilities of the DNF gate

4. Conclusion

This study addresses the challenge of post-transcriptional protein regulation in synthetic biology by developing scalable, cooperative-inducible-protein-heterodimer (CIPHR) logic gates (AND, OR, NAND, NOR, XNOR, NOT). Using de novo-designed heterodimers with high affinity, modularity, and cooperativity, these gates enable precise control of protein interactions and gene expression. Validated in yeast and human T cells, this approach expands the potential for advanced biological regulation with applications in complex logic systems. In this project, we were able to simulate the key findings of the paper. However, future work could focus on developing a more comprehensive simulation by unifying the gate functions on just one model with predefined K-values corresponding to the different affinities. These changes promise a more robust simulation that is more generalizable and easier for future investigation.

References

[1] Z. Chen et al., “De novo design of protein logic gates,” Science, vol. 368, no. 6486, pp. 78–84, Apr. 2020, doi: https://doi.org/10.1126/science.aay2790.

[2] Author links open overlay panelStefano Gianni 1 et al., “How fast is protein–ligand association?,” Trends in Biochemical Sciences, https://www.sciencedirect.com/science/article/pii/S0968000417301603