PGIS RESCON RESEARCH CONGRESS 2022

MATHEMATICAL MODELING OF CELL-FREE PROTEIN SYNTHESIS

Presented By: M.A.A. Aqeel
Supervised By: Dr. S.P. Rajapaksha

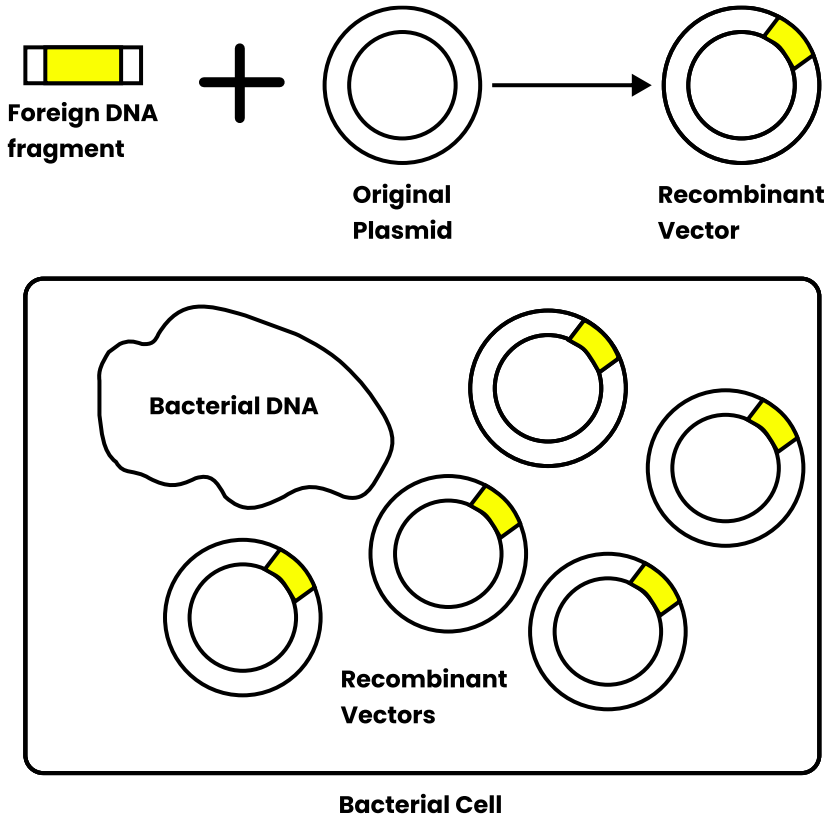
University of Sri Jayewardenepura

INTRODUCTION

INTRODUCTION TO CELL-FREE PROTEIN SYNTHESIS

Genetic Engineering

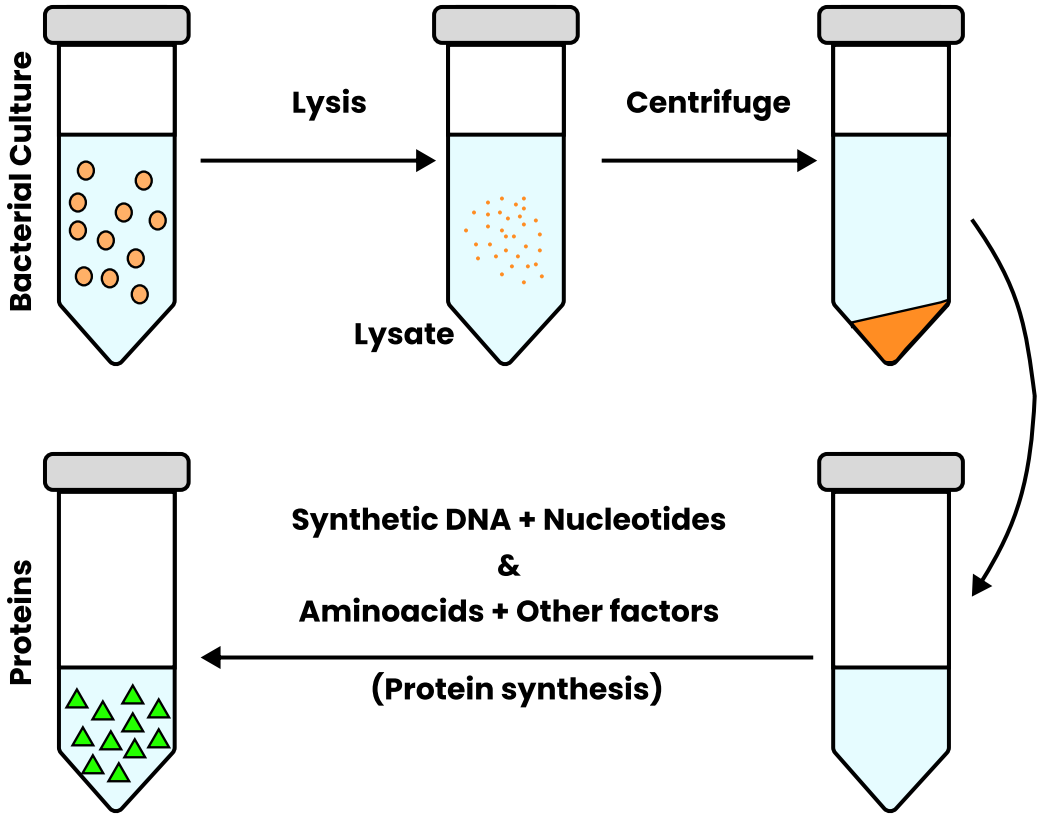
- Most therapeutic proteins are difficult to manufacture with available synthetic capabilities
- Microbial cultures can be utilized through *genetic engineering* to produce such complex molecules
- However, the product yield will be changed according to the nutritional and environmental conditions confronted by cells/culture



INTRODUCTION TO CELL-FREE PROTEIN SYNTHESIS

Cell-free protein synthesis (CFPS)

- Extracting and assembling the necessary molecular components of protein synthesis into a vessel can avoid confounding results associated with cell-based systems
- Executing DNA programs in a cell-free format can open new avenues for biomanufacturing and metabolic engineering



INTRODUCTION TO CELL-FREE PROTEIN SYNTHESIS

Applications of CFPS

- Monoclonal antibody production
- Vaccines
- Analyzing metabolic and genetic disorders
- Synthesis of non-canonical proteins
- Synthesis of niche drugs
- Membrane proteins
- Prototyping metabolic circuits
- Antimicrobial peptides

Limitations of CFPS

- Cost of synthesizing desired DNA template
- Resource exhaustion
- Inherent ribonuclease and protease activity
- Short active duration
- Small batch size

OBJECTIVES

General Objective

- To develop a minimalistic mathematical model that grasps the basic mechanics of protein synthesis in CFPS systems

Specific Objectives

- To understand the CFPS system towards DNA template loading capacity
- To understand the model behavior towards the consumption of biological nutrients
- To check the influence of inherent factors of the extract on active duration
- To provide better designs for CFPS Design-Build-Test-Learn (CFPS-DBTL) workflow

MODEL DEVELOPMENT

MODEL DEVELOPMENT

STEP 1

Literature survey on cell-free protein synthesis

OPEN

High-yield production of "difficult-to-express" proteins in a continuous exchange cell-free system based on CHO cell lysates

Received: 13 January 2017
Accepted: 6 September 2017
Published online: 15 September 2017

Lena Thoring[1,2], Srujan K. Dondapati[1], Marlitt Stech[1], Doreen A. Wüstenhagen[1] & Stefan Kubick[1]

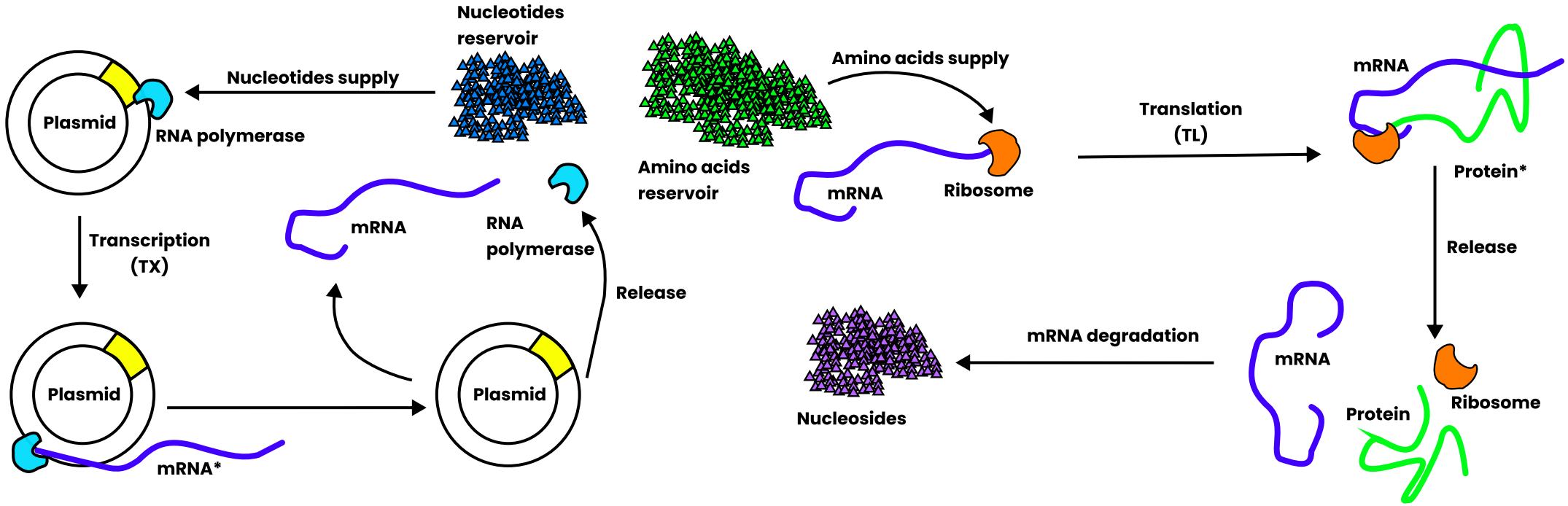
Quantitative modeling of transcription and translation of an all-*E. coli* cell-free system

Ryan Marshall & Vincent Noireaux

MODEL DEVELOPMENT

STEP 2

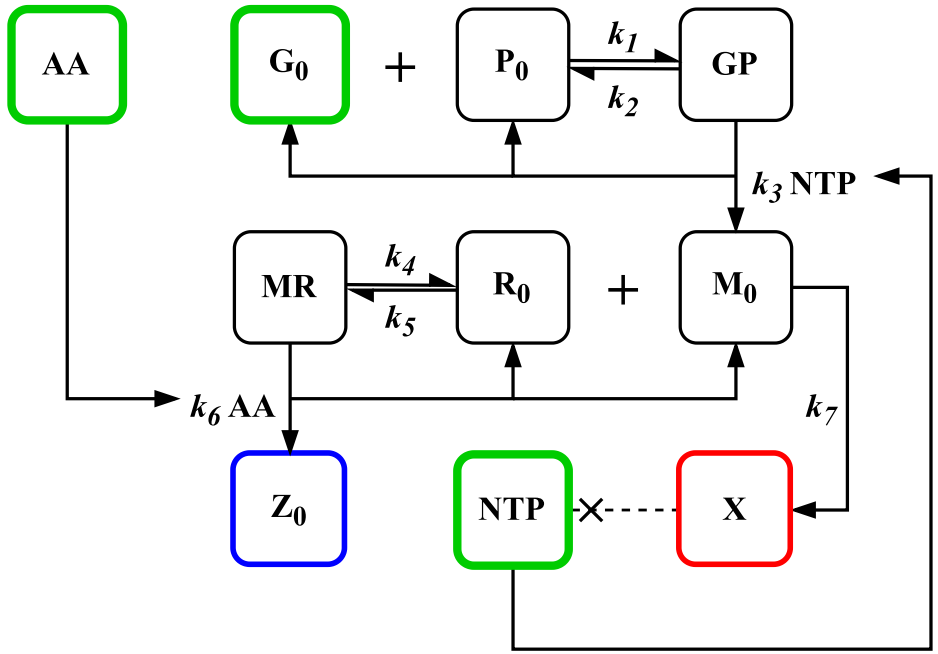
Identify the minimal molecular components required for protein synthesis



MODEL DEVELOPMENT

STEP 3

Construct the chemical reaction network based on literature findings



G_0 – plasmid

P_0 – RNA polymerase (RNAP)

GP – RNAP-plasmid complex

M_0 – mRNA

R_0 – ribosome

MR – mRNA-ribosome complex

NTP – nucleotide reservoir

AA – amino acids reservoir

Z_0 – reporter protein

k1 – RNAP-promoter association

k2 – RNAP-promoter dissociation

k3 – mRNA production

k4 – mRNA-ribosome association

k5 – mRNA-ribosome dissociation

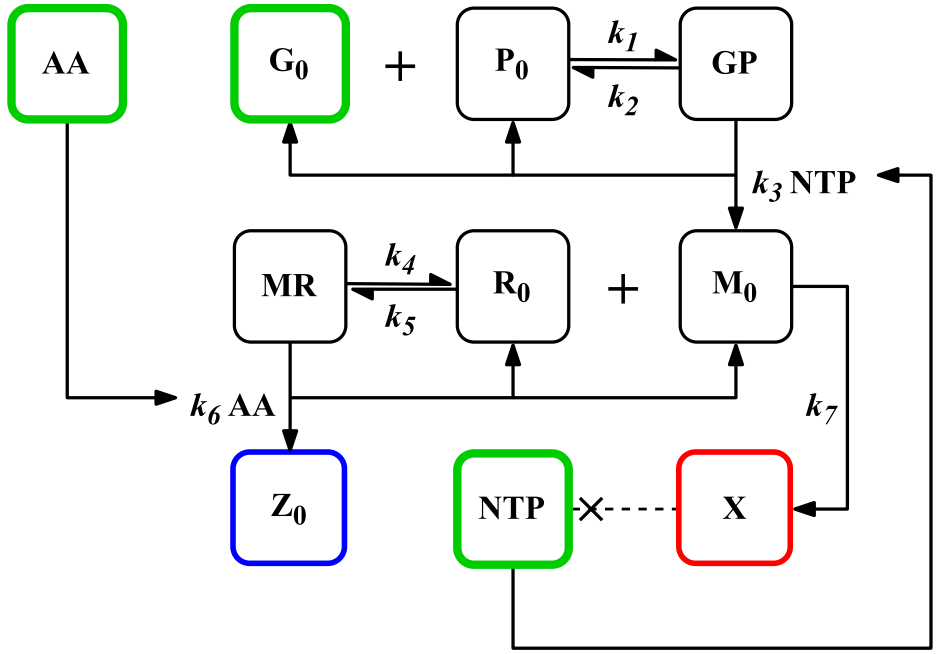
k6 – protein production

k7 – mRNA degradation

MODEL DEVELOPMENT

STEP 4

Develop mass action-based equations for each molecular component



Differential Equations	
$[\dot{G}_0] = -k_1[G_0][P_0] + k_2[GP] + k_3[NTP][GP]$	
$[\dot{P}_0] = -k_1[G_0][P_0] + k_2[GP] + k_3[NTP][GP]$	
$[\dot{GP}] = k_1[G_0][P_0] - k_2[GP] - k_3[NTP][GP]$	
$[\dot{M}_0] = k_3[NTP][GP] - k_m[M_0] - k_4[M_0][R_0] + k_5[MR] + k_6[AA][MR]$	
$[\dot{R}_0] = -k_4[M_0][R_0] + k_5[MR] + k_6[AA][MR]$	
$[\dot{MR}] = k_4[M_0][R_0] - k_5[MR] - k_6[AA][MR]$	
$[\dot{NTP}] = -k_3[NTP][GP]$	
$[\dot{AA}] = -k_6[AA][MR]$	$[\dot{Z}_0] = k_6[AA][MR]$

MODEL DEVELOPMENT

STEP 5

Carry out simulation for a collection of time points using a computational software (*R* and the package *deSolve*)

Initial Concentrations			Rate constants		Other parameters
G_0 – 1 nmol l^{-1}	P_0 – 1 nmol l^{-1}		k1 – 6 x 10^9 mol^{-1} l min^{-1}	k2 – 600 min^{-1}	Simulation time – 30 min
R_0 – 1 nmol l^{-1}	NTP – 1 nmol l^{-1}		k3 – 1 x 10^9 mol^{-1} l min^{-1}	k5 – 135 min^{-1}	Numerical integrator – BDF
AA – 1 nmol l^{-1}	GP – 0	M_0 – 0	k4 – 6 x 10^9 mol^{-1} l min^{-1}	k7 – 18 min^{-1}	Time step – 0.001 min^{-1}
MR – 0	Z_0 – 0		k6 – 1 x 10^{10} mol^{-1} l min^{-1}		Test gene – β galactosidase

Code Availability

https://github.com/zachariah-ibrahim/cell-free-protein-expression

Parameter availability

Kierzek, A. M., Zaim, J., & Zielenkiewicz, P. (2001). The effect of transcription and translation initiation frequencies on the stochastic fluctuations in prokaryotic gene expression. The Journal of Biological Chemistry, 276(11), 8165–8172. doi:10.1074/jbc.M006264200

RESULTS & DISCUSSION

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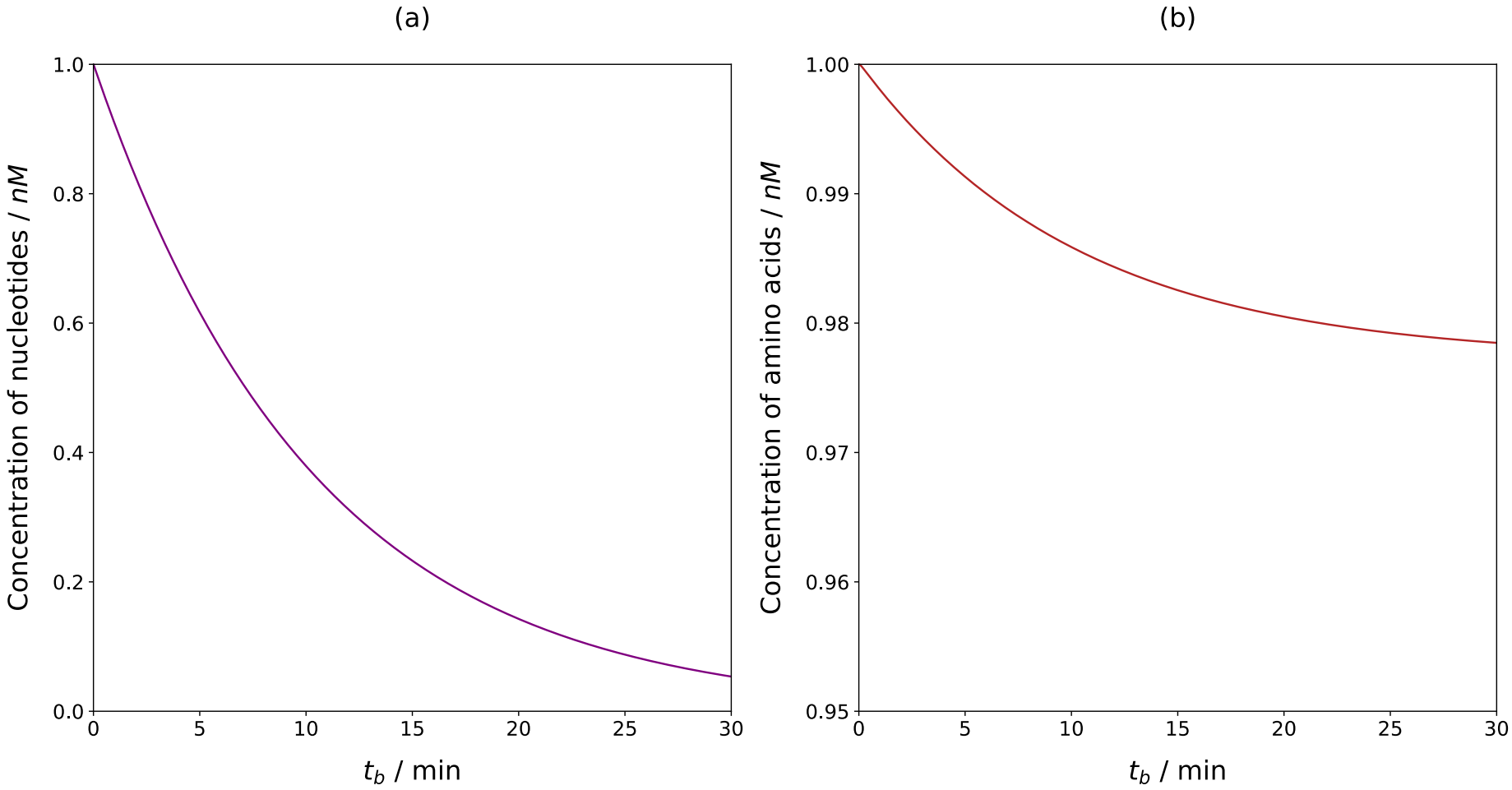
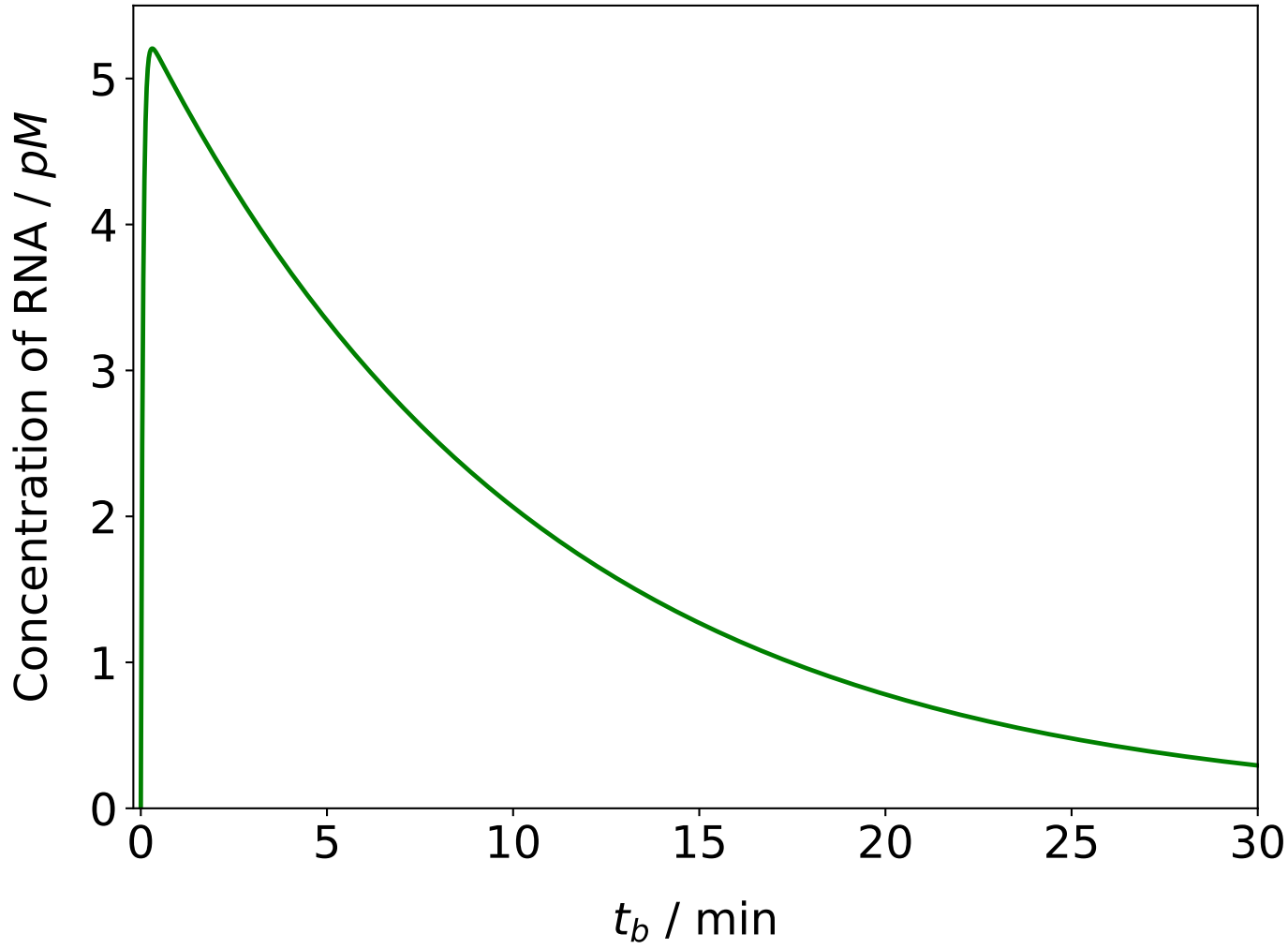


a) **The consumption of NTP is do not correlate with mRNA production**

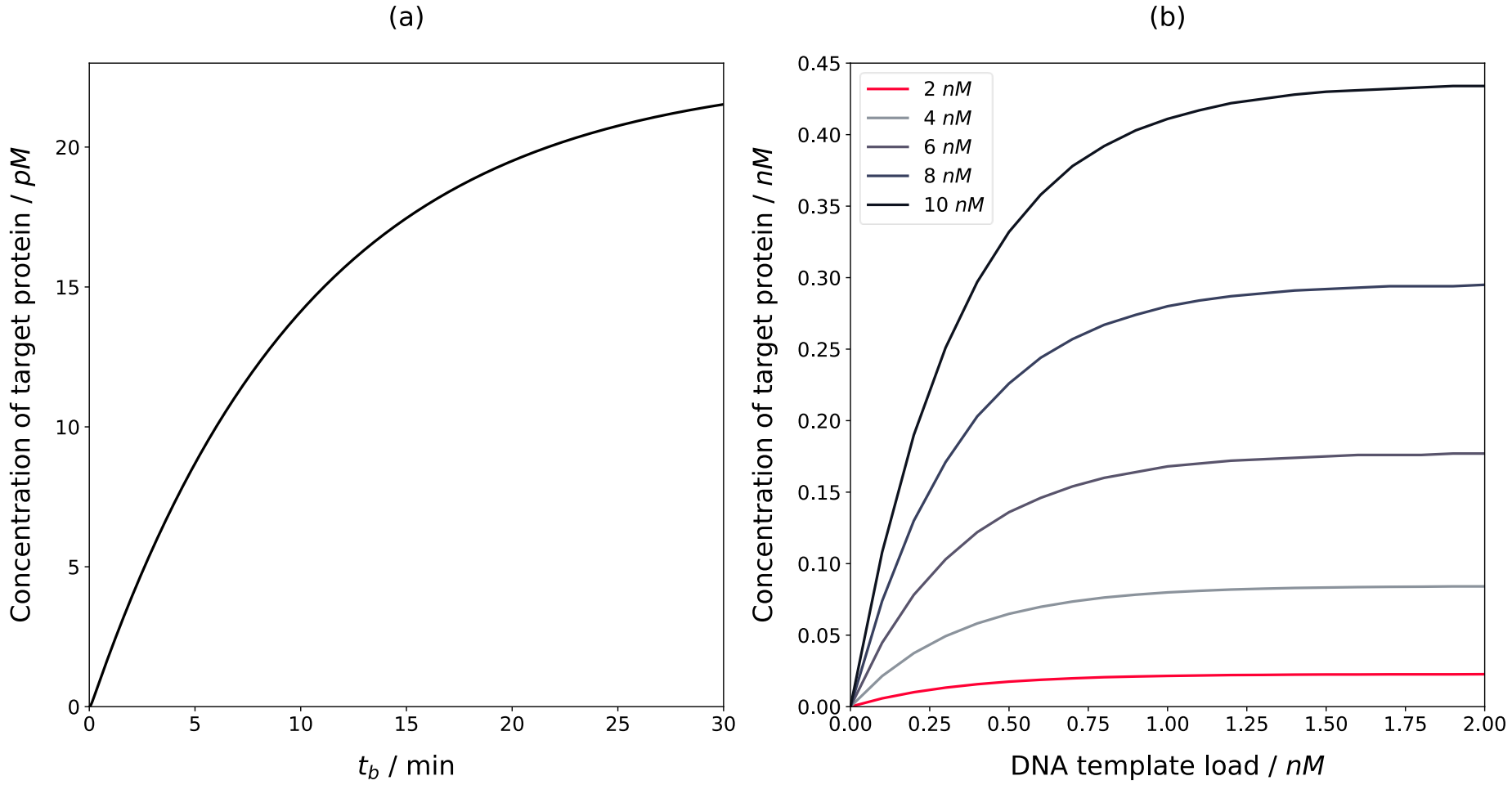
b) **In contrast, the amino acids consumption correlate with protein production**

RESULTS & DISCUSSION

- **The mRNA concentration goes through a maxima and decays exponentially**



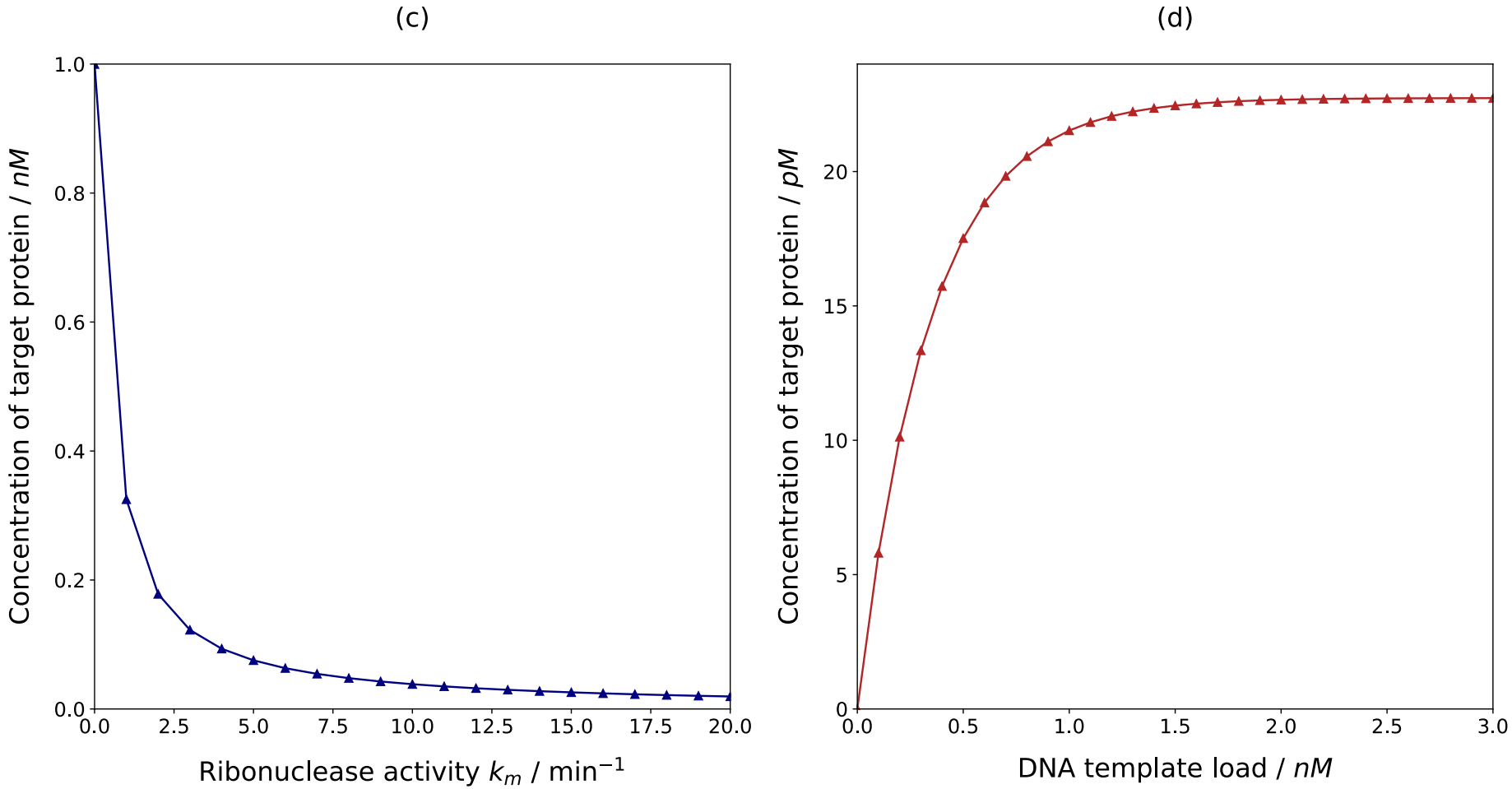
RESULTS & DISCUSSION



a) **For the reaction time of 30 min, the maximum concentration of reporter protein that can be obtained is 21 pM**

b) **Increasing the biochemical resources available to CFPS system can increase the protein yield from 21 pM to 0.4 nM**

RESULTS & DISCUSSION



c) Suppressing the ribonuclease activity can improve the target protein yield significantly

d) The DNA loading indicates that the yield increases linearly and plateaus after a critical DNA concentration of 2.0 nM

CONCLUSION

- The CFPS system require constant supply of resources to perpetuate
- Inheriting detrimental components during extract preparation affects the performance of CFPS systems
- In the absence of detrimental activities, protein yield increases proportionally with the resources supplied
- The simulation studies will be useful for the communities that are involved in gene circuit engineering and biomanufacturing

REFERENCES

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THANK YOU