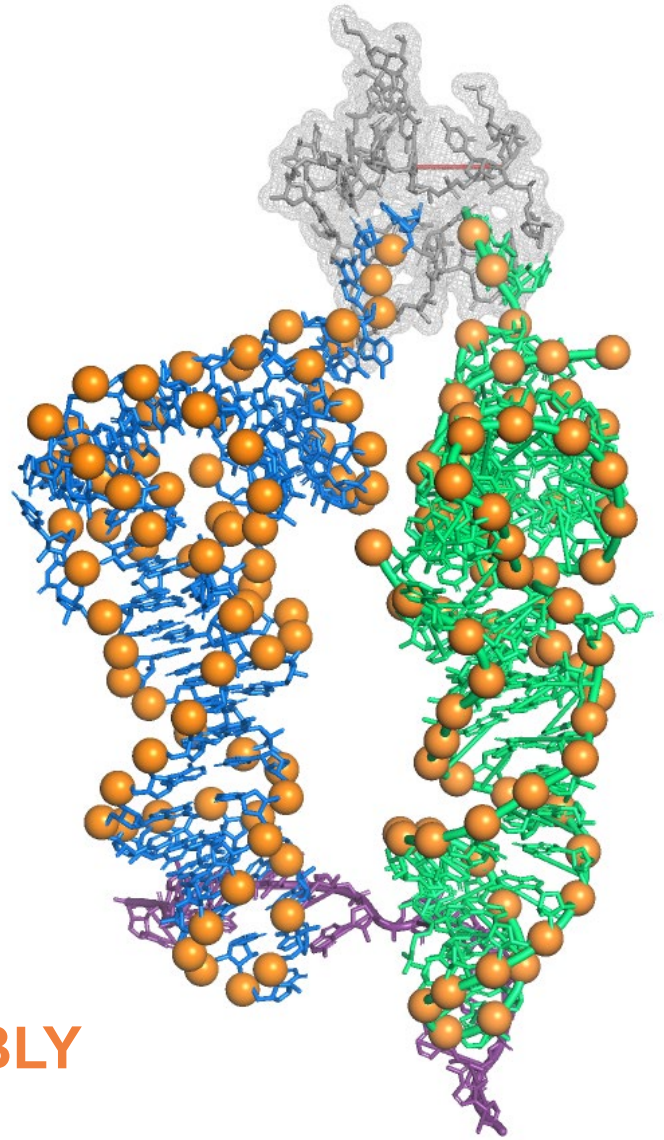
Ribosome Cruise Control:

How tRNA Pools Regulate mRNA Translation



Marc Joiret

The BELGIAN BIOPHYSICAL SOCIETY

One-day symposium on **PROTEIN FOLDING & ASSEMBLY**

LIÈGE université Sciences Appliquées

biomech UNIVERSITY OF LIÈGE RESEARCH UNIT

LIÈGE université GIGA Molecular and Computational Biology

August 29th, 2025

Outline

1. Methodological note
   Agent-Based Modeling
2. Protein synthesis by ribosomes
3. Problem & Research questions
4. Keys to address the problem
5. Implementation
   `Ribosomer` framework
6. Model outputs
7. Sensitivity & Uncertainty
8. Discussion & perspectives

Agent-Based Model (ABM)

A model is an invention, not a discovery.
Massoud *et.al*, cited in [Viceconti and Emili 2024]

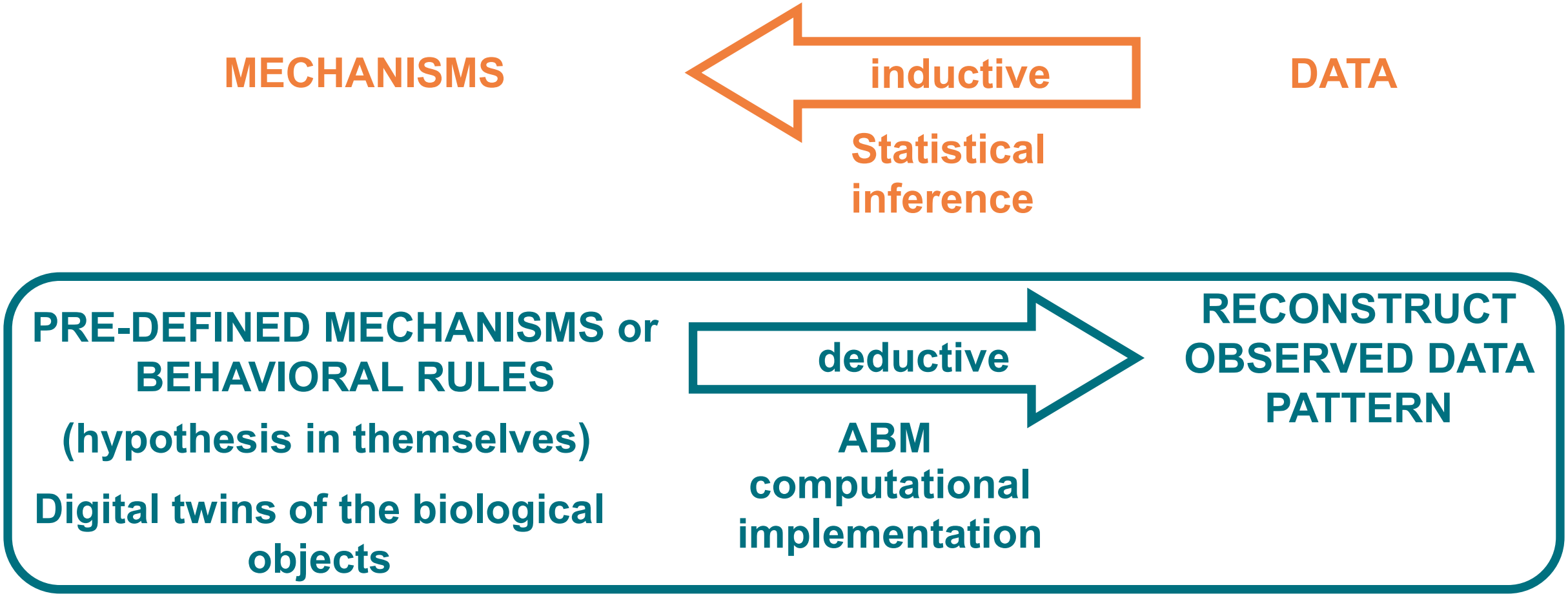
Modeling Mindset

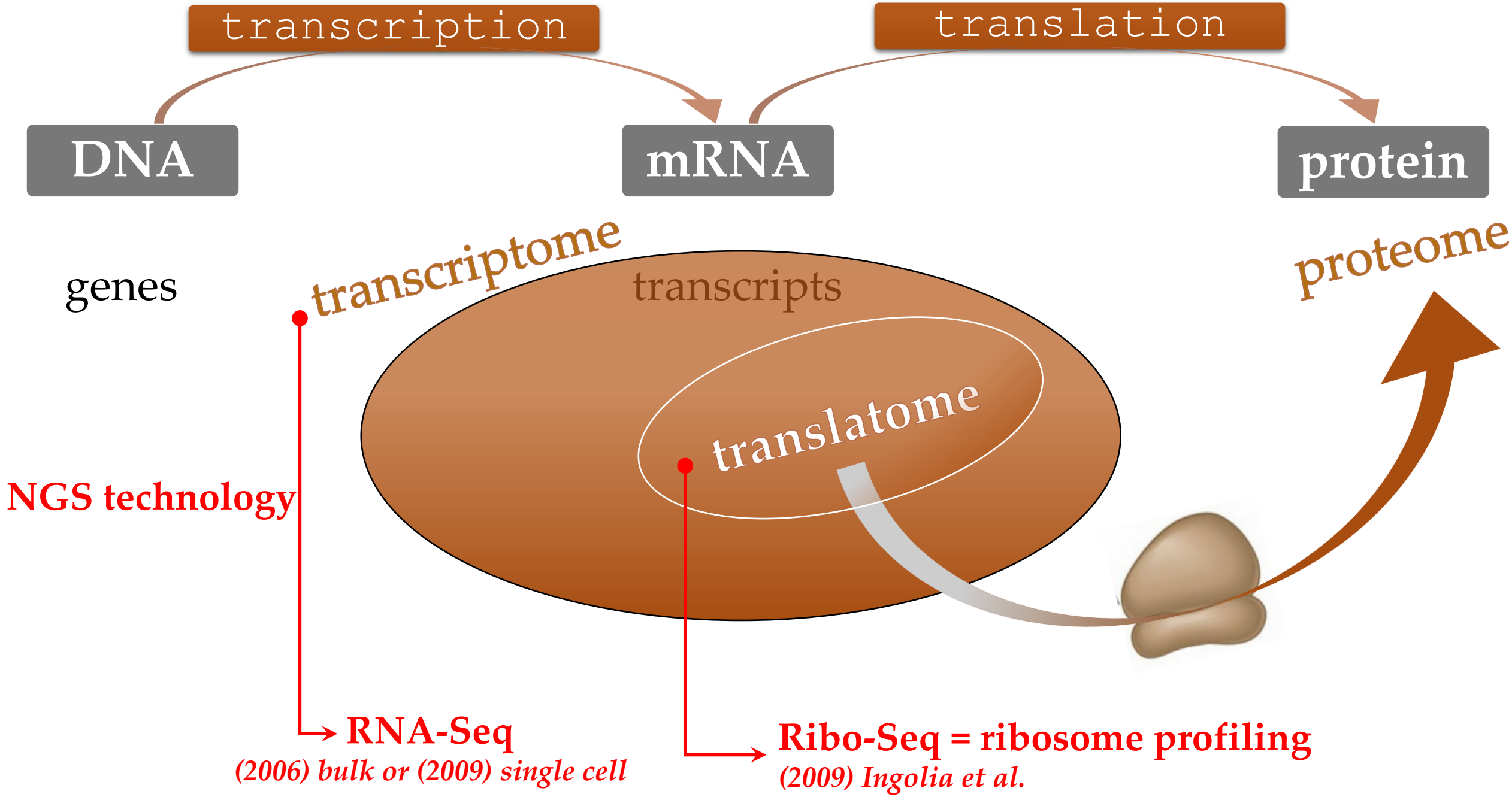
"*What I cannot create, I do not understand*"

Feynman, R. (1988). Blackboard note from Richard Feynman's office, Pasadena.

Theory construction & Mechanism elucidation

Agent-Based Model (ABM)




transcription
translation
DNA
mRNA
protein
genes
transcriptome
transcripts
translatome
proteome
NGS technology
RNA-Seq
(2006) bulk or (2009) single cell
Ribo-Seq = ribosome profiling
(2009) Ingolia et al.

transcription

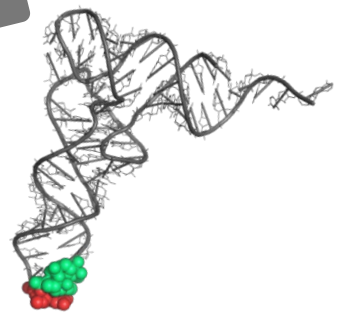
translation

DNA

4 nucleotides A,T, C, G

mRNA

51 tRNAs



protein

20 amino acids

genes

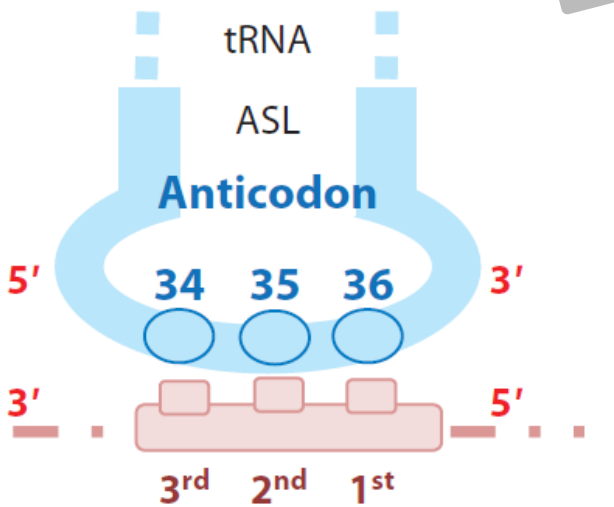
transcripts

64 codons

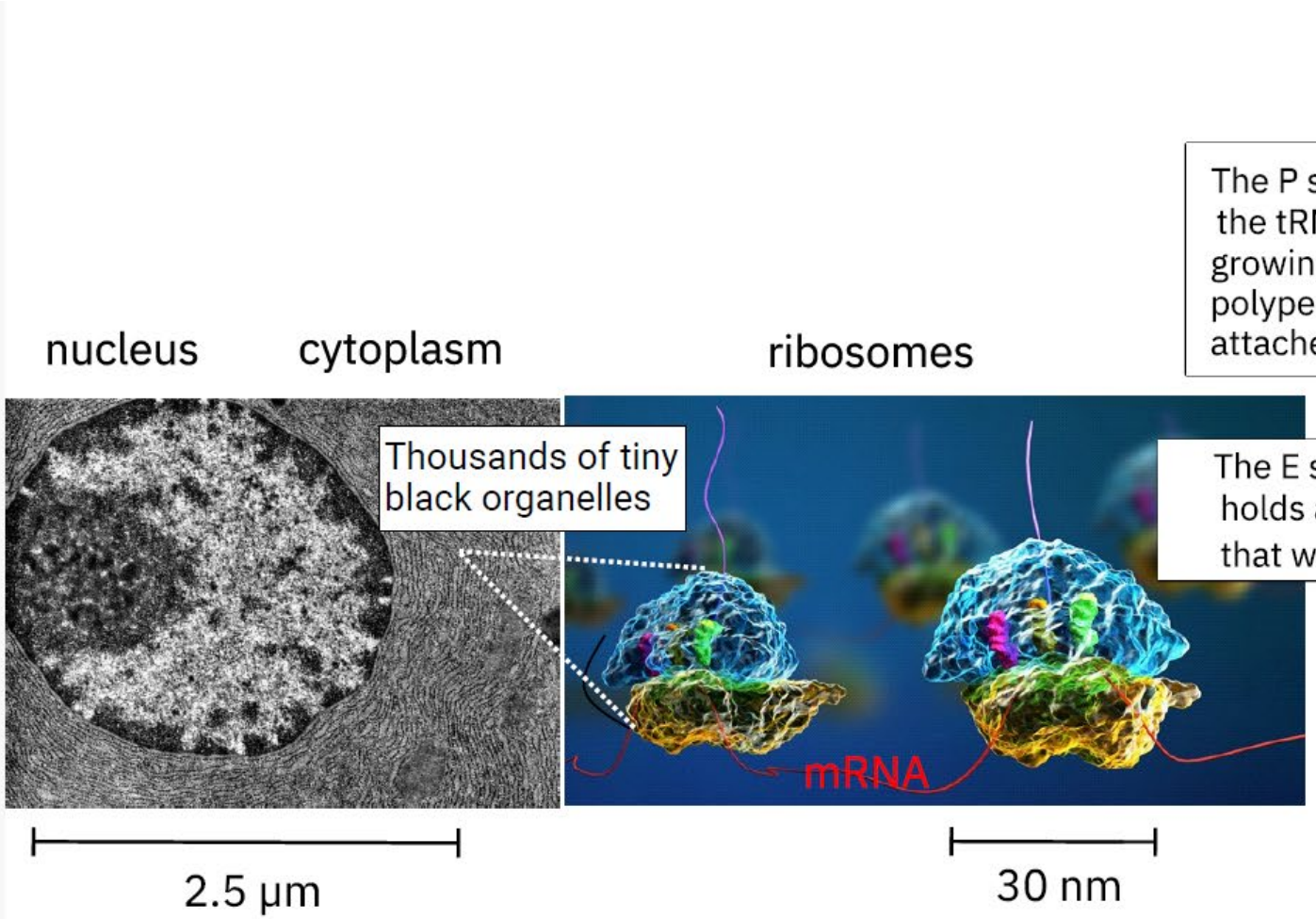
1st base

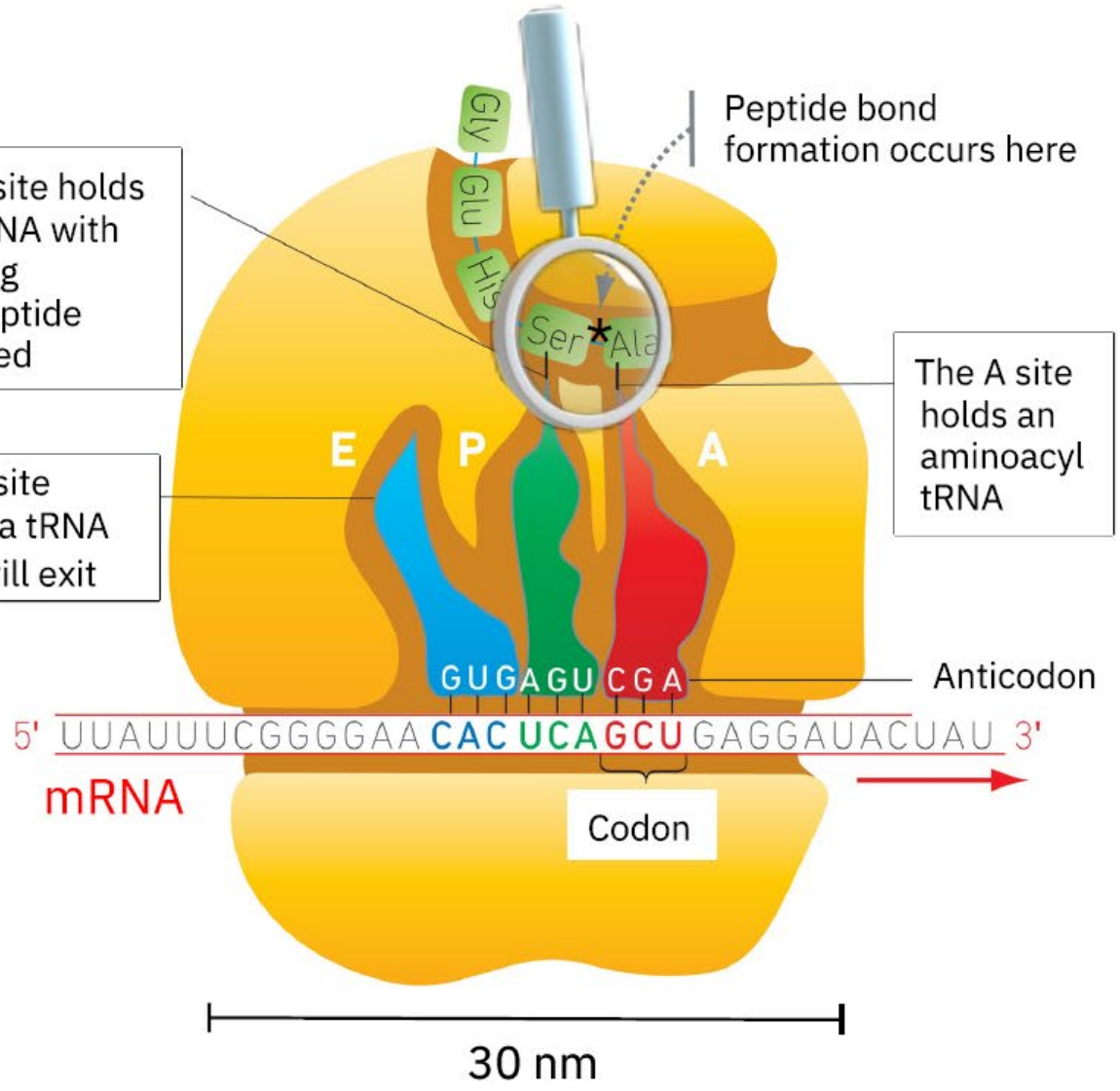
2nd base	U		C		A		G		3rd base
U	UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cysteine	U
	UUC	Phenylalanine	UCC	Serine	UAC	Tyrosine	UGC	Cysteine	C
	UUA	Leucine	UCA	Serine	UAA	Stop	UGA	Stop	A
	UUG	Leucine	UCG	Serine	UAG	Stop	UGG	Tryptophan	G
C	CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine	U
	CUC	Leucine	CCC	Proline	CAC	Histidine	CGC	Arginine	C
	CUA	Leucine	CCA	Proline	CAA	Glutamine	CGA	Arginine	A
	CUG	Leucine	CCG	Proline	CAG	Glutamine	CGG	Arginine	G
A	AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine	U
	AUC	Isoleucine	ACC	Threonine	AAC	Asparagine	AGC	Serine	C
	AUA	Isoleucine	ACA	Threonine	AAA	Lysine	AGA	Arginine	A
	AUG	Methionine (Start)	ACG	Threonine	AAG	Lysine	AGG	Arginine	G
G	GUU	Valine	GCU	Alanine	GAU	Aspartic Acid	GGU	Glycine	U
	GUC	Valine	GCC	Alanine	GAC	Aspartic Acid	GGC	Glycine	C
	GUA	Valine	GCA	Alanine	GAA	Glutamic Acid	GGA	Glycine	A
	GUG	Valine	GCG	Alanine	GAG	Glutamic Acid	GGG	Glycine	G

Nonpolar, aliphatic Polar, uncharged Aromatic Positively charged Negatively charged

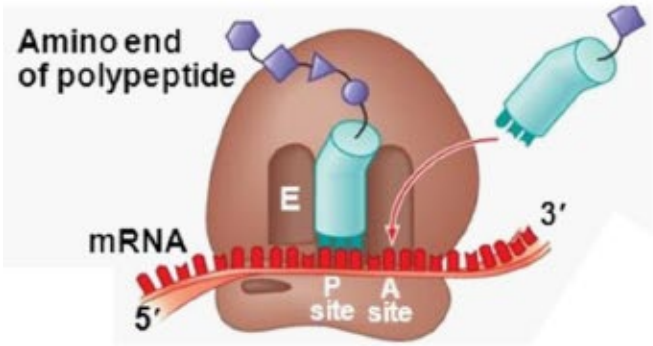


2 Protein Synthesis by Ribosomes

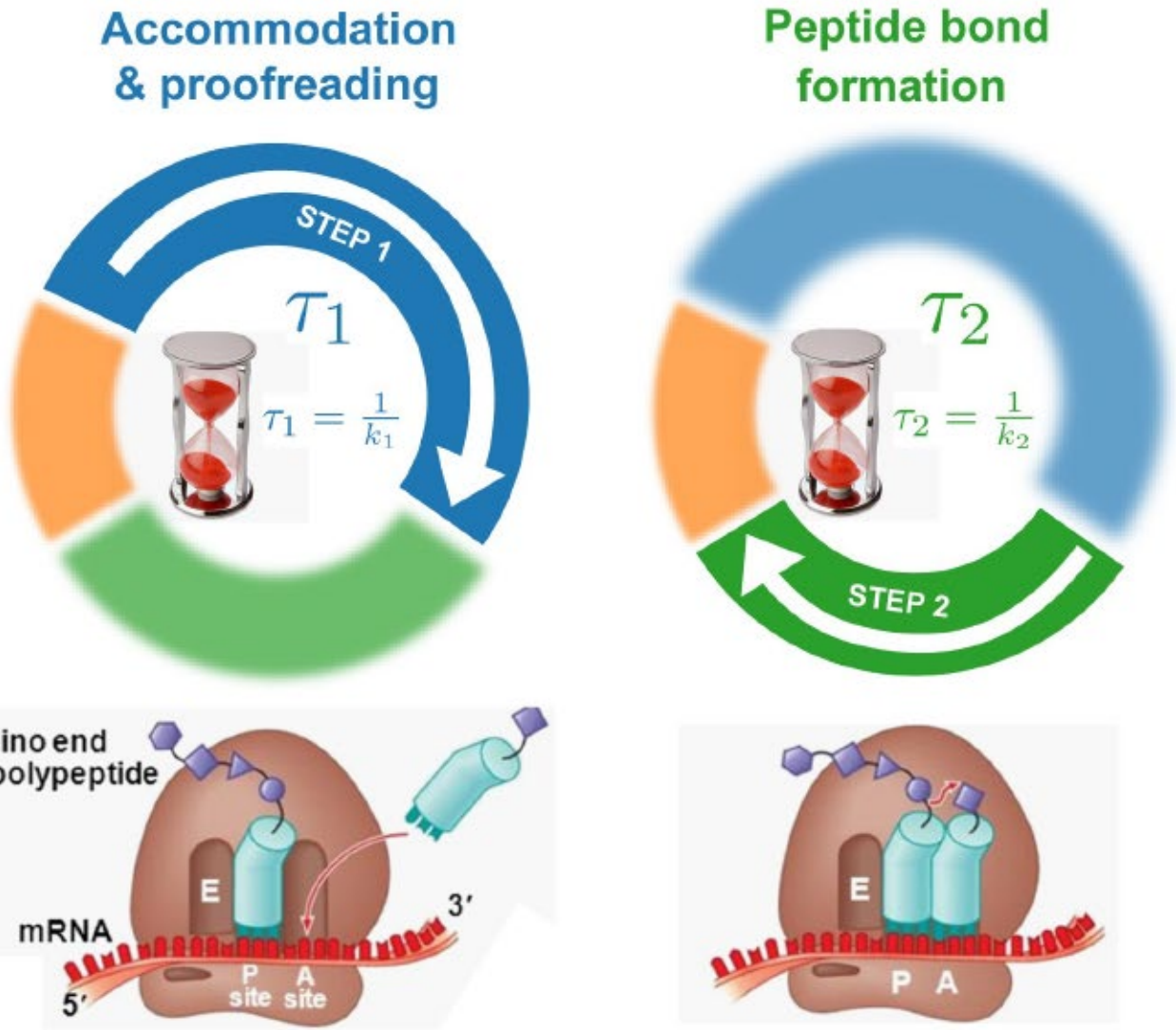




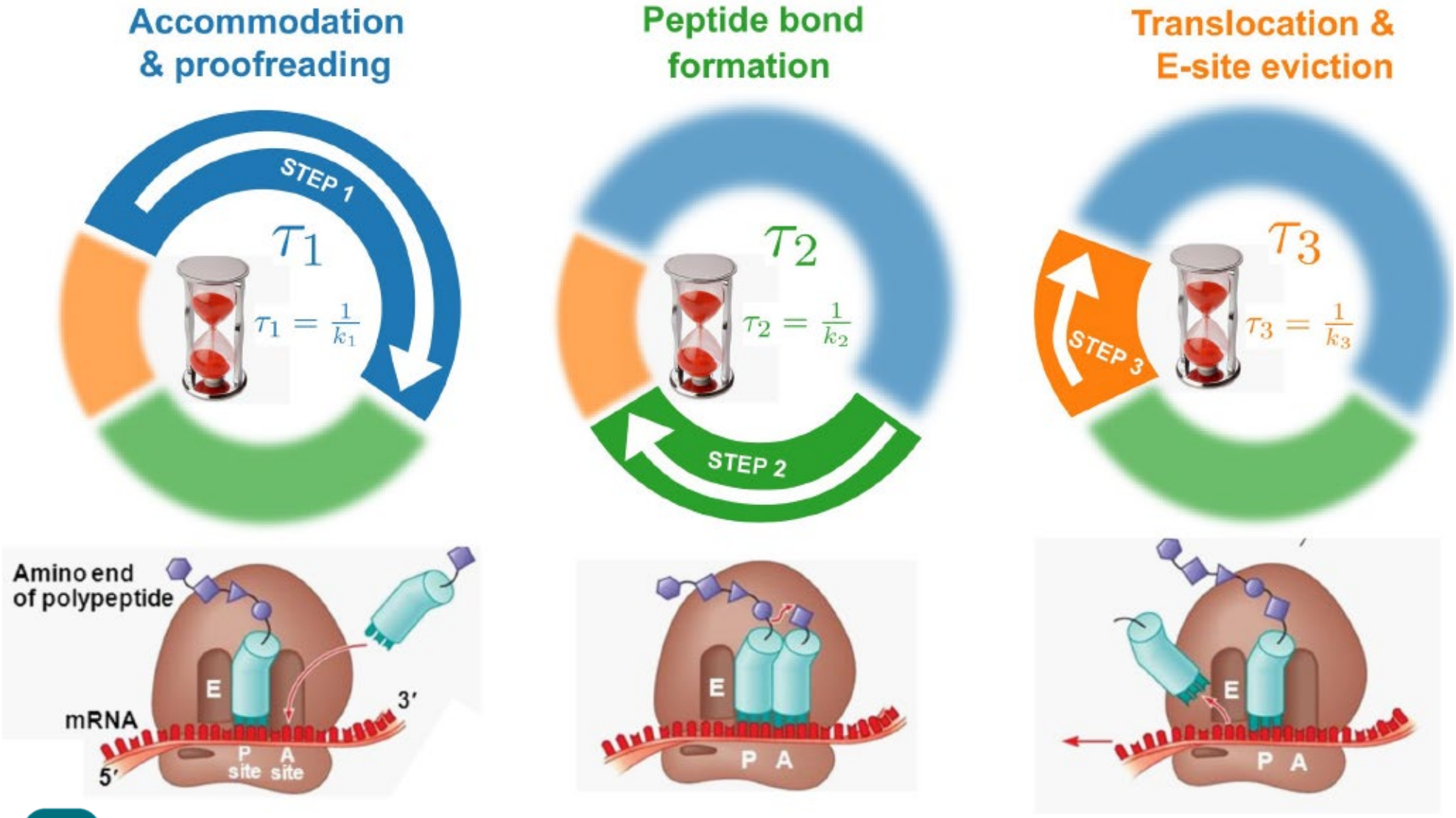
2 Protein elongation cycle by a ribosome

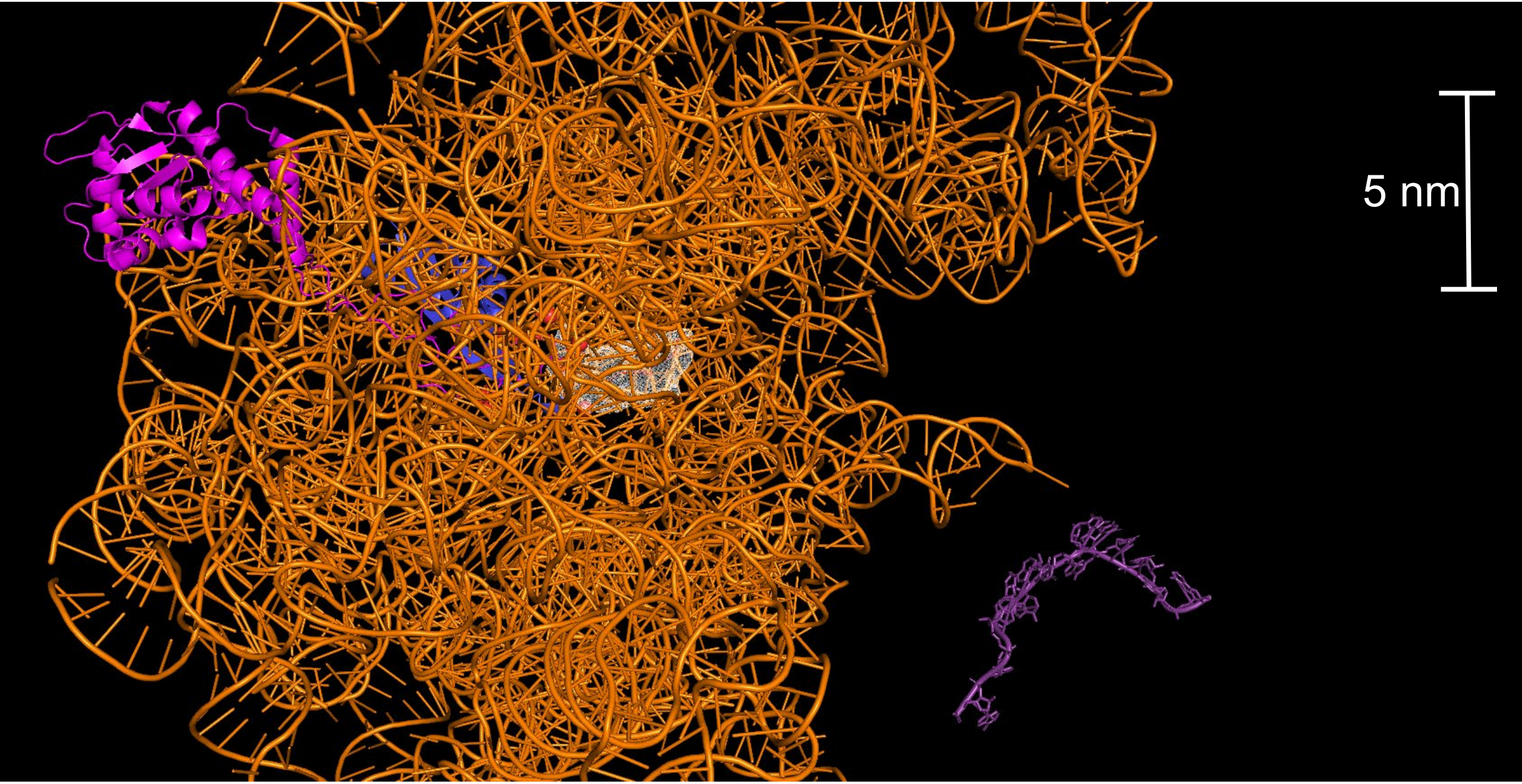


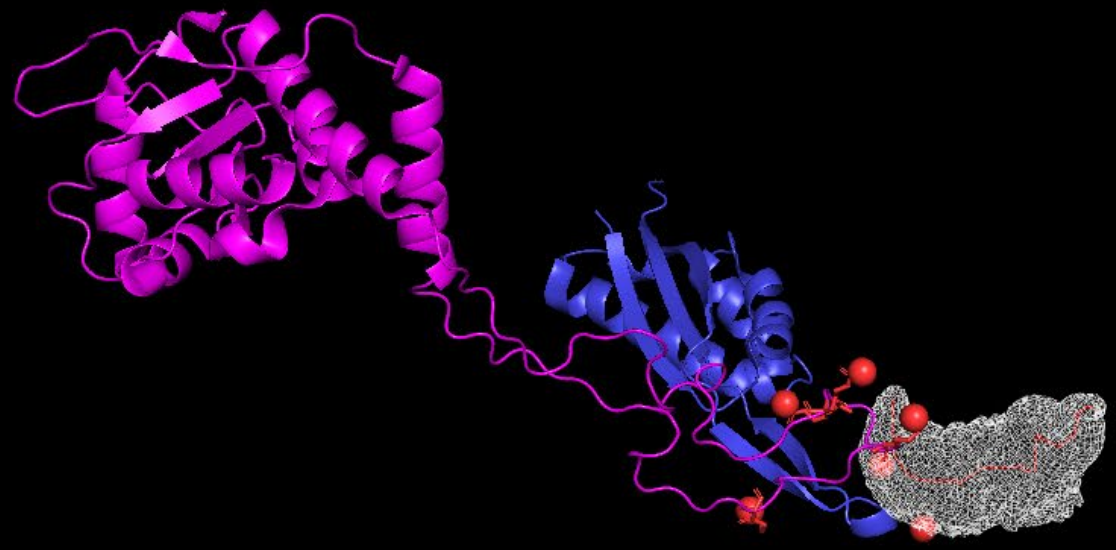
1 Protein elongation cycle by a ribosome

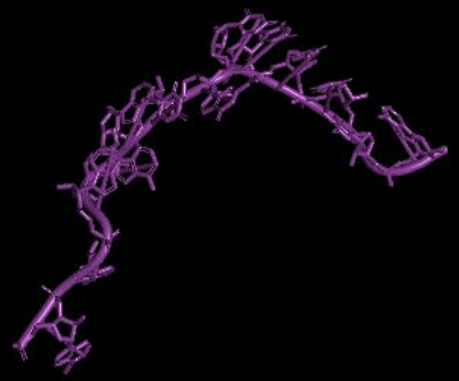


1 Protein elongation cycle by a ribosome

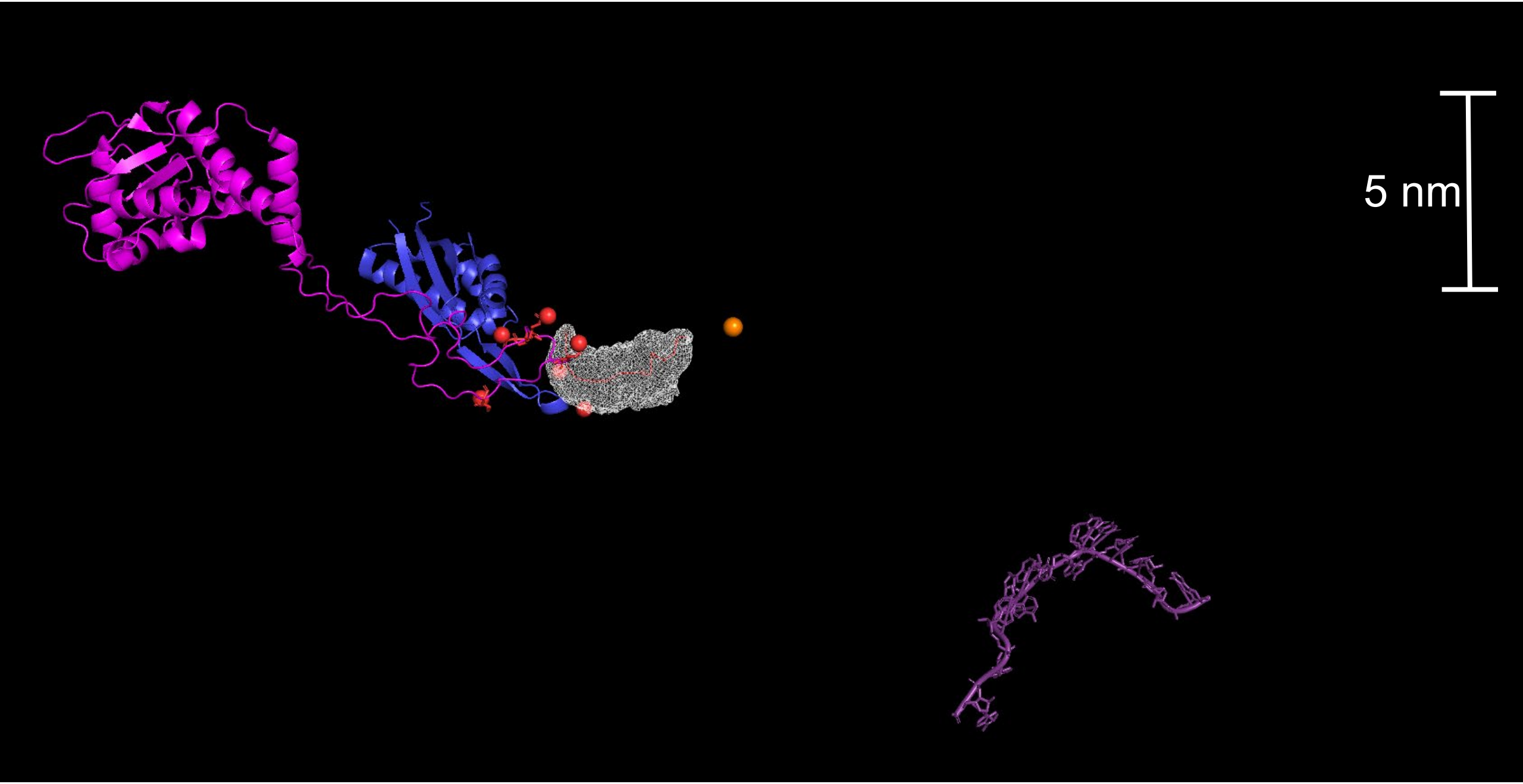


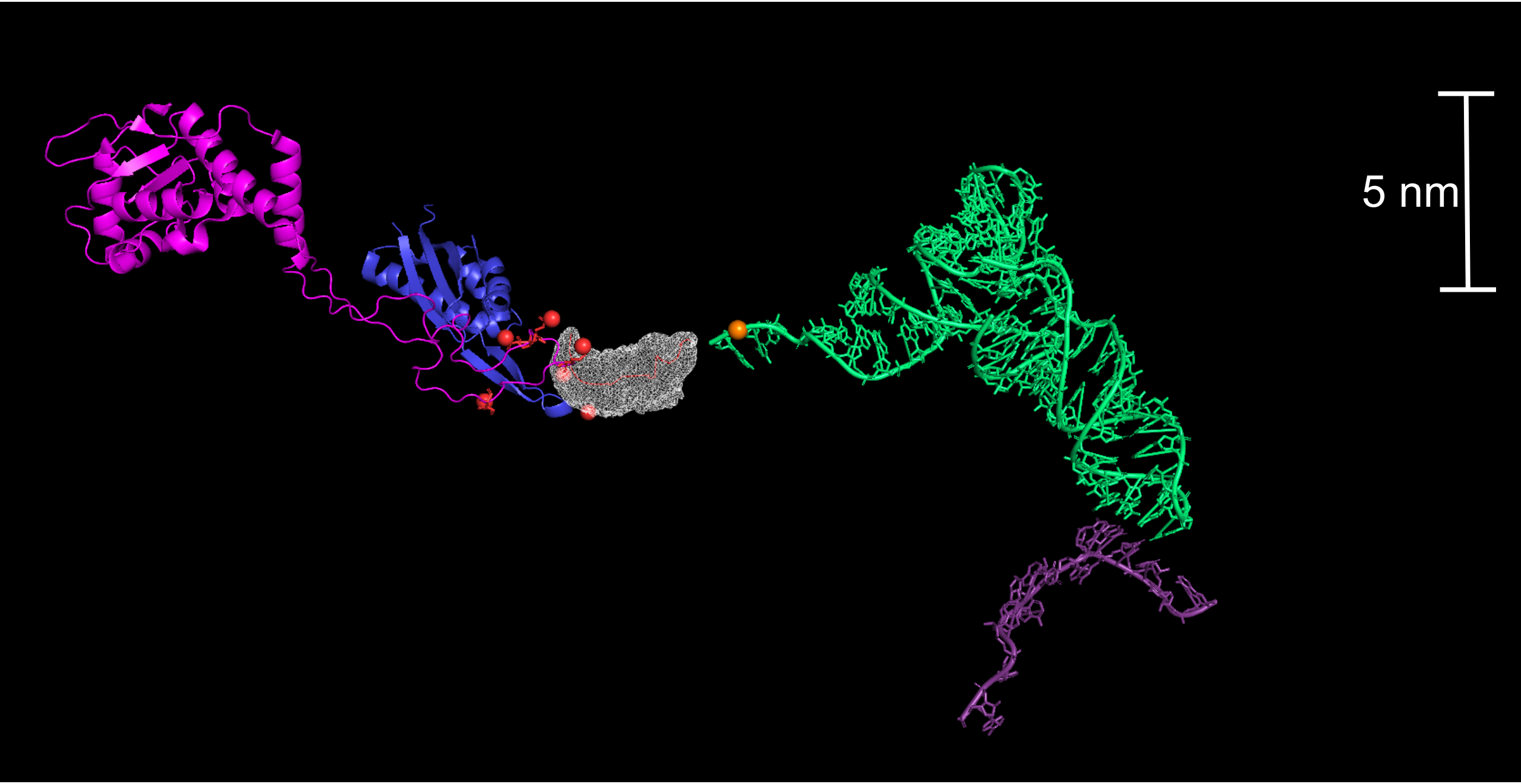

5 nm

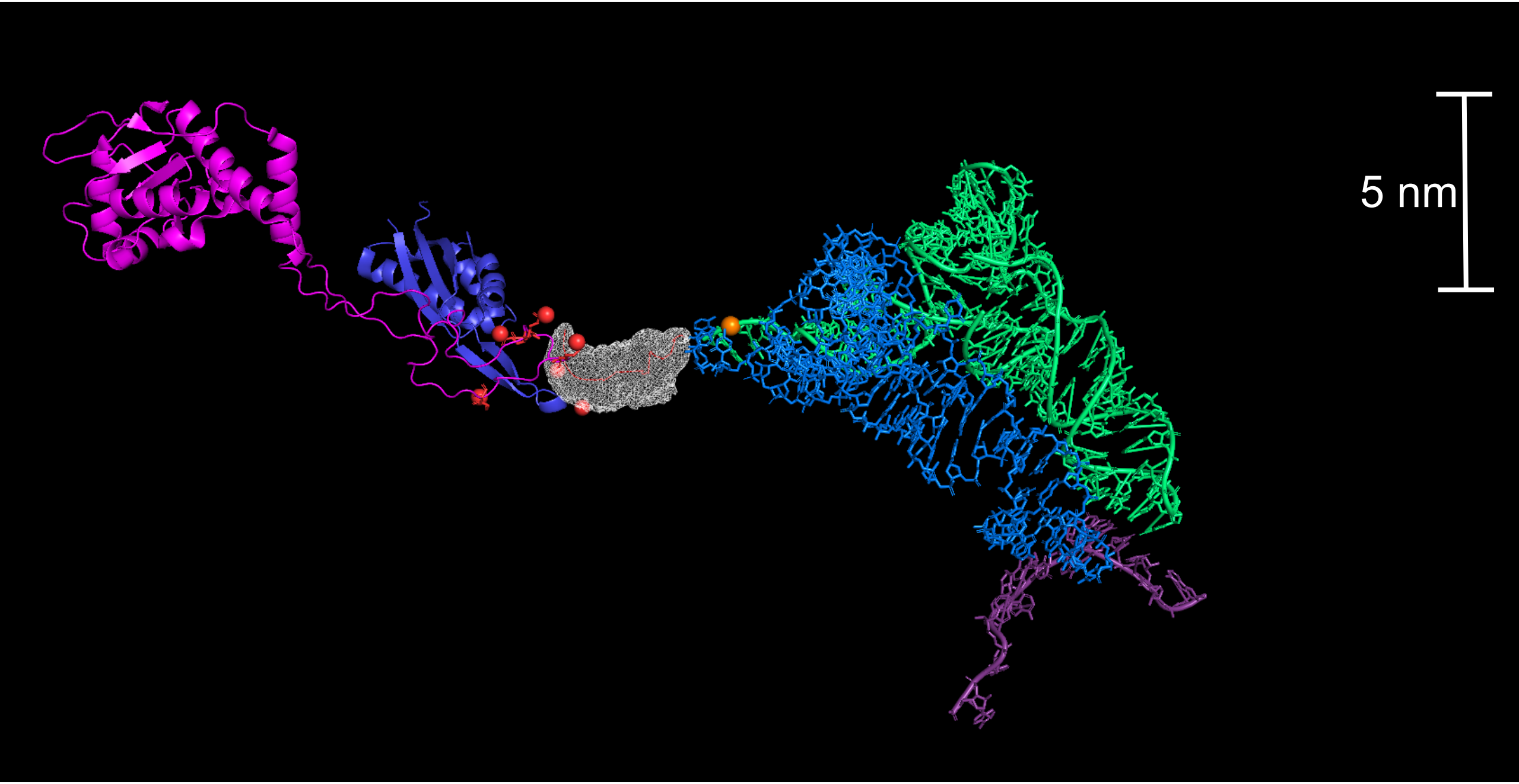


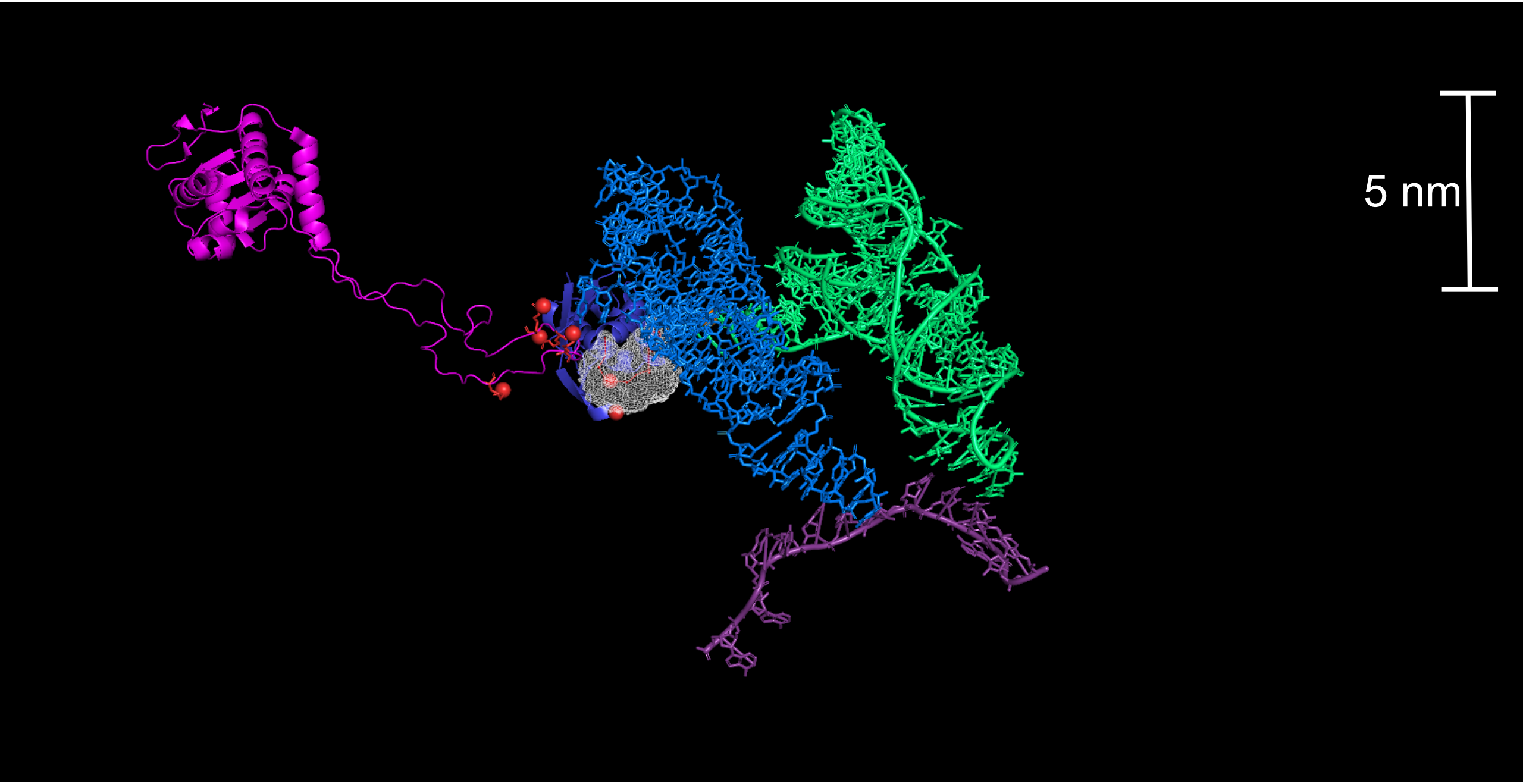


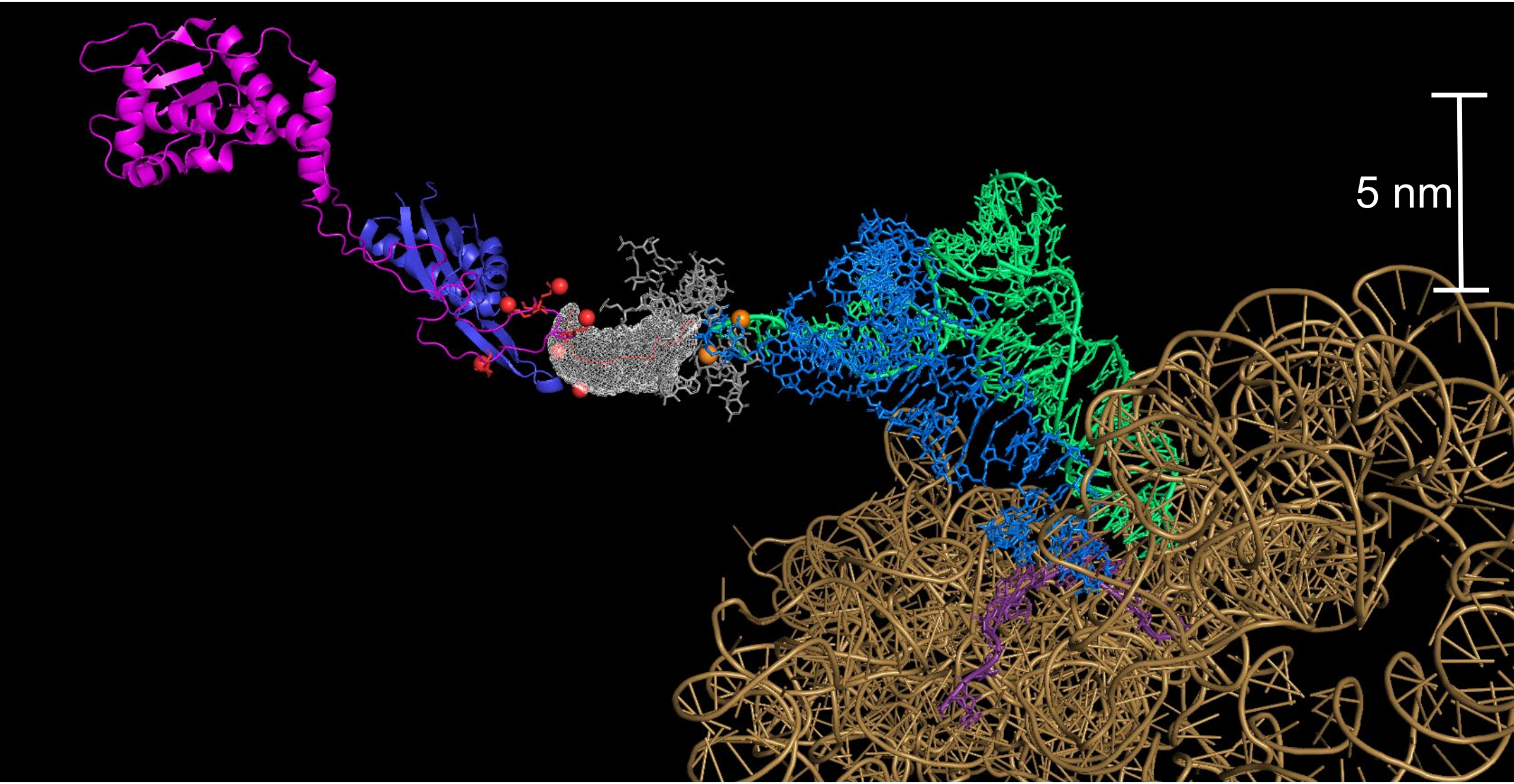
5 nm

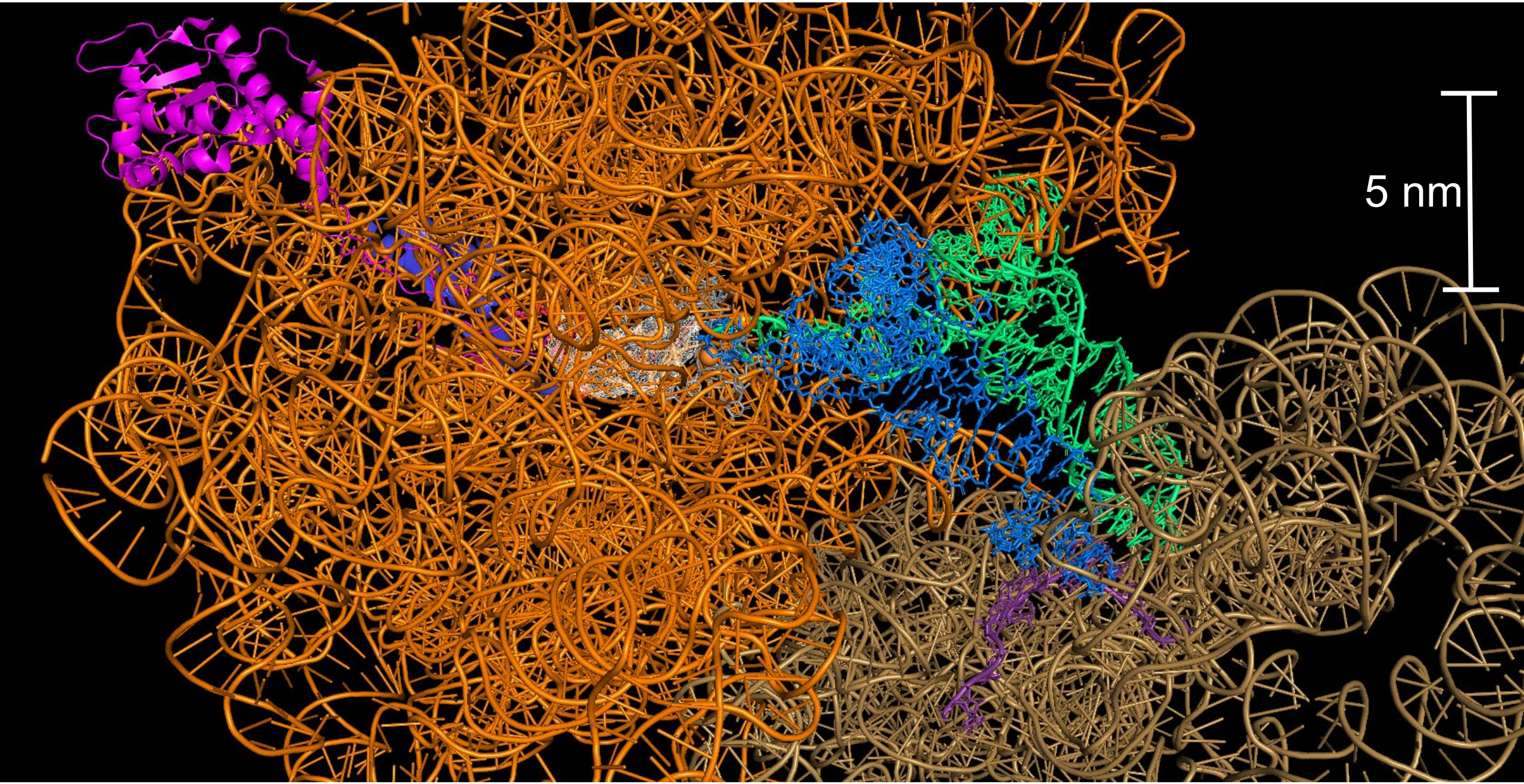

5 nm


5 nm


5 nm


5 nm

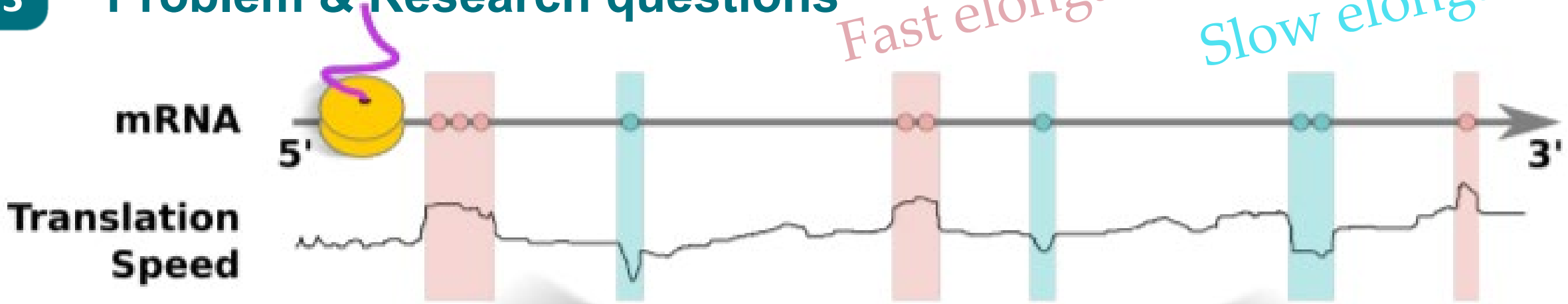

5 nm

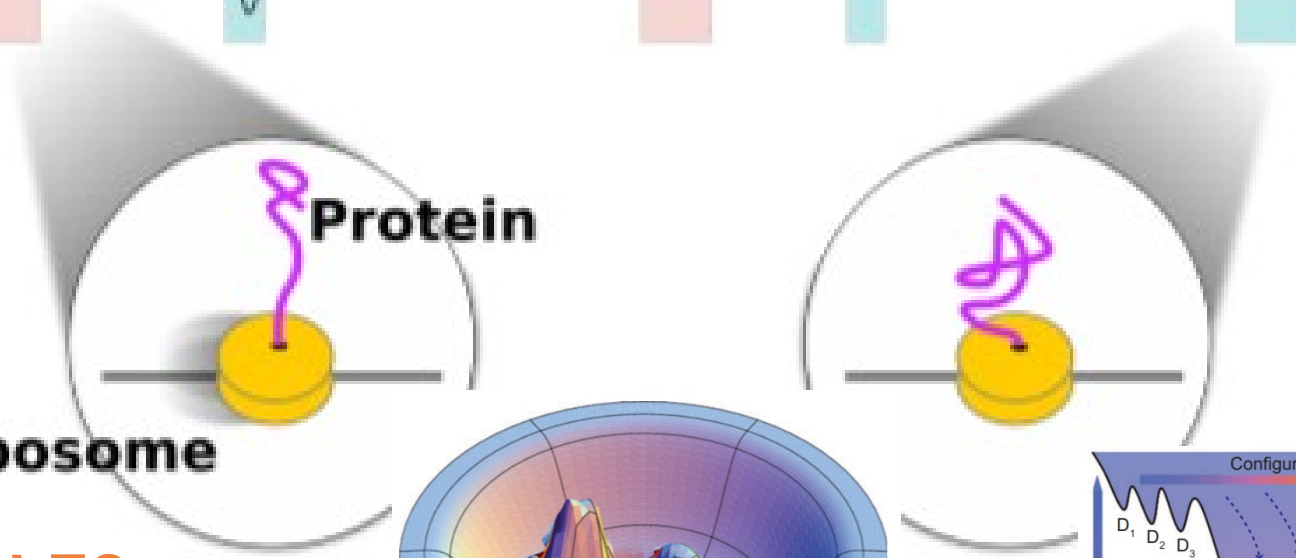

5 nm

3 Problem & Research questions

Fast elongation

Slow elongation

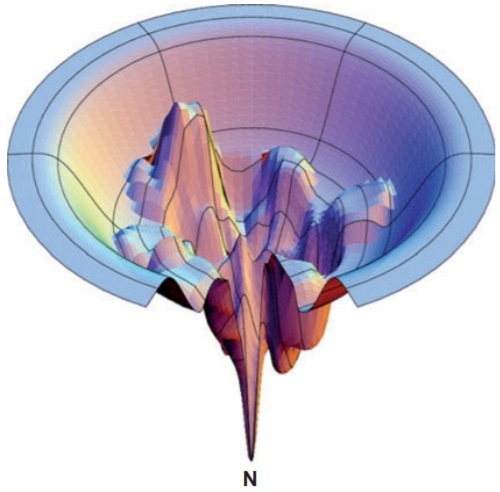


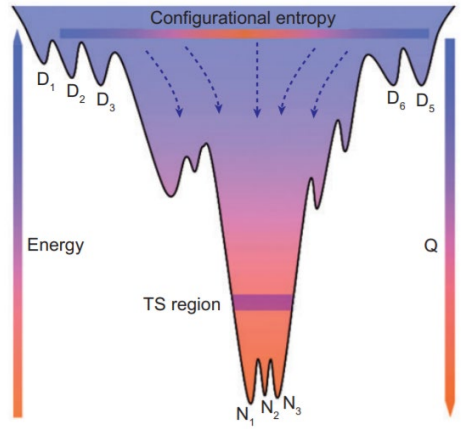


FUNCTIONAL ROLE?

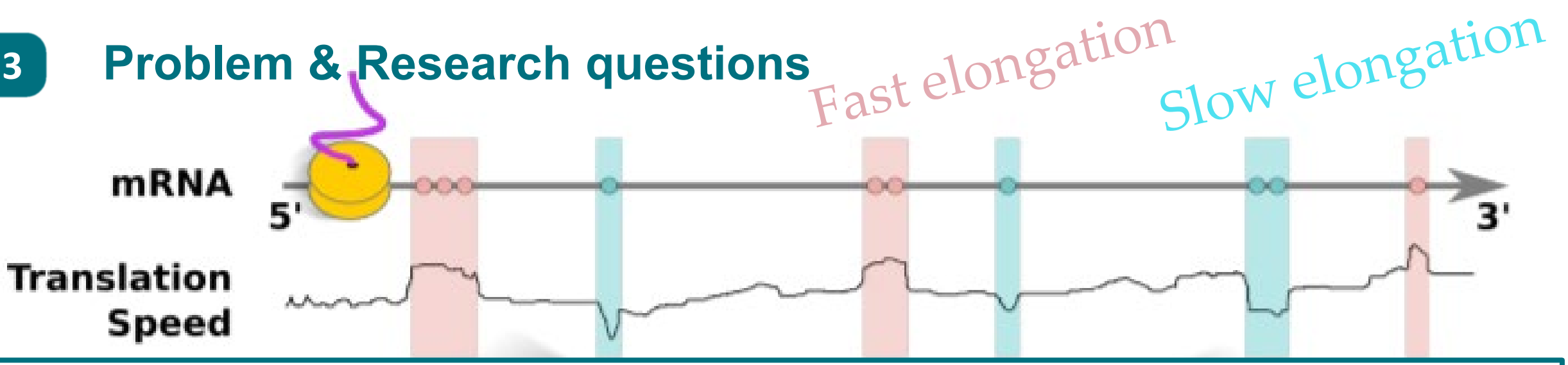
3D CONFIGURATION (FOLDING)

PROTEOME INTEGRITY





3 Problem & Research questions



WHAT ARE THE FACTORS THAT DETERMINE THE ELONGATION RATE?

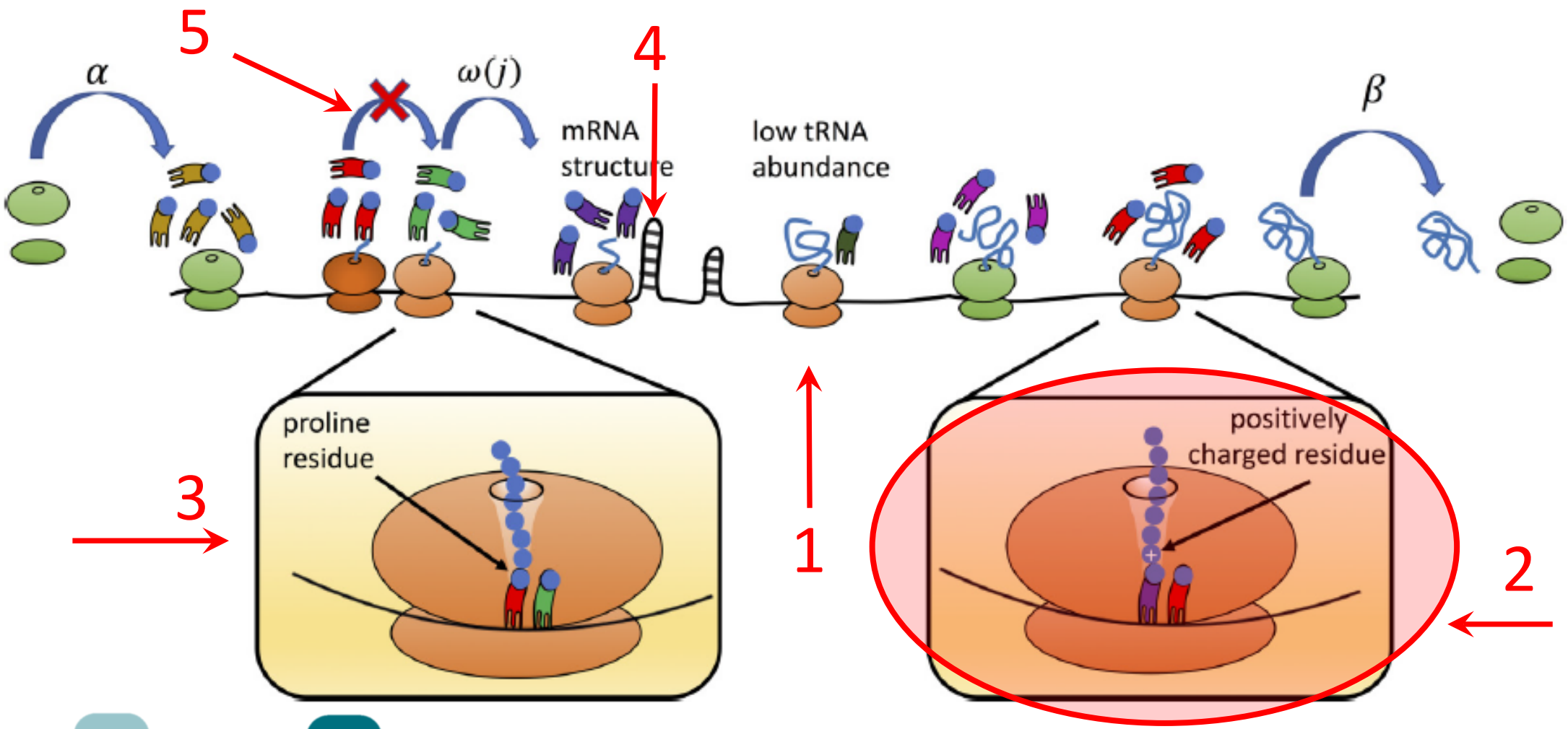
HOW DO THEY CONTRIBUTE?

IF THEY ARE INTERTWINED, DISENTANGLE THEM !

UNCOVER EMERGENT BEHAVIOR

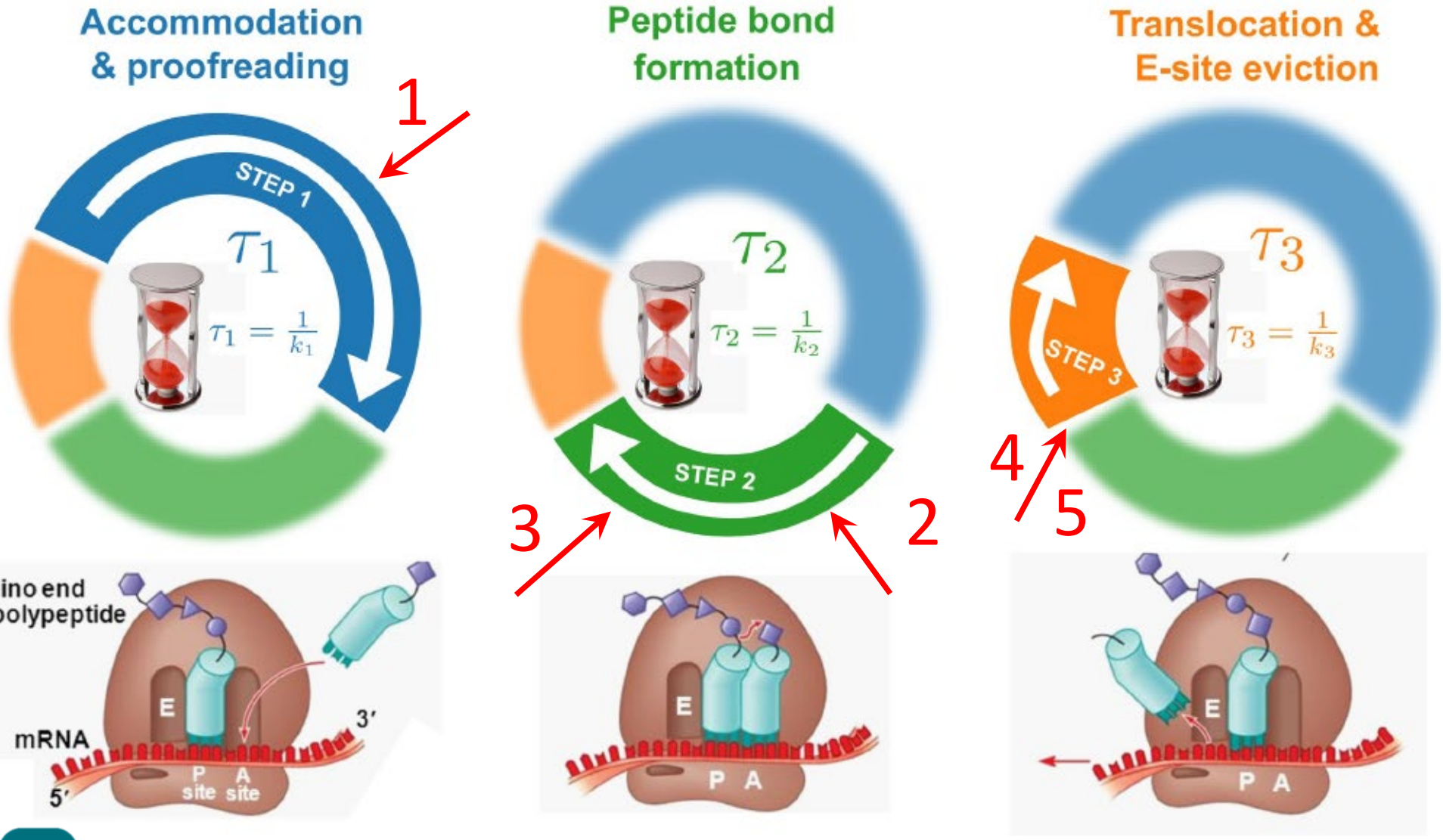
3 Problem & Research questions

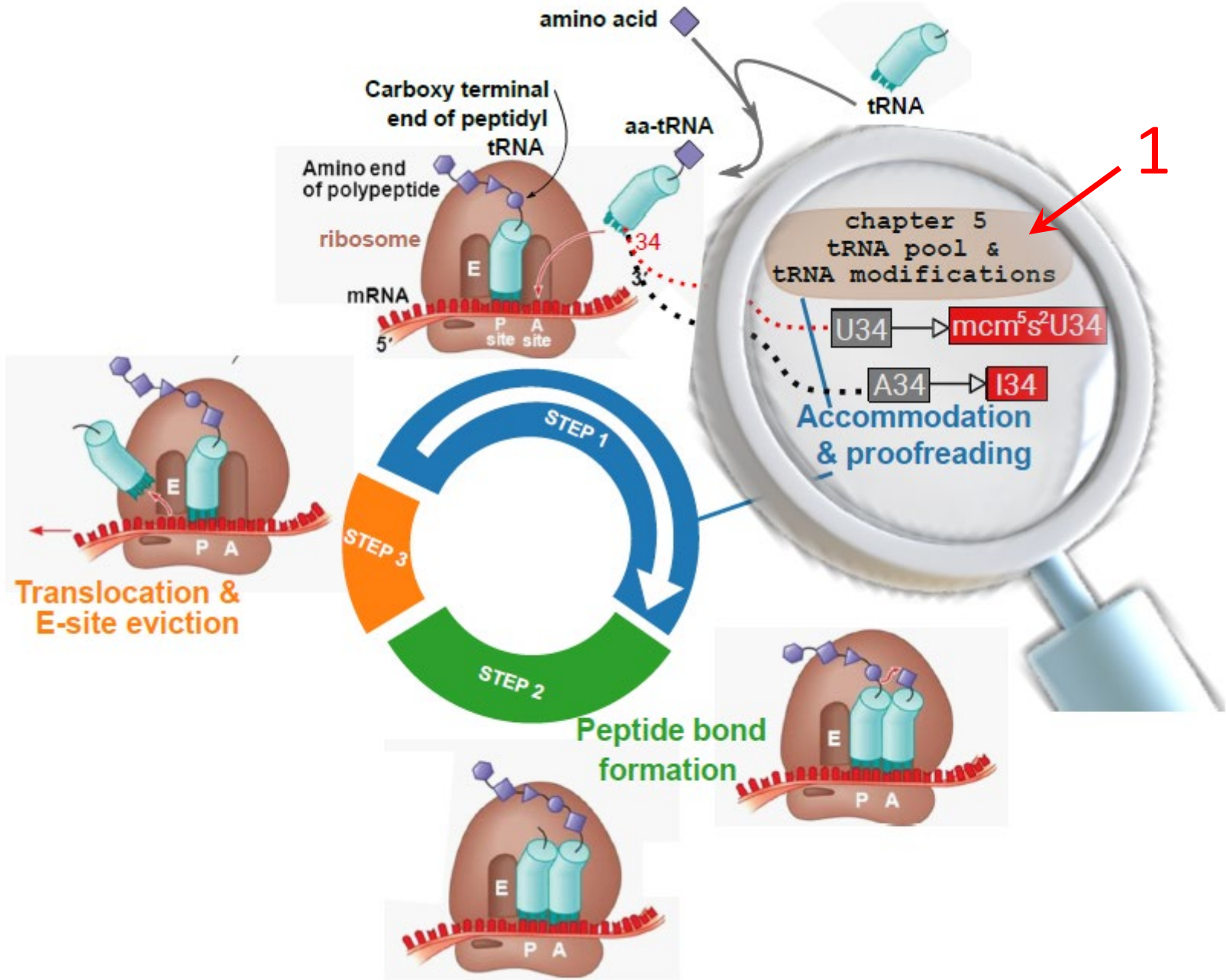
Contextual factors ?

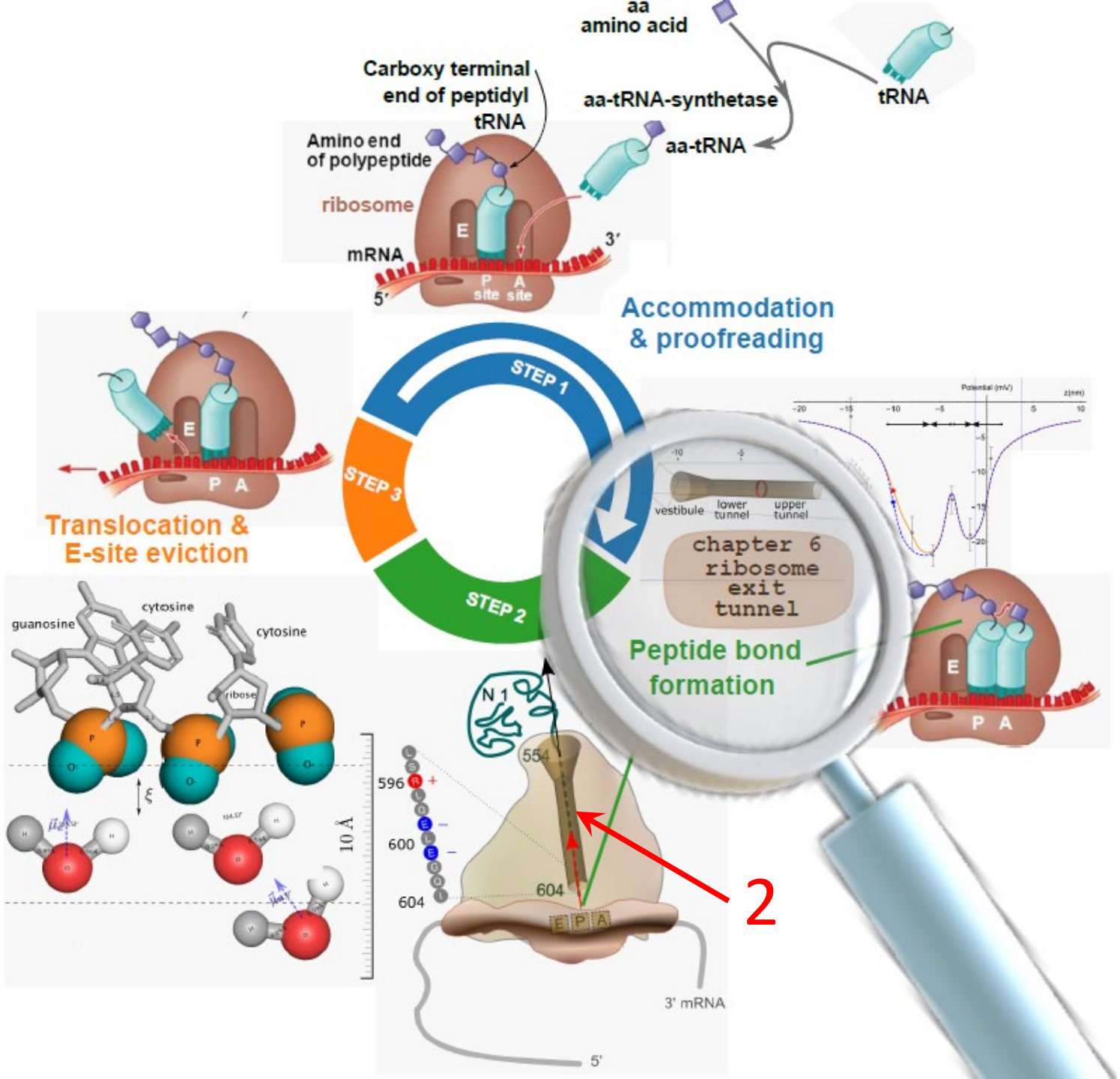


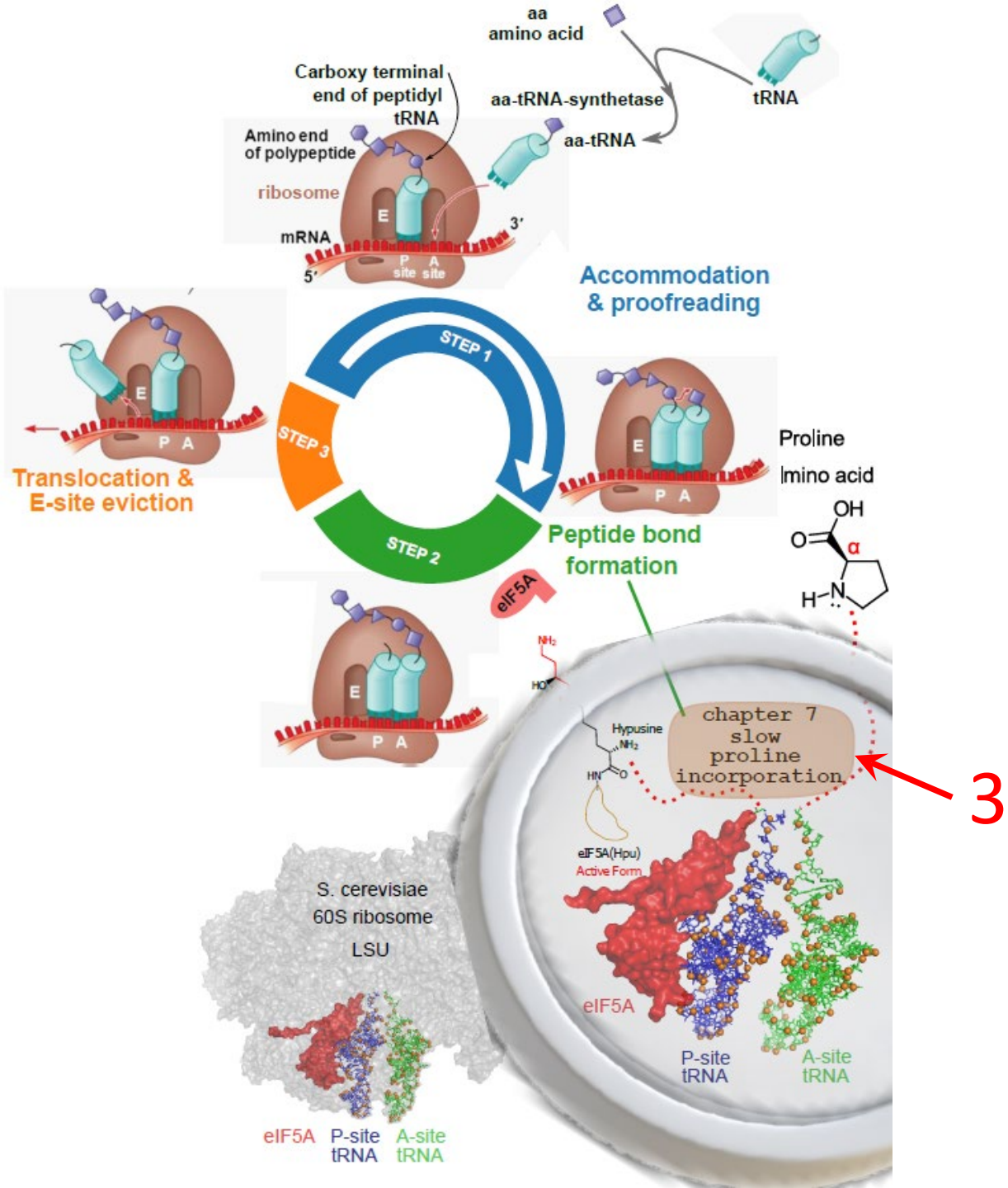
Sharma, A. K., Ahmed, N., & O'Brien, E. P. (2018). Determinants of translation speed are randomly distributed across transcripts resulting in a universal scaling of protein synthesis times. *Physical Review E, 97*(2), 022409-20.

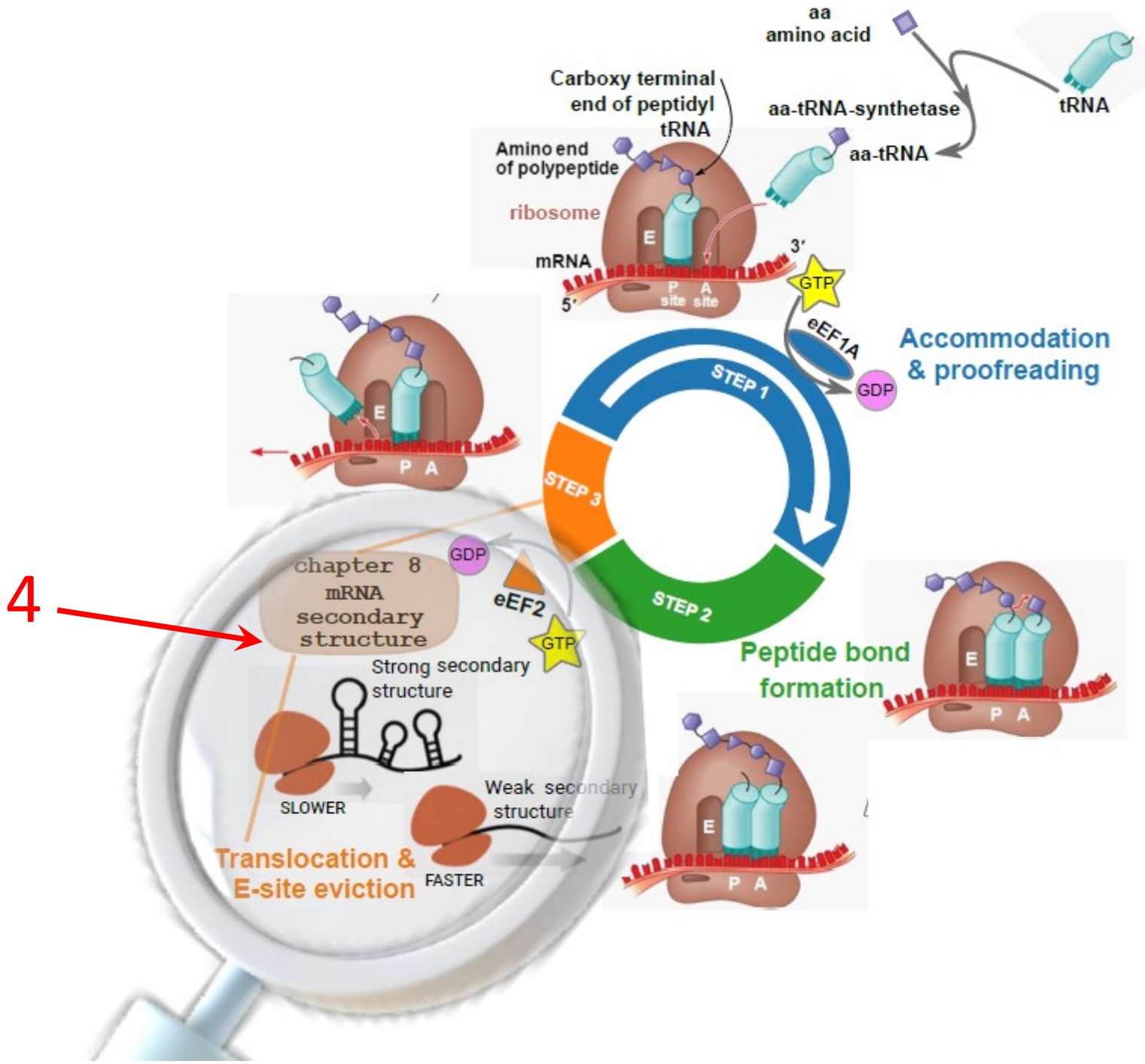
3 Problem & Research question



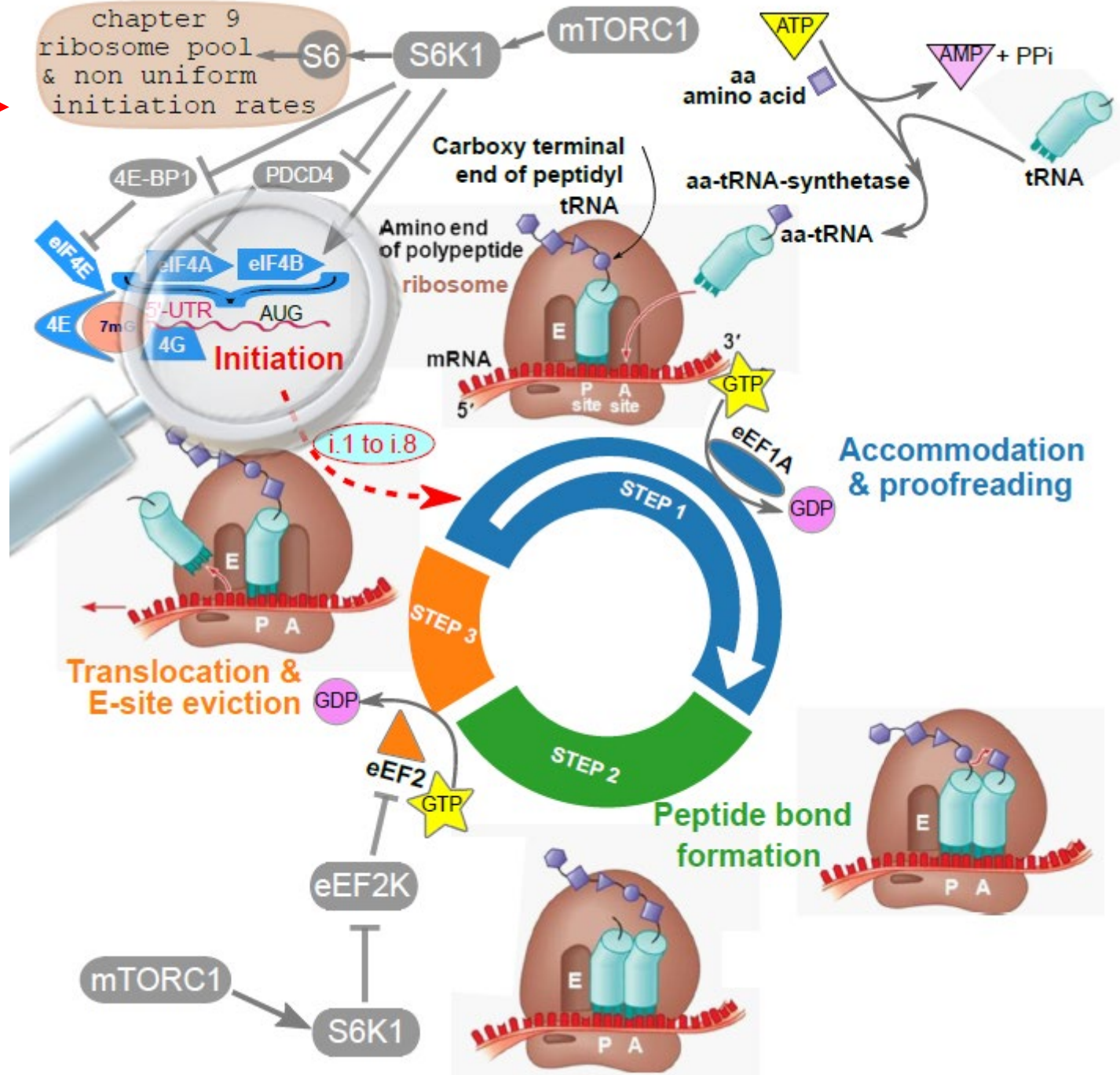

amino acid
tRNA
Carboxy terminal
end of peptidyl
tRNA
aa-tRNA
Amino end
of polypeptide
ribosome
mRNA
E
P A
site site
5'
3'
34
1
chapter 5
tRNA pool &
tRNA modifications
U34
mcm5s2U34
A34
I34
Accommodation
& proofreading
STEP 1
STEP 2
STEP 3
Translocation &
E-site eviction
Peptide bond
formation


aa
amino acid
tRNA
aa-tRNA-synthetase
aa-tRNA
Carboxy terminal
end of peptidyl
tRNA
Amino end
of polypeptide
ribosome
mRNA
E
P A
site site
5′
3′
Accommodation
& proofreading
STEP 1
STEP 2
STEP 3
Translocation &
E-site eviction
chapter 6
ribosome
exit
tunnel
vestibule
lower
tunnel
upper
tunnel
Peptide bond
formation
guanosine
cytosine
cytosine
ribose
10 Å
N1
554
596
600
604
604
E P A
3' mRNA
5'
2


aa
amino acid
tRNA
aa-tRNA-synthetase
aa-tRNA
Carboxy terminal
end of peptidyl
tRNA
Amino end
of polypeptide
ribosome
mRNA
5′
3′
E
P A
site site
Accommodation
& proofreading
STEP 1
STEP 2
STEP 3
Translocation &
E-site eviction
Peptide bond
formation
eIF5A
Proline
Imino acid
OH
O
α
H
N
chapter 7
slow
proline
incorporation
Hypusine
eIF5A(Hpu)
Active Form
eIF5A
P-site
tRNA
A-site
tRNA
S. cerevisiae
60S ribosome
LSU
eIF5A
P-site
tRNA
A-site
tRNA
3


aa
amino acid
tRNA
aa-tRNA-synthetase
aa-tRNA
Carboxy terminal
end of peptidyl
tRNA
Amino end
of polypeptide
ribosome
mRNA
5′
3′
E
P A
site site
GTP
eEF1A
GDP
Accommodation
& proofreading
STEP 1
STEP 2
STEP 3
Peptide bond
formation
4
chapter 8
mRNA
secondary
structure
GDP
eEF2
GTP
Strong secondary
structure
SLOWER
Weak secondary
structure
FASTER
Translocation &
E-site eviction

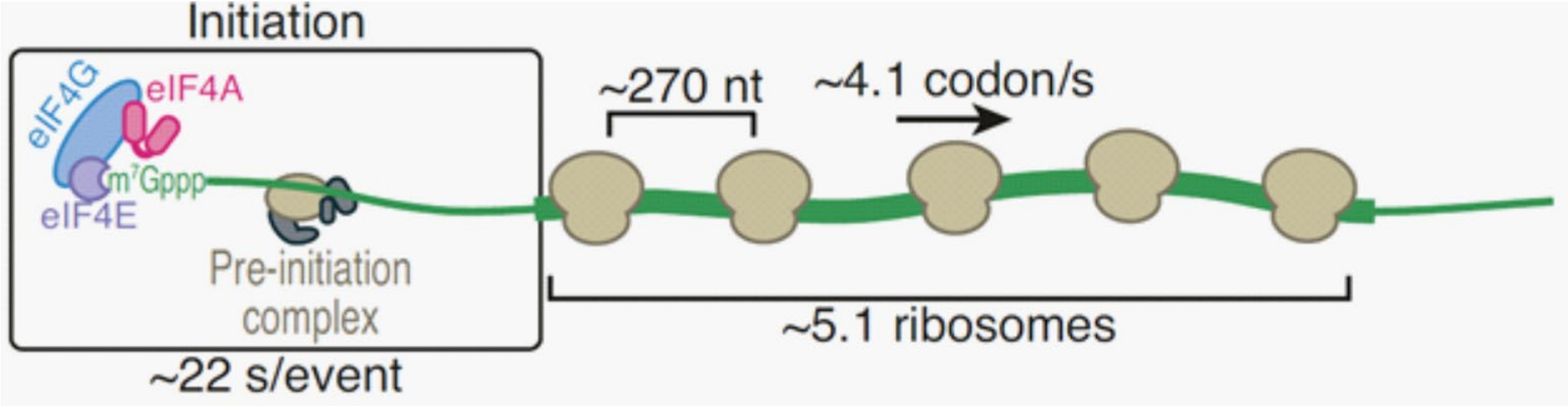
5, 6, 7…


chapter 9
ribosome pool
& non uniform
initiation rates
S6
S6K1
mTORC1
ATP
aa
amino acid
AMP + PPi
tRNA
aa-tRNA-synthetase
aa-tRNA
Carboxy terminal
end of peptidyl
tRNA
Amino end
of polypeptide
ribosome
4E-BP1
PDCD4
eIF4E
eIF4A
eIF4B
4E
7mG
5'-UTR
AUG
4G
Initiation
mRNA
3'
5'
E
P A
site site
GTP
eEF1A
GDP
Accommodation
& proofreading
i.1 to i.8
STEP 1
STEP 2
STEP 3
Translocation &
E-site eviction
eEF2
GTP
Peptide bond
formation
eEF2K
mTORC1
S6K1

3 Problem & Research questions

5, 6, 7… → **Initiation & Elongation dynamics ?**

Ribosome pool resources ?



1 2 3

Tomuro, K., Mito, M., Toh, H., Kawamoto, N., Miyake, T., Chow, S., Doi, M., Ikeuchi, Y., Shichino, Y., & Iwasaki, S. (2024, August). Calibrated ribosome profiling assesses the dynamics of ribosomal flux on transcripts. *Nature Communications, 15*

4 Keys to address the problem

1) Mechano-biochemistry in Henry Eyring's Catalysis Theory

Guldberg & Waage's law of mass action (1864)

$$X + Y \xrightarrow[v_1]{k} Z \qquad v_1 = \frac{d[X]}{dt}$$

In-"Avogadro"

k *= rate constant*

Law of mass action

$$\frac{d[X]}{dt} = -k[X][Y]$$

First order kinetic

$$\frac{d[X]}{dt} = -k \cdot [X]$$

if [Y] is constant

Exponential decay

$$X(t) = X(t_0) \cdot e^{-k \cdot (t - t_0)}$$

if AUC normalized to 1

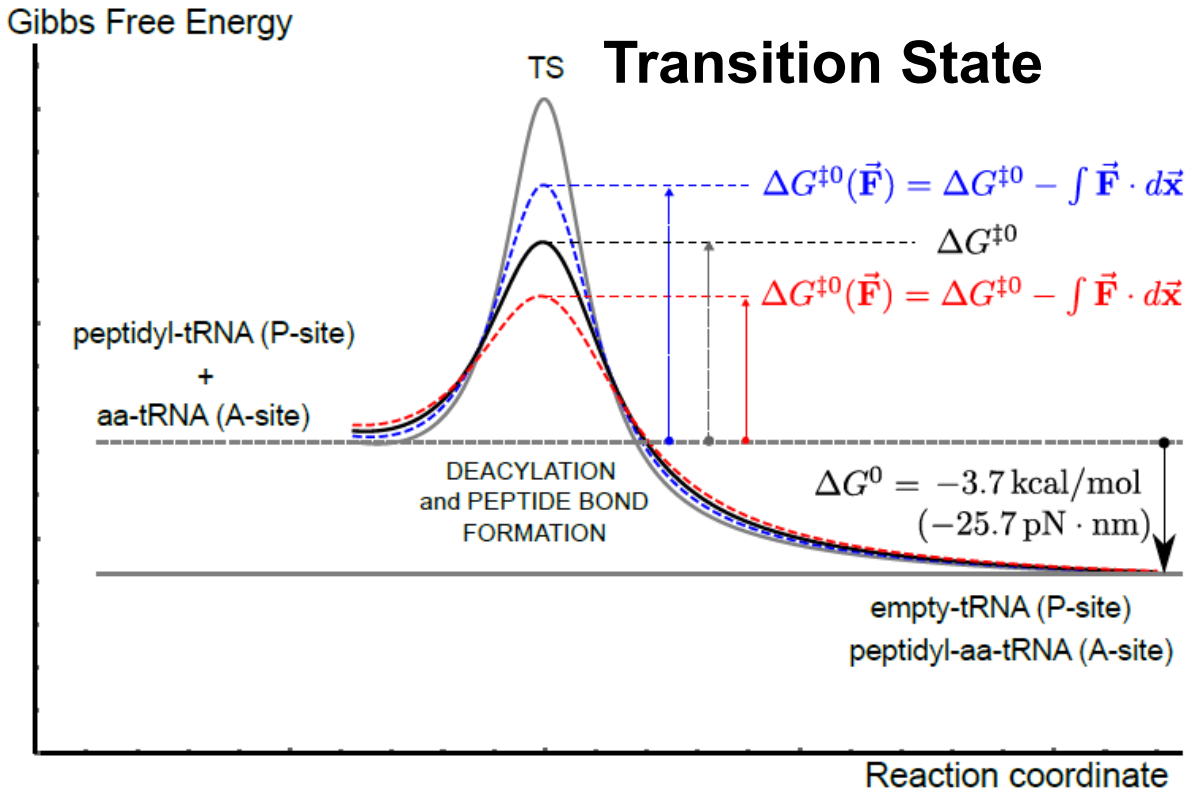
PDF of an exponentially distributed random variable

$$f_{\mathrm{EXP}}(t) = \lambda e^{-\lambda \cdot t}$$

$\lambda = k$ $\qquad \tau_{1/2} = \frac{\ln 2}{k}$

in-singulo $\tau_{1/2} = \frac{\ln 2}{k}$ *= median "queueing" time*

Mechano-biochemistry in Henry Eyring's Catalysis Theory



$$\Delta G^{\ddagger 0}(\vec{\mathbf{F}}) = \Delta G^{\ddagger 0}(\mathbf{0}) - \int \vec{\mathbf{F}} \cdot \mathbf{d}\vec{\mathbf{x}}$$

Eyring's Relation (1934)

$$k(\vec{\mathbf{F}}) = \kappa \cdot \left(\frac{k_B \cdot T}{h}\right) \cdot e^{-\Delta G^{\ddagger 0(\vec{\mathbf{F}})}/N\,k_B\,T}$$

$$k(\vec{\mathbf{F}}) = \kappa \cdot \left(\frac{k_B \cdot T}{h}\right) \cdot e^{-\left(\frac{\Delta G^{\ddagger 0(\mathbf{0})}}{N\,k_B\,T} - \frac{\int \vec{\mathbf{F}} \cdot d\vec{\mathbf{x}}}{k_B\,T}\right)} = k(\mathbf{0}) \cdot e^{\frac{\int \vec{\mathbf{F}} \cdot d\vec{\mathbf{x}}}{k_B\,T}}$$

$$\underbrace{\text{peptidyl tRNA}}_{P-site} + \underbrace{\text{aminoacyl tRNA}}_{A-site} \longrightarrow \underbrace{\text{empty tRNA}}_{P-site} + \underbrace{\text{peptidyl(+1) tRNA}}_{A-site}.$$

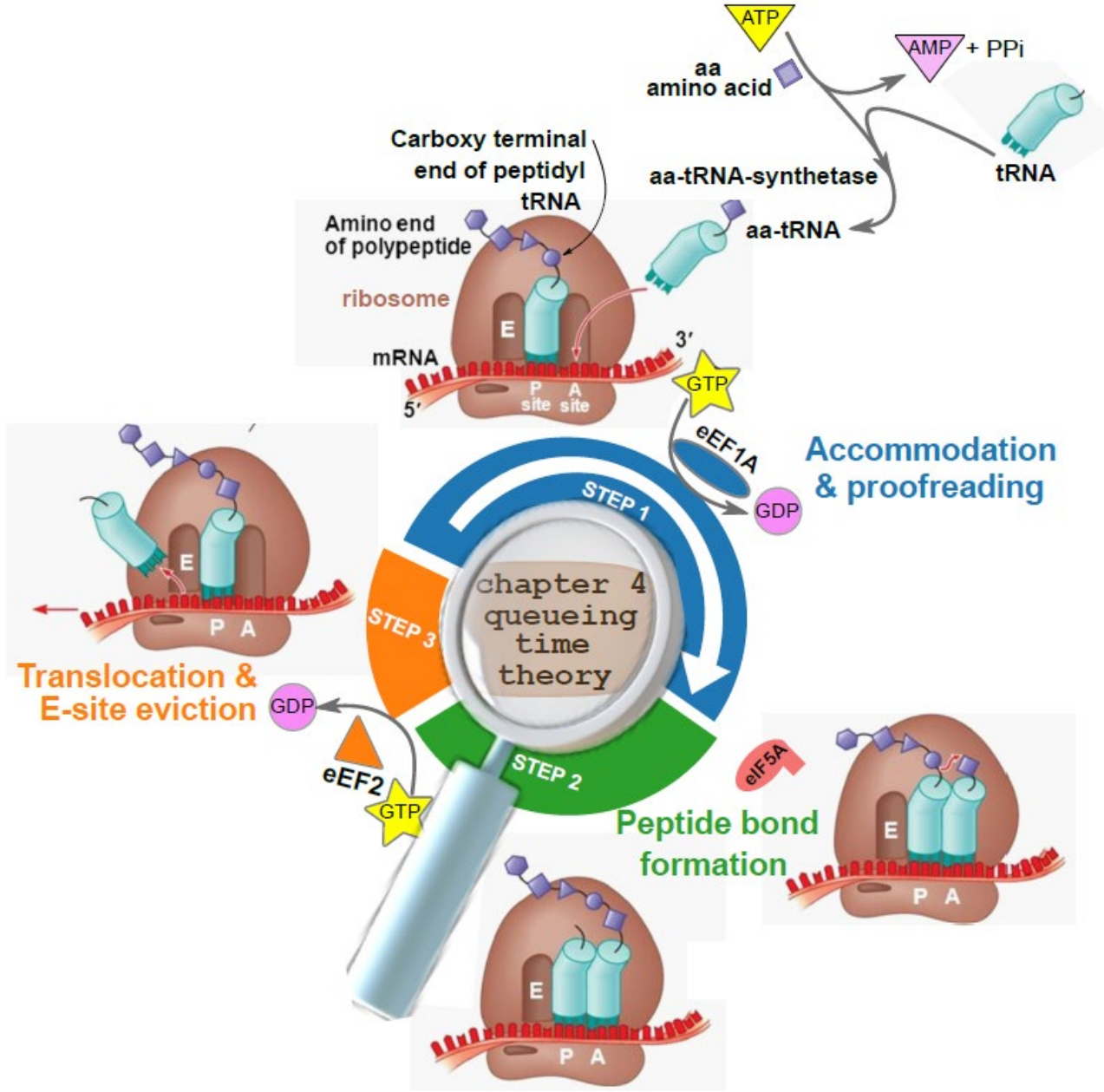
1 2 3 4

Joiret, M., Kerff, F., Rapino, F., Close, P., & Geris, L. (2023). A simple geometrical model of the electrostatic environment around the catalytic center of the ribosome and its significance for the elongation cycle kinetics. *Computational and Structural Biotechnology Journal, 21*, 3768–3795.

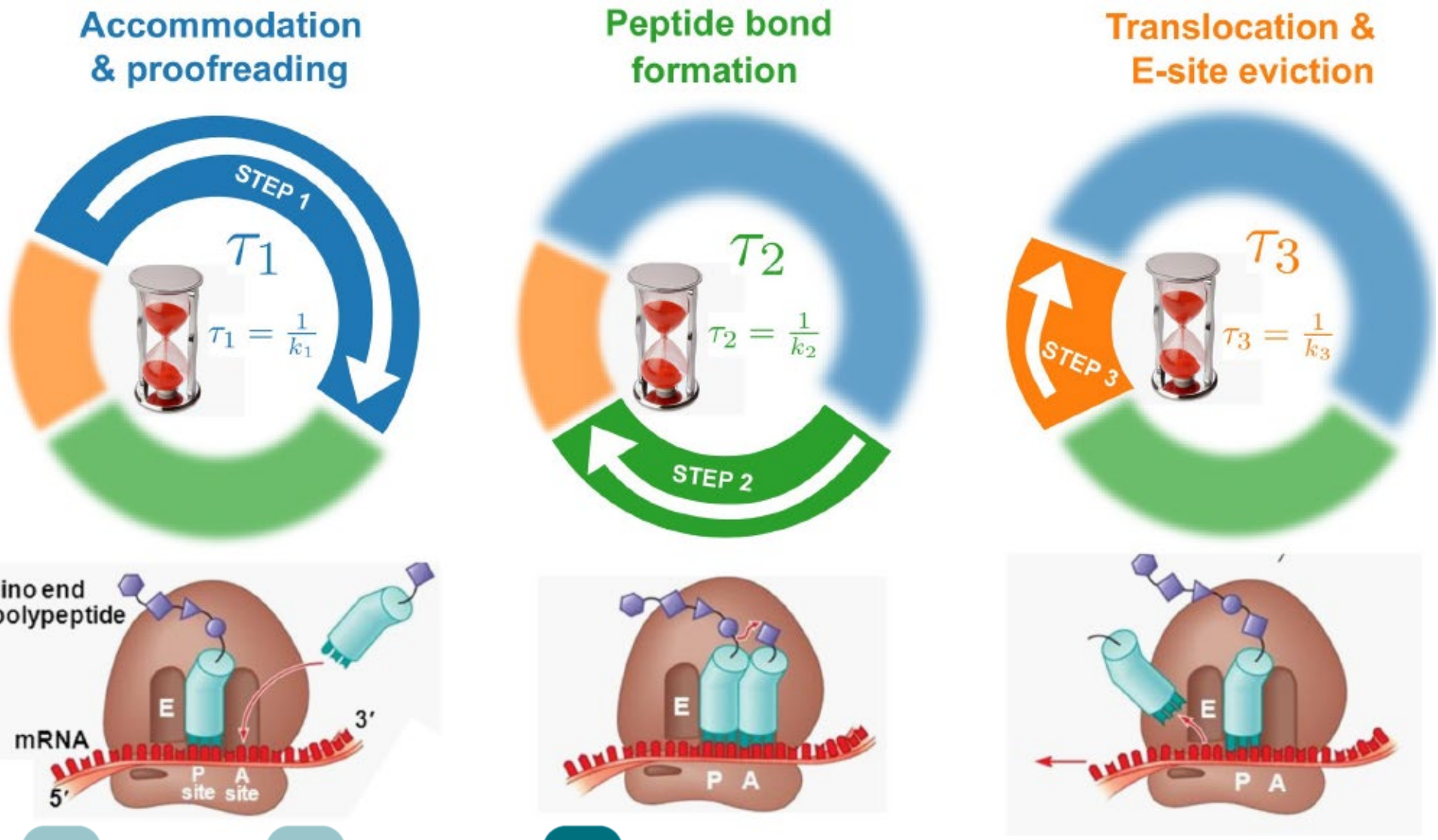
4 Keys to address the problem

1) Mechano-biochemistry In Henry Eyring's Catalysis Theory

2) Queueing Time Theory & Convolution of Multiple Random Variables


ATP
AMP + PPi
aa
amino acid
tRNA
aa-tRNA-synthetase
aa-tRNA
Carboxy terminal
end of peptidyl
tRNA
Amino end
of polypeptide
ribosome
mRNA
5′
3′
E
P site
A site
GTP
eEF1A
GDP
Accommodation
& proofreading
STEP 1
STEP 2
STEP 3
chapter 4
queueing
time
theory
Translocation &
E-site eviction
GDP
eEF2
GTP
eIF5A
Peptide bond
formation
P A

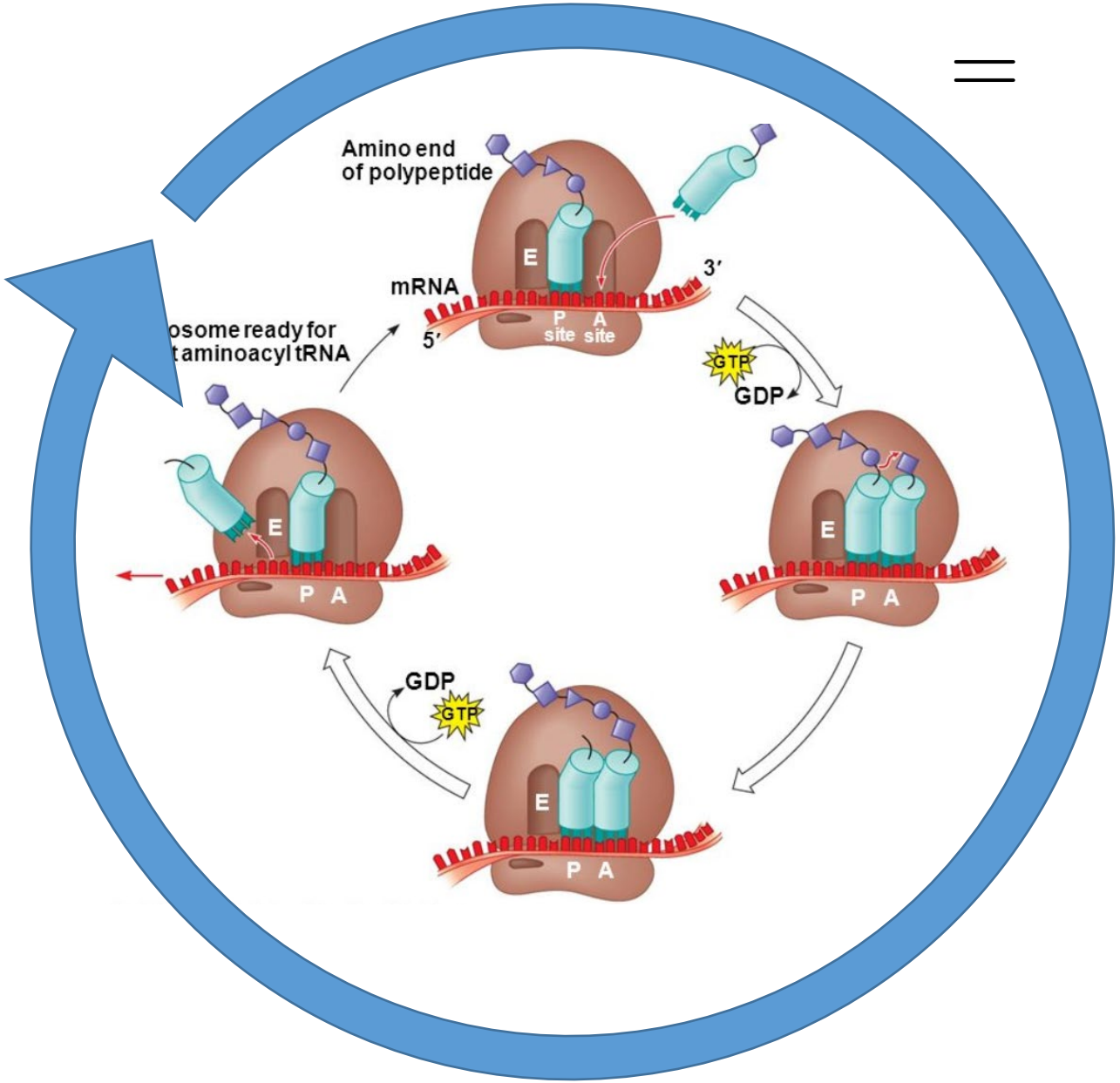
Consider each sequential step as a queueing step with a random queueing time



- **The 3 queueing times**

$$\tau_{tot} = \tau_1 + \tau_2 + \tau_3$$

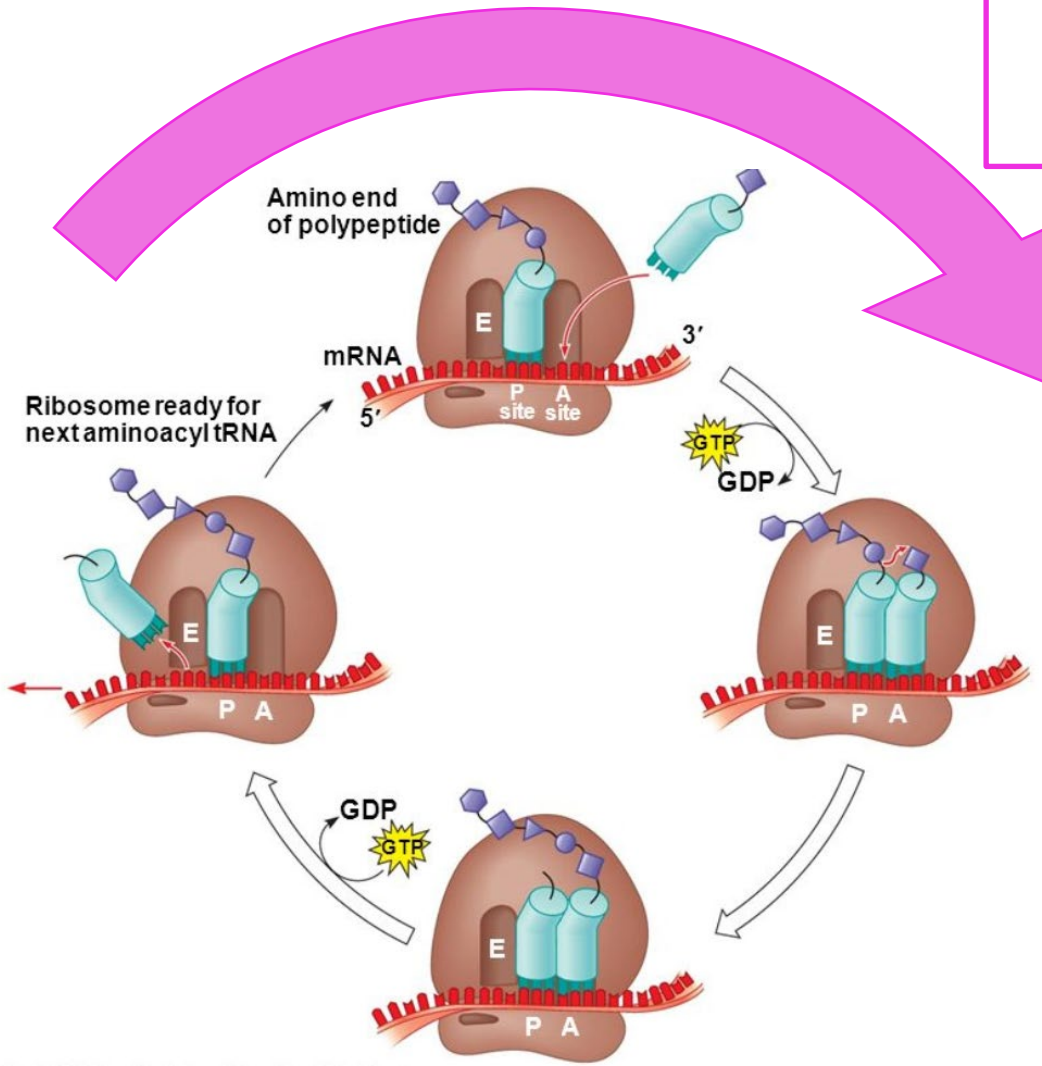
$$= 180\,\mathrm{ms}$$



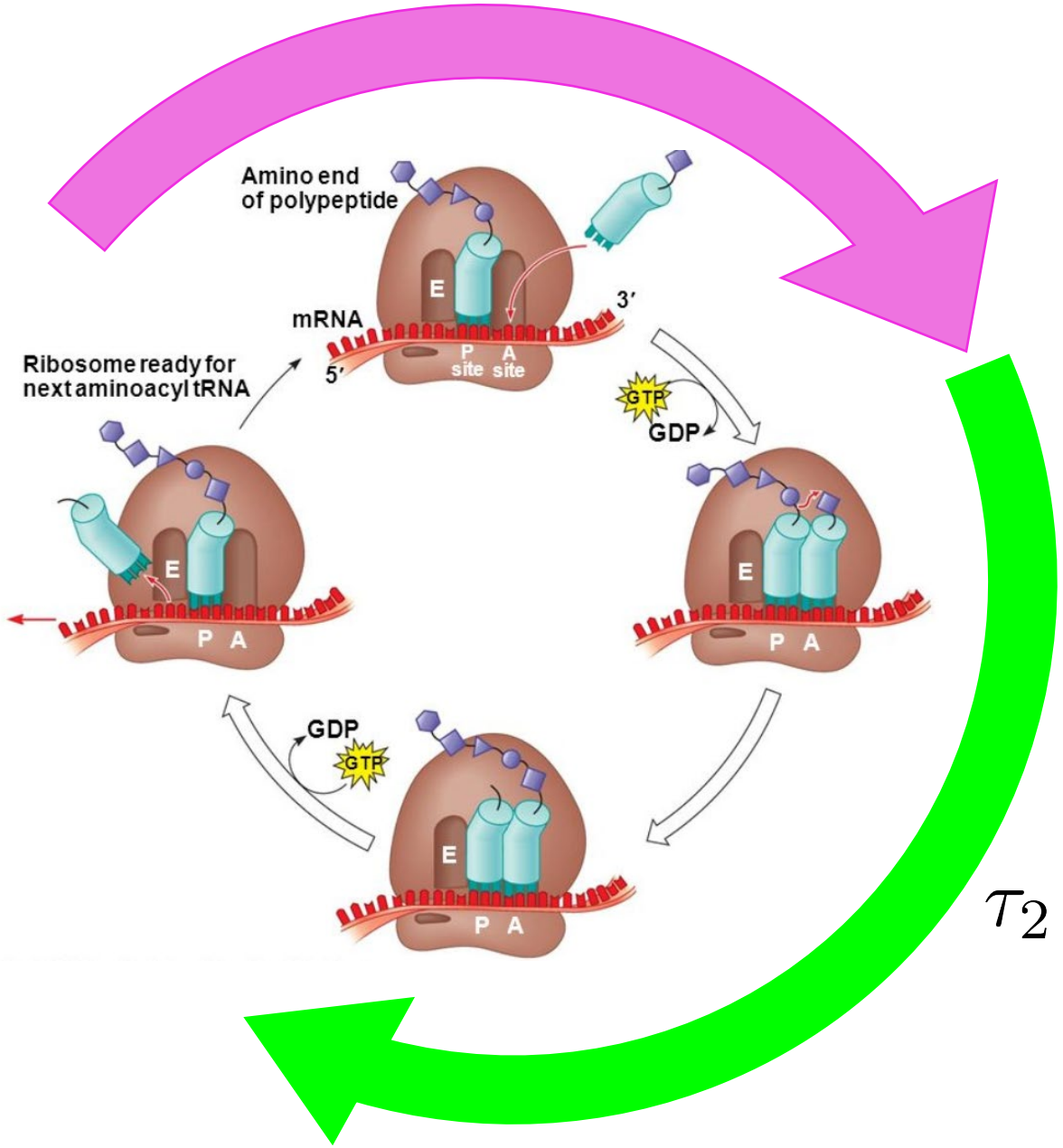
Average turnover 5.6 amino acids per second

- **The 3 queueing times** $\tau_1 = 100 - 350 \quad \mathrm{ms}$

aa-tRNA-ACCOMMODATION & PROOFREADING



- **The 3 queueing times**



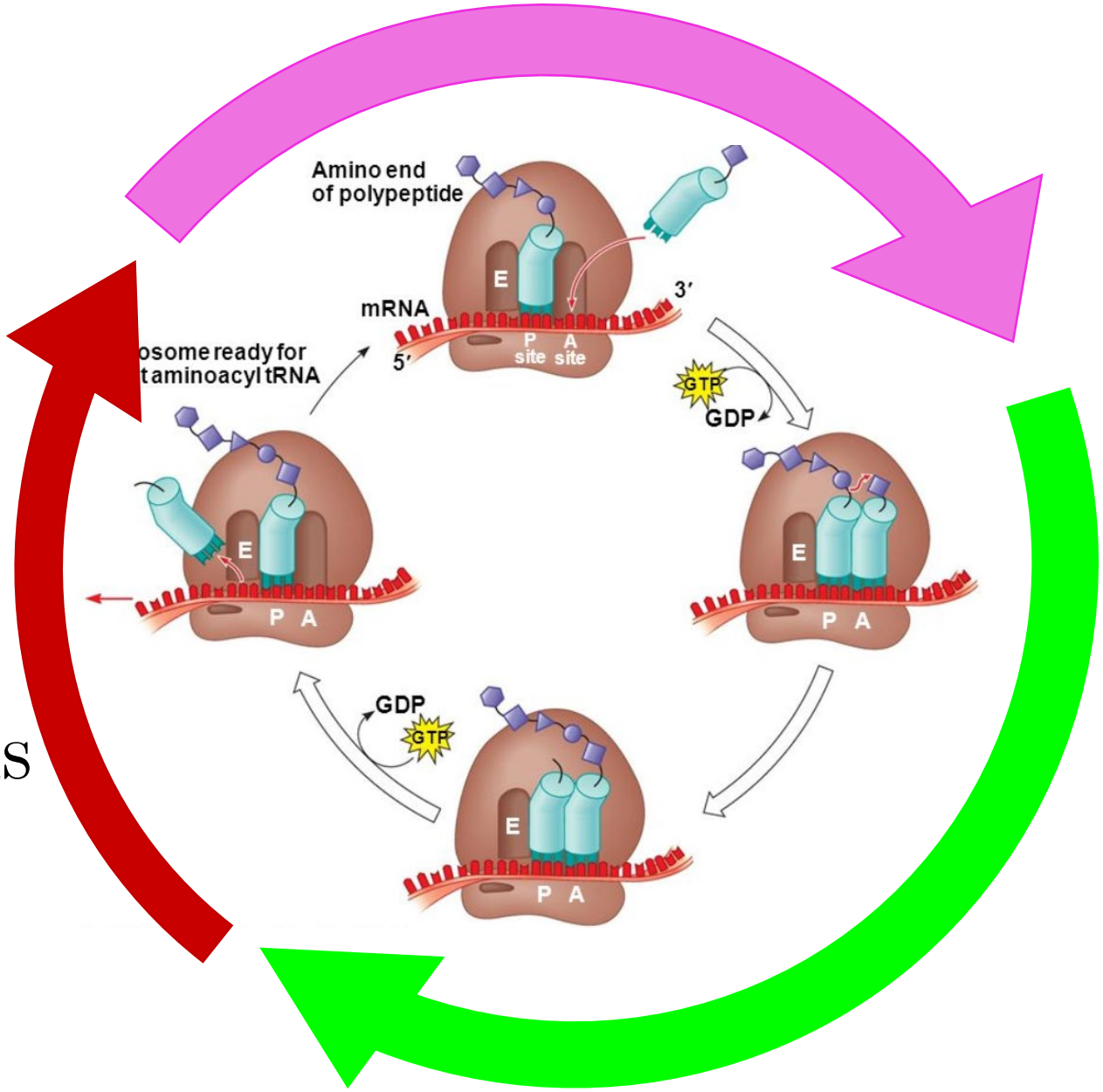
DE-ACYLATION
&
PEPTIDE BOND
FORMATION

$$\tau_2 = 0.4 - 60 \quad \text{ms}$$

- **The 3 queueing times**

TRANSLOCATION

$$\tau_3 = 10 - 18 \quad \text{ms}$$



Prob & Stats

Sum of random variables ?

$$X \sim P(X = x) = f(x)$$

$$Y \sim P(Y = x) = g(x)$$

What is the pdf for X + Y ?

$$Z = X + Y$$

$$Z \sim ?$$

$$Z = X + Y + W$$

$$Z \sim ?$$

Prob & Stats

Sum of 3 random EXP variables ?

$$X1_{EXP} = X2_{EXP} = X3_{EXP} \sim f_{X_i\,EXP}(x_i) = \lambda e^{-\lambda x_i}$$
$$Z \sim P(Z = z = x_1 + x_2 + x_3)$$

What is the pdf for X + Y + Z ?

If the rates are equal: GAMMA

If the rates are pairwise distinct: $\mathrm{HYPO} - \mathrm{EXP}$

EMG

$$f_{EMG}(x) = \frac{\lambda}{2} e^{\frac{\lambda^2\sigma^2}{2}} e^{-\lambda(x-\mu)} \operatorname{Erfc}\left[\frac{1}{\sqrt{2}}\left(\lambda\sigma - \frac{x-\mu}{\sigma}\right)\right]$$

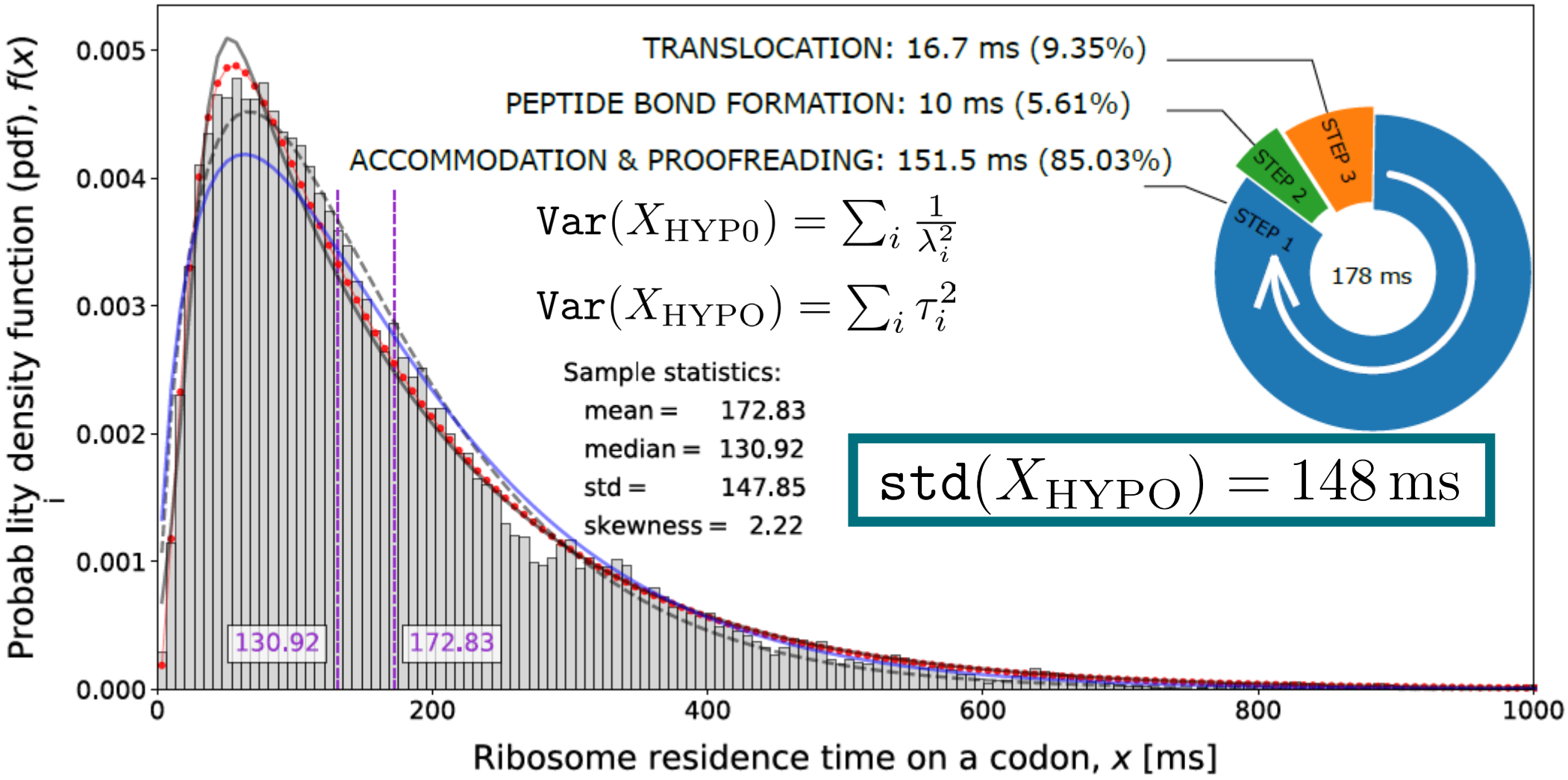
GAMMA

$$f_{X_{GAMMA}}(x;\alpha,\beta) = \begin{cases} \frac{\lambda^\alpha}{\Gamma(\alpha)} x^{\alpha-1} e^{-\lambda x} & x \geq 0 \\ \frac{1}{\beta^\alpha \Gamma(\alpha)} x^{\alpha-1} e^{-x/\beta} & x \geq 0 \\ 0 & x < 0 \end{cases}$$

HYPO – EXP

$$f_{HYPO}(t) = \lambda_1\lambda_2\lambda_3\left(\frac{e^{-\lambda_1 t}}{(\lambda_3-\lambda_1)(\lambda_2-\lambda_1)} + \frac{e^{-\lambda_2 t}}{(\lambda_3-\lambda_2)(\lambda_1-\lambda_2)} + \frac{e^{-\lambda_3 t}}{(\lambda_2-\lambda_3)(\lambda_1-\lambda_3)}\right).$$

Queueing time theory



1 2 3 4

Joiret, M., Kerff, F., Rapino, F., Close, P., & Geris, L. (2023). A simple geometrical model of the electrostatic environment around the catalytic center of the ribosome and its significance for the elongation cycle kinetics. *Computational and Structural Biotechnology Journal, 21*, 3768–3795.

Stochasticity

Mechanobiochemistry in the elongation cycle and queueing time statistical theory of the ribosome on a codon

The inherent probabilistic nature means that we can never precisely predict stochastic outcomes. This might be why we opt to use the more pretentious-sounding word "stochastic" instead of just saying "random". At least in common parlance, "random" has connotations of hopelessness. To try to understand something random feels futile; it's just random. But of course, we can understand many things about stochastic-random processes, despite this inherent unpredictability, by studying the probability distributions.

Professor Karen Abbott
Case Western Reserve University, Department of Biology, Theoretical Ecology and Evolution, Cleveland , Ohio, USA.

1 2 3 4

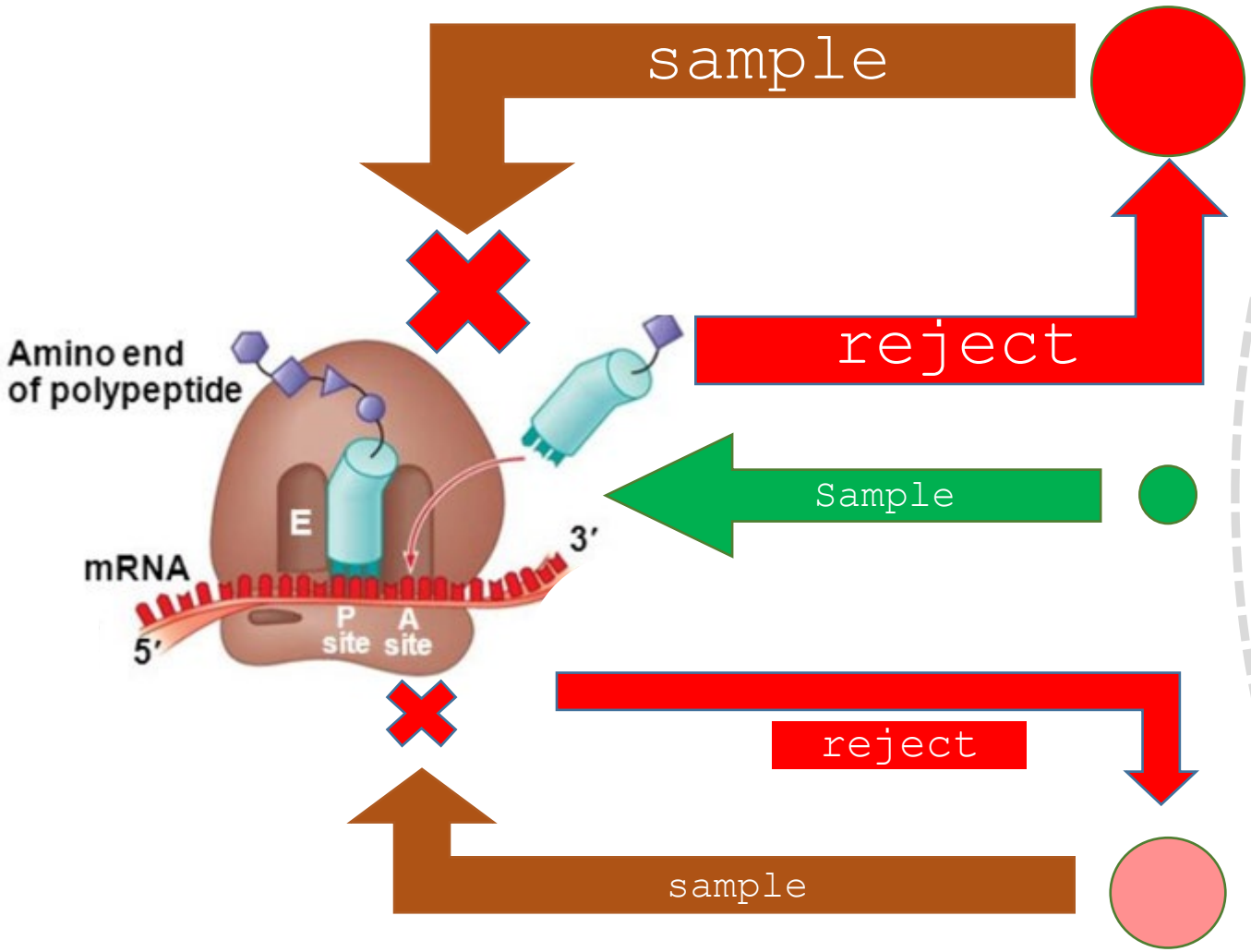
Joiret, M., Kerff, F., Rapino, F., Close, P., & Geris, L. (2023). A simple geometrical model of the electrostatic environment around the catalytic center of the ribosome and its significance for the elongation cycle kinetics. *Computational and Structural Biotechnology Journal, 21*, 3768–3795.

Stochasticity

- **Mathematical origin and description:**
  a sequence of 3 queueing times exponentially distributed

Joiret, M., Kerff, F., Rapino, F., Close, P., & Geris, L. (2023). A simple geometrical model of the electrostatic environment around the catalytic center of the ribosome and its significance for the elongation cycle kinetics. *Computational and Structural Biotechnology Journal, 21*, 3768–3795.

tRNAs pool

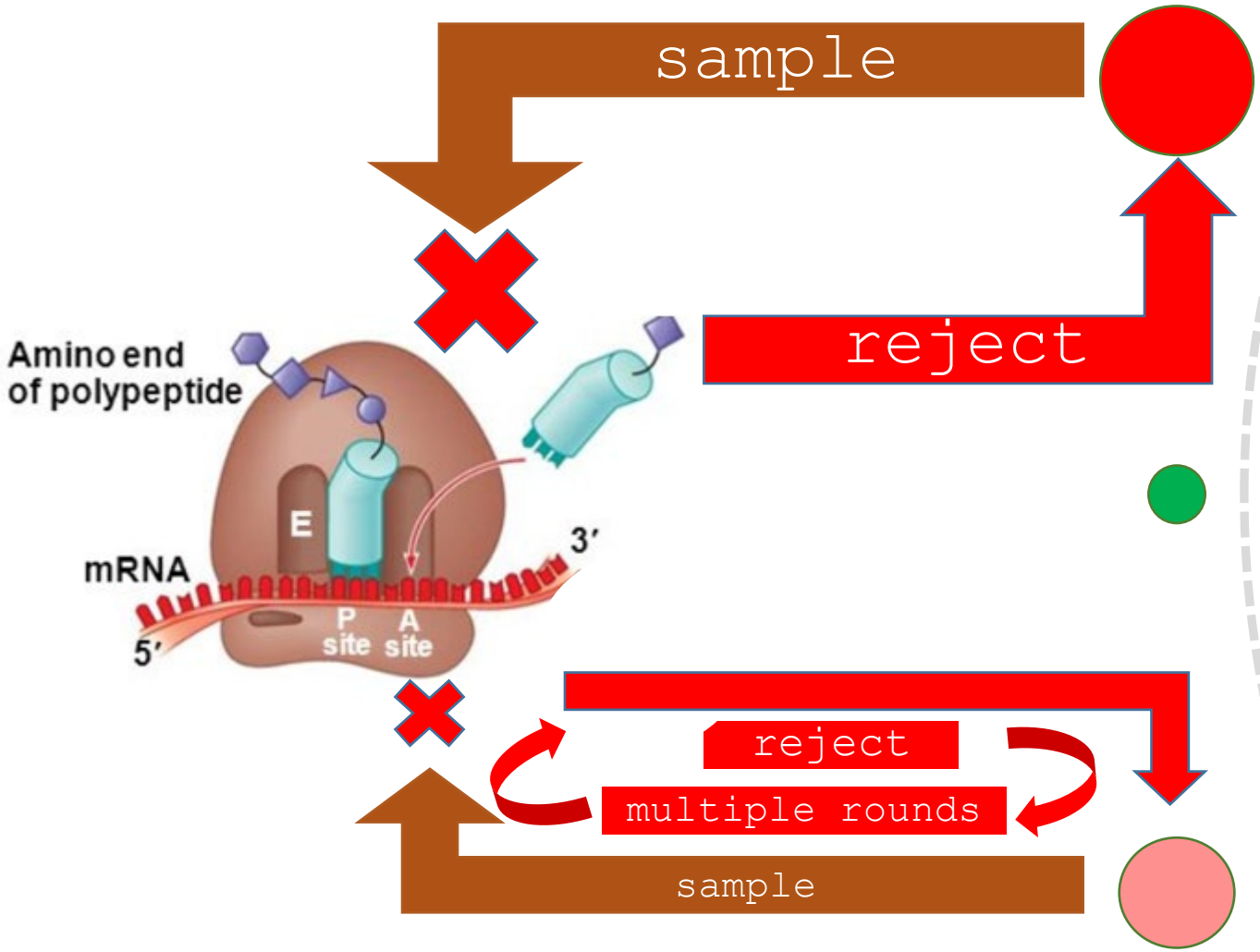


Non-cognate tRNAs

Cognate tRNAs

Near-cognate tRNAs

tRNAs pool



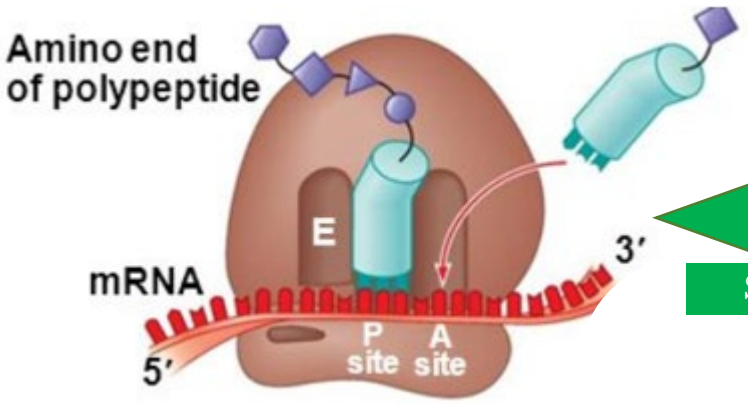
Non-cognate tRNAs

Cognate tRNAs

Near-cognate tRNAs

tRNAs pool

Non-cognate tRNAs



Sample & accept

Cognate tRNAs

The queuing time of a ribosome before translocation depends on the codon and on its number of near/non-cognate tRNAs

Inherently stochastic

Near-cognate tRNAs

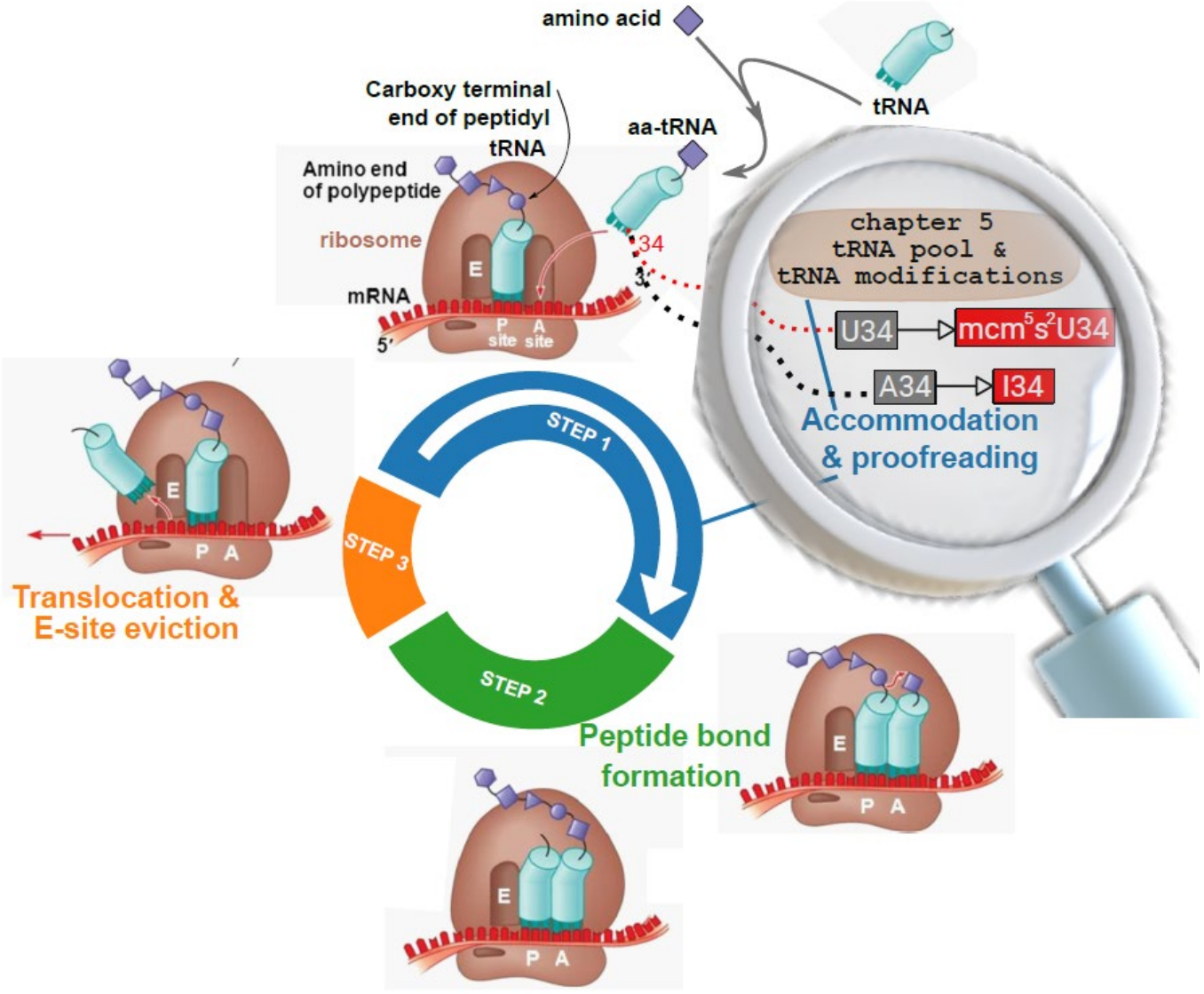
Stochasticity

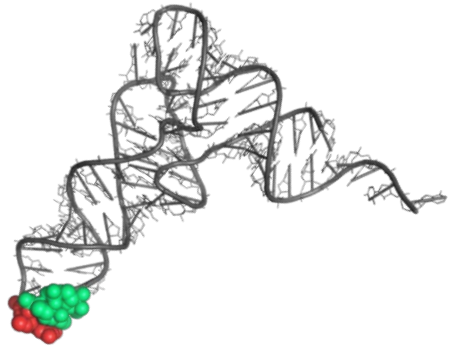
- **Mathematical origin and description:**
  a sequence of 3 queueing times exponentially distributed

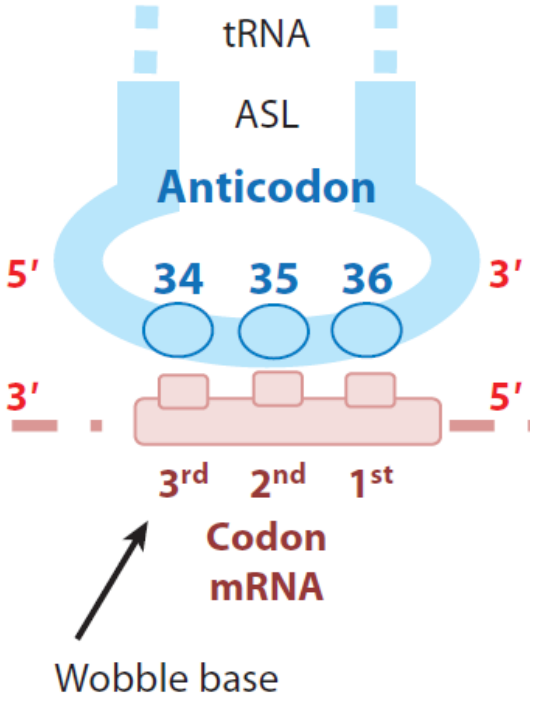
- **Biochemical origin and description:**
  sample and reject until cognate tRNA accepted

Joiret, M., Kerff, F., Rapino, F., Close, P., & Geris, L. (2023). A simple geometrical model of the electrostatic environment around the catalytic center of the ribosome and its significance for the elongation cycle kinetics. *Computational and Structural Biotechnology Journal, 21*, 3768–3795.

3 Problem & Research questions







Wobble Base Rule

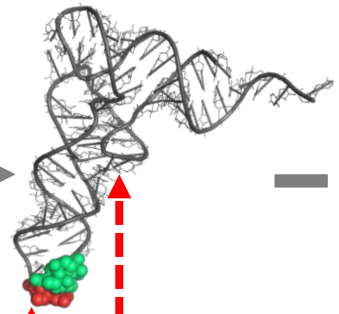
First anticodon base	Third codon base
C	G
A	U
U	A or G
G	U or C
I	U, C or A

3 Problem & Research questions

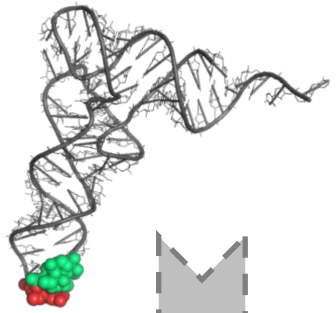
tRNAs (in humans)

51 tRNAs

512 tRNA genes
(of which 434 are unique)



modified tRNAs
(more than 120 modifications are known)

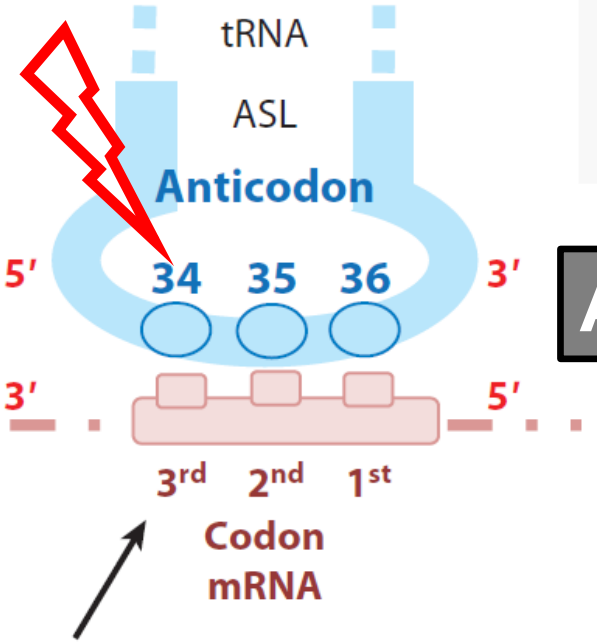


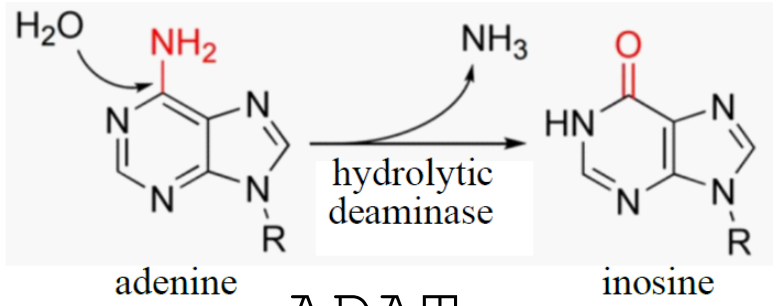
Modified proteome and/or modified elongation rates depending on codon usage

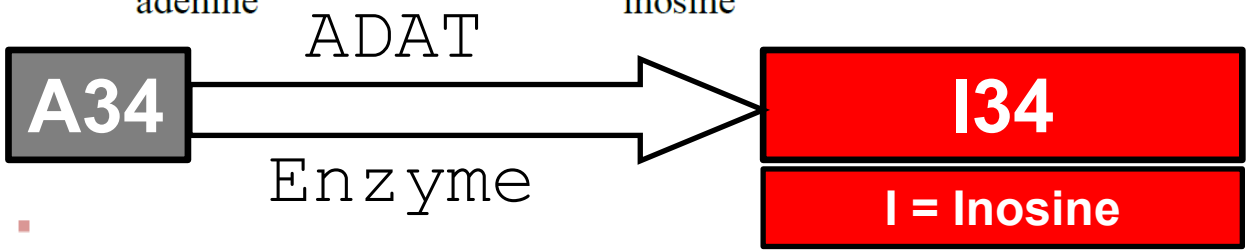
tRNA modifying enzyme genes

tRNA modifying enzymes (ELP3, ADAT,…)

3 Problem & Research questions







A34

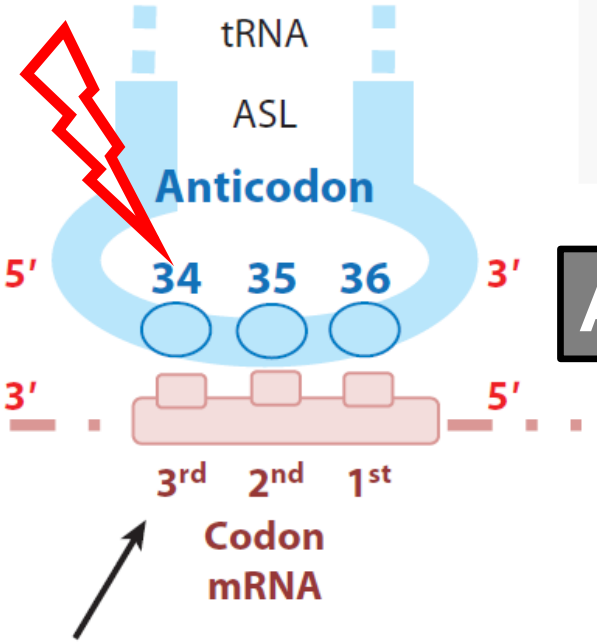
Wobble Base Rule

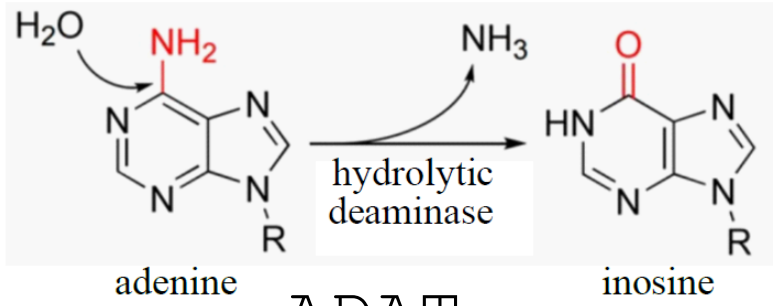
First anticodon base	Third codon base
C	G
A	U
U	A or G
G	U or C
I	U, C or A

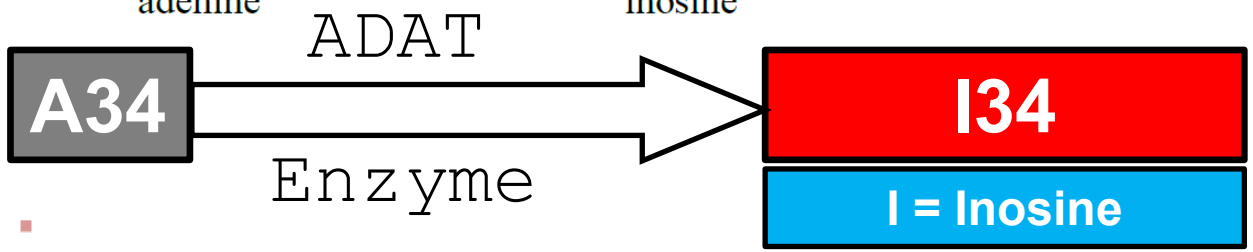
Alanine has 4 synonymous codons

tRNA gene copy number	0	29	5	3
tRNA anti-codon	3’ CGG 5’	3’ CGA 5’	3’ CGC 5’	3’ CGU 5’
mRNA codon	5’ GCC 3’	5’ GCU 3’	5’ GCG 3’	5’ GCA 3’
Codon usage in ‰	36.0	21.1	17.3	12.6

3 Problem & Research questions







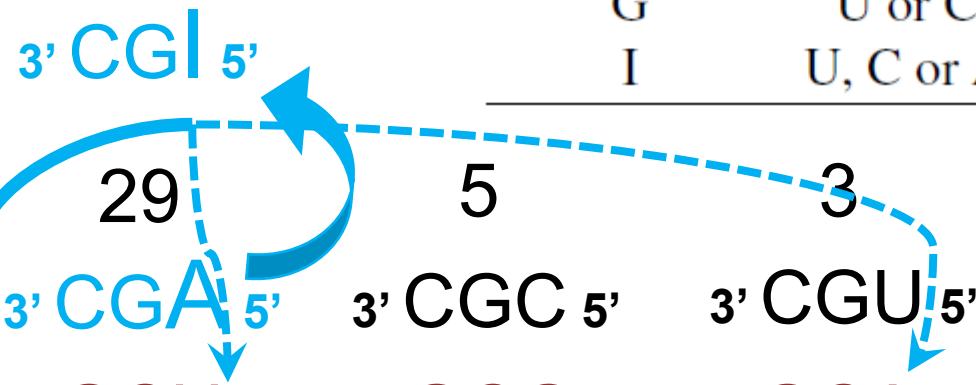
A34

Wobble Base Rule

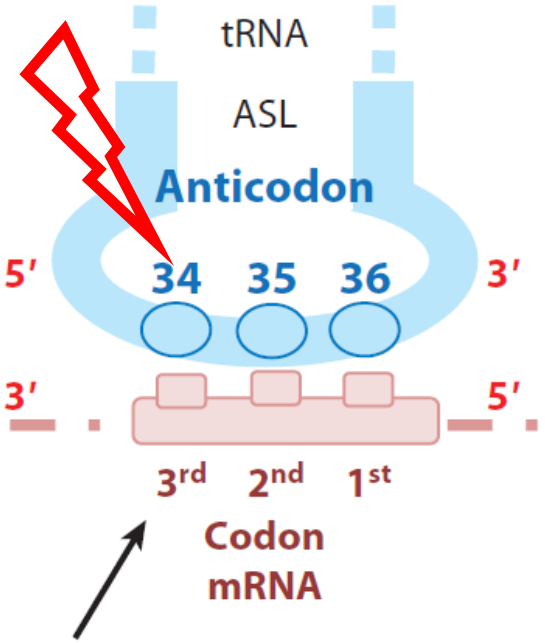
First anticodon base	Third codon base
C	G
A	U
U	A or G
G	U or C
I	U, C or A

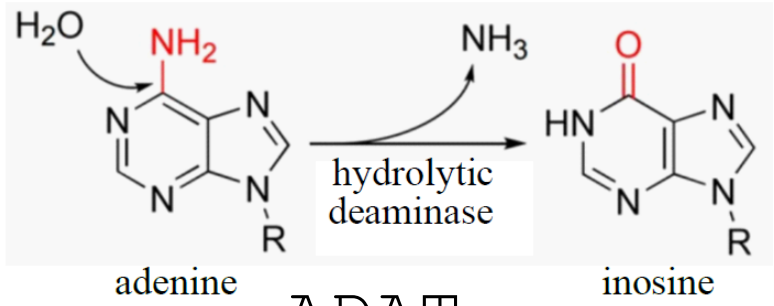
Alanine has 4 synonymous codons

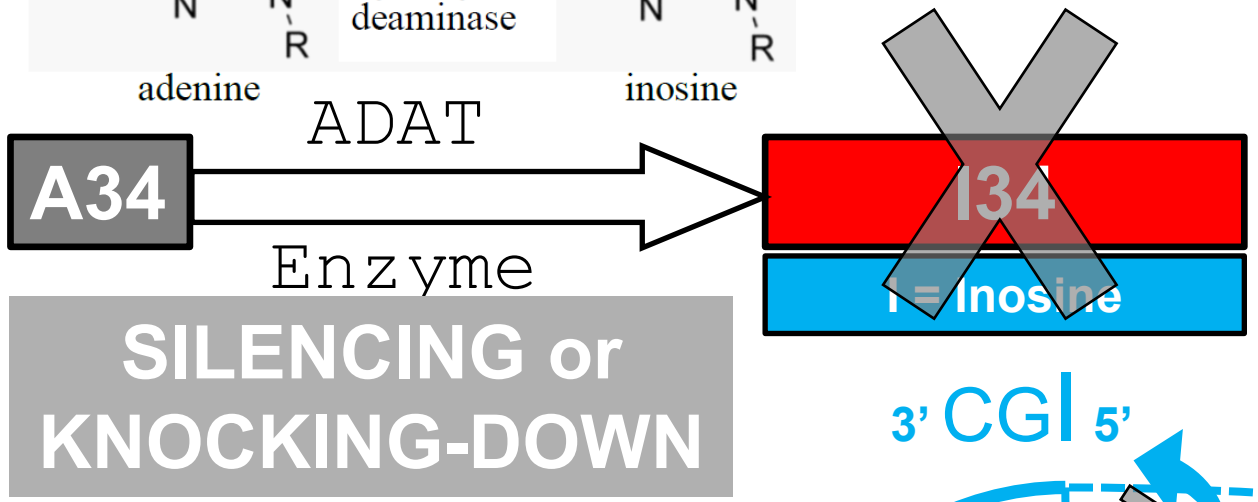
tRNA gene copy number	0	29	5	3
tRNA anti-codon	~~3’ CGG 5’~~	3’ CGA 5’	3’ CGC 5’	3’ CGU 5’
mRNA codon	5’ GCC 3’	5’ GCU 3’	5’ GCG 3’	5’ GCA 3’
Codon usage in ‰	36.0	21.1	17.3	12.6
	much faster	**slower**		**faster**



3 Problem & Research questions







SILENCING or KNOCKING-DOWN

A34

Wobble Base Rule

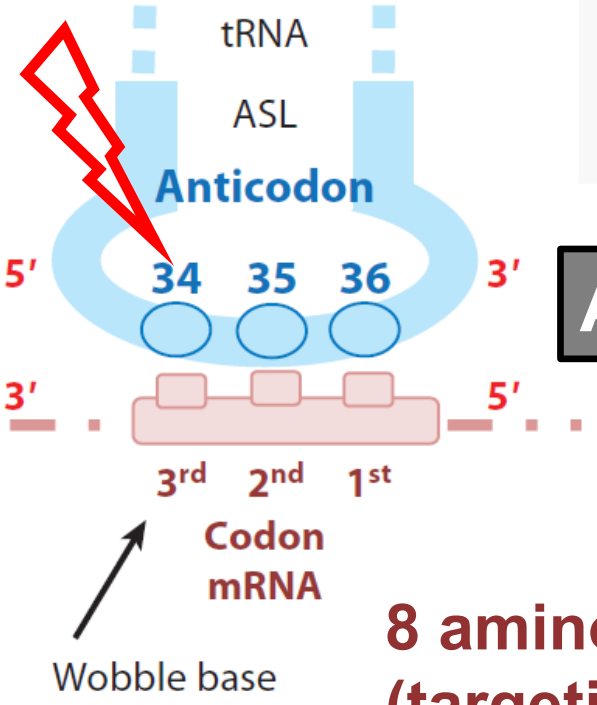
First anticodon base	Third codon base
C	G
A	U
U	A or G
G	U or C
I	U, C or A

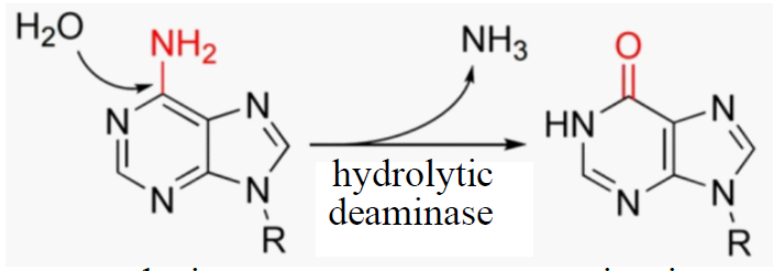
Alanine has 4 synonymous codons

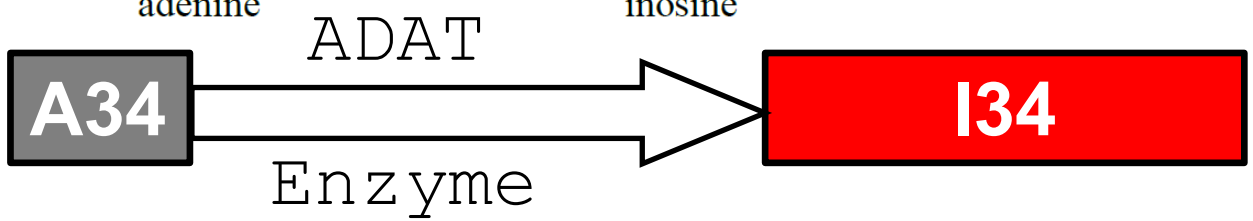
tRNA gene copy number	0	29	5	3
tRNA anti-codon	~~3’ CGG 5’~~	3’ CGA 5’	3’ CGC 5’	3’ CGU 5’
mRNA codon	5’ GCC 3’	5’ GCU 3’	5’ GCG 3’	5’ GCA 3’
Codon usage in ‰	36.0	21.1	17.3	12.6
	much slower	faster		slower

Lyu, X., Yang, Q., Li, L., Dang, Y., Zhou, Z., Chen, S., & Liu, Y. (2020). Adaptation of codon usage to tRNA I34 modification controls translation kinetics and proteome landscape. *PLOS Genetics, 16*, e1008836.

3 Problem & Research questions







A34

Wobble Base Rule

First anticodon base	Third codon base
C	G
A	U
U	A or G
G	U or C
I	U, C or A

8 amino acids are sensitive to enzyme modification by ADAT (targeting A34 tRNAs): T, A, P, S, L, I, V, R

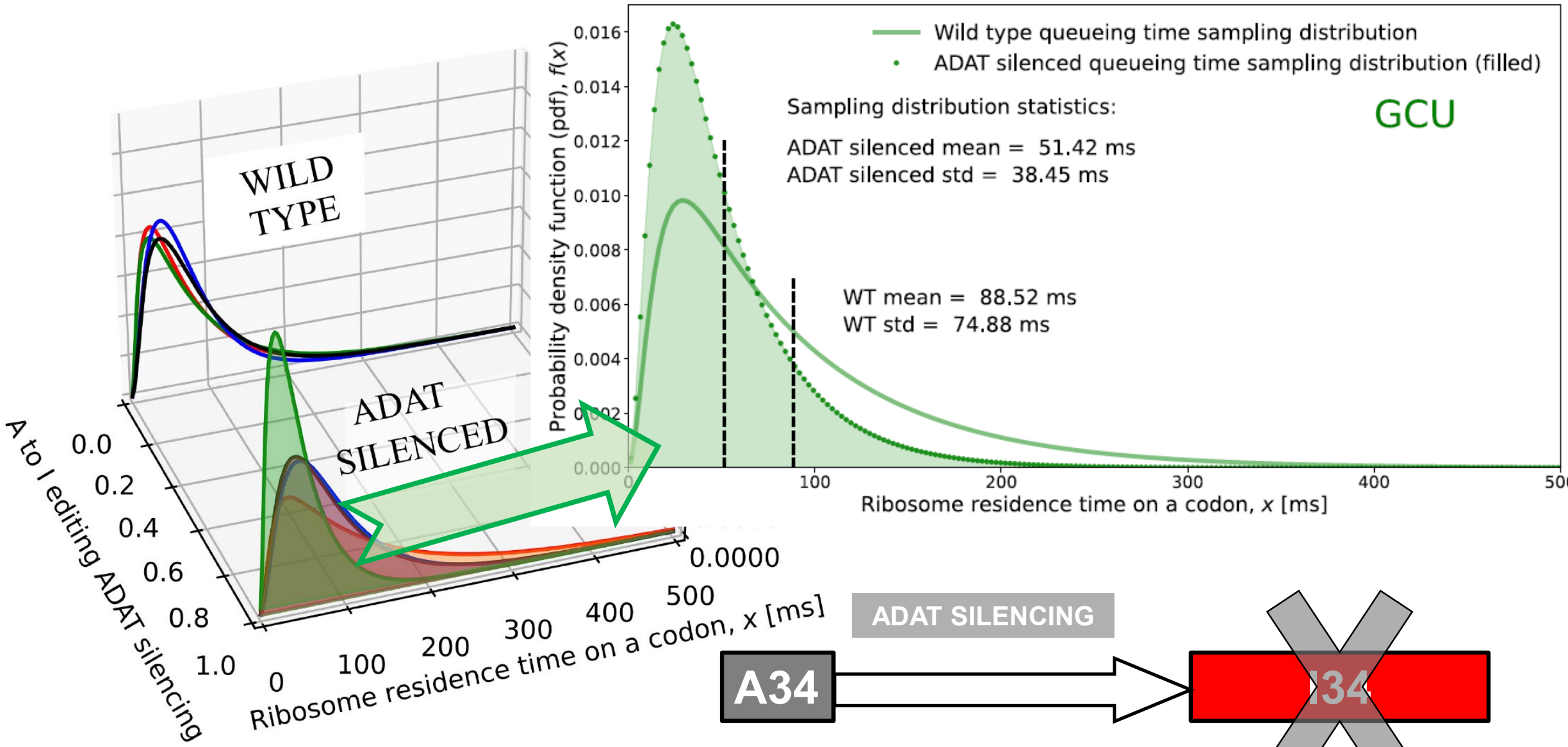
The elongation rates of 37 codons are affected by a single ADAT tRNA modification

1 2 3

Lyu, X., Yang, Q., Li, L., Dang, Y., Zhou, Z., Chen, S., & Liu, Y. (2020). Adaptation of codon usage to tRNA I34 modification controls translation kinetics and proteome landscape. *PLOS Genetics, 16*, e1008836.

3 Problem & Research questions

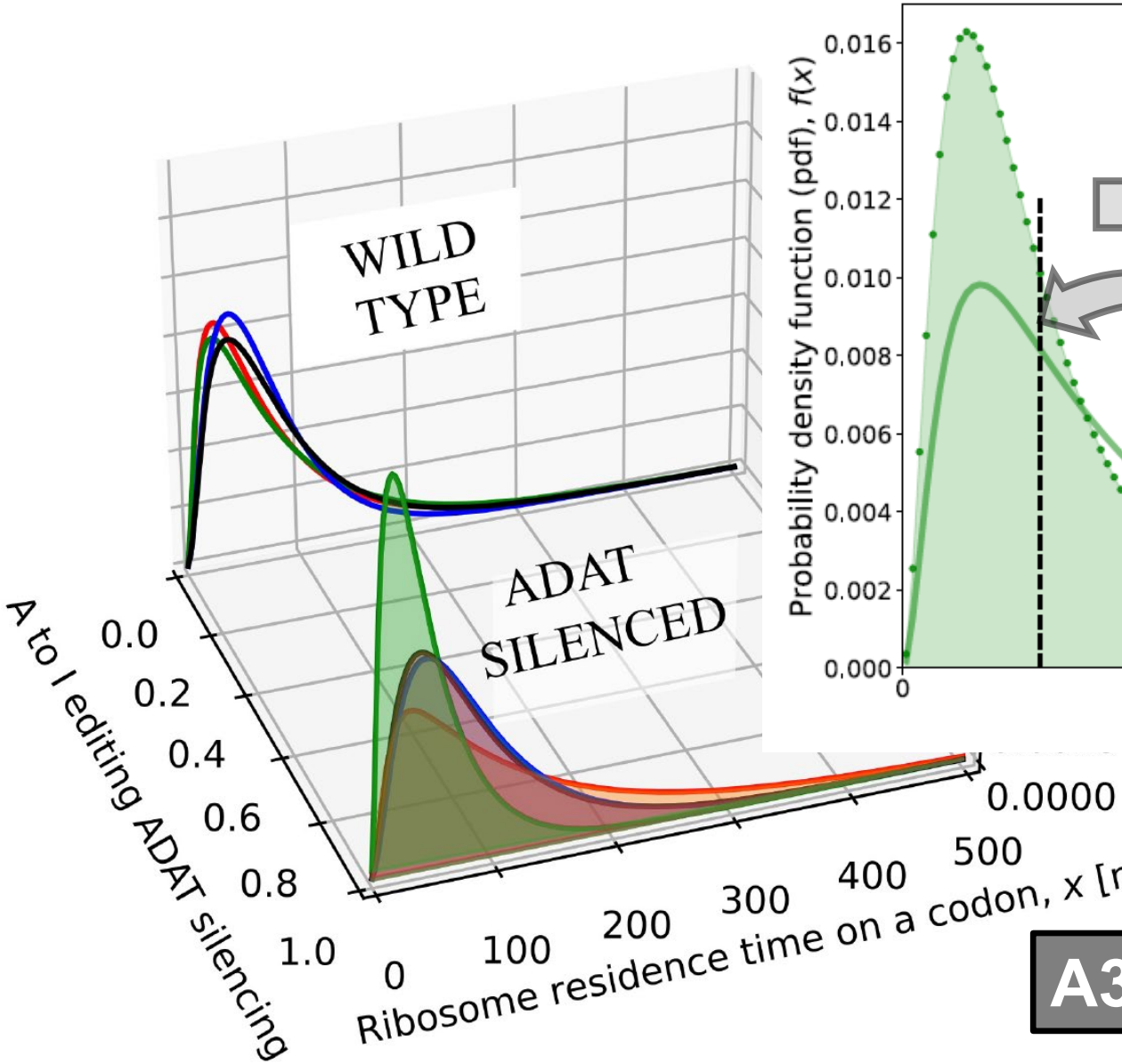
A34

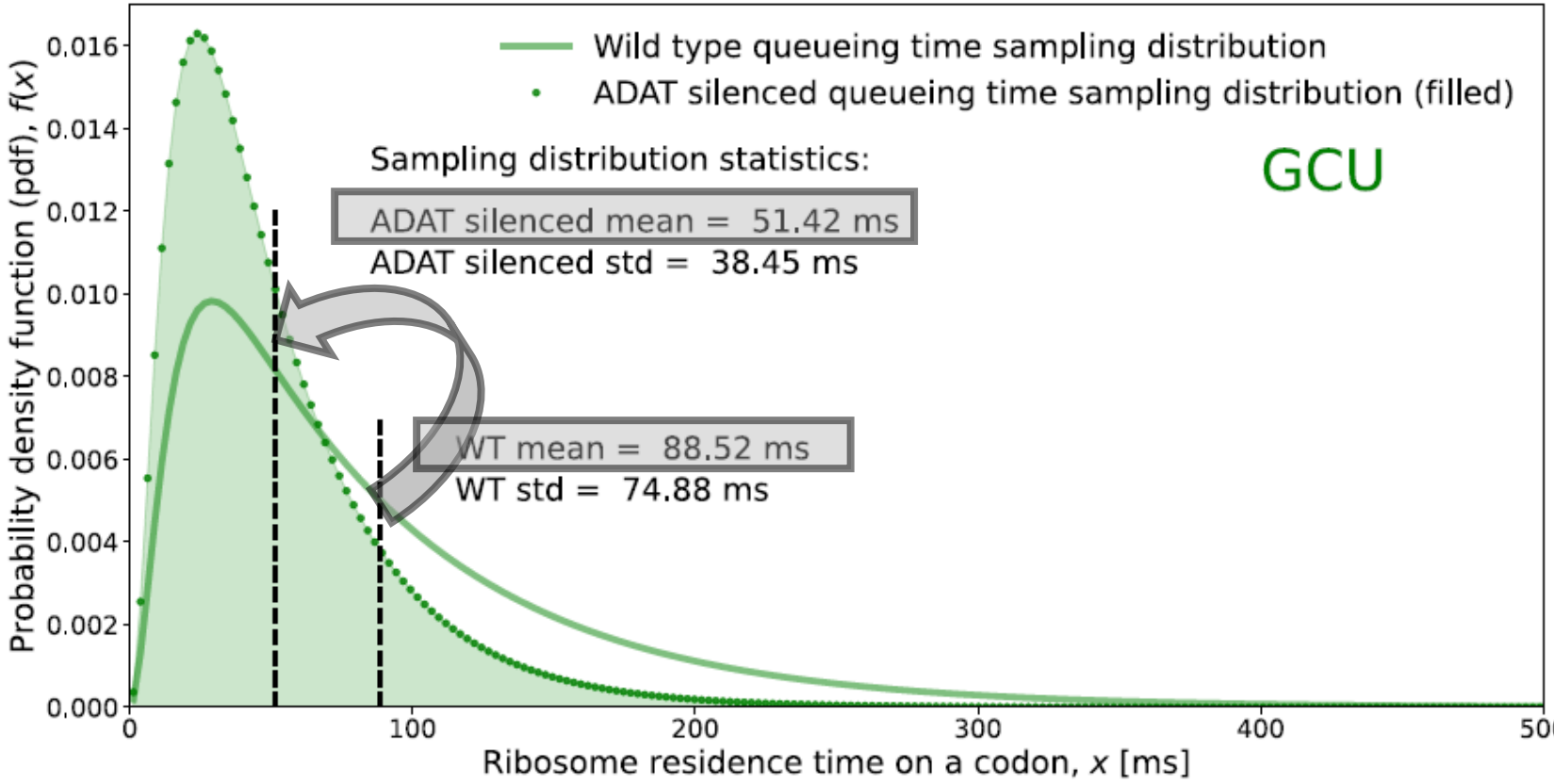


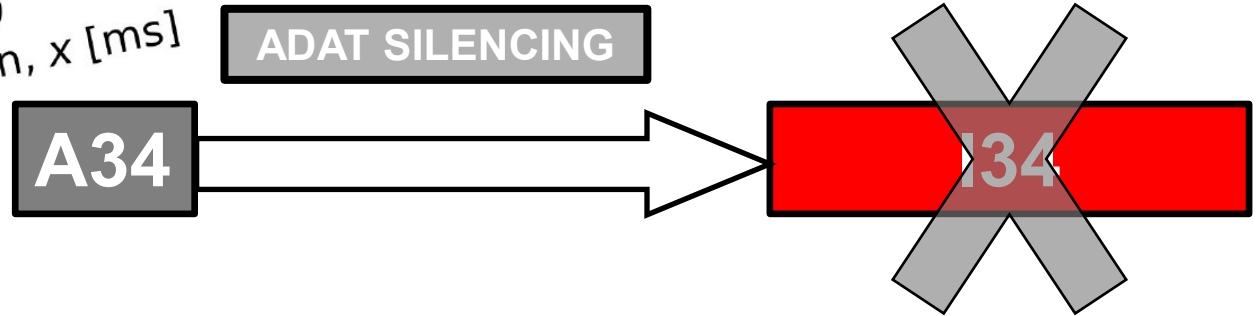
ADAT SILENCING

3 Problem & Research questions

A34

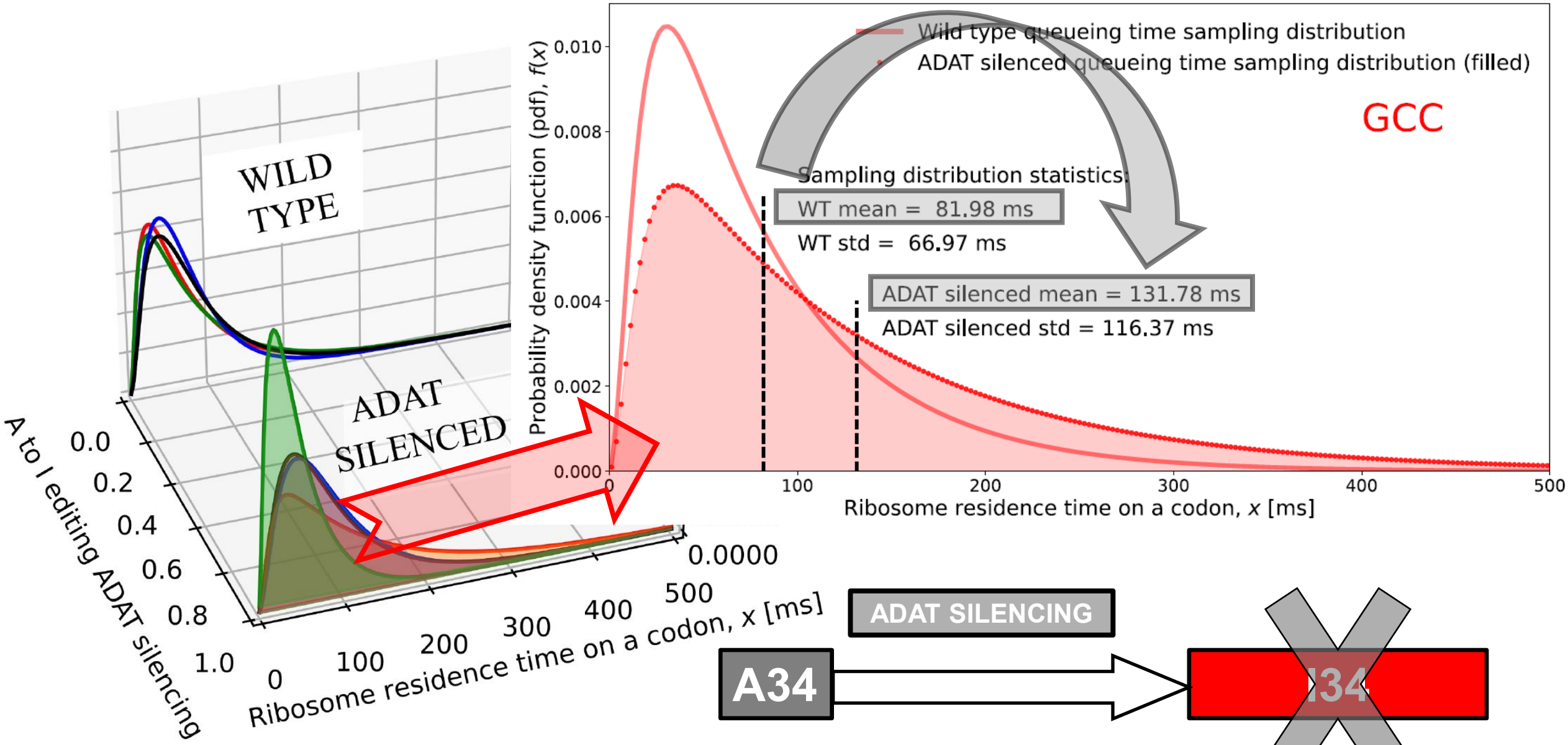






3 Problem & Research questions

A34



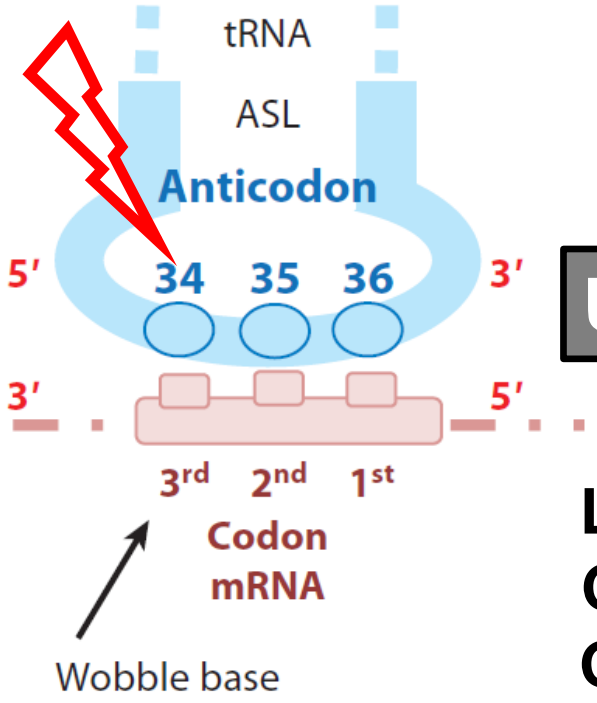
ADAT SILENCING

A34

I34

3 Problem & Research questions

U34



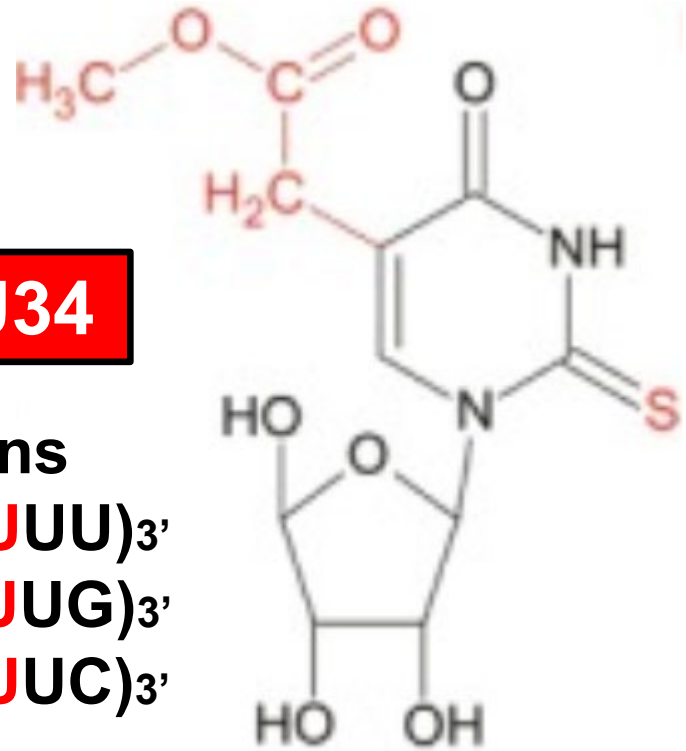
Enzymes
ELP3 URM1

U34 → mcm^{5}s^{2}U34

Codons
Lys (AAA)
Gln (CAA)
Glu (GAA)

are decoded faster

Anti-codons
Lys tRNA- 5’(UUU)3’
Gln tRNA- 5’(UUG)3’
Glu tRNA- 5’(UUC)3’



5-methoxy-carbonyl-methyl, 2-thio-uridine

1 2 3

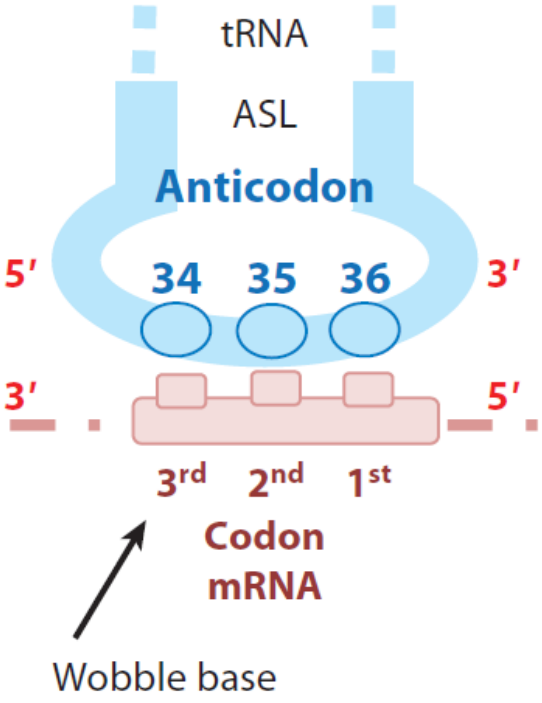
Nedialkova, D., & Leidel, S. (2015). Optimization of codon translation rates via tRNA modifications maintains proteome integrity. *Cell, 161*(6), 1–13.

Rapino, F. *et al.* (2021, April). Wobble tRNA modification and hydrophilic amino acid patterns dictate protein fate. *Nature Communications*, *12*(1), 2170.

3 Problem & Research questions

U34

SILENCING or KNOCKING-DOWN



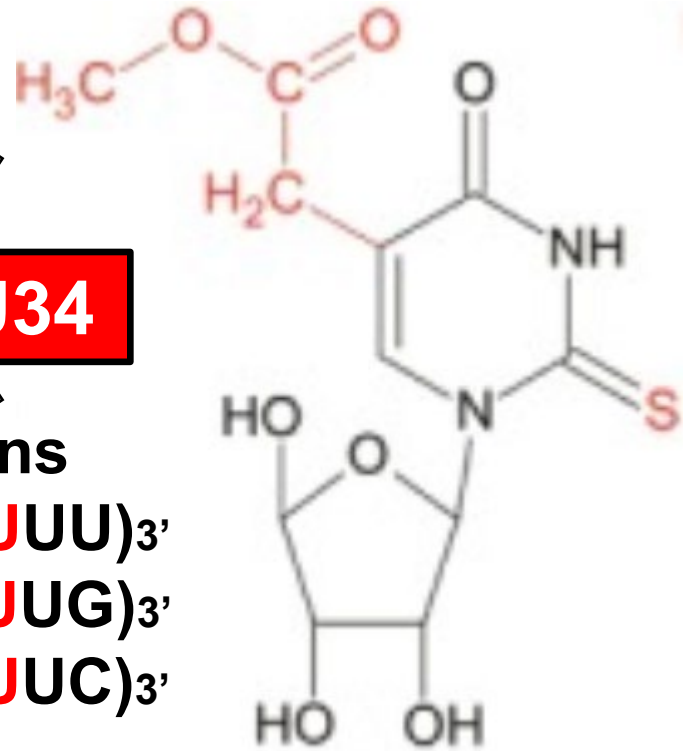
Enzymes
ELP3 URM1

U34 → mcm^{5}s^{2}U34

Codons
Lys (AAA)
Gln (CAA)
Glu (GAA)

are decoded faster

Anti-codons
Lys tRNA- 5’(UUU)3’
Gln tRNA- 5’(UUG)3’
Glu tRNA- 5’(UUC)3’

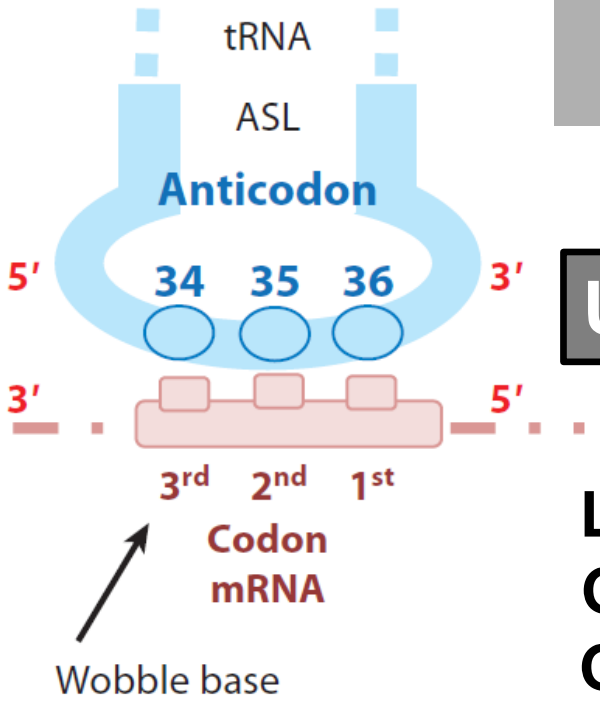


5-methoxy-carbonyl-methyl, 2-thio-uridine

are decoded slower Slower Elongation Rate
Longer Ribosome Residence Time (RRT) on these codons

3 Problem & Research questions

U34



SILENCING or KNOCKING-DOWN

Enzymes
ELP3 URM1

U34 → mcm^{5}s^{2}U34

Codons
Lys (AAA)
Gln (CAA)
Glu (GAA)

are decoded faster

Anti-codons
Lys tRNA- 5′(UUU)3′
Gln tRNA- 5′(UUG)3′
Glu tRNA- 5′(UUC)3′

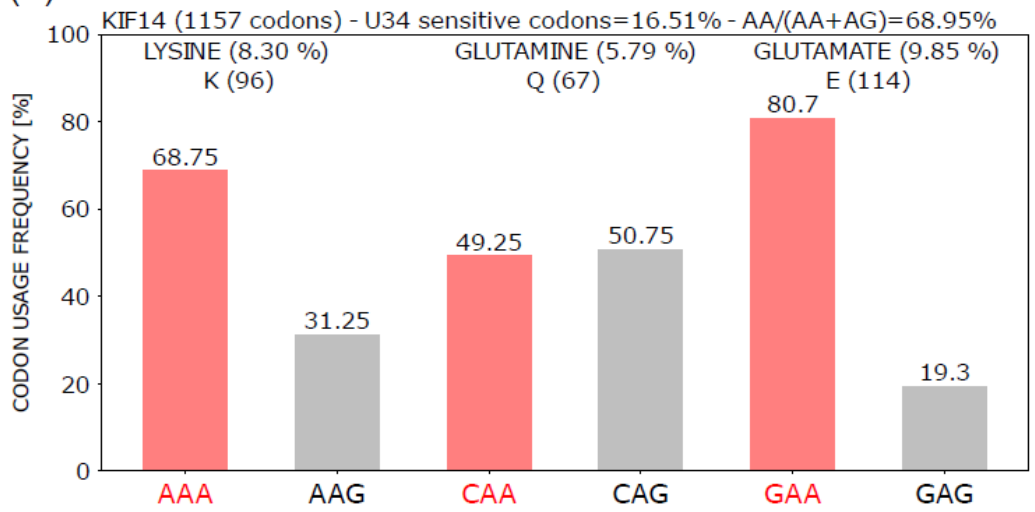
are decoded slower Slower Elongation Rate
Longer Ribosome Residence Time (RRT) on these codons

Problem & Research questions

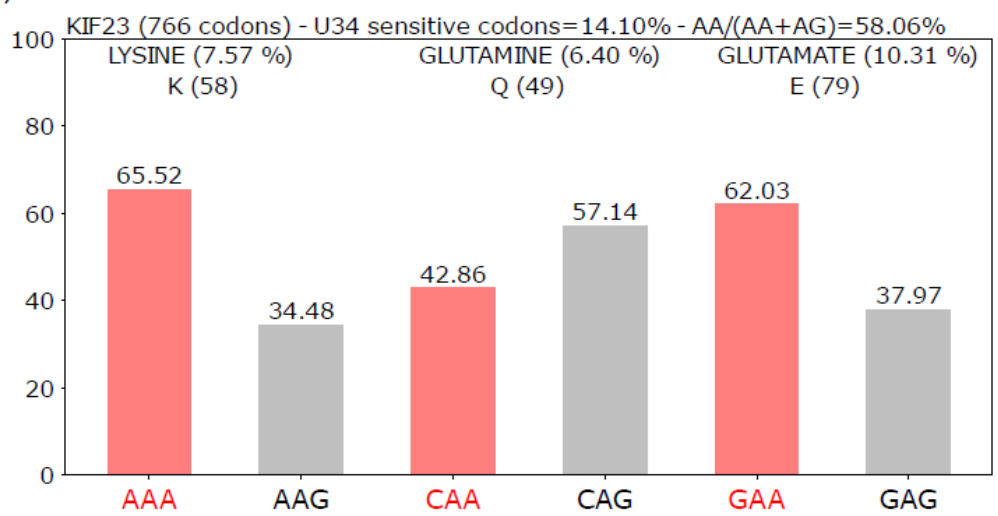
Impact of Codon Usage ?

U34

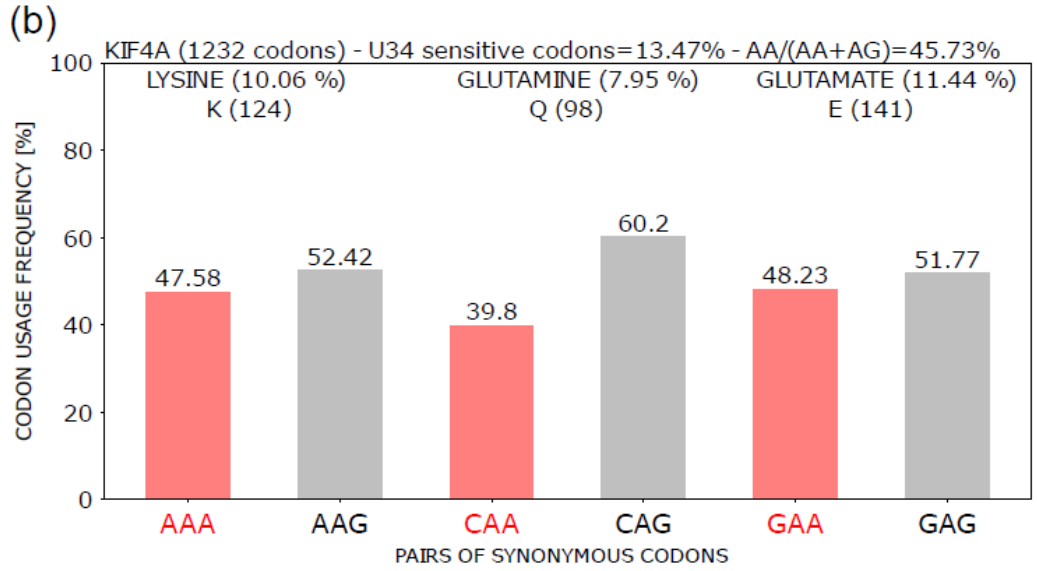
(a)



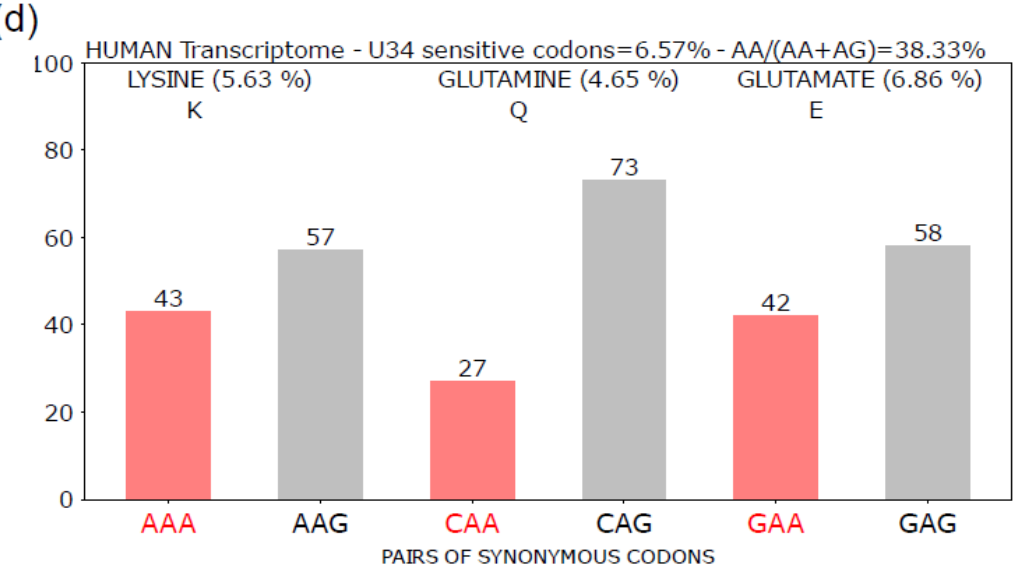
(c)



(b)



(d)



4 Keys to address the problem

1) Mechano-biochemistry In Henry Eyring's Catalysis Theory

2) Queueing Time Theory & Convolution of Multiple Random Variables

- **The parameter calibration of tRNA and tRNA modifications**

- The parameter calibration of RRT and tRNA modifications

RRT calibration

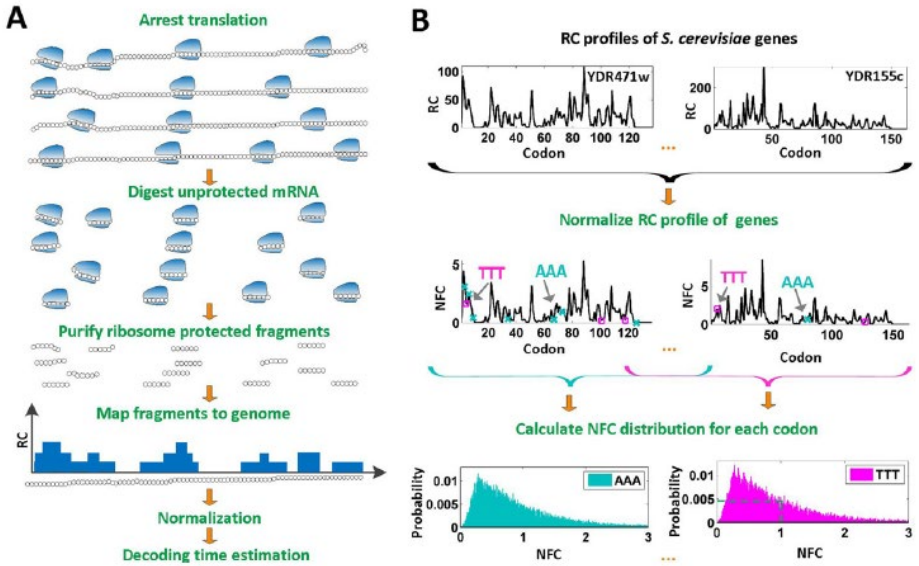
RiboSeq profile per codon in YEASTS:

The effect of tRNA levels on decoding times of mRNA codons

Alexandra Dana and Tamir Tuller*

Department of Biomedical Engineering, Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv 69978, Israel

Received April 10, 2014; Revised July 02, 2014; Accepted July 3, 2014

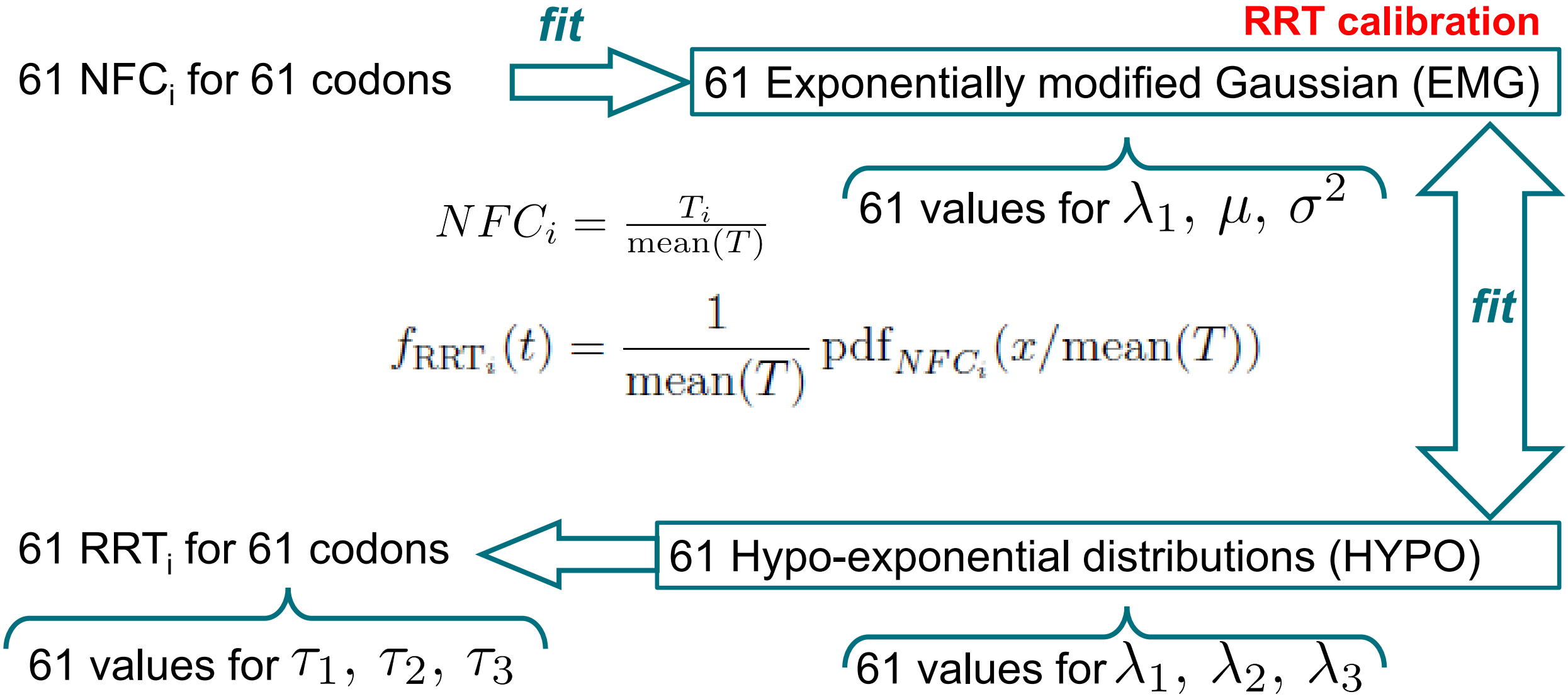


been performed in a previous study (11)). Therefore this normalization enables measuring the relative time a ribosome spends translating each codon in a specific gene relative to other codons in it, while considering the total number of codons in the gene, resulting in its normalized footprint count (NFC):

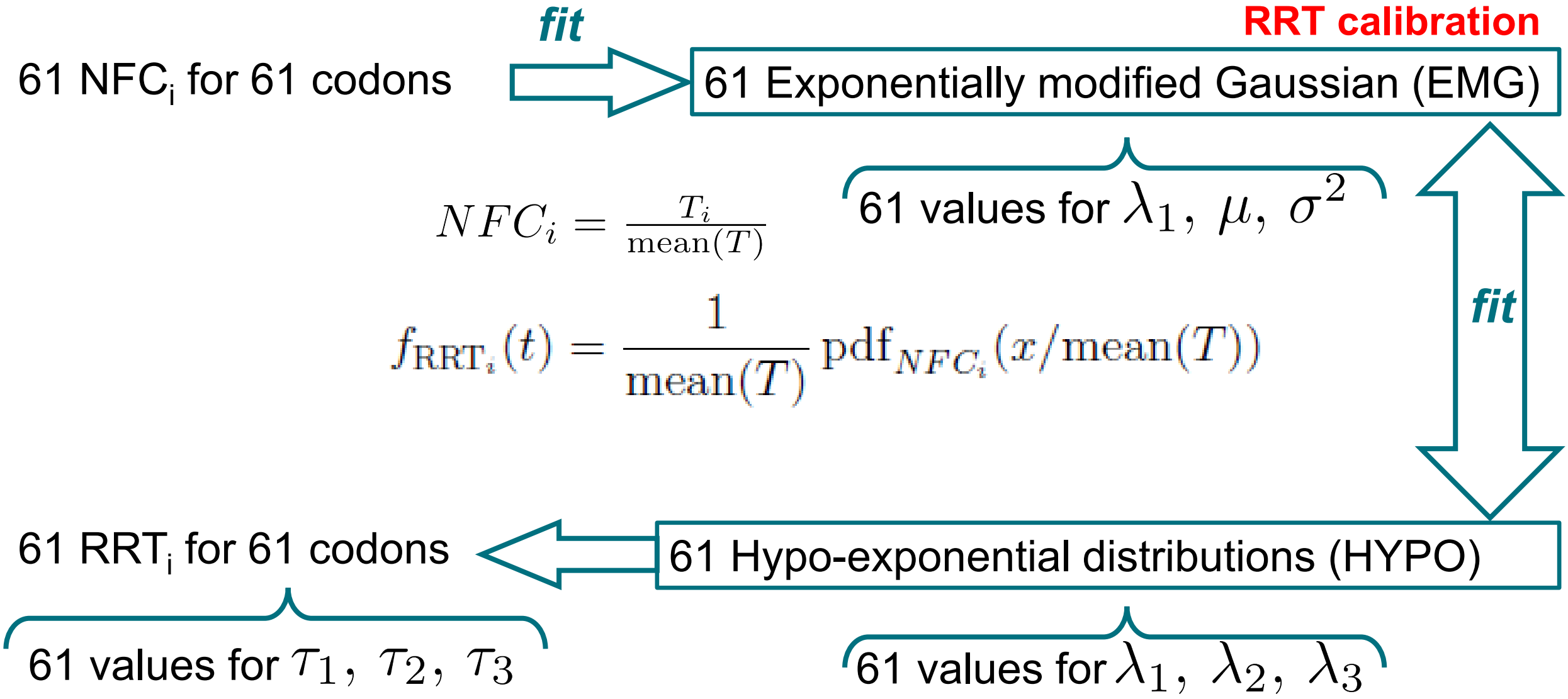
$$NFC_j = \frac{RC_j}{\frac{1}{J-40}(RC_{21} + RC_{22} + \cdots + RC_{J-20})}, \quad j = 21..J-20$$

where J is the number of codons in the gene and j is the index of a codon; see also Figure 1B.

- **The parameter calibration of RRT and tRNA modifications**



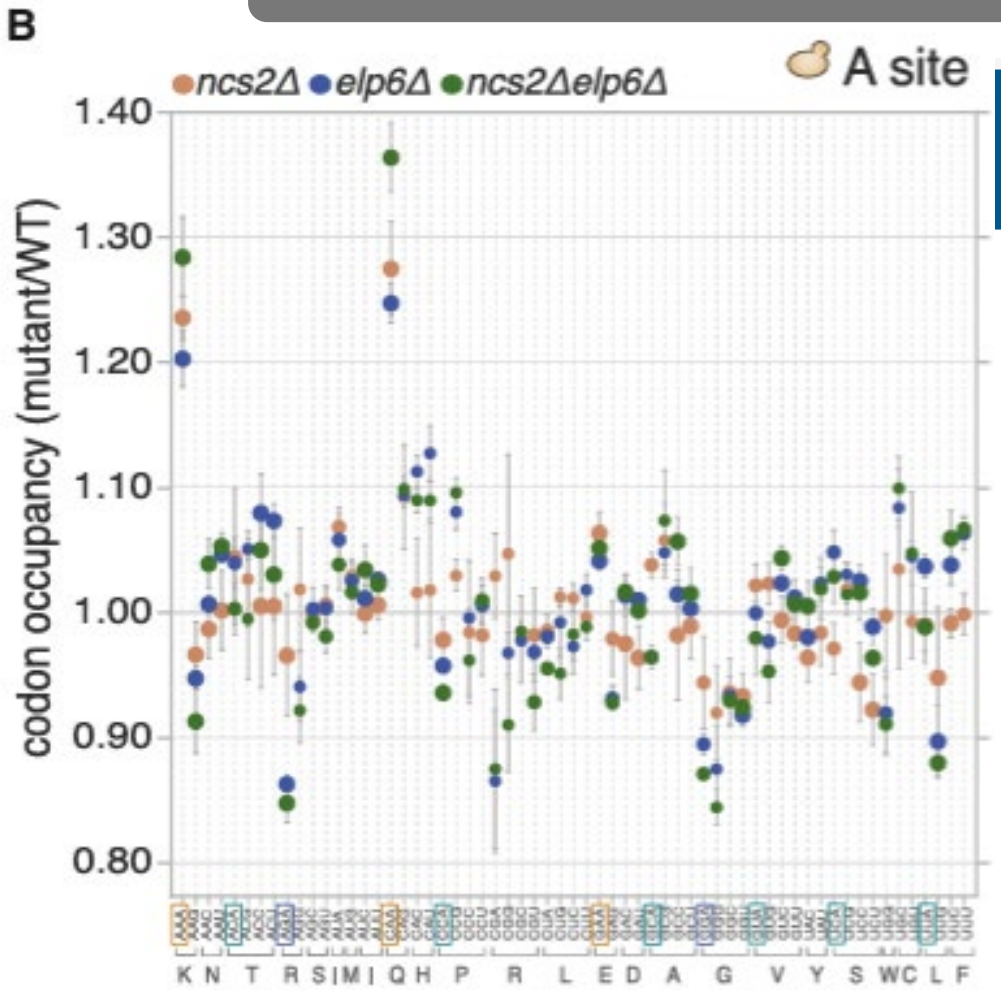
- **The parameter calibration of RRT and tRNA modifications**



- **The parameter calibration of RRT and tRNA modifications**

tRNA modification calibration

RiboSeq profile per codon in YEASTS:



Cell

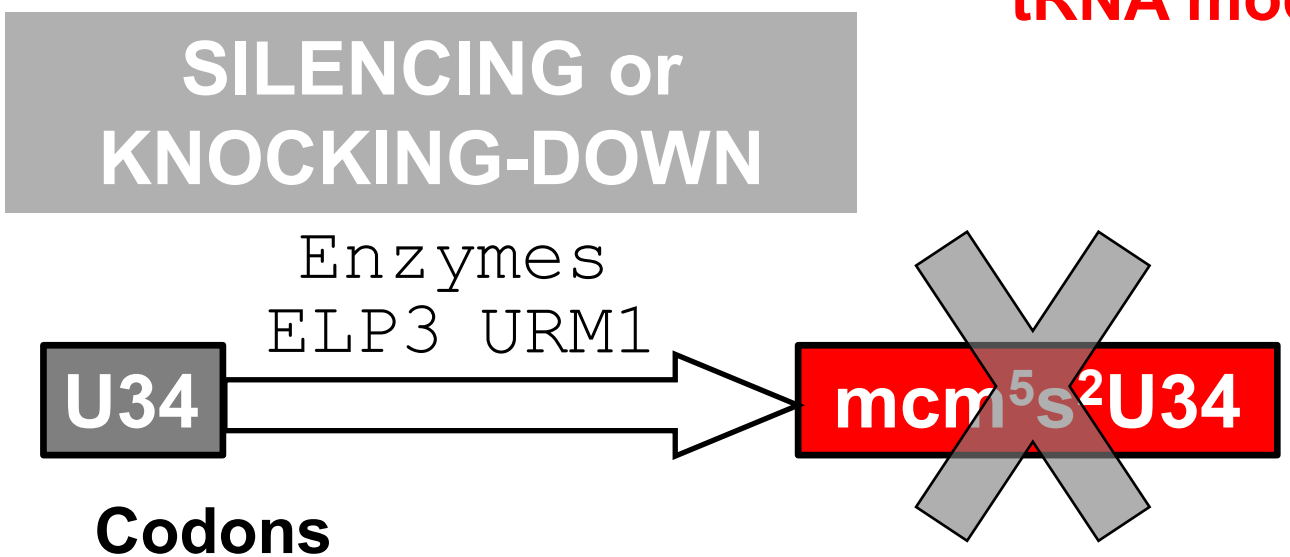
Article

Optimization of Codon Translation Rates via tRNA Modifications Maintains Proteome Integrity

Danny D. Nedialkova[1,2] and Sebastian A. Leidel[1,2,3,*]
[1]Max Planck Research Group for RNA Biology, Max Planck Institute for Molecular Biomedicine, Von-Esmarch-Strasse 54, 48149 Muenster, Germany
[2]Cells-in-Motion Cluster of Excellence, University of Muenster, 48149 Muenster, Germany
[3]Faculty of Medicine, University of Muenster, Albert-Schweitzer-Campus 1, 48149 Muenster, Germany
*Correspondence: sebastian.leidel@mpi-muenster.mpg.de
http://dx.doi.org/10.1016/j.cell.2015.05.022

- **The parameter calibration of RRT and tRNA modifications**

tRNA modification calibration



Codons
Lys (AAA)
Gln (CAA)
Glu (GAA)

$$\tau_{1,\mathrm{MUTANT}} = \tau_{1,\mathrm{WT}} \times \mathrm{fold\,change}$$

5 ABM implementation

TASEP approach

Meet « Ribosomer »

« Ribosomer » didactical implementation (javascript, p5.js)

« Ribosomer » for HPC implementation (Python)

5 ABM implementation

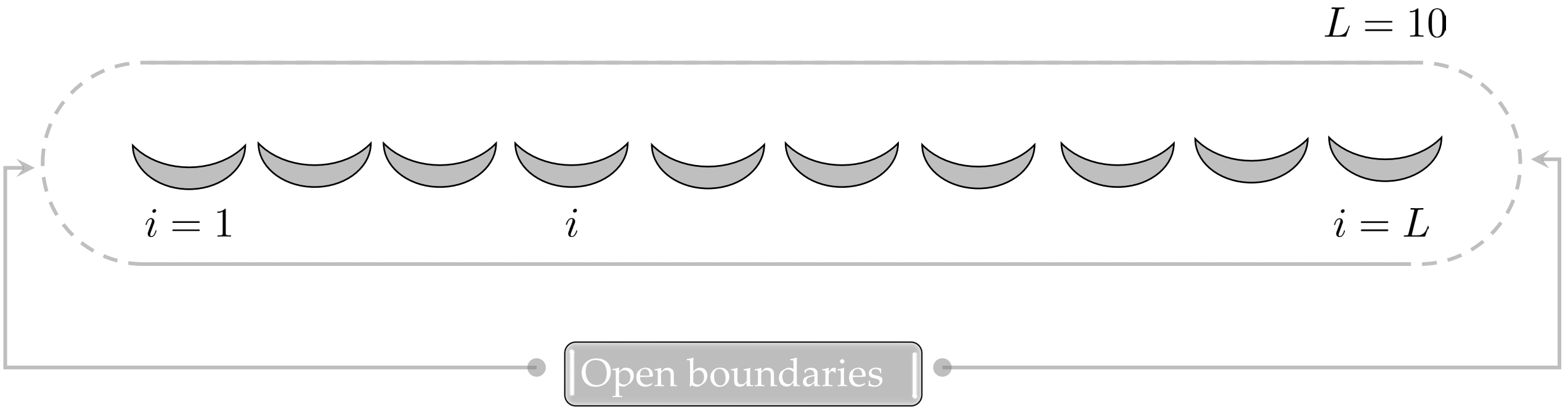
The TASEP approach

Particle transport model on a 1D lattice

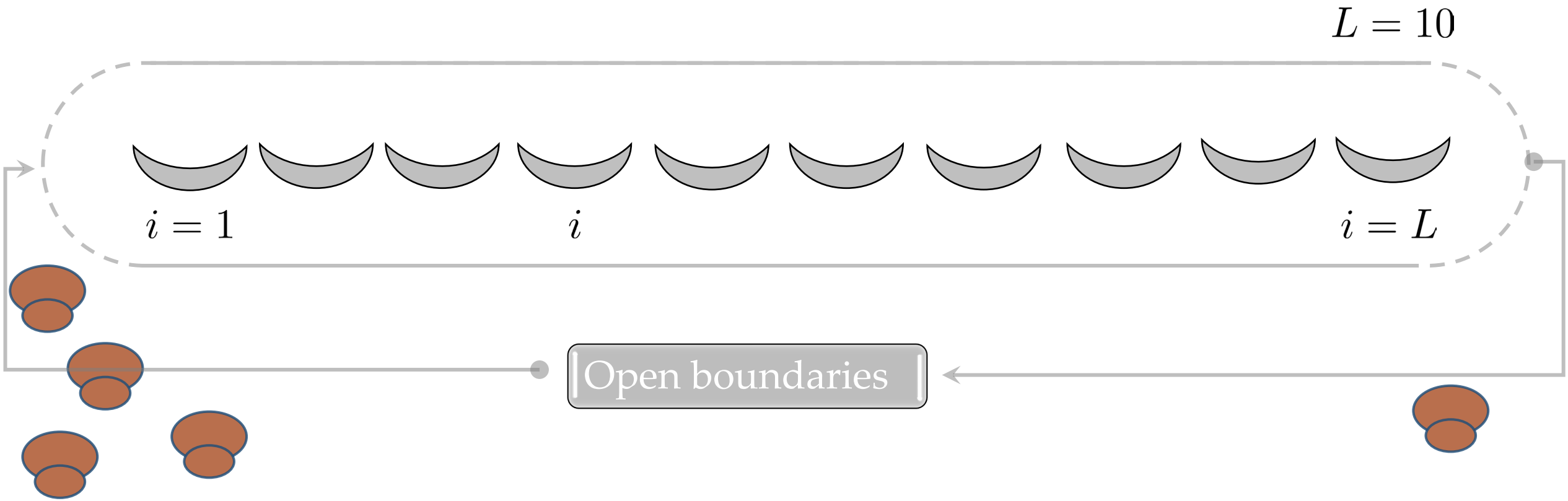
$L = 10$

$i = 1$ i $i = L$

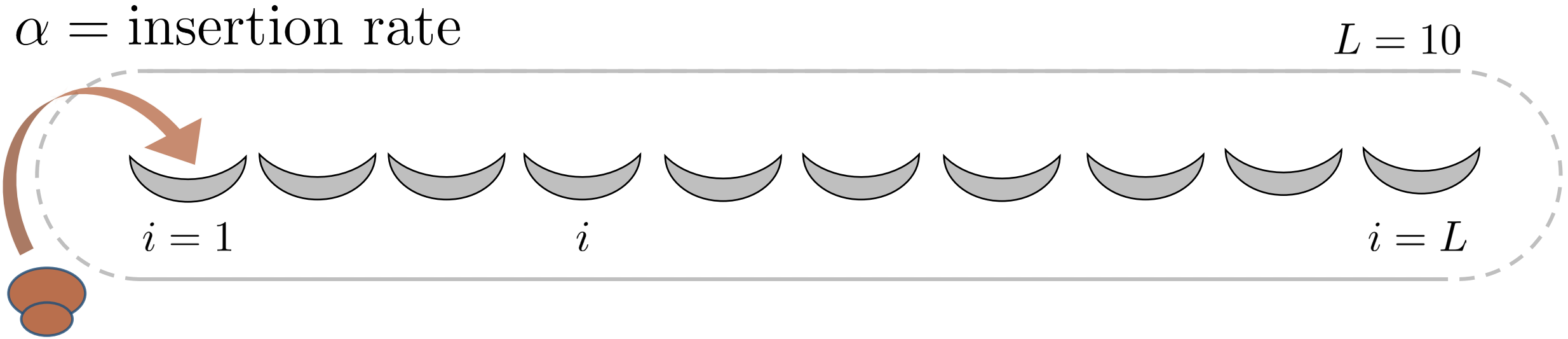
Particle transport model on a 1D lattice



Particle transport model on a 1D lattice



Particle transport model on a 1D lattice

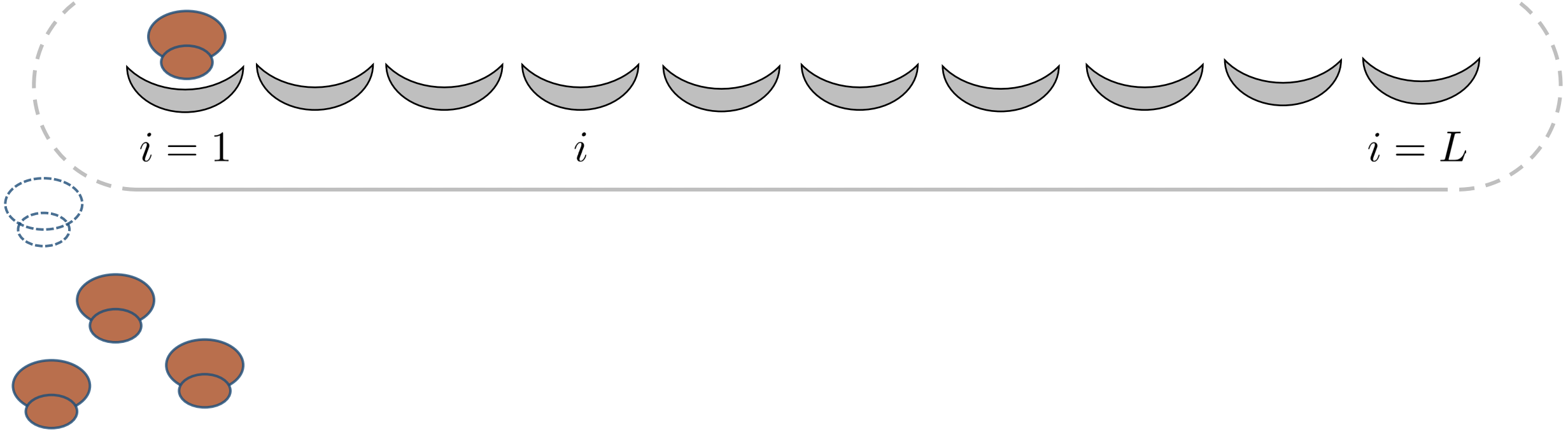


Particles may move from left to right only.
There is no way back

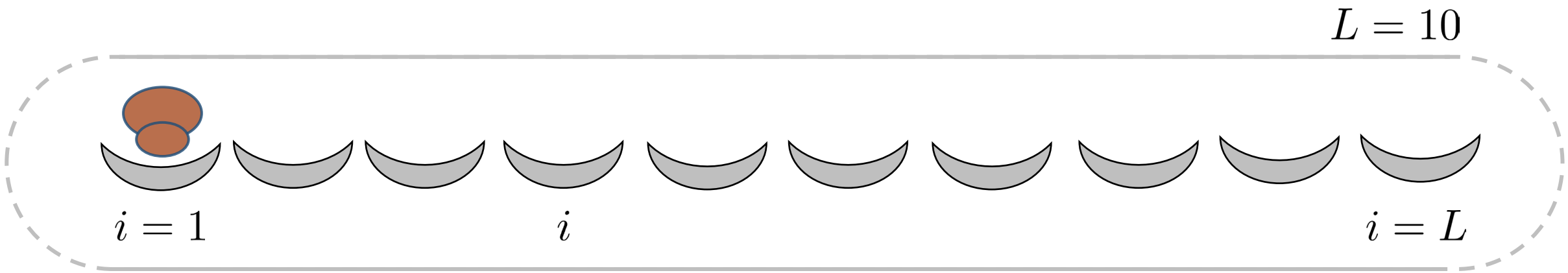
This is … **totally asymmetric**

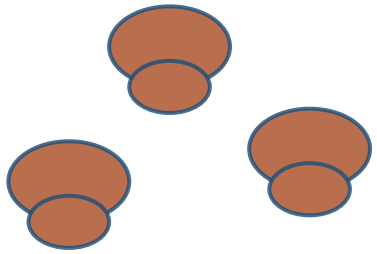
Particle transport model on a 1D lattice

$\alpha =$ insertion rate

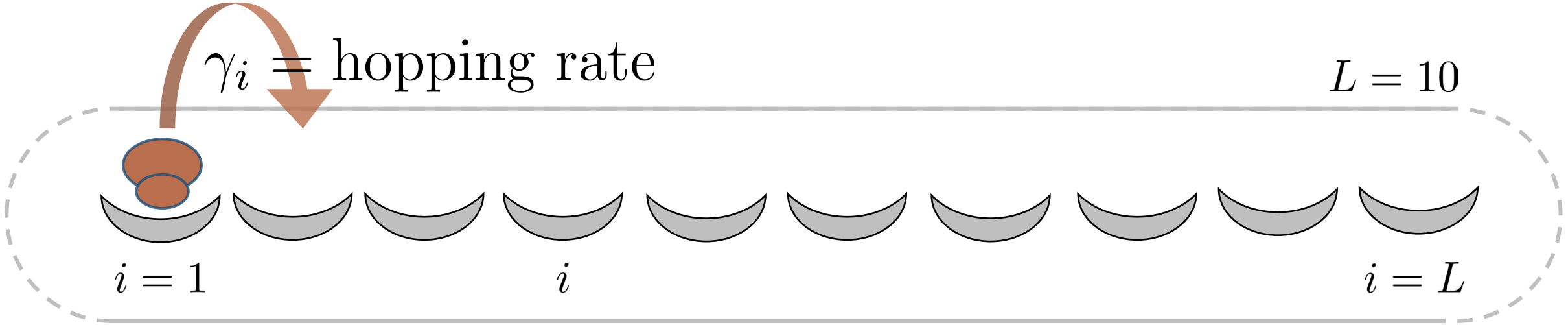


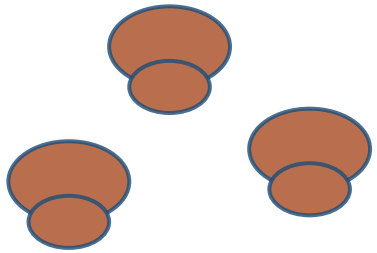
Particle transport model on a 1D lattice





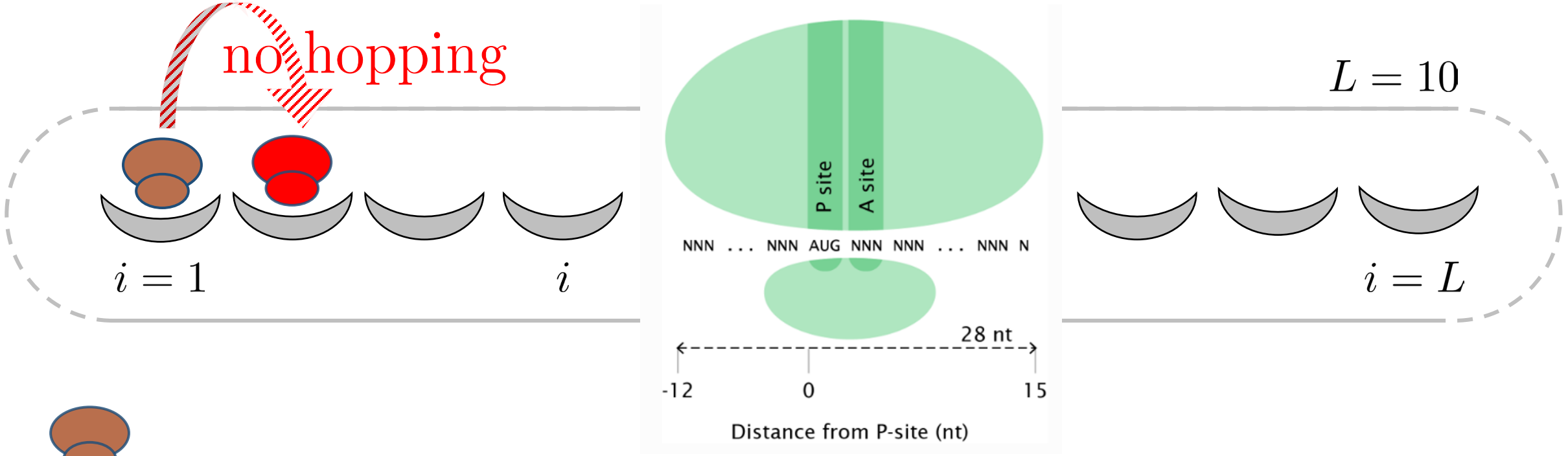
Particle transport model on a 1D lattice

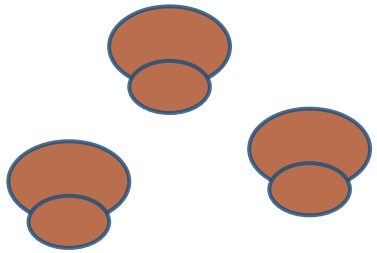




Hopping allowed from site *i* to *i+1* if site *i+1* is empty

Particle transport model on a 1D lattice



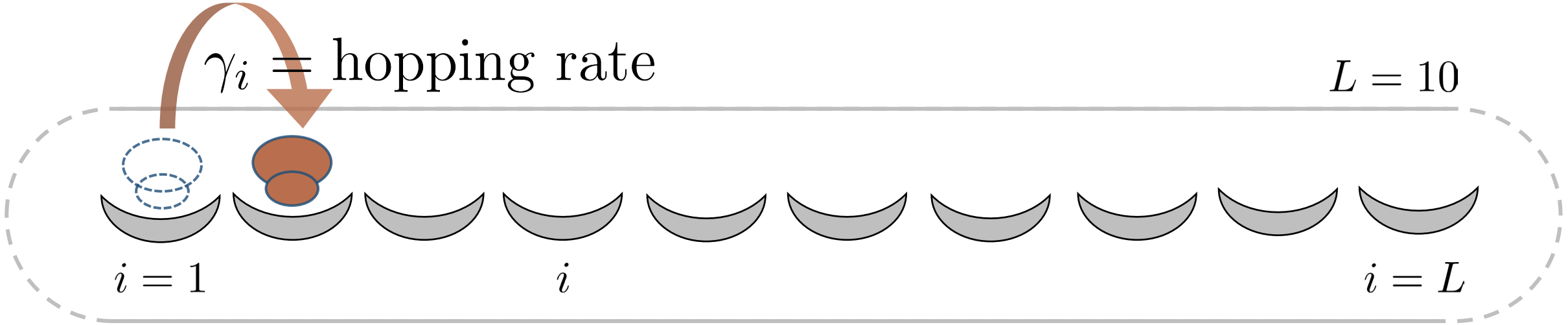


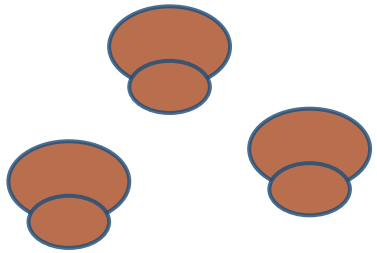
No hopping from *i* to *i+1* if site *i+1* is not empty

This is called the **simple exclusion**

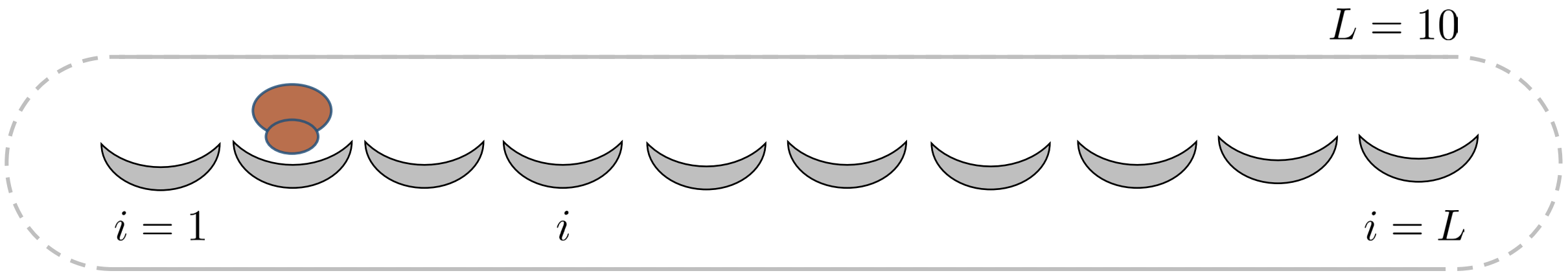
Real ribosomes have an extended footprint on the 'lattice'

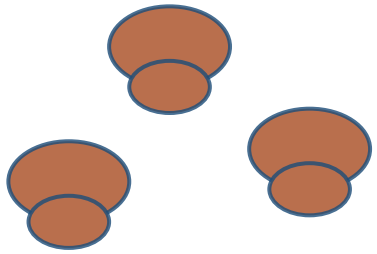
Particle transport model on a 1D lattice



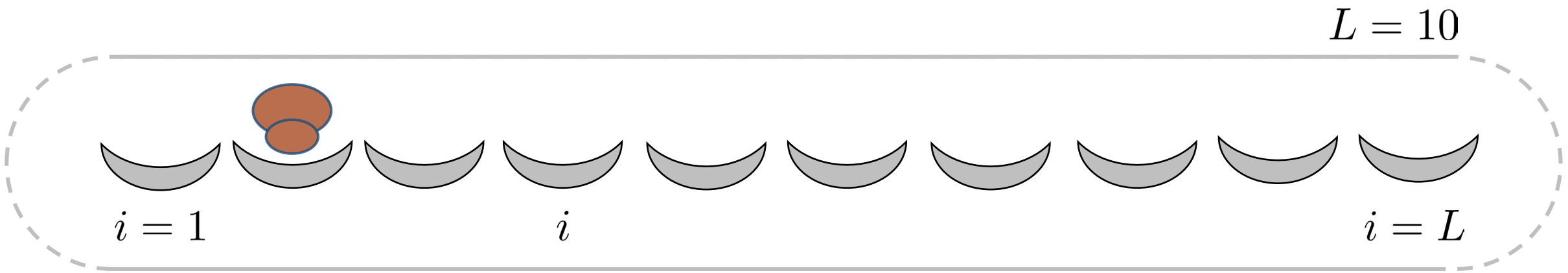


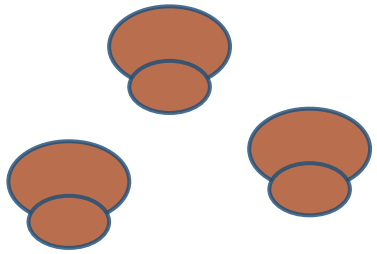
Particle transport model on a 1D lattice



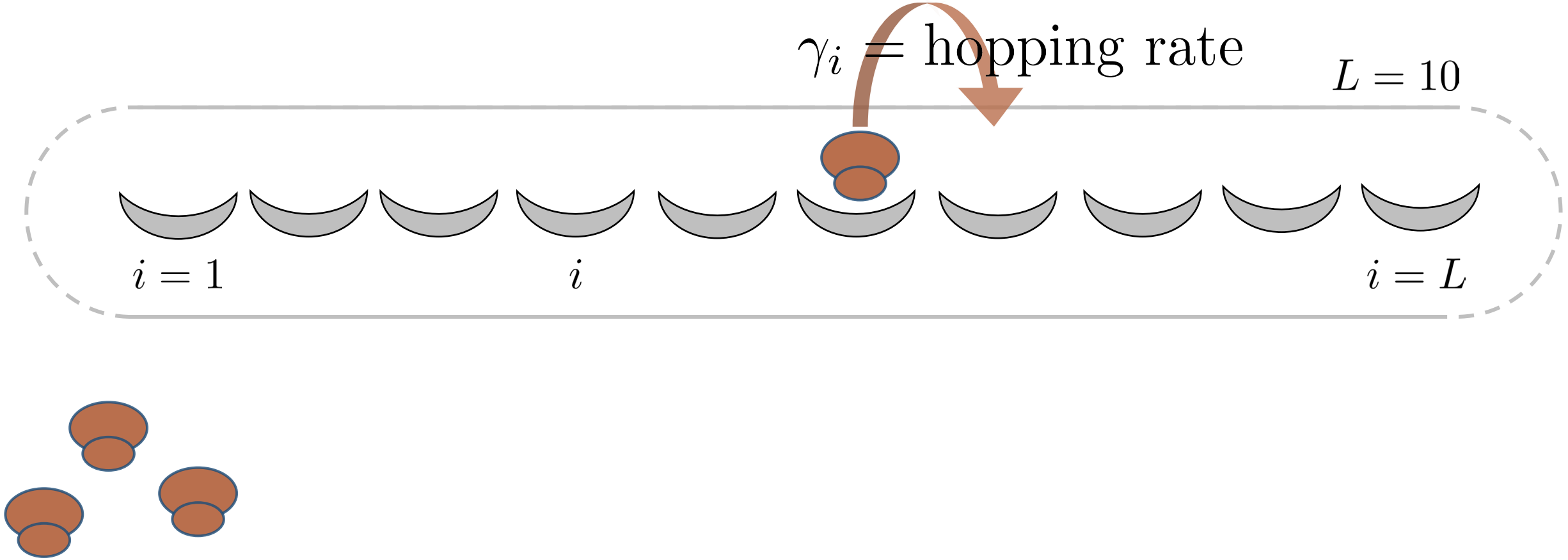


Particle transport model on a 1D lattice

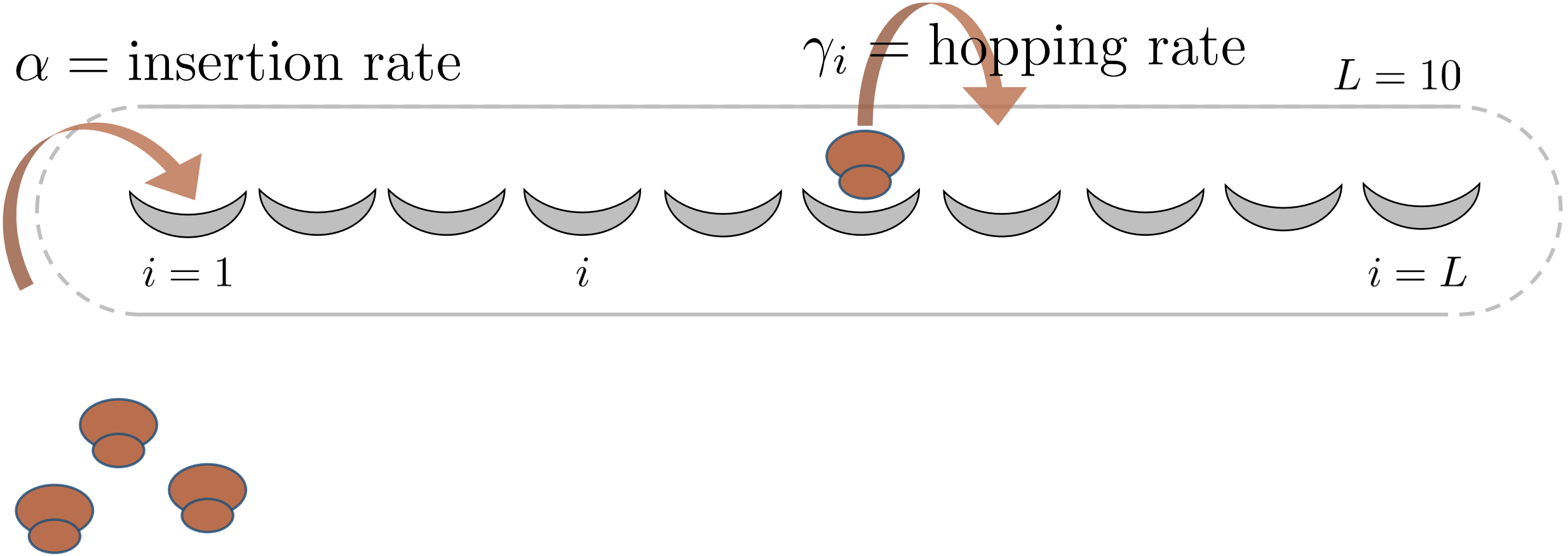




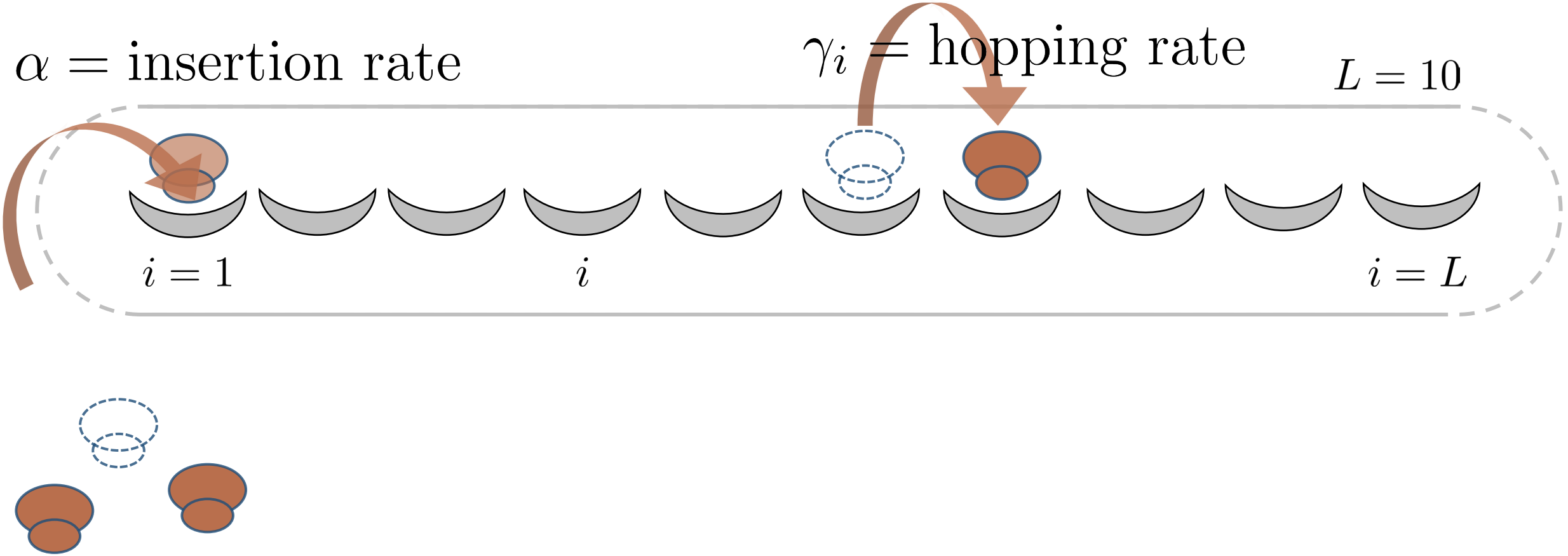
Particle transport model on a 1D lattice



Particle transport model on a 1D lattice



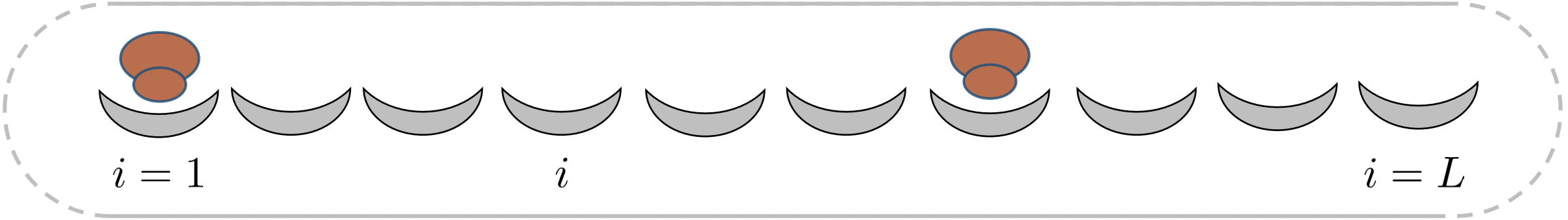
Particle transport model on a 1D lattice

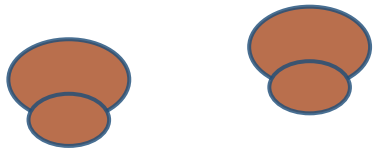


Particle transport model on a 1D lattice

$\alpha =$ insertion rate

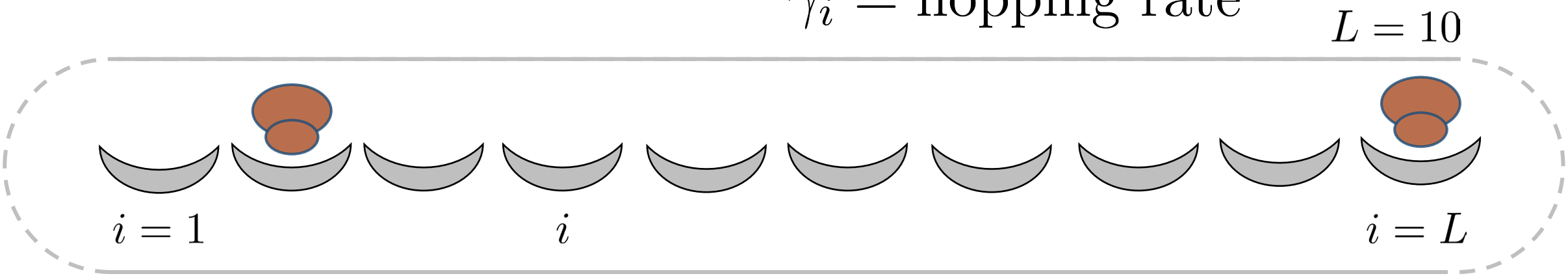
$\gamma_i =$ hopping rate

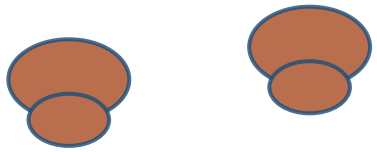




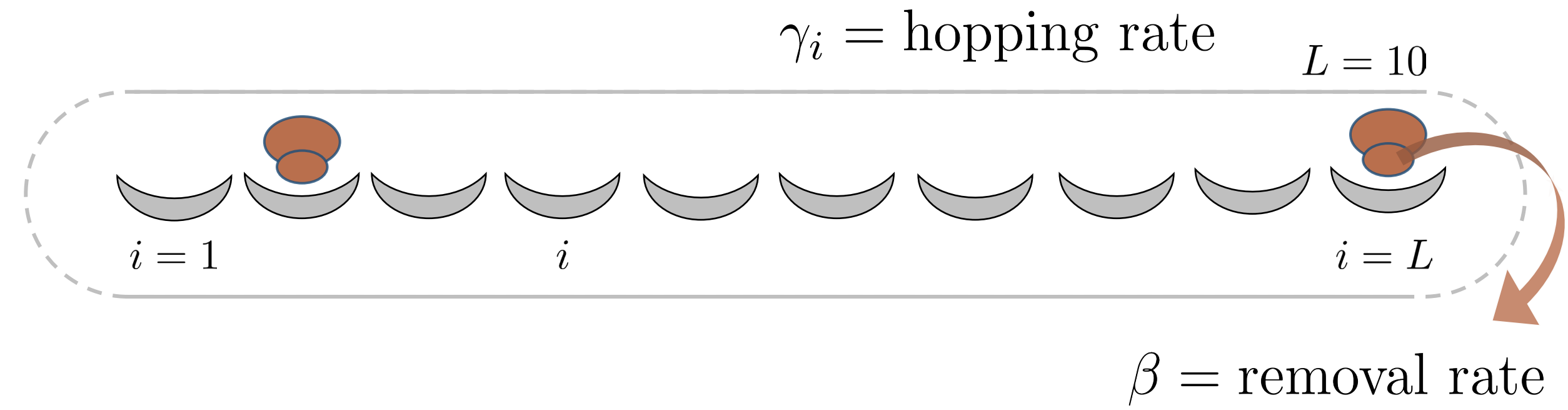
Particle transport model on a 1D lattice

$\gamma_i =$ hopping rate

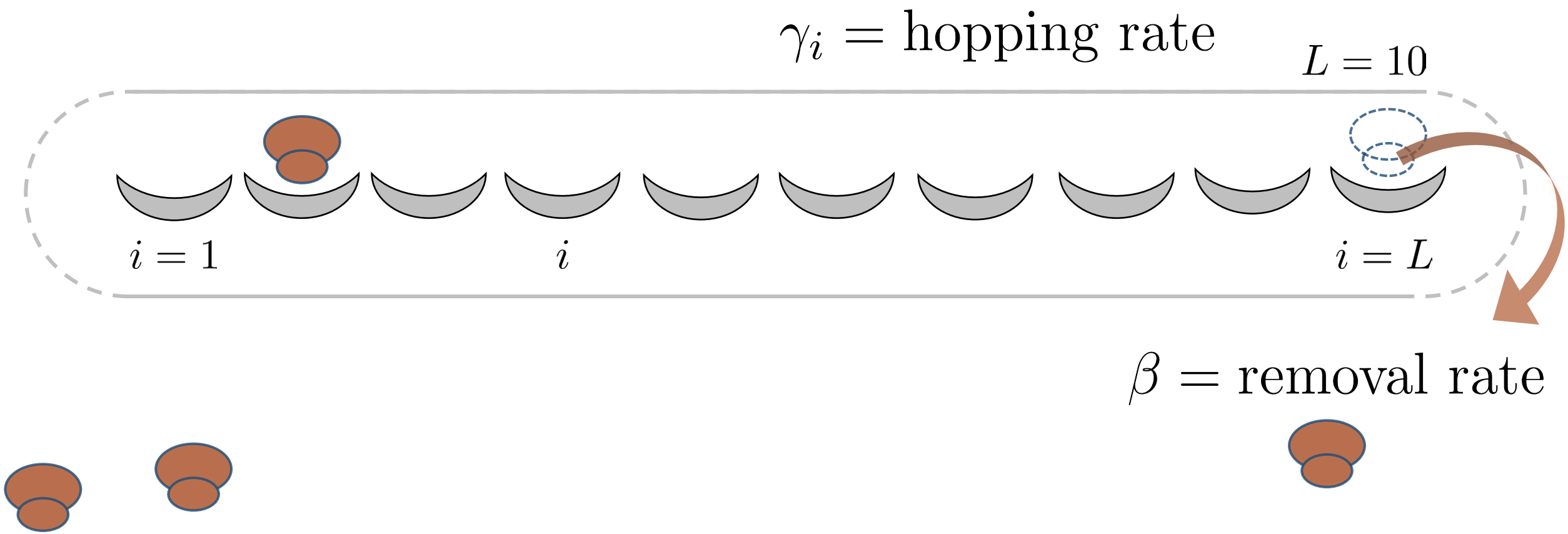




Particle transport model on a 1D lattice



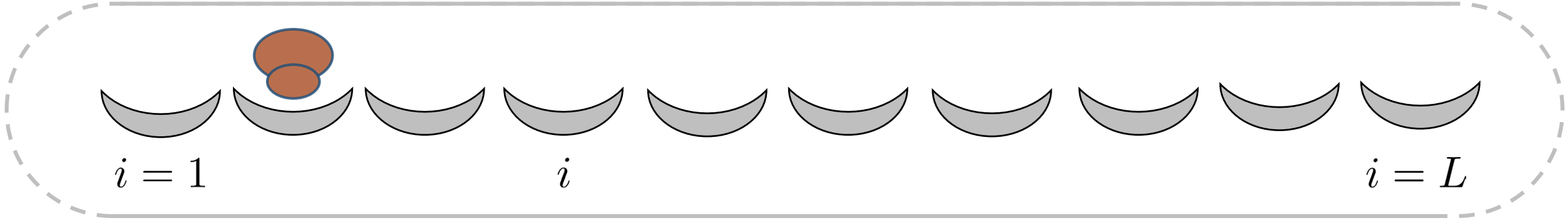
Particle transport model on a 1D lattice

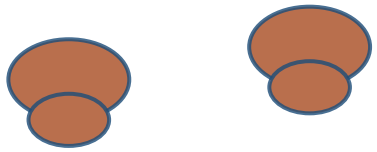


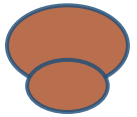
Particle transport model on a 1D lattice

$\gamma_i =$ hopping rate

$L = 10$

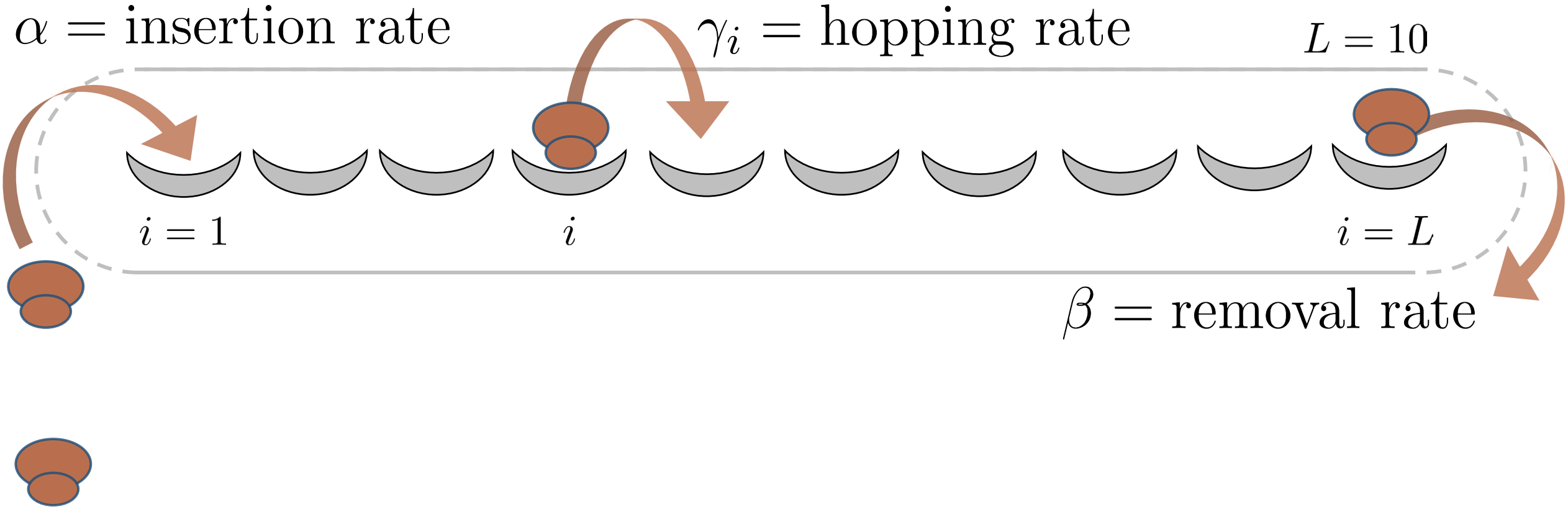






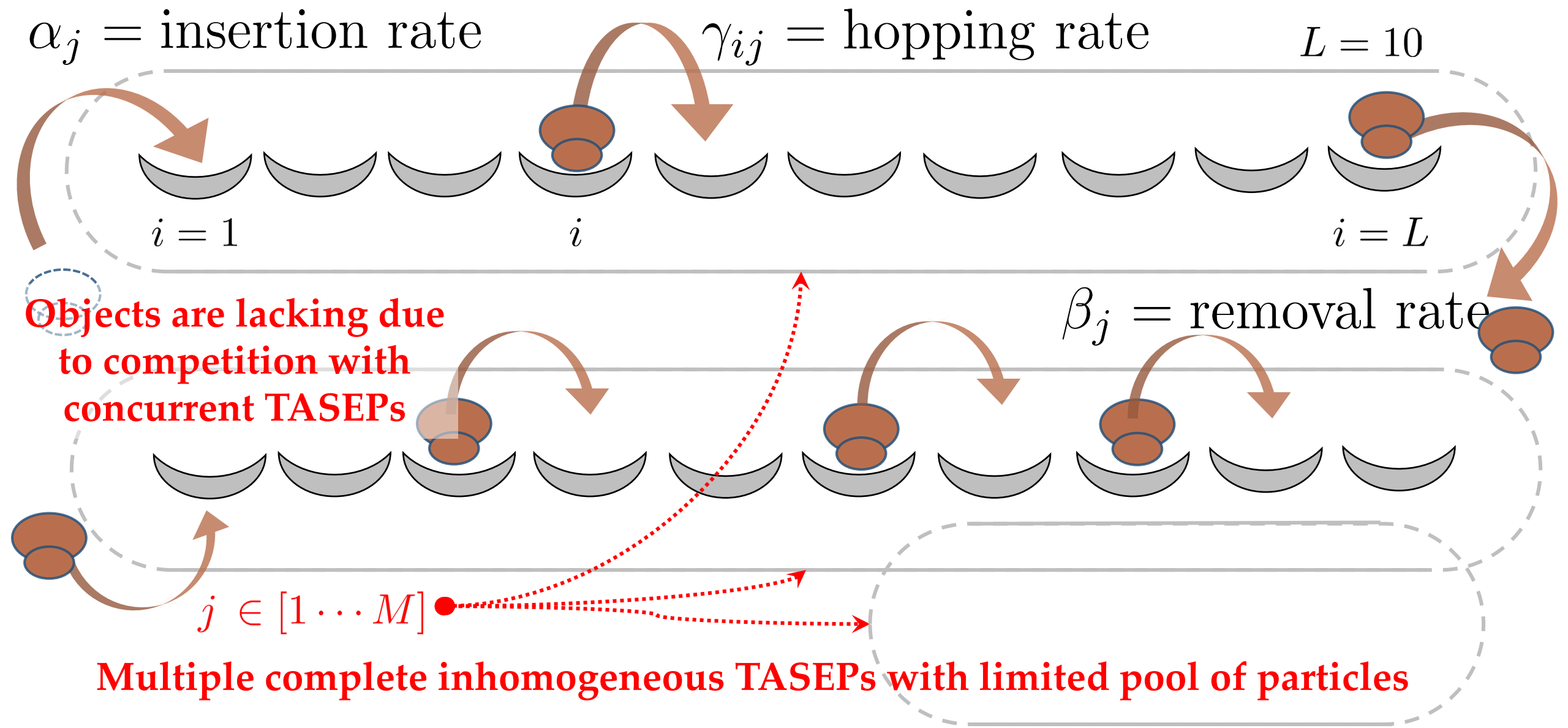
1 particle has proceeded through all sites

Particle transport model on a 1D lattice



Complete inhomogeneous TASEP: all γ_i are different

More than one TASEP … and a limited pool of particles



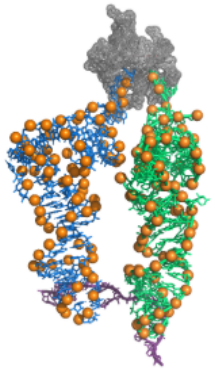
5 ABM implementation

TASEP approach

Building an INTEGRATIVE framework « Ribosomer »

Ribosomer - TASEP modeling platform for mRNA translation simulations

Ribo-Seq Predictor



- Home
- Input data: mRNA sequences, tRNA abundance or rate table and ribosome resources settings
- Ribosomer: TASEP modeling bench
- Ribo-Seq occupancy maps predictions
- Relative protein levels and translation efficiency (TE) predictions

Welcome to the RIBOSOMER protein synthesis modeling bench, providing in-silico translatomics simulations, predicting ribosome density profiles of your transcripts, protein relative expression levels, pausing or stalling sites, translation efficiency using an extended Totally Asymmetric Simple Exclusion Process (TASEP) based on:

- primary sequences of transcripts (and their codon usage)
- relative abundance of transcripts (mRNA counts from RNA-Seq data)
- possible non-uniform relative initiation rates of ribosomes on transcripts
- tRNAs relative abundance in a given species or cell type expression vector with or without tRNA enzymatic modifications (e.g. N34 wobble base modifications)
- ribosomal electrostatic interaction in the protein exit tunnel
- transcript secondary structures
- proline incorporation or stalling codon pairs events or EF-P/eIF5A elongation factor depletion
- average initiation rate of ribosomes on transcripts
- limited or unlimited pool of ribosome resources

Ribosomer - TASEP modeling platform for mRNA translation simulations

Resources settings

drop your transcripts variants file here: in FASTA format with correct CDS (without UTRs)
as retrieved from NCBI or Ensembl repositories

Click the button(s) to activate (GREEN) / desactivate (RED) the desired factor(s) combination

modification on U34-tRNA (ELP3- URM1-) | mRNA secondary structure | Proline slowdown | Non-uniform mRNA relative abundance | Limited pool of ribosomes

silencing A to I tRNAs editing (ADAT-) | Exit tunnel electrostatic interaction | EF-P or eIF5A depleted | Non-uniform initiation rates

Initiation time rate in (s) = 40 040.0
Initiation half-time in (s) = 027.7

Limited or unlimited pool of ribosomes: use the slider to change the ratio of available ribosomes over transcripts counts:
Ratio of ribosomes cardinality / transcripts cardinality (range = [0.1 - 10]):

Ratio #Ribosomes / #Transcripts
3

drop your transcripts copy number file as derived from RNA-seq reads count (within sample reads counts).
Use a tab delimited format: geneID tags '\tab' transcript copy counts ['\tab' optional relative initiation rates]'\n'

Elongation cycle sub-steps rates are dependent on tRNA relative abundance, tRNA gene copy numbers of the expression vector for your transcripts. Click the button to select the expression vector species.

E. coli | Yeast | Worm | Zebrafish | Mouse | Human

Here is the list of the first 16 provided transcripts variants (capitalized letters gene ID tags):		ORF	last codon	peptide length	mRNA length	read copy #	init. rate fold change
COL1A1	NM_000088.4:119-4513 Homo sapiens collagen type I alpha 1 chain (COL1A1), mRNA	yes	TAA	1464	4395	4	01.0
RPL4	NM_000968.4:66-1349 Homo sapiens ribosomal protein L4 (RPL4), mRNA	yes	TAA	427	1284	3	02.0
RPL22	NM_000983.4:23-409 Homo sapiens ribosomal protein L22 (RPL22), mRNA	yes	TAA	128	387	3	01.0
EIF5A	NM_001970.5:147-611 Homo sapiens eukaryotic translation initiation factor 5A (EIF5A), transcript variant B, mRNA	yes	TAA	154	465	1	01.0
BGN	Biglycan Homo sapiens (Bgn), transcript variant, mRNA 1107 nt 368 aa	yes	TAG	368	1107	1	01.0
KIF4A	NM_012310.5:69-3767 Homo sapiens kinesin family member 4A (KIF4A), mRNA	yes	TGA	1232	3699	1	01.0
KIF14	NM_001305792.1:1501-4974 Homo sapiens kinesin family member 14 (KIF14), transcript variant 2, mRNA	yes	TGA	1157	3474	3	01.0
KIF23	NM_001281301.2:311-2611 Homo sapiens kinesin family member 23 (KIF23), transcript variant 3, mRNA	yes	TGA	766	2301	2	01.0
RAF1	NM_001354689.3:332-2338 Homo sapiens Raf-1 proto-oncogene, serine/threonine kinase (RAF1), transcript variant 1, mRNA	yes	TAG	668	2007	4	01.0
HIF1A	NM_001530.4:293-2773 Homo sapiens hypoxia inducible factor 1 subunit alpha (HIF1A), transcript variant 1, mRNA	yes	TGA	826	2481	2	01.0
LCFA	AF394059.1:70-3783 Pyrocystis lunula luciferase (lcfA) mRNA, complete cds	yes	TGA	1237	3714	1	01.0
OMD	Osteomodulin Homo sapiens (Omd) cds:protein_coding 1266 nt 421 aa	yes	TAG	421	1266	5	01.0
DCN	Decorin Homo sapiens (Dcn), transcript variant, mRNA 1080 nt 359 aa	yes	TAA	359	1080	1	01.0
ASPN	Asporin ASPN-202 Homo sapiens (Aspn) cds:protein_coding	yes	TAA	380	1143	1	01.0

Home Settings Ribosomer Ribo-Seq output Protein levels

Ribosomer - TASEP modeling platform for mRNA translation simulations

Resources settings

drop your transcripts variants file here: in FASTA format with correct CDS (without UTRs)
as retrieved from NCBI or Ensembl repositories

Click the button(s) to activate (GREEN) / desactivate (RED) the desired factor(s) combination

modification on U34-tRNA (ELP3- URM1-) | mRNA secondary structure | Proline slowdown | Non-uniform mRNA relative abundance | Limited pool of ribosomes

silencing A to I tRNAs editing (ADAT-) | Exit tunnel electrostatic interaction | EF-P or eIF5A depleted | Non-uniform initiation rates

Initiation time rate in (s) = 40 40
Initiation half-time in (s) = 027.7

Limited or unlimited pool of ribosomes: use the slider to change the ratio of available ribosomes over transcripts counts:
Ratio of ribosomes cardinality / transcripts cardinality (range = [0.1 - 10]):

Ratio #Ribosomes / #Transcripts
10

drop your transcripts copy number file as derived from RNA-seq reads count (within sample reads counts).
Use a tab delimited format: geneID tags '\tab' transcript copy counts ['\tab' optional relative initiation rates]'\n'

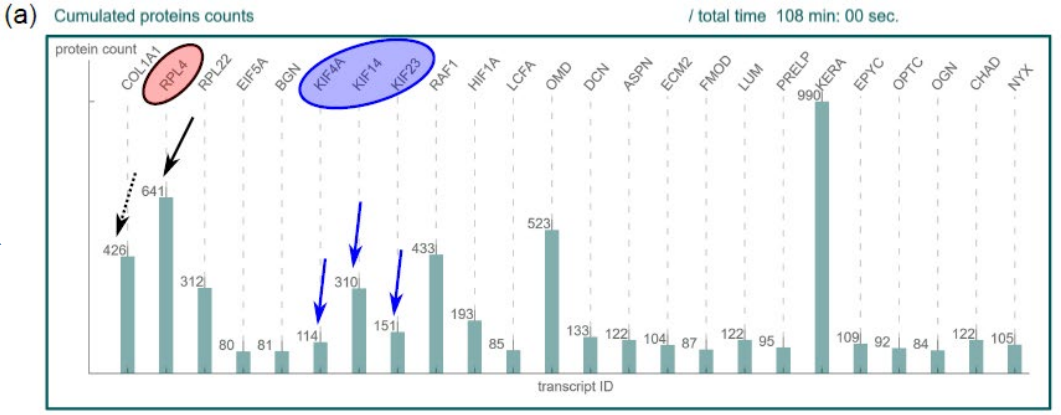
Elongation cycle sub-steps rates are dependent on tRNA relative abundance, tRNA gene copy numbers of the expression vector for your transcripts. Click the button to select the expression vector species.

E. coli | Yeast | Worm | Zebrafish | Mouse | Human

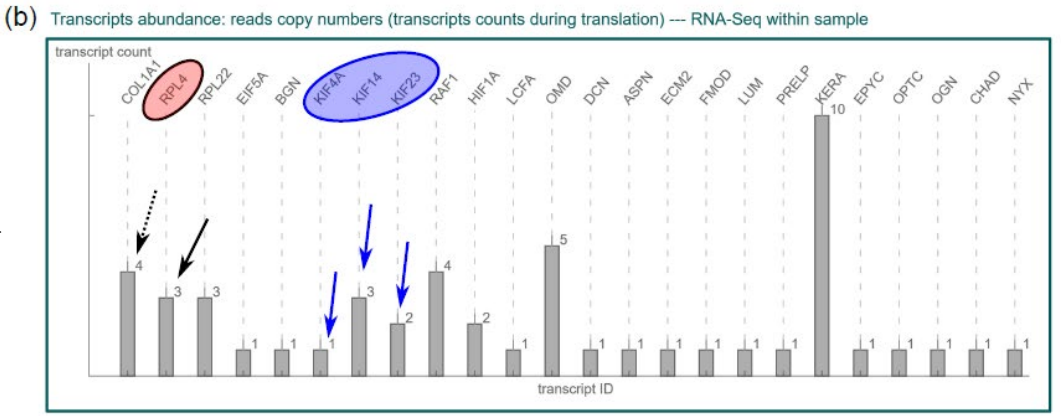
STATUS: waiting for transcripts...

6 Model Output

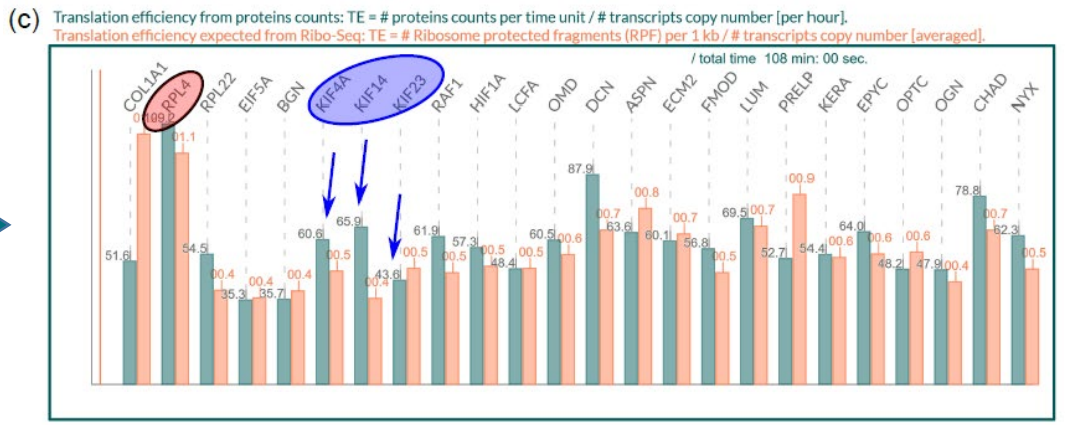
Output: Protein counts



Input: Transcript relative abundances

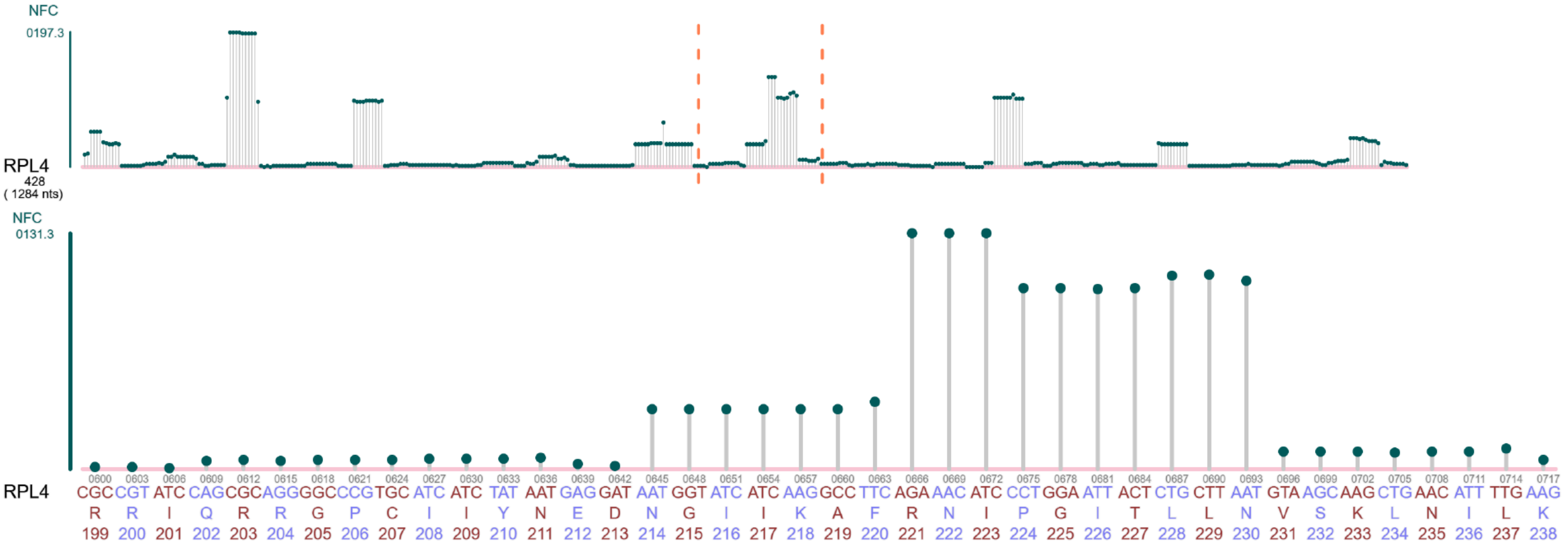


Output: Translation efficiencies



6 Model Output

Predicted ribosome occupancy map (P-site reference frame)



Number of ribosome footprint snapshots = 648
Number of ribosome footprint fragments = 46442
Averaged number of ribosomes on all transcripts per snapshot = 071.6
Exact Ribo-Seq coverage = 13.9

7 Uncertainty & Sensitivity Analysis

Parameter space exploration

Fully crossed factorial design with balanced randomized replicates

8 by 8 by n replicates (embarrassingly parallel mode on HPC cluster)

- **6000 core.hours of computational runs**

NIC5 @ ULiège

4672 CPUs

256GB …1TB RAM

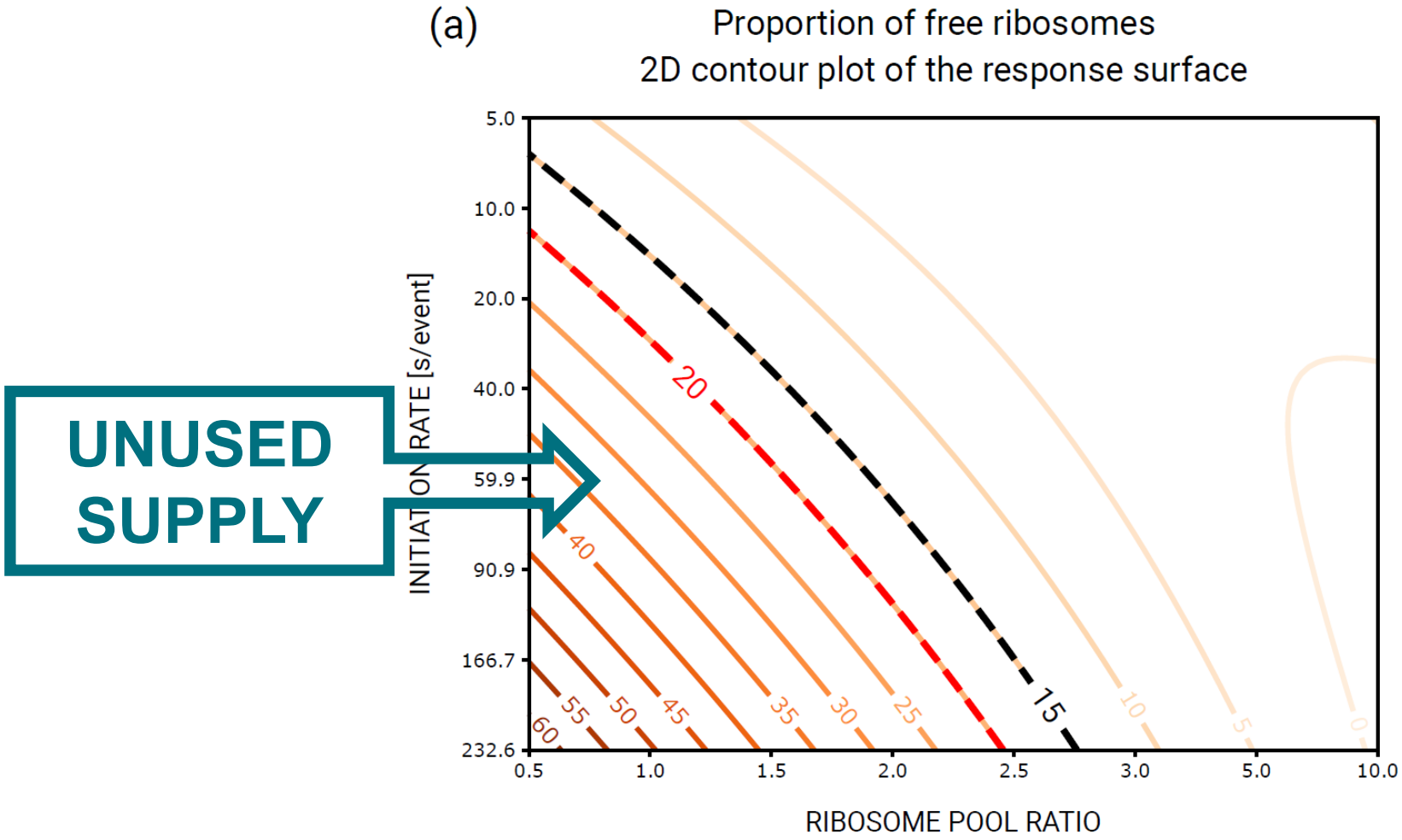
2.9 GHz

C.E.C.I

7

Uncertainty & Sensitivity Analysis

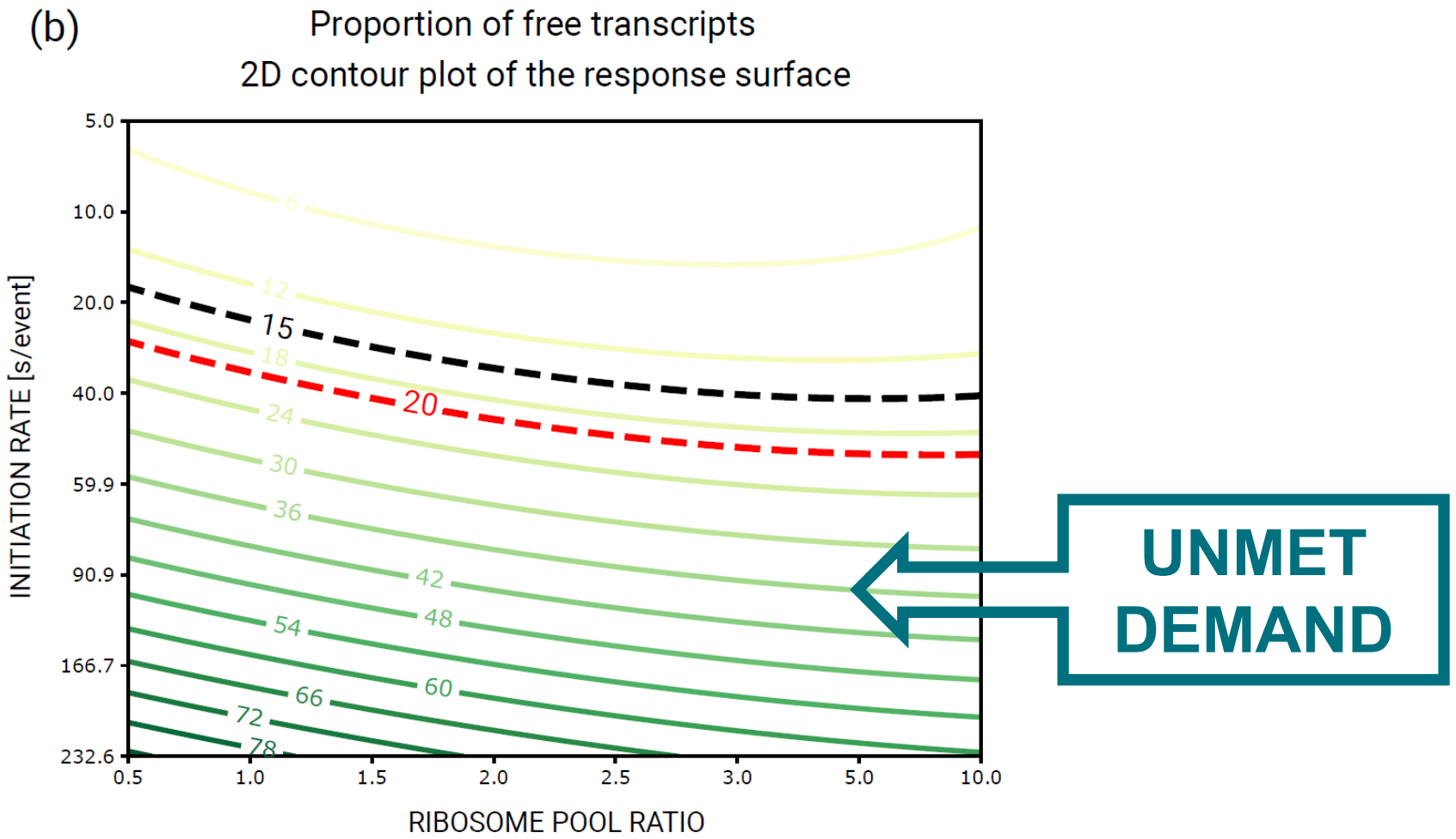
Ribosome ratio & Global initiation rate



UNUSED SUPPLY

Uncertainty & Sensitivity Analysis

Ribosome ratio & Global initiation rate



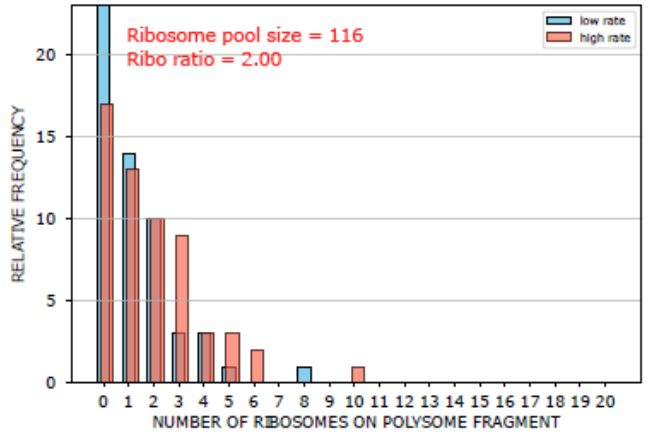
UNMET DEMAND

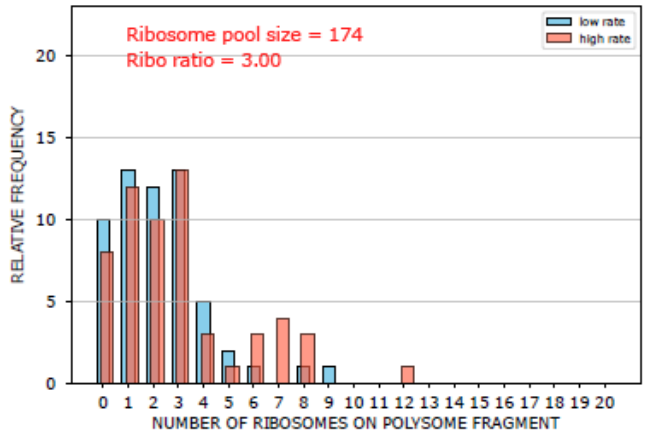
7 Uncertainty & Sensitivity Analysis

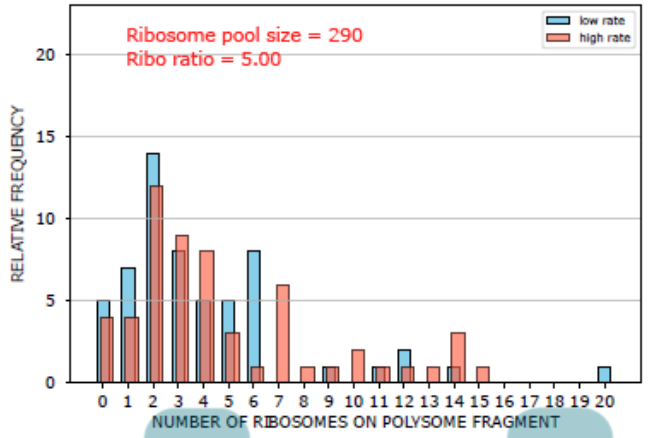
Polysome fragmentation

Polysome multiplicity increases much more with ribosome pool size than with initiation rate

Large dispersion of polysome multiplicity







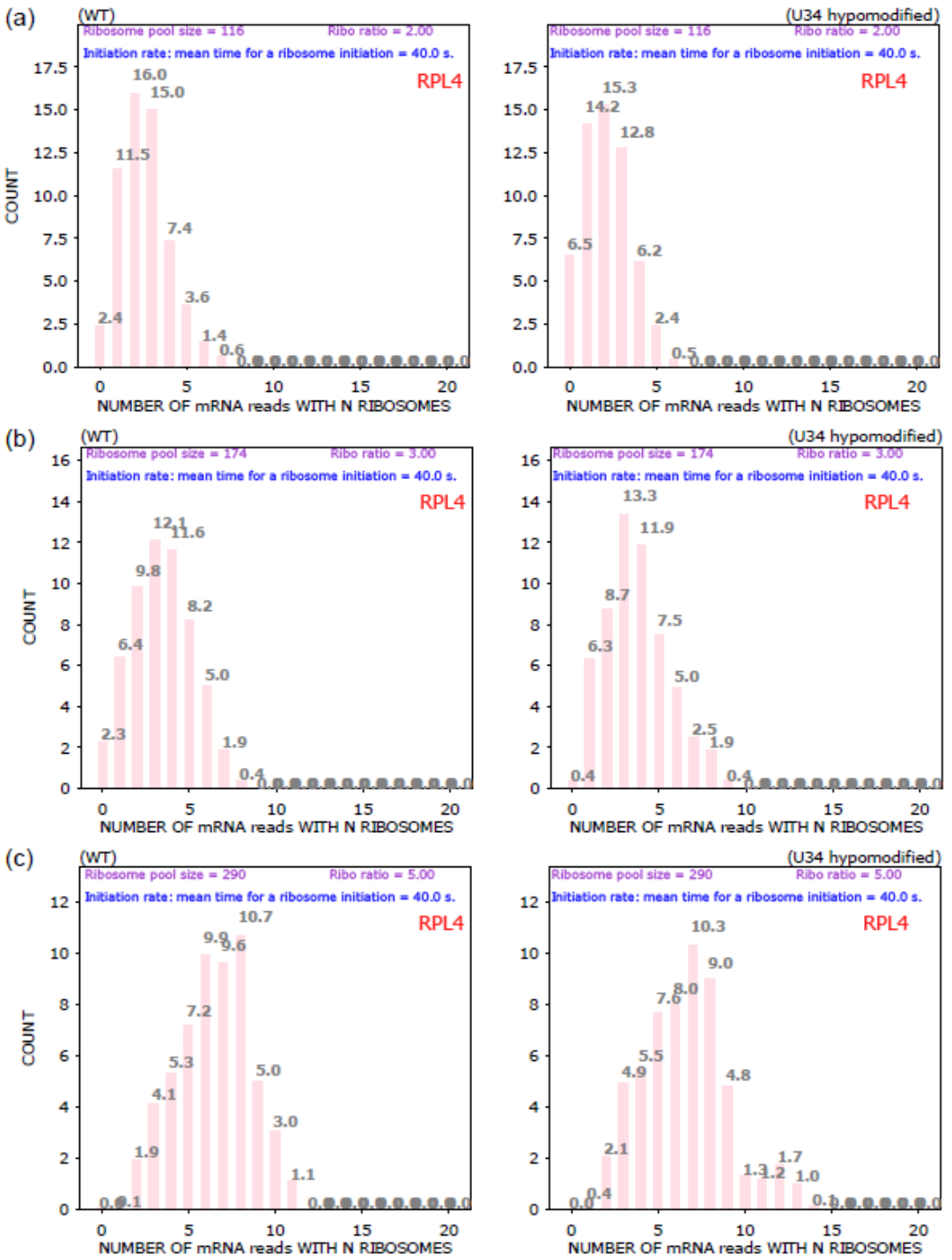
7 Uncertainty & Sensitivity Analysis

Polysome fragmentation per transcript

Polysome multiplicity shifts to the right with ribosome pool size

Large dispersion of polysome multiplicity

tRNA modification effects are overridden



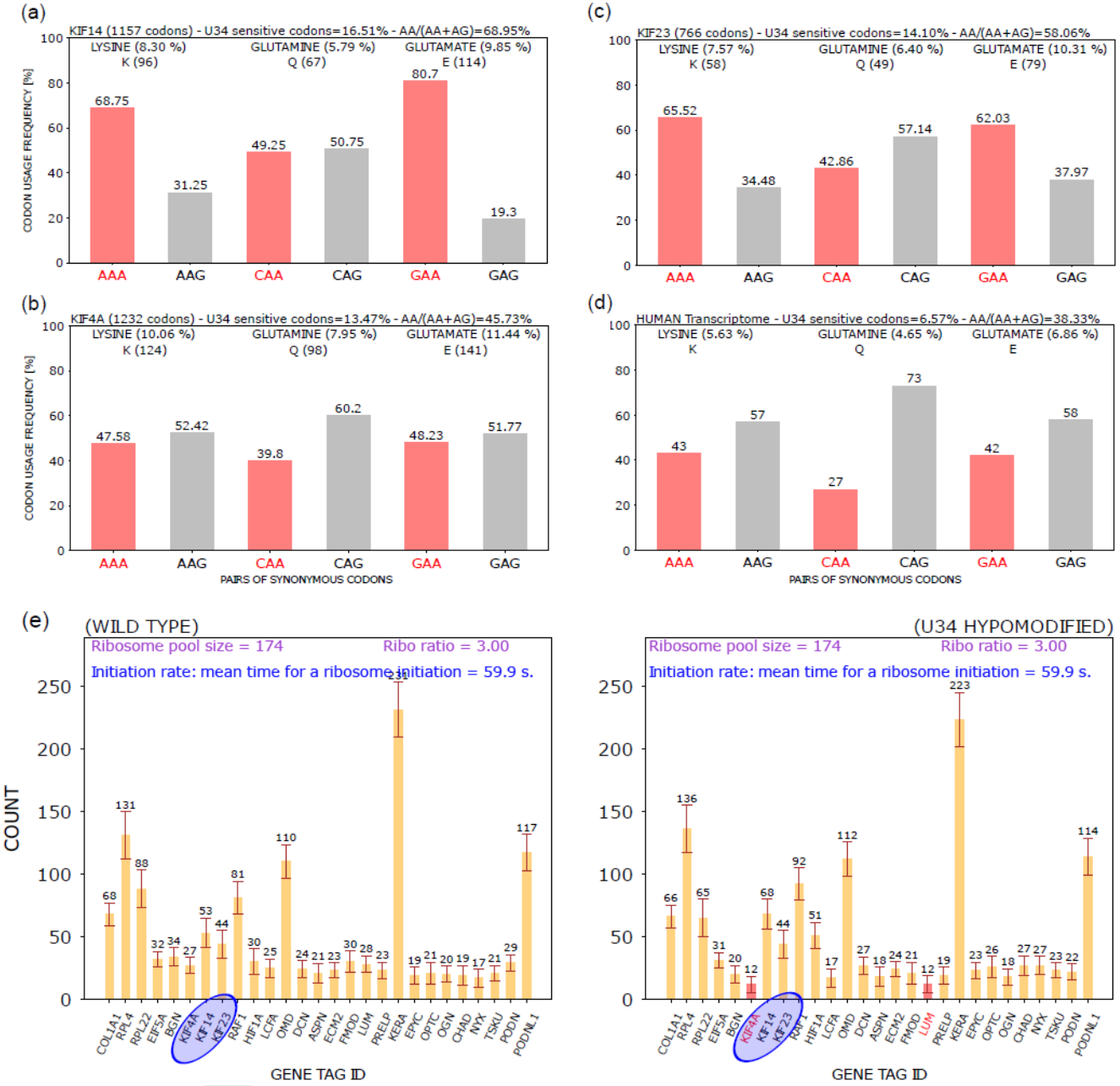
7

Uncertainty & Sensitivity Analysis

U34 tRNA hypo-modification

Even with a 2-fold enrichment in sensitive codons

tRNA modification effects undectable on the protein relative abundance



7 Uncertainty & Sensitivity Analysis

Privileged ribosome recruitment on a subset of transcripts

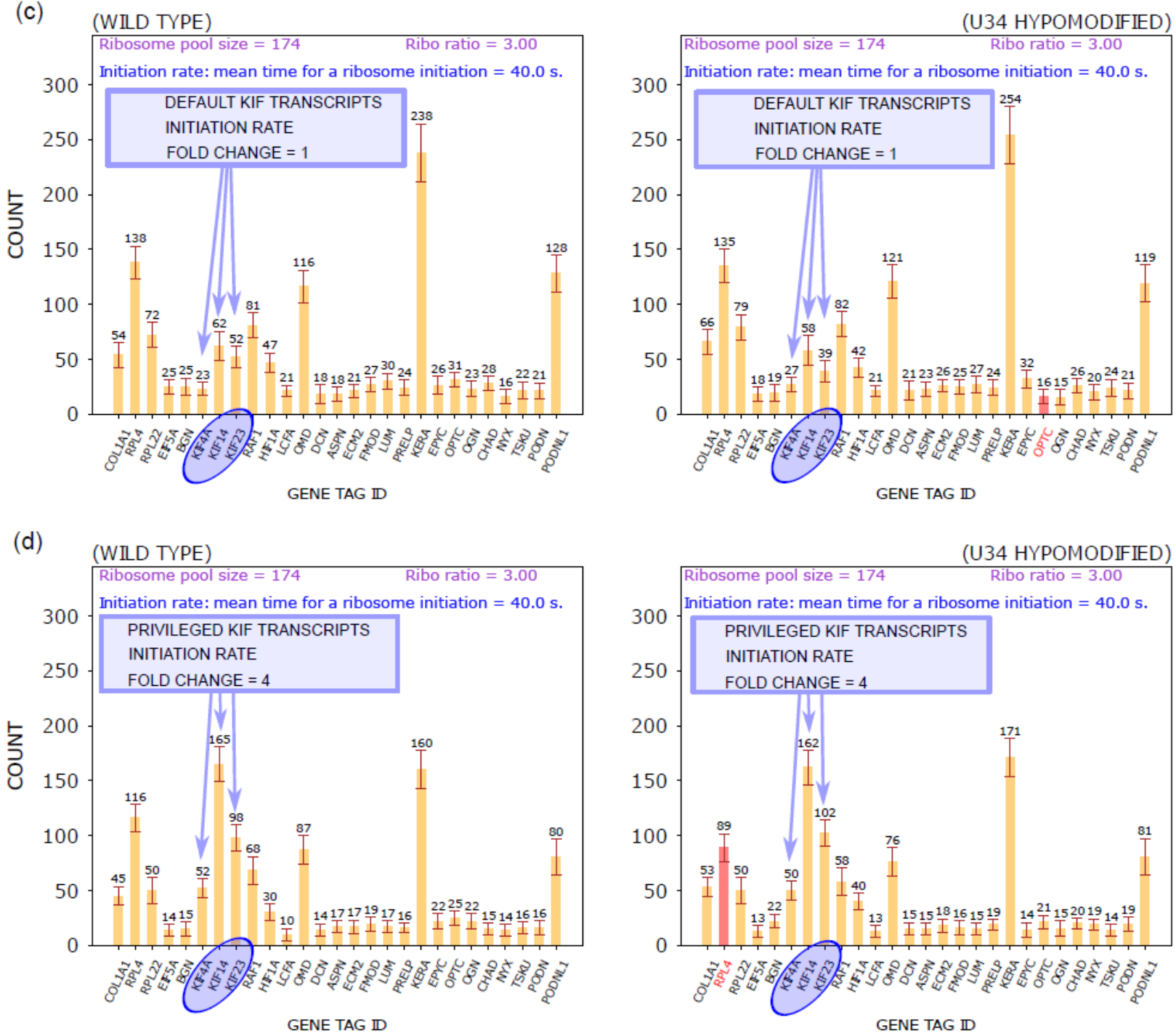
= Privileged individual initiation rates

The privileged ribosome recruitment on a specific subset of transcripts confounds the interpretation of protein relative abundance

Emergent behavior due to limited ribosome pool

Transcripts do compete for ribosome recruitment

tRNA modification effects overridden by confounding factors



8 Discussion & Future Perspectives

INTEGRATIVE framework « Ribosomer » offers high flexibility

Sources of stochasticity / uncertainty identified and quantified

Modularity allowed to disentangle contextual intertwined factors thanks to the ABM implementation

Allows to work with incomplete knowledge, build theory progressively, elucidate mechanisms progressively

Emergent behaviors uncovered

Transcripts compete for ribosomes under limited ribosome resources

I acknowledge Prof. Liesbet Geris for providing funding support through the FNRS-FWO EOS grant n°30480119 (Join-t-against-Osteoarthritis) , the ERC grant n°772418 (INSITE) and the ERC grant n°101088919 (INStant CARMA) which enabled this PhD thesis.