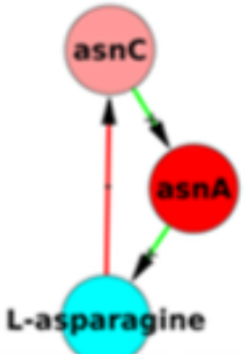
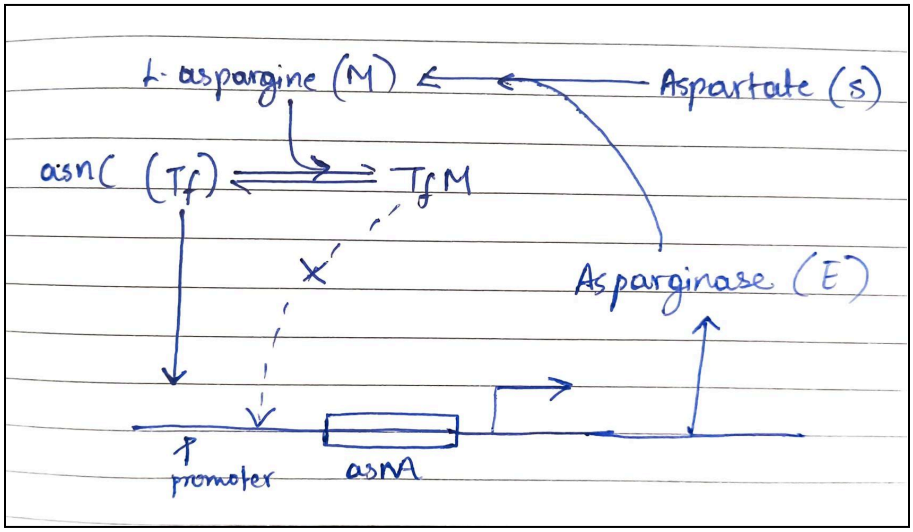
Mathematical modeling of motifs

Regulatory network

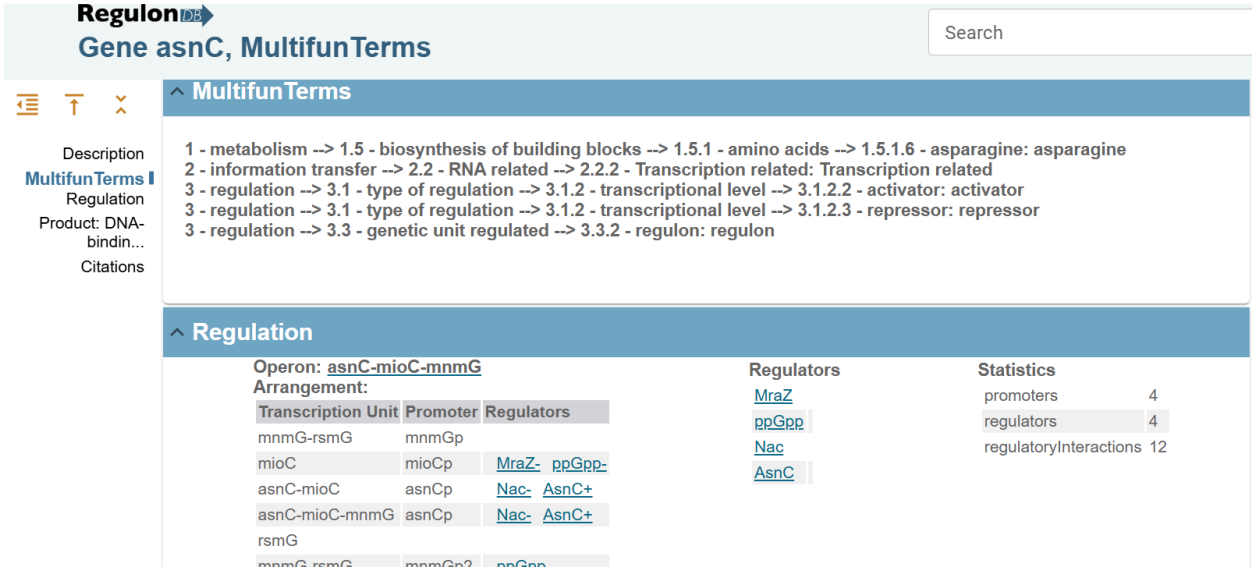


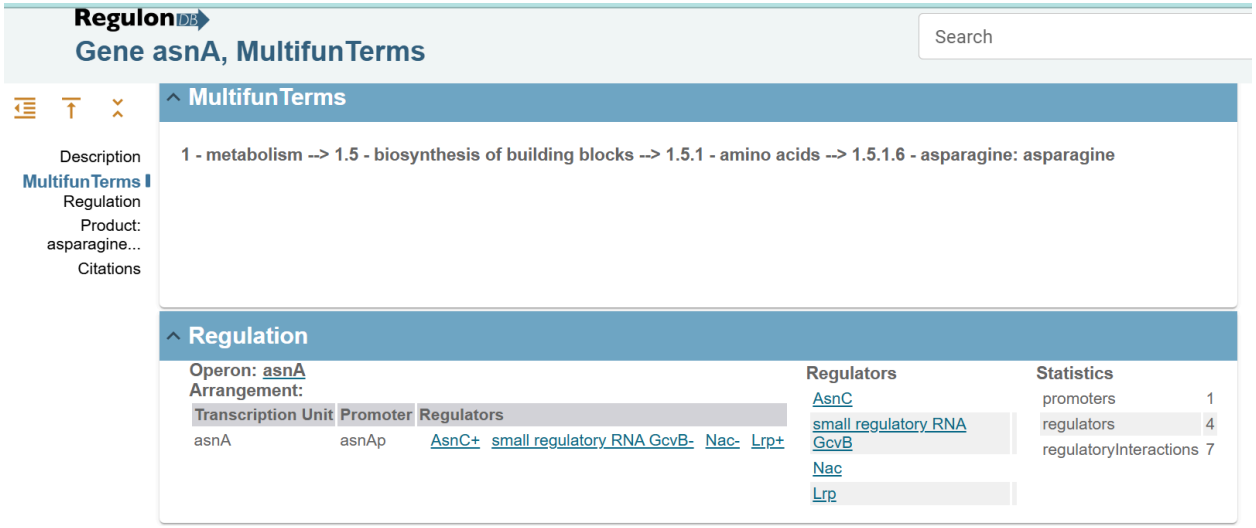
Source: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0203311#sec025

In the E. coli system, the transcription factor asnC (Tf) activates the asnA promoter, driving the production of the enzyme asparaginase (E). Asparaginase catalyzes the conversion of the substrate aspartate (S) into the metabolite L-asparagine (M). The produced L-asparagine binds to the transcription factor asnC, forming a complex (asnC-asparagine, TfM). This complex loses its ability to activate the asnA promoter, effectively downregulating the production of asparaginase in response to increased levels of L-asparagine. This forms a negative feedback loop, where the accumulation of the metabolite L-asparagine self-regulates its production by inhibiting further enzyme synthesis (AsnC: an autogenously regulated activator of asparagine synthetase A transcription in Escherichia coli).



AsnC is a transcriptional regulator that activates the expression of *asnA*, a gene involved in the synthesis of asparagine Kolling R., et al. 1985[4], de Wind N., et al. 1985[11], However, when asparagine is present the activation of *asnA* is turned off. On the other hand, AsnC is negatively autoregulated, but the presence of asparagine does not affect this regulation Kolling R., et al. 1985[4],





Both the genes asnC and asnA work to build the amino acid - asparagine.

Gensor Unit AsnC

Summary:

Molecular Biology Level:

- **Detailed:** In the absence of L-asparagine, AsnC negatively regulates its own expression and the expression of genes required for tRNA methylation and positively regulates the expression of genes required for L-asparagine biosynthesis. Under these conditions, AsnC remains unmodified, binds its target sites preventing its own expression and the expression of some of its regulated genes and at other promoters enabling the expression of its regulated gene.
- **General:** In the absence of L-asparagine, AsnC negatively regulates the expression of genes needed for tRNA methylation and positively regulates the expression of genes necessary for L-asparagine biosynthesis.

Physiology Level:

- **Detailed:** The presence of L-asparagine induces the expression of genes involved with tRNA methylation and inhibits the expression of genes required for L-asparagine biosynthesis. Under this condition the genes necessary for modification of tRNA uridine to tRNA methyl uridine are expressed, and the genes necessary for conversion of L-aspartate to L-asparagine are not expressed.
- **General:** The presence of L-asparagine induces the expression of genes needful for modification of uridine tRNA to methyl uridine tRNA, and inhibits the expression of genes required for conversion of L-aspartate to L-asparagine.

Functional Groups: Energy production and conversion, Amino acid transport and metabolism, Translation, ribosomal structure and biogenesis, Transcription

Source: https://regulondb.ccg.unam.mx/gu/RDBECOLITFC00002

The role of **AsnC**, a regulatory protein involved in asparagine metabolism, is context-dependent, with its effects determined by the presence or absence of **L-asparagine**.

In the absence of L-asparagine, AsnC remains unmodified and binds to its target sites on the DNA. This binding prevents transcription of its own gene and genes required for tRNA methylation. At promoters of biosynthetic genes, AsnC binds in a way that enables transcription, allowing the synthesis of enzymes required to produce L-asparagine.

The presence of L-asparagine alters AsnC's behavior, likely by a conformational change upon binding L-asparagine. This prevents AsnC from binding to biosynthetic gene promoters, thereby inhibiting their expression. L-asparagine induces the expression of genes for tRNA methylation and suppresses genes required for L-asparagine biosynthesis.

Conformations

Type	Class	Name
regulatoryComplex	active	DNA-binding transcriptional dual regulator AsnC
regulatoryComplex	inactive	AsnC-L-asparagine

Source: https://regulondb.ccg.unam.mx/regulon/RDBECOLITFC00002

Kinetic equations/ODEs

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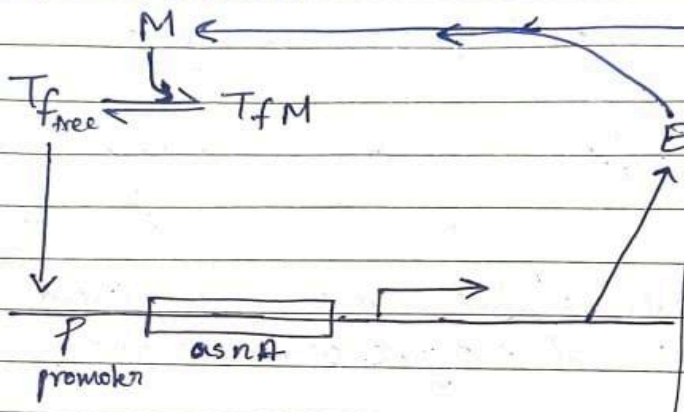
Reaction network:



M = L-asparagine
Tf = asnC
TfM = asnC-asparagine complex
E = Asparaginase
S = Aspartate

① $Tf + M \underset{k_2}{\overset{k_1}{\rightleftharpoons}} TfM$

Binding of metabolite (M) to free transcription factor (Tf)

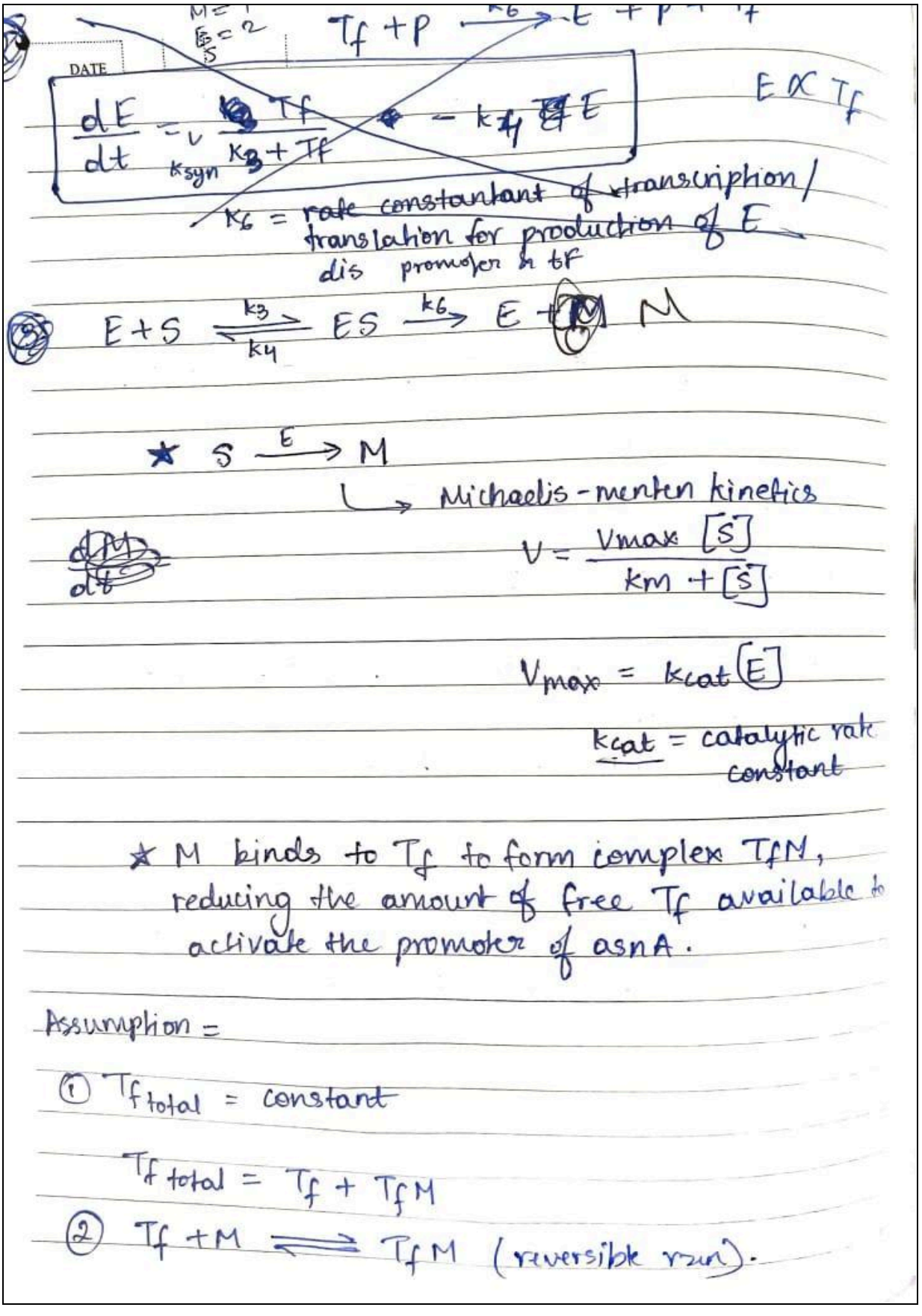
k_1 = forward binding rate constant for $Tf + M \rightarrow TfM$

k_2 = reverse (unbinding) rate constant $TfM \rightarrow Tf + M$

① $$\frac{d[Tf]}{dt} = -k_1[Tf][M] + k_2[TfM]$$

⇒ conc. of Tf_{free} ↓↓ when it binds to M & ↑↑ when TfM dissociates.

② $$\frac{d[TfM]}{dt} = k_1[Tf][M] - k_2[TfM]$$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$\frac{dE}{dt} = \nu_{ksyn} \frac{Tf}{K_3 + Tf} - k_4 E$
$E \propto Tf$
k_6 = rate constant of transcription / translation for production of E
dis promoter is tF
$E + S \underset{k_4}{\overset{k_3}{\rightleftharpoons}} ES \xrightarrow{k_6} E + M$
$S \xrightarrow{E} M$
Michaelis-menten kinetics
$V = \frac{V_{max}[S]}{K_M + [S]}$
$V_{max} = k_{cat}[E]$
k_{cat} = catalytic rate constant
M binds to Tf to form complex TfM, reducing the amount of free Tf available to activate the promoter of asnA.
Assumption =
① Tf_{total} = constant
$Tf_{total} = Tf + TfM$
② $Tf + M \rightleftharpoons TfM$ (reversible rxn).

DATE

∴ M is produced from S through the catalysis of E, & is consumed by binding with T_f :

$$\text{(3)}\quad \frac{dM}{dt} = k_{cat}[E]\frac{[S]}{K_m + [S]} - k_1[T_f][M] + k_2[T_fM]$$

k_{cat} = catalytic rate constant of E

K_m = Michaelis constant.

$k_1 = T_f + M \longrightarrow T_fM$

$k_2 = T_fM \longrightarrow T_f + M$.

$$\text{(4)}\quad \frac{dS}{dt} = -k_{cat}[E]\frac{[S]}{K_m + [S]}$$

Substrate is consumed by the enzymatic reaction.

$$\text{(5)}\quad \frac{dE}{dt} = k_{syn} \times \left(\frac{T_f}{K_3 + T_f}\right) - K_4 E \quad (\text{decay})$$

k_3 = dissociation constant of the promoter & transcription factor.

Reactions:

1. Binding of free transcription factor (Tf) with metabolite (M):

$$\mathrm{Tf} + \mathrm{M} \xrightarrow{k_1} \mathrm{TfM}$$

For free transcription factor (Tf, $X[0]$):

$$\frac{d[\mathrm{Tf}]}{dt} = -k_1[\mathrm{Tf}][\mathrm{M}] + k_2[\mathrm{TfM}]$$

---

2. Dissociation of the TfM complex:

$$\mathrm{TfM} \xrightarrow{k_2} \mathrm{Tf} + \mathrm{M}$$

For TfM complex (TfM, $X[1]$):

$$\frac{d[\mathrm{TfM}]}{dt} = k_1[\mathrm{Tf}][\mathrm{M}] - k_2[\mathrm{TfM}]$$

---

3. Catalysis by enzyme (E) to convert substrate (S) into metabolite (M):

Synthesis of Enzyme (E):

$$\mathrm{Tf} \xrightarrow{k_{\mathrm{syn}}} \mathrm{E}$$

Degradation of Enzyme (E):

$$\mathrm{E} \xrightarrow{k_4} \emptyset$$

For enzyme (E, $X[2]$):

$$\frac{d[\mathrm{E}]}{dt} = \frac{k_{\mathrm{syn}}[\mathrm{Tf}]}{k_3 + [\mathrm{Tf}]} - k_4[\mathrm{E}]$$

---

4. Consumption and production of metabolite (M) during binding and catalysis:

Formation of Metabolite (M) from Substrate (S):

$$\mathrm{S} + \mathrm{E} \xrightarrow{k_{\mathrm{cat}}} \mathrm{M} + \mathrm{E}$$

For metabolite (M, $X[3]$):

$$\frac{d[\mathrm{M}]}{dt} = k_{\mathrm{cat}} \frac{[\mathrm{E}][\mathrm{S}]}{k_m + [\mathrm{S}]} - k_1[\mathrm{Tf}][\mathrm{M}] + k_2[\mathrm{TfM}]$$

---

5. Consumption of the substrate (S) in the enzyme-catalyzed reaction by (E):

For substrate (S, $X[4]$):

$$\frac{d[\mathrm{S}]}{dt} = -k_{\mathrm{cat}} \frac{[\mathrm{E}][\mathrm{S}]}{k_m + [\mathrm{S}]}$$

Components of the network:

Tf = Free transcription factor (asnC)
TfM = Transcription factor bound with metabolite (asnC-aspargine complex)
E = Enzyme (asparaginase)
M = Metabolite (L-Aspargine)
S = Substrate (Aspartate)

Parameters:

k1 = forward binding rate constant for Tf+M--> TfM
k2 = reverse (unbinding) rate constant for TfM-->Tf+M
k3 = dissociation constant of the promoter and transcription factor
k4 = decay constant of asparaginase
kcat = catalytic rate constant of asparaginase
km = michaelis menten constant for the enzymatic reaction
k_syn = synthesis rate constant of the enzyme

Simulation code (Python):

```
#!/usr/bin/env python3
# -*- coding: utf-8 -*-
"""
Created on Mon Dec 12 17:37:29 2022
Modified on Tue Nov 26 11:55:50 2024

@author: bingalls, vishrutp
"""
import numpy as np
import matplotlib.pyplot as plt
from scipy.integrate import odeint

###Full model#####

# Decide how to encode your system.
# A State vector [X] is conc. of all the components at a particular time.
# The order in which they are specified needs to remain fixed.
## Here is the example system of an enzymatic reaction:
## Free transcription factor - asnC (Tf)
## Transcription factor and metabolite complex - asnC-aspargine (TfM)
## Enzyme - Asparaginase (E)
## Metabolite - L-aspargine (M)
## Substrate - aspartate (S)
## X0 is the initial state/conditions
# Set initial conditions State vector is [Tf, TfM , E , M , S]

init_state=np.array([10**-1,0,0,0,1]) #µM

# Declare your ODE model in the same order
## dX[0] = dTf/dt, dX[1] = dTfM/dt, dX[2] = dE/dt, dX[3] = dM/dt, dX[4] = dS/dt
def diffEq(X,t):
    # generate a list to store derivatives,
    # The number should be the no. of variables in your system
    dX= np.zeros(5)
    dX[0]= (-k1*X[0]*X[3]) + (k2*X[1]) #-k1*[Tf]*[M]+k2*[TfM]
    dX[1]= (k1*X[0]*X[3]) - (k2*X[1]) #k1*[Tf]*[M]-k2*[TfM]
    dX[2]= (k_syn * (X[0])/(k3+X[0])) -k4*X[2] #(k6*Tf/(k6+Tf))-K7*E #k6
changed to k3 and k7 changed to k4
    dX[3]=((kcat*X[2])*(X[4]/(km+X[4])))-k1*X[0]*X[3]+k2*X[1]
#(kcat*E)*([S]/(Km+[S]))-k1*[Tf][M]+k2*[TfM]
    dX[4]=-((kcat*X[2])*(X[4]/(km+X[4]))) #-(kcat*E)*([S]/(Km+[S]))
    return dX
```

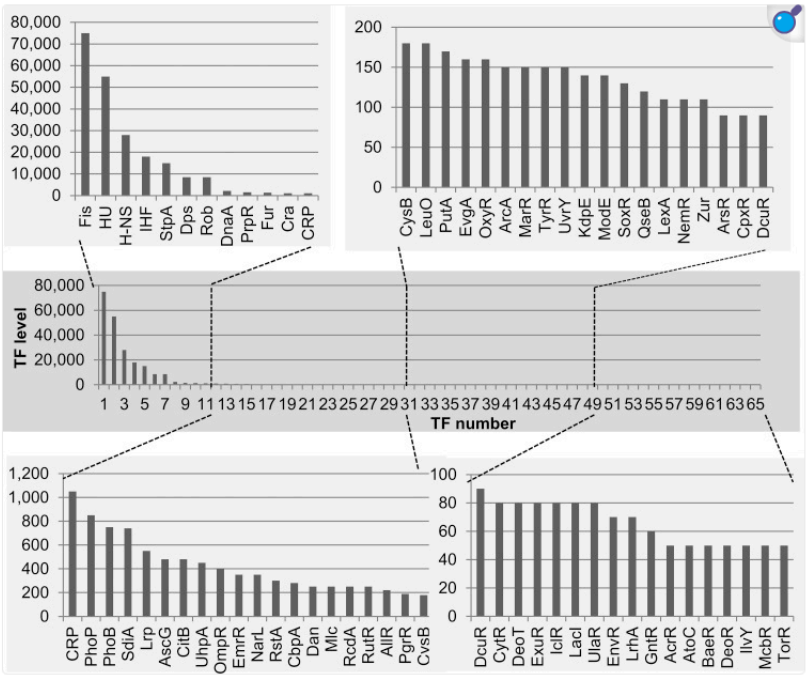
```
## Tf = X[0]
## TfM = X[1]
## E = X[2]
## M = X[3]
## S = X[4]

#assign parameter values

k1=10**8 #forward binding rate constant for Tf+M--> TfM
k2=10**7 #reverse (unbinding) rate constant for TfM-->Tf+M
k3=10**-1 #dissociation constant of the promoter and transcription factor
k4=0.83 #decay constant of asparagine synthase
kcat=600 #catalytic rate constant of asparaginase
km=3500 #Km michaelis menten constant for the enzymatic reaction
k_syn = 1 #synthesis rate constant of the enzyme

#set time_grid for simulation
t_min=0; t_max=300; dt=0.001
times=np.arange(t_min, t_max+dt, dt) #generate time-grid list

X=odeint(diffEq, init_state, times) #run simulation

# Plot simulation
plt.figure()  # Generate figure
plt.plot(times, X[:, 0], label="Tf (Free asnC)", linewidth=2)  # Free
transcription factor conc.
plt.plot(times, X[:, 1], label="TfM (asnC-Aspargine Complex)", linewidth=2)  #
Complex conc.
plt.plot(times, X[:, 2], label="E (Aspargine Synthase)", linewidth=2)  # Enzyme
conc.
plt.plot(times, X[:, 3], label="M (L-Aspargine)", linewidth=2)  # Metabolite
conc.
plt.plot(times, X[:, 4], label="S (Aspartate)", linewidth=2)  # Substrate conc.
plt.xlabel("Time (s)")
plt.ylabel("Concentration (µM)")
plt.legend()
plt.title("Time Evolution of Transcription Factor, Complex, Enzyme, Metabolite,
and Substrate")
plt.grid(True)
plt.show()
```

Justification for using the parameter values:

1. Tf initial = 10^-1 μM

The transcription factor concentrations in *E. coli* range from:

~16.6 nM (low TFs) to ~0.12 μM (high TFs).



Intracellular concentration of TFs in exponential-phase cells of *E. coli* K-12 W3110. Cells were grown in LB-glucose medium at 37°C with shaking. In the middle of exponential phase (optical density at 600 nm [OD_{600}], 0.4), cells were harvested and the cell lysate was prepared by the standard method (27–29). The intracellular concentration was determined for a total of 65 species of TF by the quantitative immunoblot method as described in Materials and Methods. The TF concentration was calculated as the relative value to that of RNA polymerase RpoA subunit and is represented as the number of molecules per genome equivalent of DNA. TF level (*y* axis) represents the number of TF molecules per genome.

Source: Intracellular Concentrations of 65 Species of Transcription Factors with Known Regulatory Functions in Escherichia coli.

2. S initial = 1 μM

This is the substrate concentration and is an independent factor that can be externally varied.

3. k1 = 10^8 μM

Binding affinities of transcription factors is generally between $1\mu M$ and 100 μM.

k2 = 10^7 μM

The unbinding affinity of the Tf and M would be in the same range as the binding, with little difference.

k3 = 10^-1 μM

The dissociation factor, often expressed in terms of the dissociation constant (kd), quantifies the binding affinity between transcription factors (TFs) and their target DNA sequences within promoters.

High-affinity binding interactions for transcription factors can have kd values in the low nanomolar range, often between 1 nM to 10 nM. This indicates a strong affinity for their target DNA sequences.

Lower-affinity interactions may exhibit kd values in the micromolar range, typically around 1 μM to 100 μM. These interactions are less stable and can be influenced by various factors, including the presence of competing molecules or changes in cellular conditions.

Source: Transcription Factor Binding Affinities and DNA Shape Readout

k4 = 0.83 sec^-1

The decay constant of asparaginase, specifically for the enzyme derived from *E. coli*, is approximately kd=1.10×10^−3 min^−1 (*kd* ≈1.83×10^−5 s^−1) at 37 °C.

Source: Molecular Characterization of a Stable and Robust L-Asparaginase from Pseudomonas sp. PCH199: Evaluation of Cytotoxicity and Acrylamide Mitigation Potential

Abstract

L-asparaginase is an important industrial enzyme widely used to treat acute lymphoblastic leukemia (ALL) and to reduce acrylamide formation in food products. In the current study, a stable and robust L-asparaginase from *Pseudomonas* sp. PCH199, with a high affinity for L-asparagine, was cloned and expressed in *Escherichia coli* BL21(DE3). Recombinant L-asparaginase (*Pg*-ASNase II) was purified with a monomer size of 37.0 kDa and a native size of 148.0 kDa. During characterization, *Pg*-ASNase II exhibited 75.8 ± 3.84 U/mg specific activities in 50.0 mM Tris-HCl buffer (pH 8.5) at 50 °C. However, it retained 80 and 70% enzyme activity at 37 °C and 50 °C after 60 min, respectively. The half-life and k_d values were 625.15 min and 1.10 × 10^{-3} min^{-1} at 37 °C. The kinetic constant K_m, V_{max}, k_{cat}, and k_{cat}/K_m values were 0.57 mM, 71.42 U/mg, 43.34 s^{-1}, and 77.90 mM^{-1} for L-asparagine, respectively. In addition, the enzyme has shown stability in the presence of mos s and protein-modifying agents. *Pg*-ASNase II was cytotoxic towards the MCF-7 cell line (breast cance estimated IC_{50} value of 0.169 U/mL in 24 h. Further, *Pg*-ASNase II treatment led to a 70% acrylamide reduction in baked foods. These findings suggest the potential of *Pg*-ASNase II in therapeutics and the food industry.

Keywords: L-ASNase II; Himalayan bacteria; enzyme; cytotoxicity; acrylamide reduction

kcat = 600 sec^-1

Escherichia coli L-asparaginase: This enzyme demonstrates a high specificity for asparagine, with a reported kcat value of approximately 770 s^-1 for one isoform (ReAIV) at 37°C, while another isoform (ReAV) shows a kcat of about 603 s^-1 under similar conditions.

Source: Biochemical characterization of L-asparaginase isoforms from Rhizobium etli—the boosting effect of zinc.

In another study,

> Low catalytic activity is a key factor limiting the widespread application of type II L-asparaginase (ASNase) in the food and pharmaceutical industries. In this study, smart libraries were constructed by semi-rational design to improve the catalytic activity of type II ASNase from *Bacillus licheniformis*. Mutants with greatly enhanced catalytic efficiency were screened by saturation mutations and combinatorial mutations. A quintuple mutant ILRAC was ultimately obtained with specific activity of 841.62 IU/mg and k_{cat}/K_m of 537.15 min^{-1}·mM^{-1}, which were 4.24-fold and 6.32-fold more than those of wild-type ASNase. The highest specific activity and k_{cat}/K_m were firstly reported in type II ASNase from *Bacillus licheniformis*. Additionally, enhanced pH stability and superior thermostability were both achieved in mutant ILRAC. Meanwhile, structural alignment and molecular dynamic

Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC9456134/

km = 3500 µM

Asparginase has an apparent K(m) for l-asparagine of 3.5 mM.

Asparagine utilization in Escherichia coli

R C Willis, C A Woolfolk

PMID: 4595199 PMCID: PMC246662 DOI: 10.1128/jb.118.1.231-241.1974

Abstract

Asparagine-requiring auxotrophs of Escherichia coli K-12 that have an active cytoplasmic asparaginase do not conserve asparagine supplements for use in protein synthesis. Asparagine molecules entering the cell in excess of the pool required for use of this amino acid in protein synthesis are rapidly degraded rather than accumulated. Supplements are conserved when asparagine degradation is inhibited by the asparagine analogue 5-diazo-4-oxo-l-norvaline (DONV) or mutation to cytoplasmic asparaginase deficiency. A strain deficient in cytoplasmic asparaginase required approximately 260 mumol of asparagine for the synthesis of 1 g of cellular protein. The cytoplasmic asparaginase (asparaginase I) is required for growth of cells when asparagine is the nitrogen source. This enzyme has an apparent K(m) for l-asparagine of 3.5 mM, and asparaginase activity is competitively inhibited by DONV with an apparent K(i) of 2 mM. The analogue provides a time-dependent, irreversible inhibition of cytoplasmic asparaginase activity in the absence of asparagine.

Source: https://pubmed.ncbi.nlm.nih.gov/4595199/

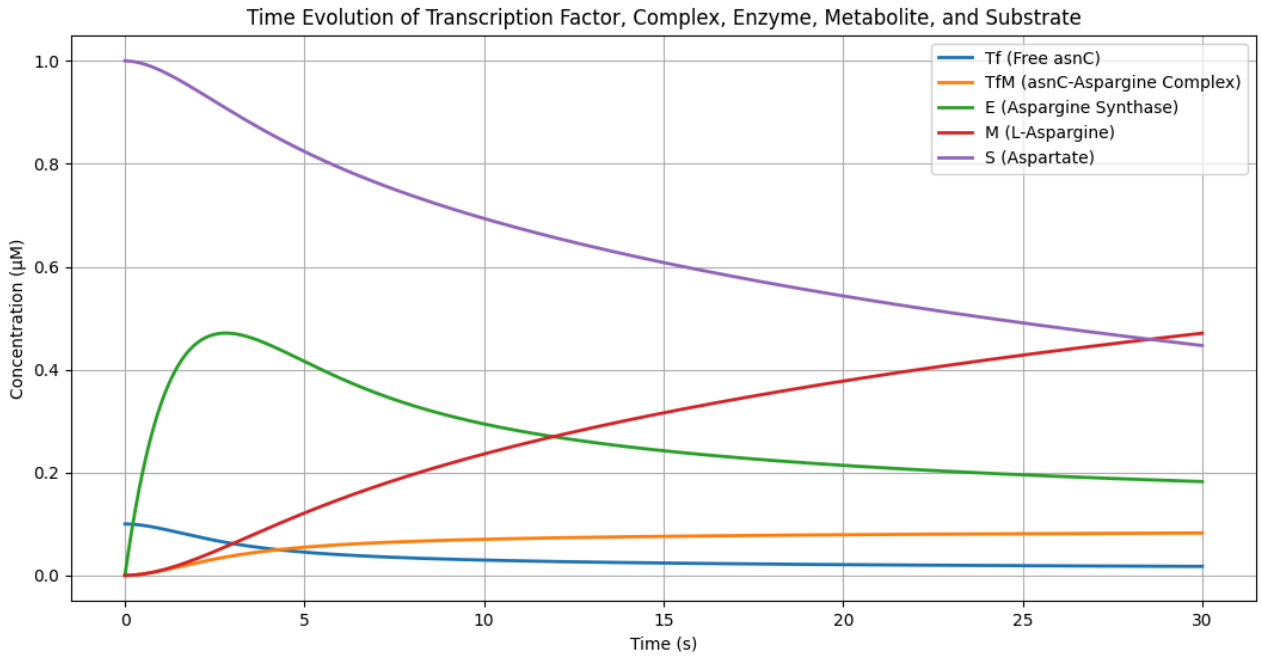
k_syn = 1 sec^-1

In **E. coli** or other bacterial systems, synthesis rates of enzymes like asparaginase might range from $10^{-2}\,sec^{-1}$ to $10^{1}\,sec^{-1}$, depending on factors like promoter strength, growth conditions, and environmental factors.

Source: Molecular Modeling and Optimization of Type II E.coli l-Asparaginase Activity by in silico Design and in vitro Site-directed Mutagenesis

Plots for different simulation times:

Simulation time: 30 sec



Aspartate (S) - Rapid initial decrease: Aspartate is the substrate for the asparagine synthase enzyme. Initially, as enzyme synthesis begins and its activity ramps up, the substrate is rapidly consumed. The catalytic efficiency (high kcat) ensures a fast reaction, leading to a sharp decline in S.

L-asparagine (M) - Sharp initial rise: The production of L-asparagine is directly proportional to the consumption of aspartate by asparagine synthase. As the enzyme becomes active, the metabolite concentration rises sharply due to the high catalytic rate (kcat) of the reaction.

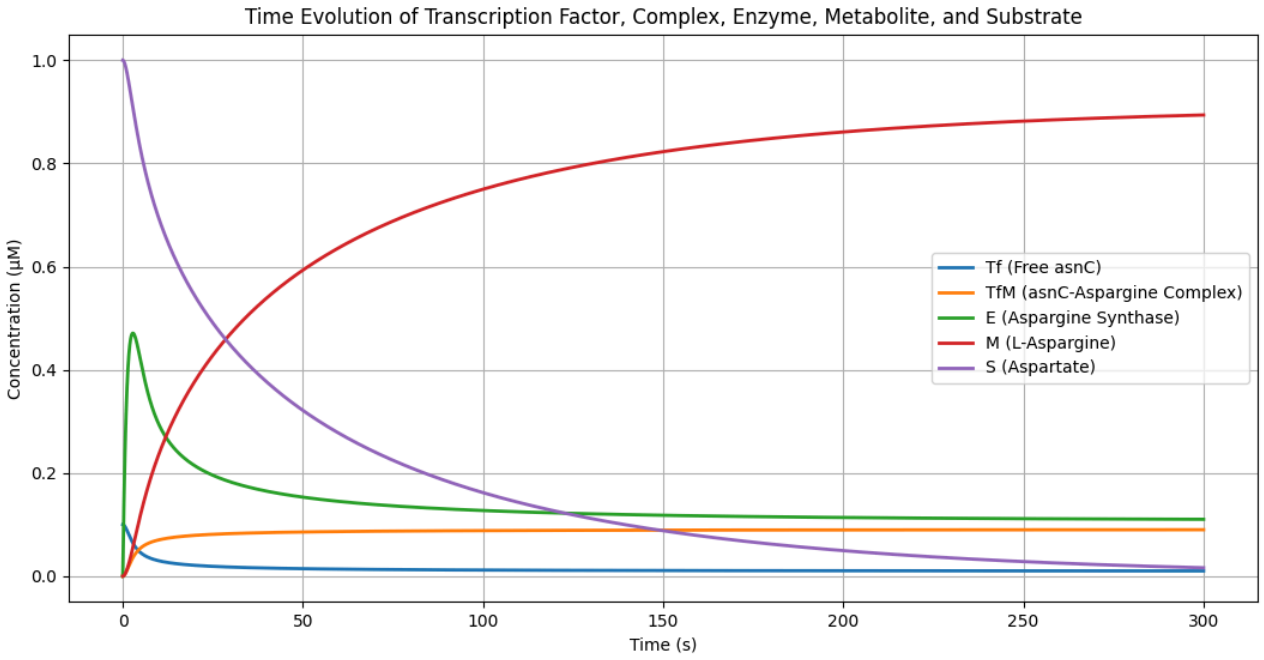
TfM Complex - Slight increase: The formation of the transcription factor-metabolite complex (TfM) depends on the interaction between free Tf and M. Initially, M is produced rapidly, and TfM starts to form, though this interaction is slower than the enzymatic conversion of S to M, leading to a more gradual increase.

Asparagine Synthase (E) - Gradual increase: The synthesis of asparagine synthase is regulated by the transcription factor Tf (asnC activates the transcription of asnA producing asparaginase). As Tf

concentration decreases, enzyme synthesis occurs at a steady rate, which reflects a delayed but gradual increase in enzyme concentration.

Free asnC (Tf) - Gradual decrease: Tf is consumed as it binds to M to form the TfM complex. Its slow decline reflects the gradual production of M, as the rate of complex formation (k1) is moderate relative to metabolite production.

Simulation time: 300 sec



Aspartate (S) - Approaches depletion: Over time, the enzymatic reaction consumes the substrate. With high kcat, the reaction rapidly depletes S to a near-zero concentration.

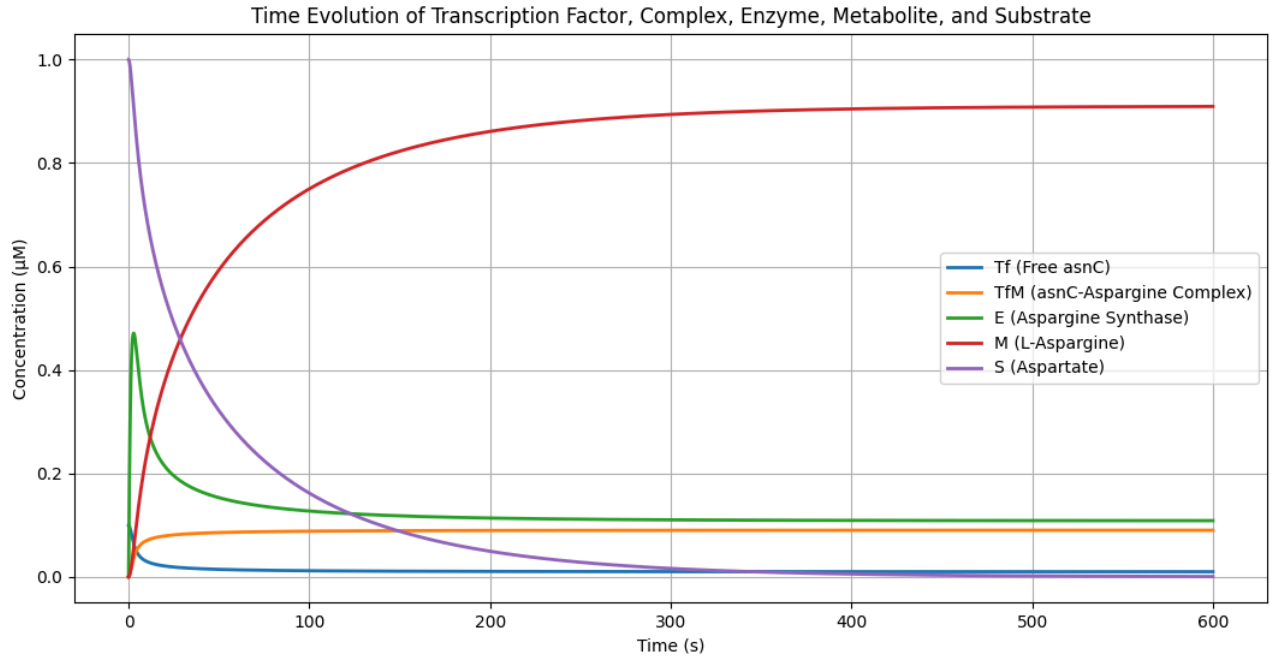
L-asparagine (M) - Steady accumulation: As S is converted to M, the metabolite accumulates. Its production eventually slows as S becomes scarce. The system approaches a steady state where M production is balanced by its consumption in TfM formation.

TfM Complex - Reaches equilibrium: The formation of the TfM complex is a reversible reaction (k1 and k2). Once the concentration of Tf and M stabilizes, the complex reaches equilibrium. This reflects dynamic binding and unbinding processes.

Asparagine Synthase (E) - Plateaus: The production of E is regulated by Tf, which stabilizes as its free concentration decreases. This transcriptional regulation leads to a constant level of enzyme production, balanced by its decay (k4).

Free asnC (Tf) - Stabilizes at a lower concentration: As Tf binds to M, its free concentration decreases. However, the rate of TfM dissociation (k2) ensures a small, stable pool of free Tf remains in equilibrium with the complex.

Simulation time: 600 sec



Aspartate (S) - Complete depletion: With sufficient time, all the substrate is consumed by the enzyme (reaches 0 concentration). The depletion reflects the complete utilization of S in producing M.

L-asparagine (M) - Maximum accumulation: M concentration reaches a maximum once S is entirely converted. As TfM formation occurs slowly relative to M production, the metabolite continues to accumulate until substrate exhaustion. The difference between 1 (max value) and the saturation level of the metabolite is how much TfM is being formed.

TfM Complex - Stabilizes: The complex reaches equilibrium as the concentrations of Tf and M stabilize. This equilibrium reflects the balance between complex formation and dissociation.

Asparagine Synthase (E) - Constant concentration: E concentration stabilizes because it is controlled by the transcriptional regulation of Tf. As Tf reaches equilibrium, the enzyme synthesis is balanced by its degradation (k4).

Free asnC (Tf) - Stable low concentration: As Tf forms a stable complex with M, its free concentration remains low. This reflects a dynamic balance between free Tf and TfM formation, with minimal unbound Tf available.

Key Reasons Why E Doesn't Become Zero!

Due to the decay constant and the Tf binding with M to form a complex, we would expect that enzyme concentration becomes zero after a particular time. However,

The rate of synthesis of E is governed by the equation:

$$\frac{dE}{dt} = \frac{k_{\text{syn}} \cdot \text{Tf}}{k_3 + \text{Tf}} - k_4 \cdot E$$

The first term represents the **rate of production** of E, which depends on the availability of Tf. Since Tf never drops to zero (due to reversible binding with M), the synthesis of E continues, but at a reduced rate when Tf is low.

The formation of the Tf complex is governed by reversible binding:

$$\text{Tf} + M \leftrightarrow \text{TfM}$$

The dissociation rate constant (k2) ensures that the TfM complex constantly dissociates back into Tf and M. As long as k2 > 0, some Tf will always be regenerated, preventing its complete depletion.

Tf is not only involved in the binding reaction but may also have a natural turnover (synthesis and degradation). In this system, if Tf synthesis exists at even a minimal rate, it would counteract any tendency toward depletion. In the absence of explicit degradation, Tf concentration will stabilize as it reaches dynamic equilibrium with TfM.

This ensures a baseline production of E.