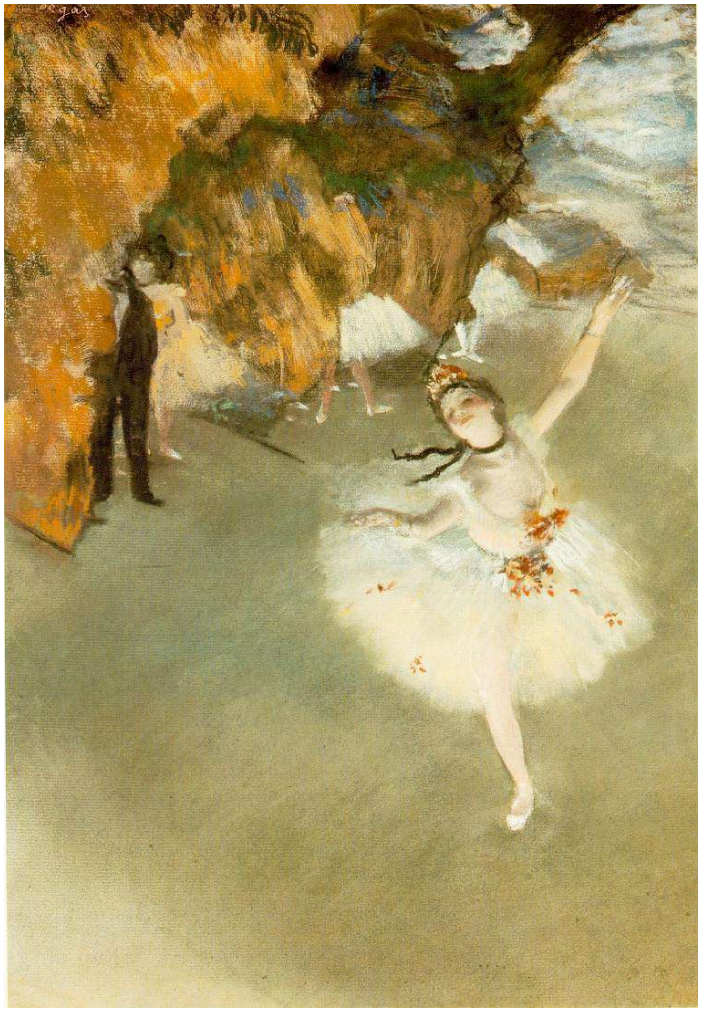
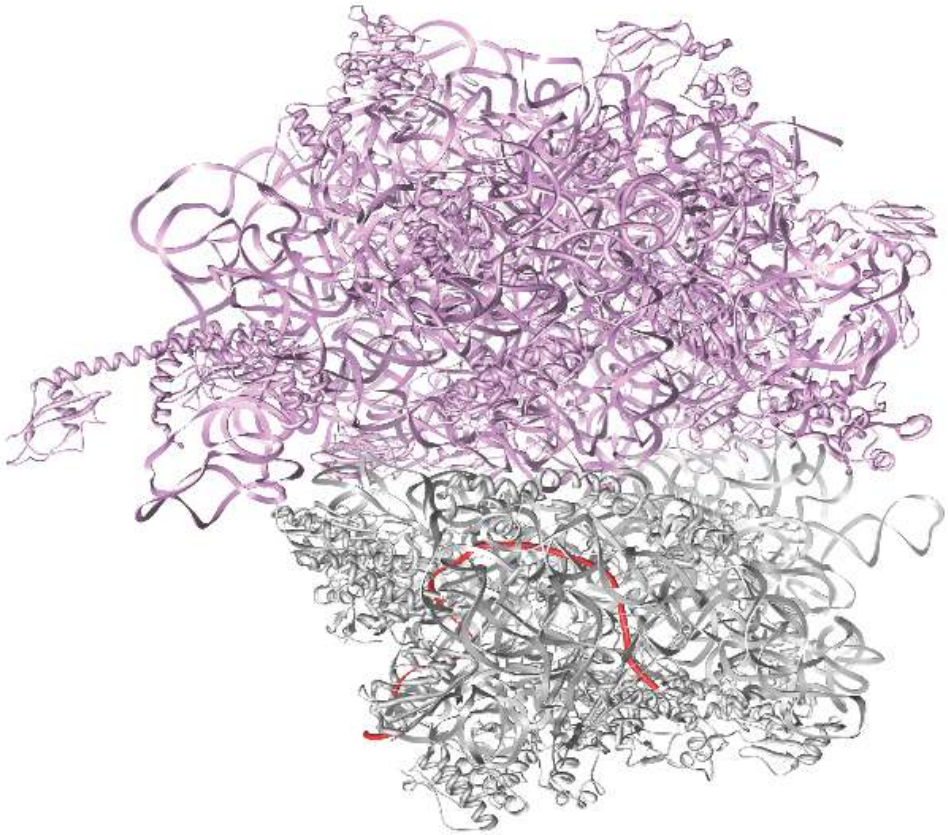
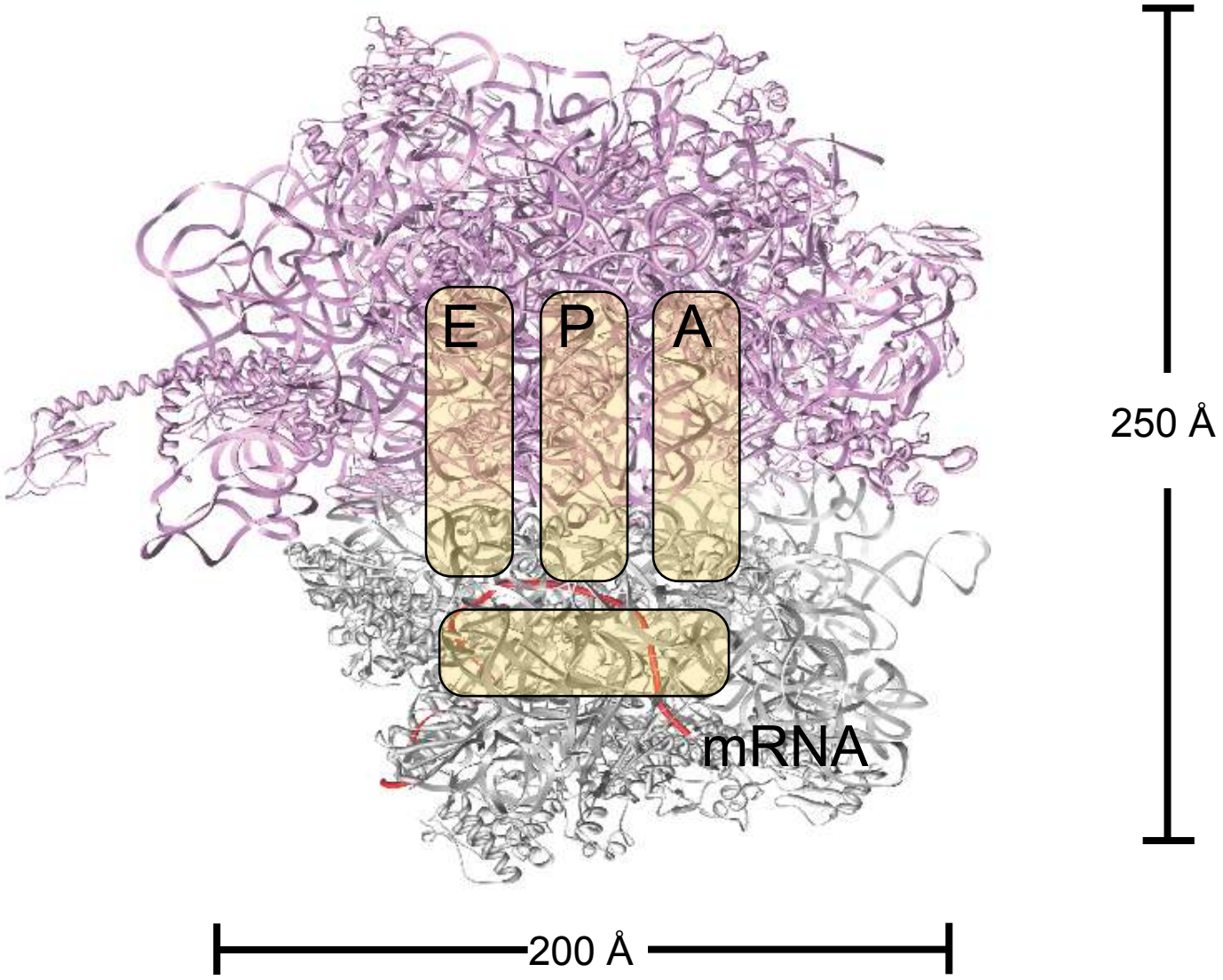
Dynamics of Translation



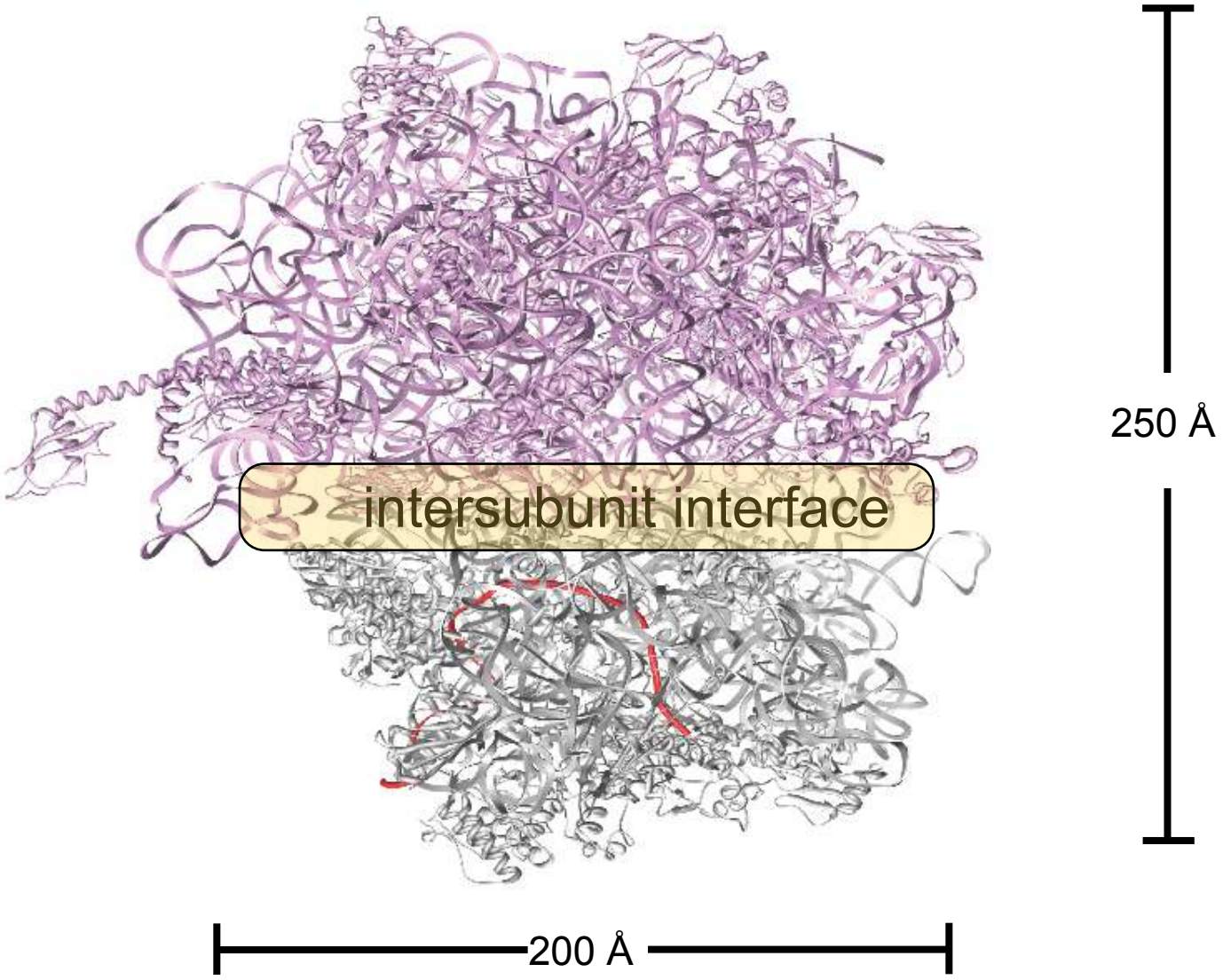


ICTS Oct 28, 2017

The Ribosome



The Ribosome



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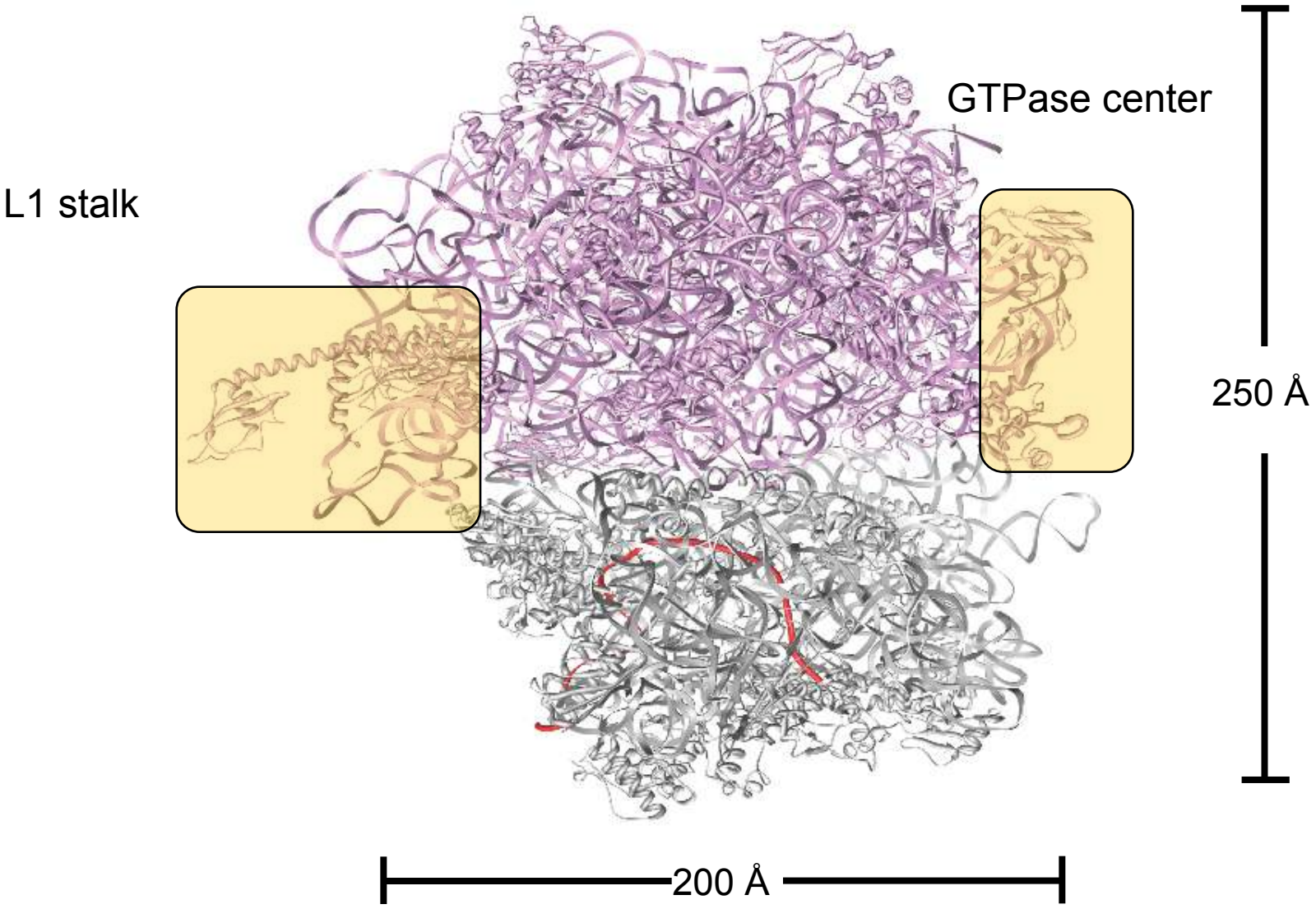

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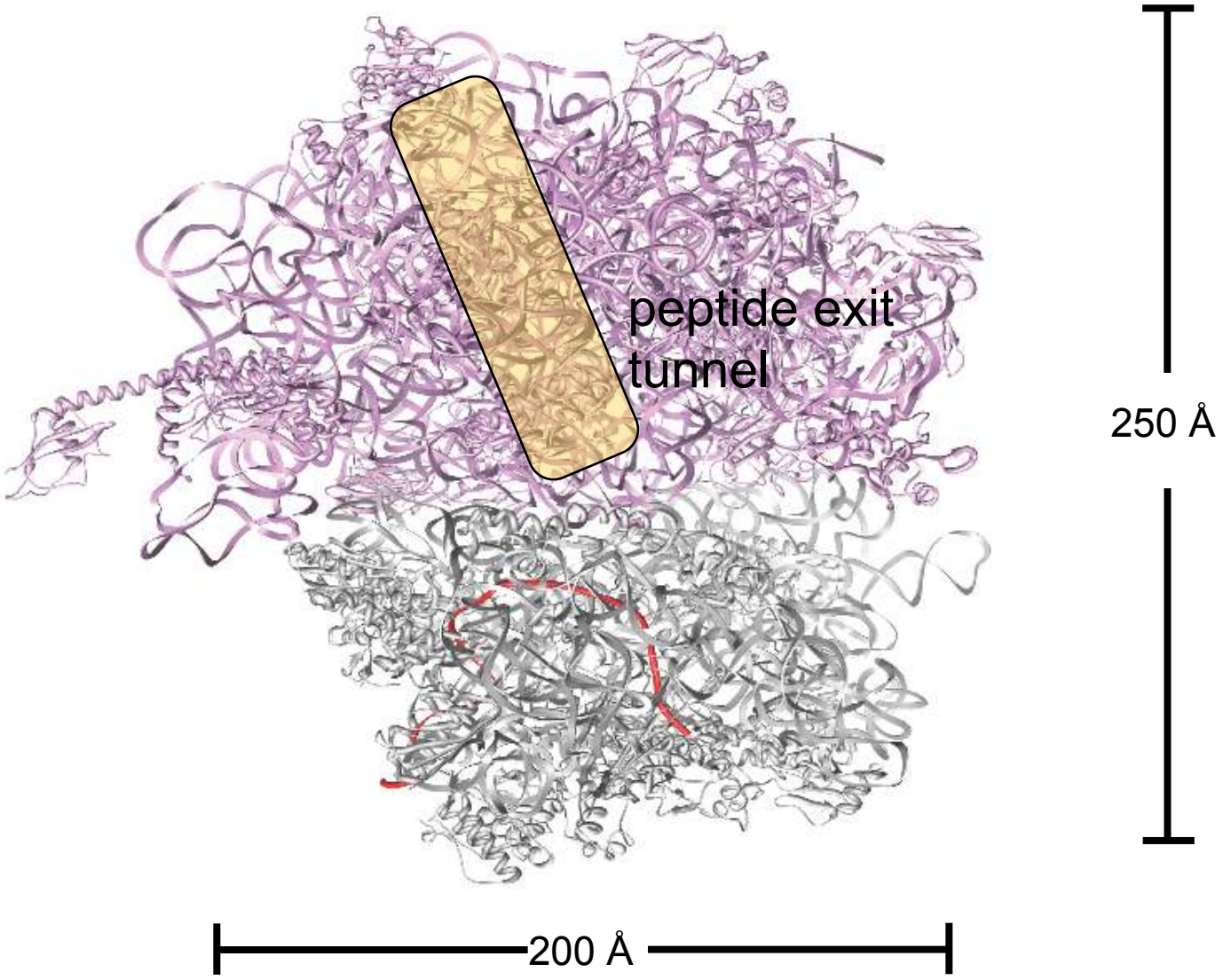
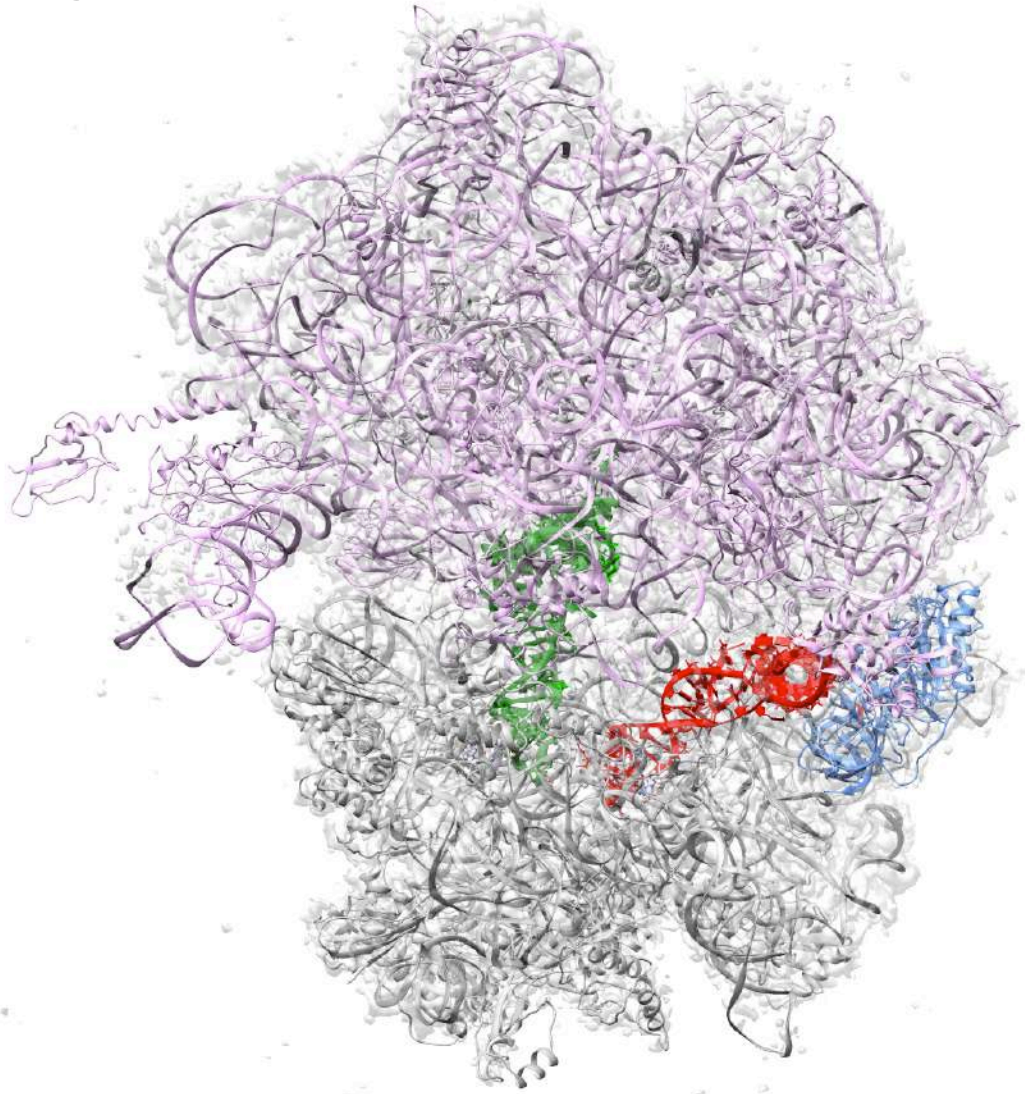
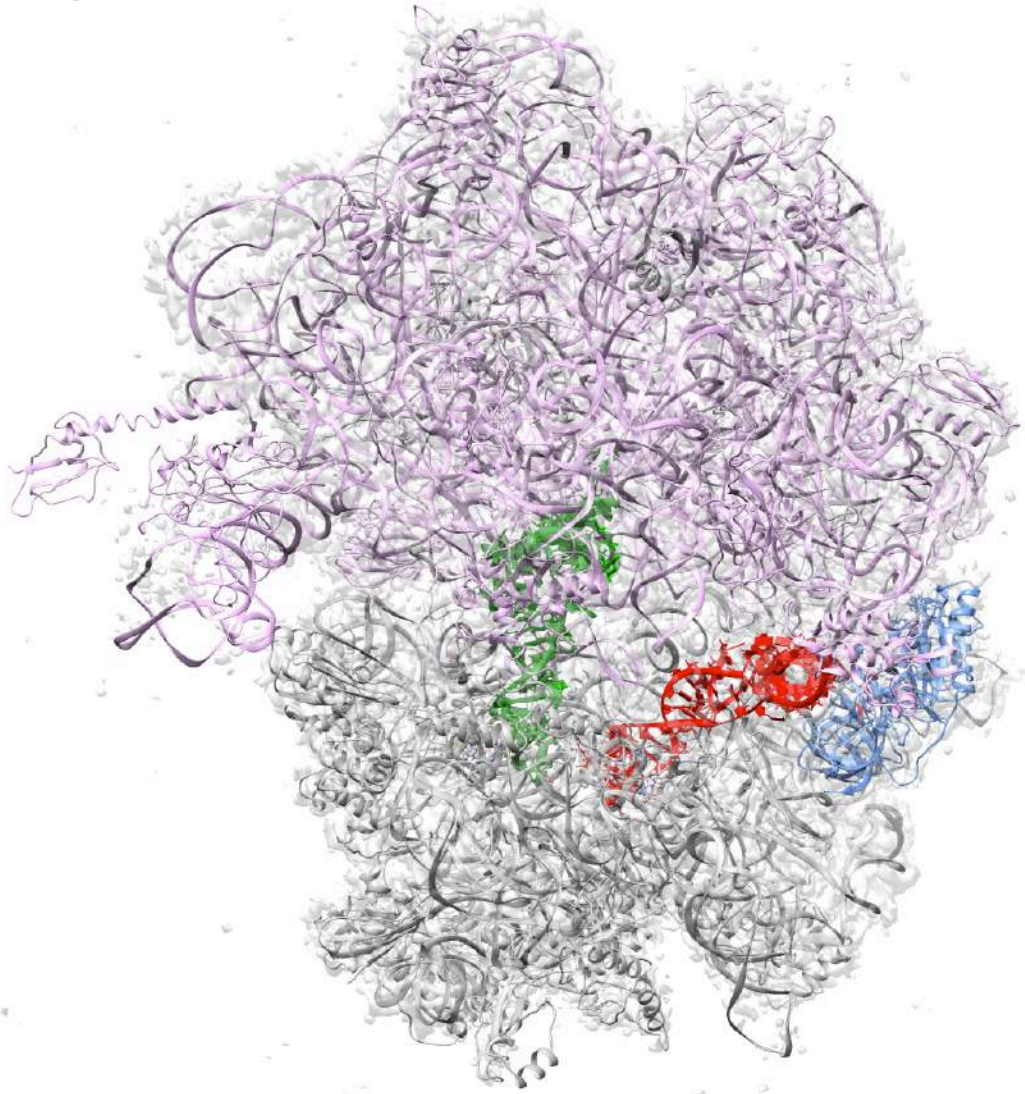


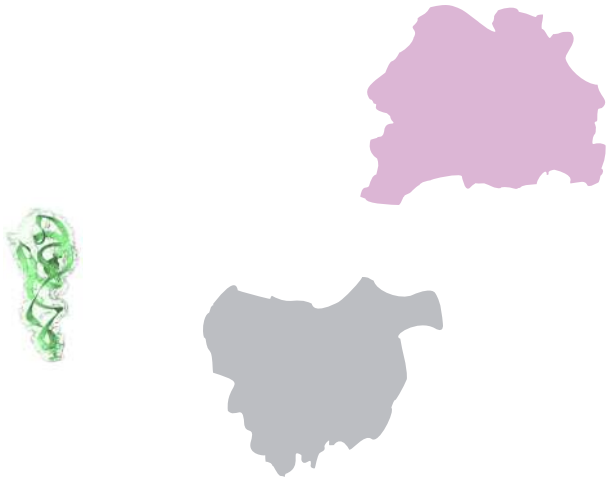
The Ribosome



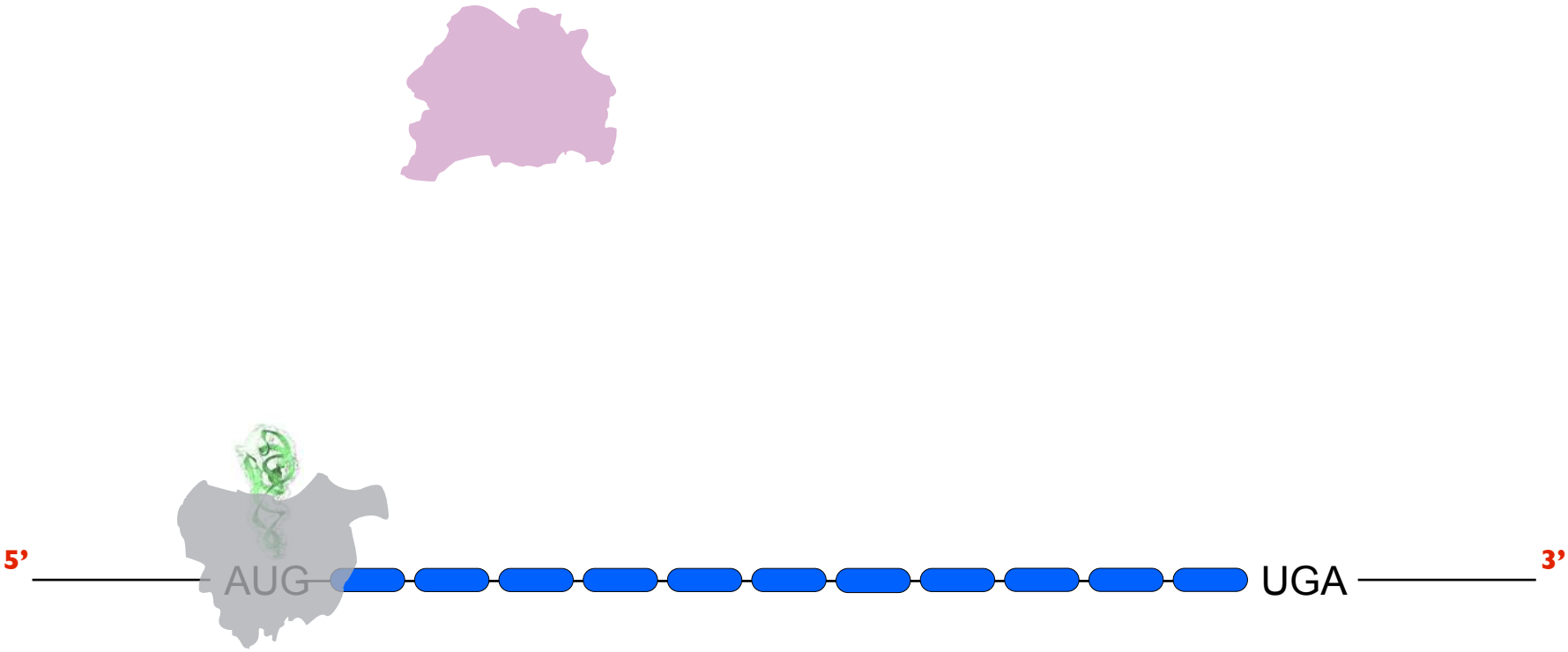
The Ribosome



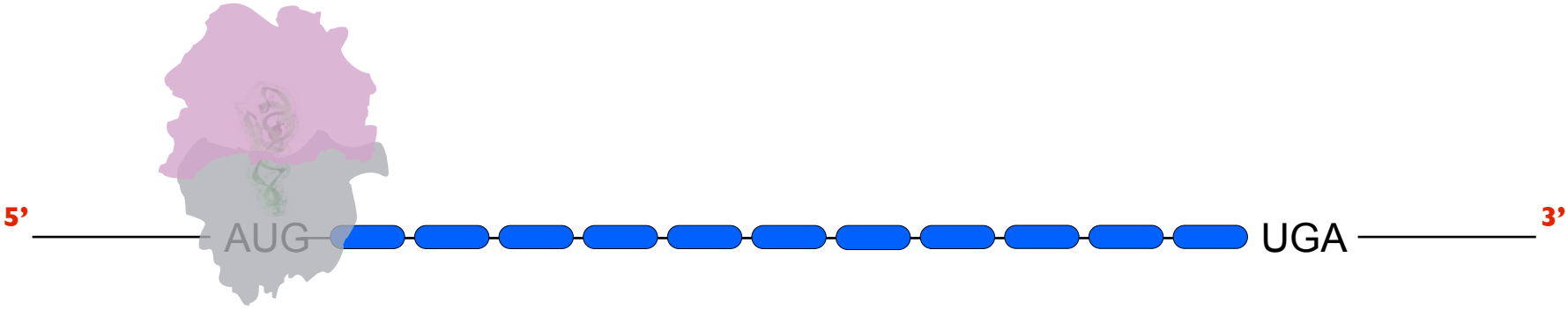
Dynamics of Translation



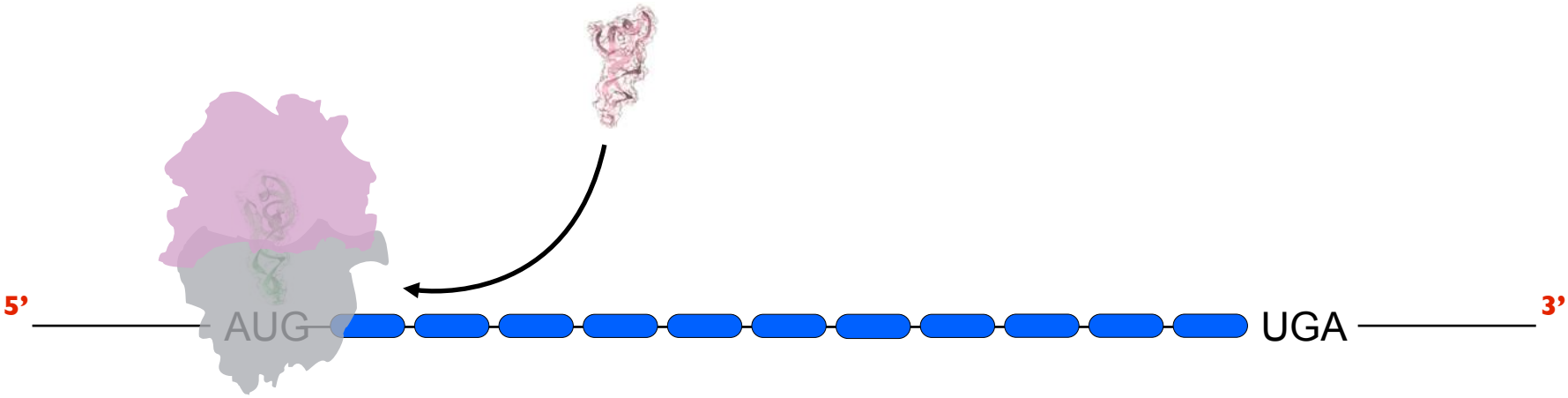
Dynamics of Translation



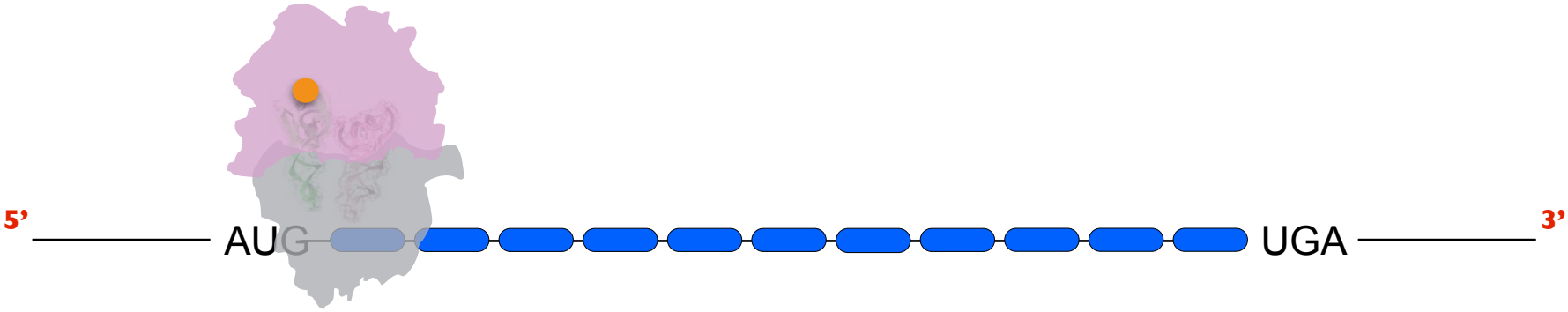
Dynamics of Translation



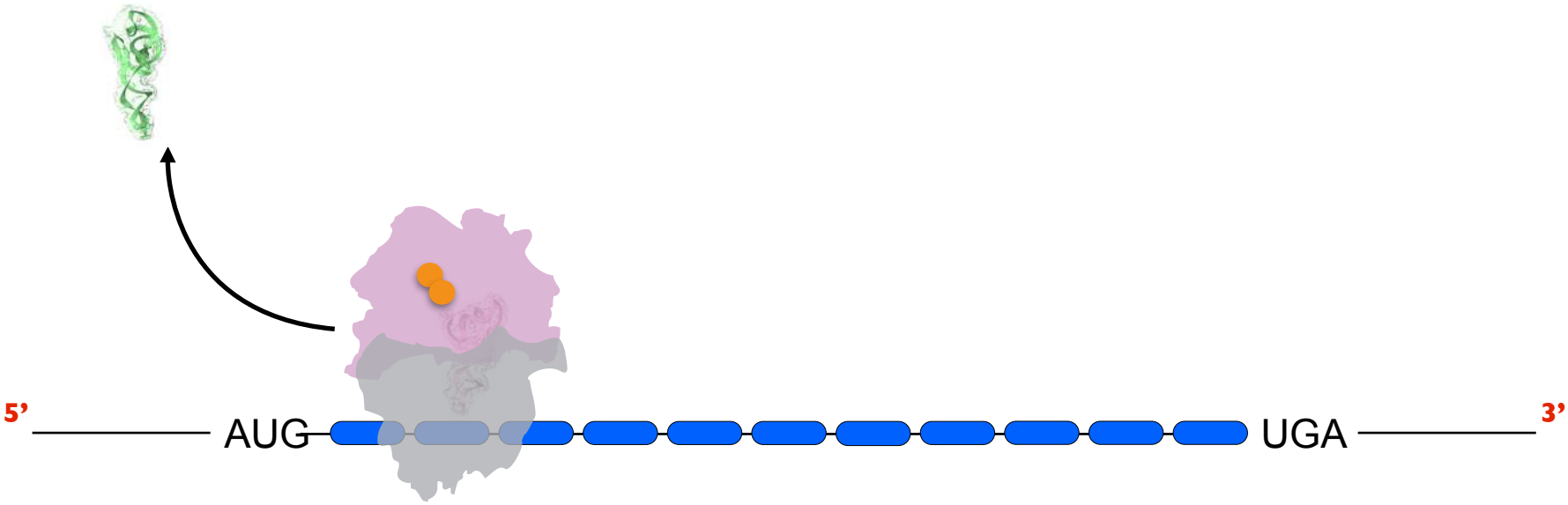
Dynamics of Translation



Dynamics of Translation



Dynamics of Translation



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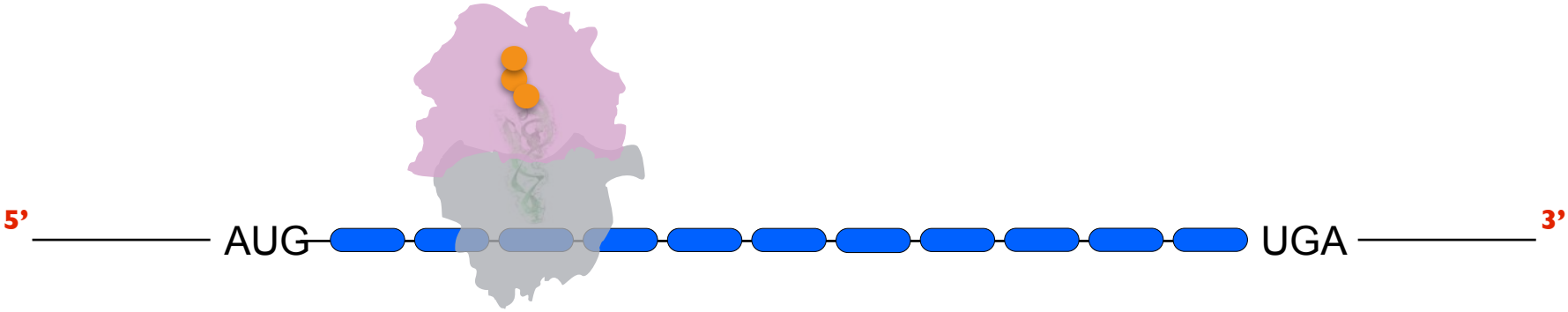

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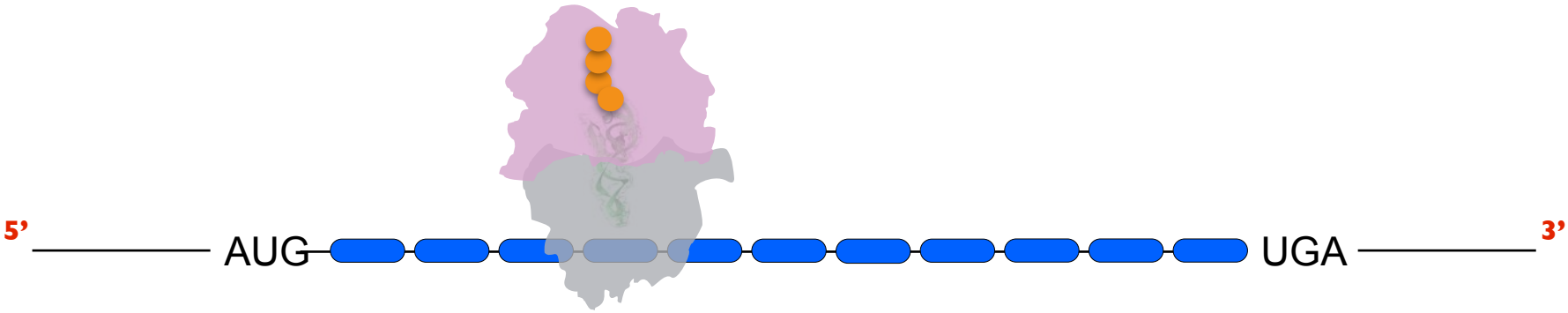

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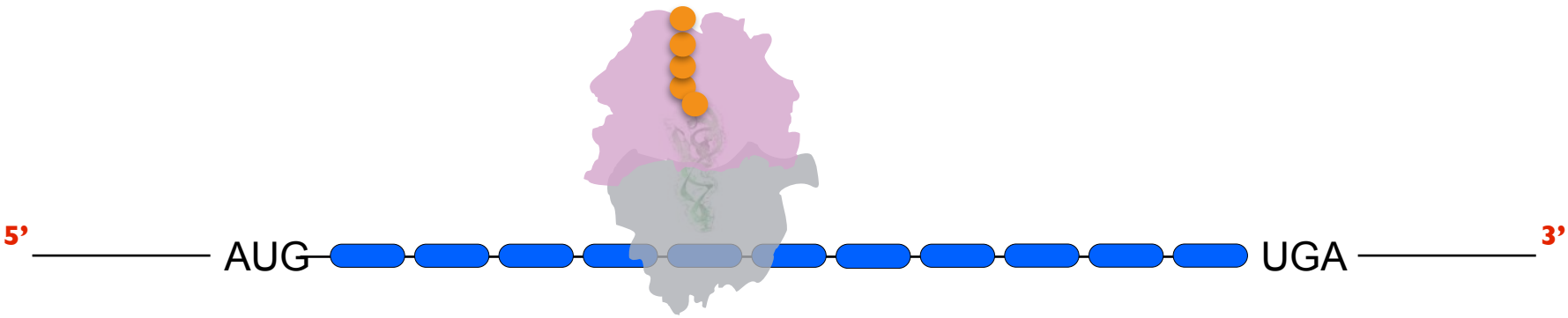

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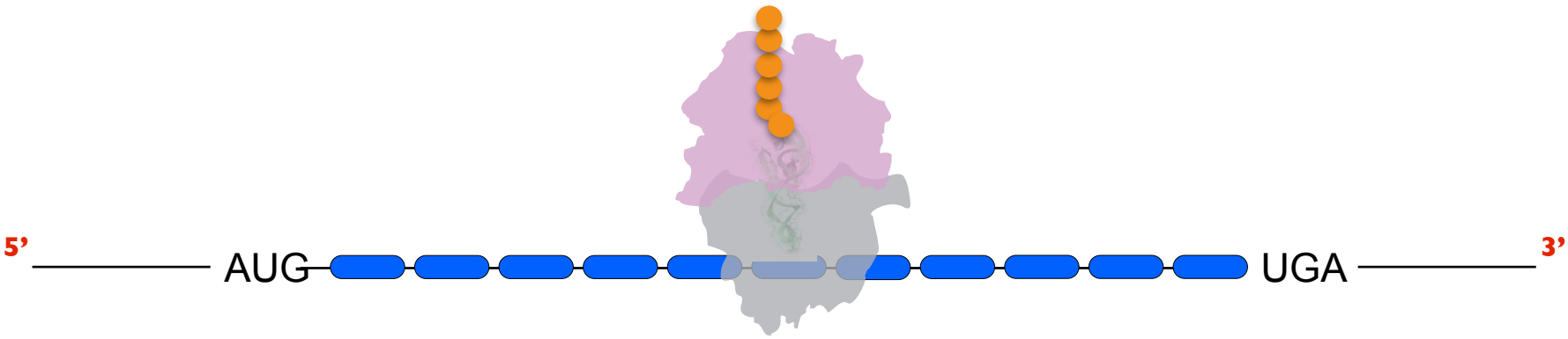

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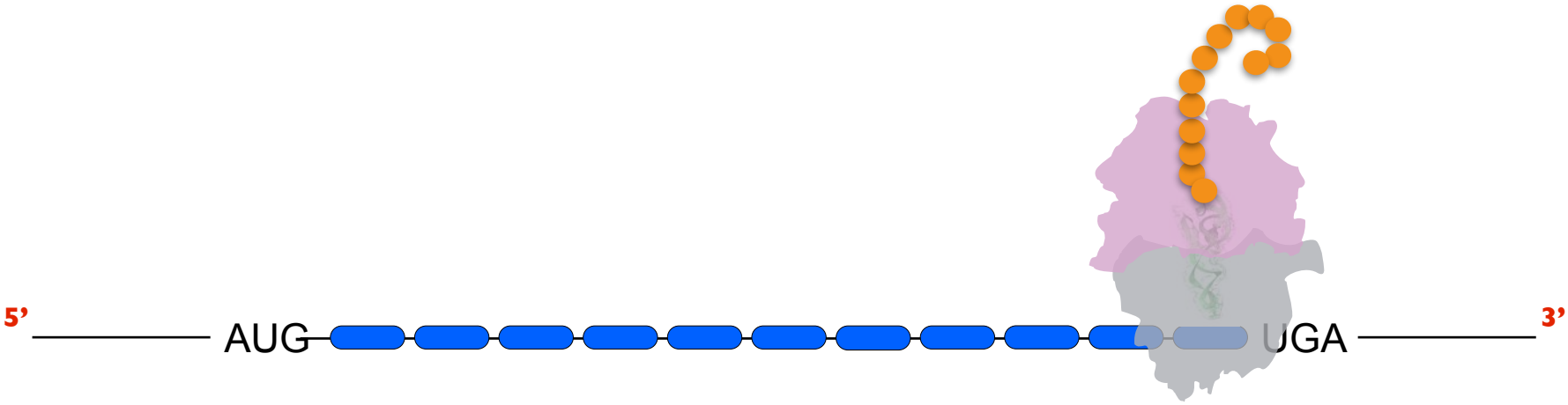
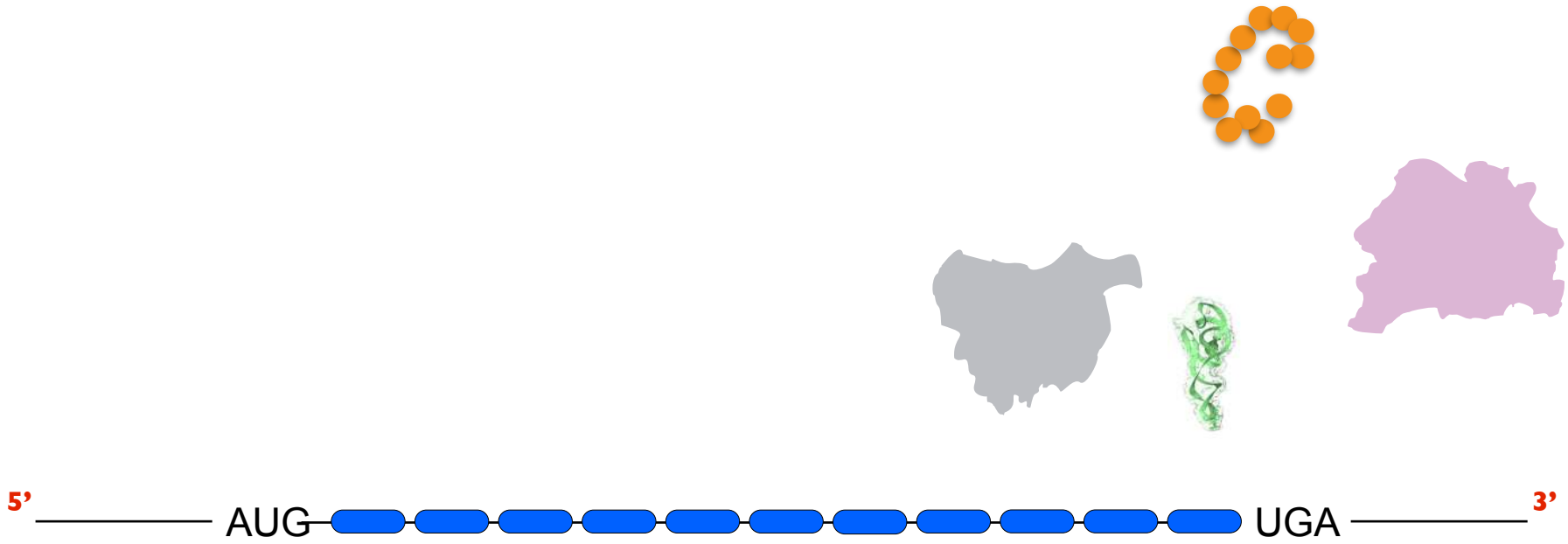
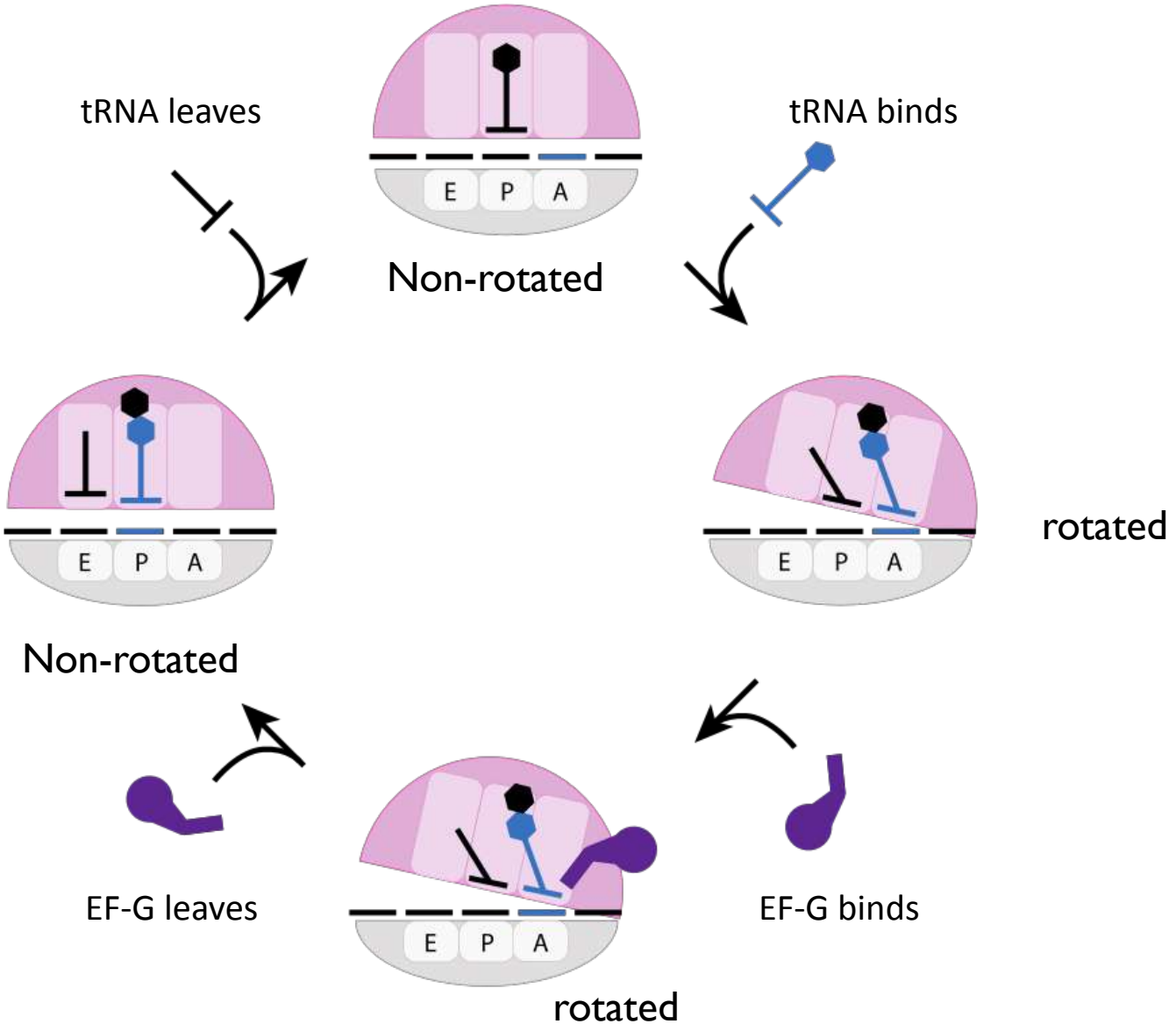


Dynamics of Translation



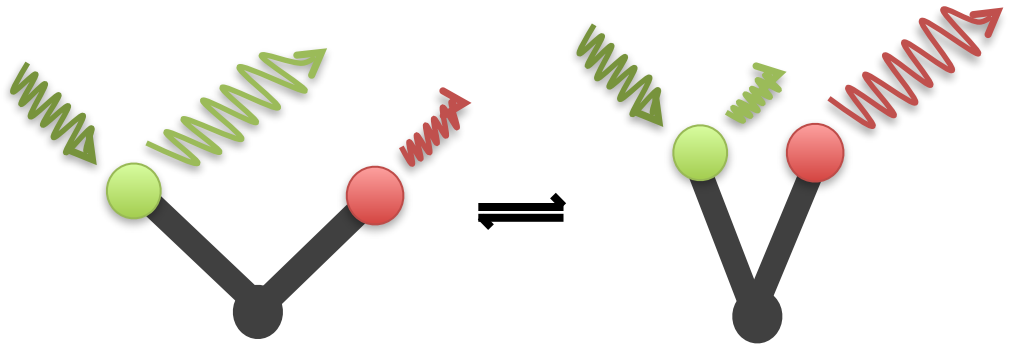
The translation elongation cycle



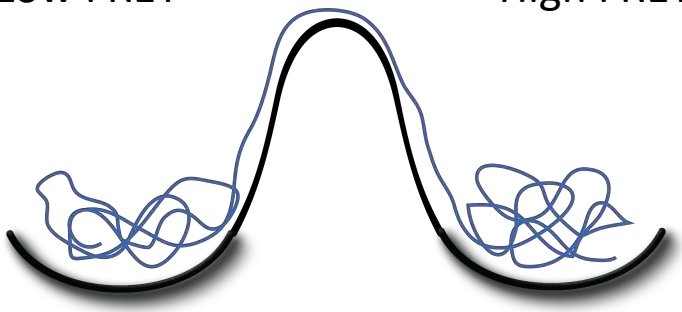
The translation elongation cycle

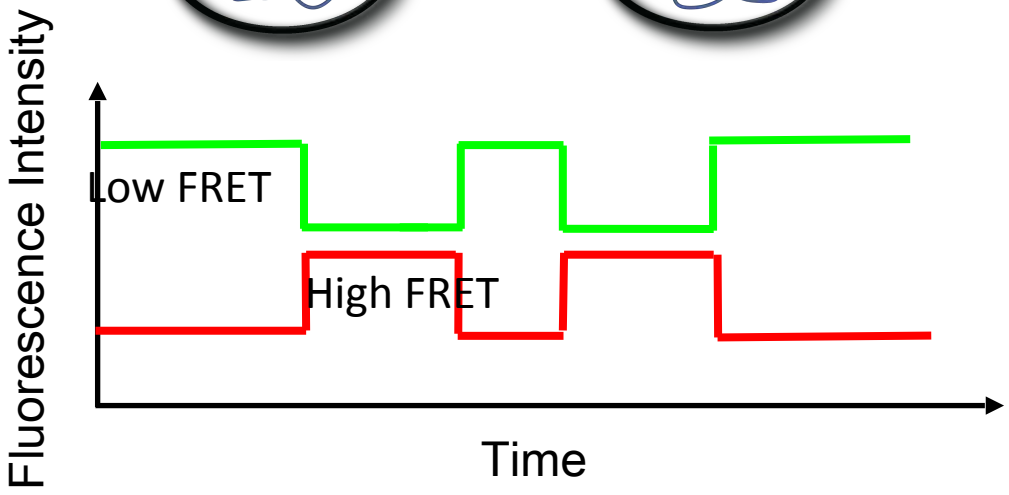
1. Speed (1-20 amino acids/sec)
2. Fidelity (1 error per 3000 aa)
3. Energy use-non wasteful
4. Regulation and modulation

Single-Molecule FRET to probe conformation

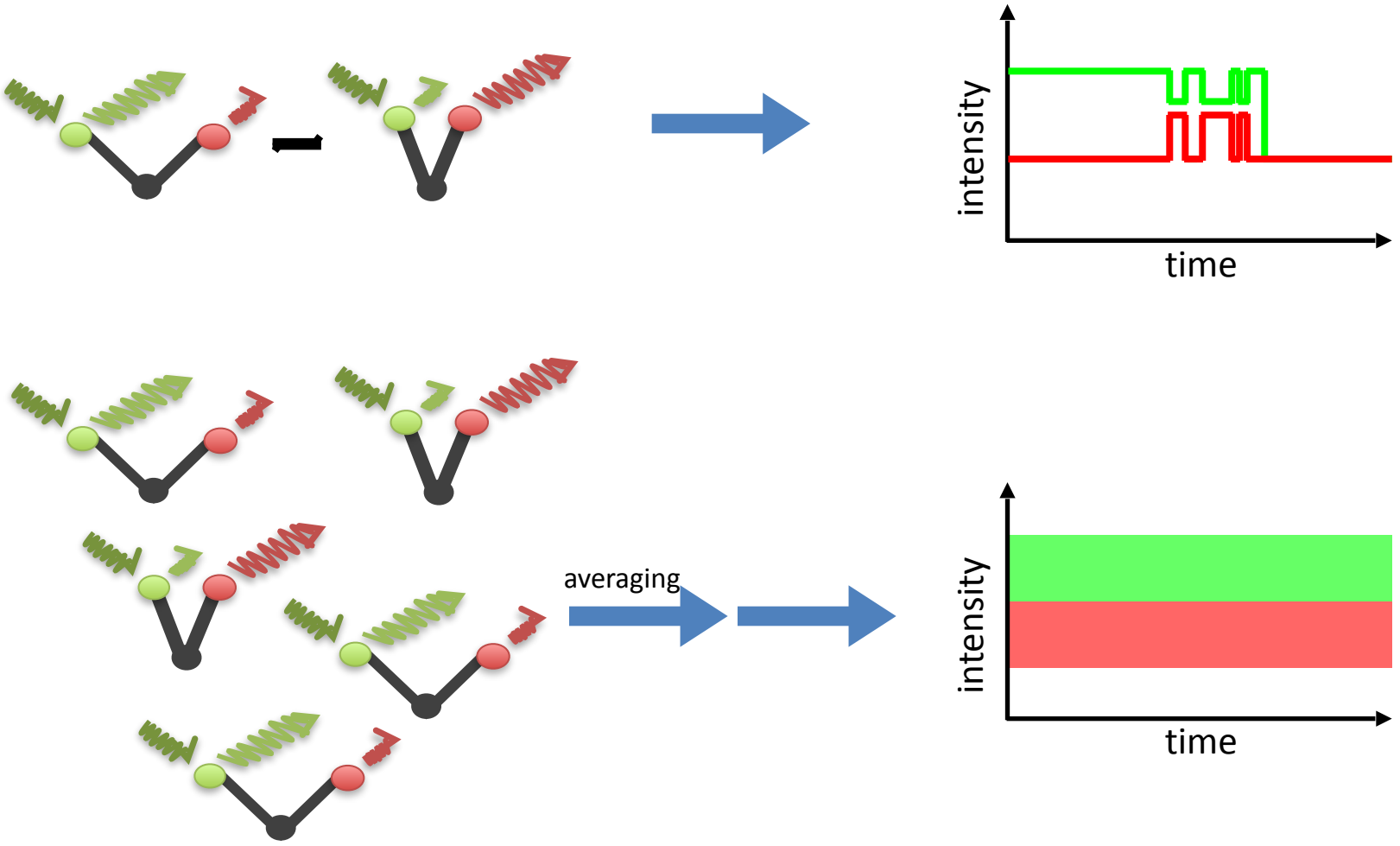


Low FRET High FRET

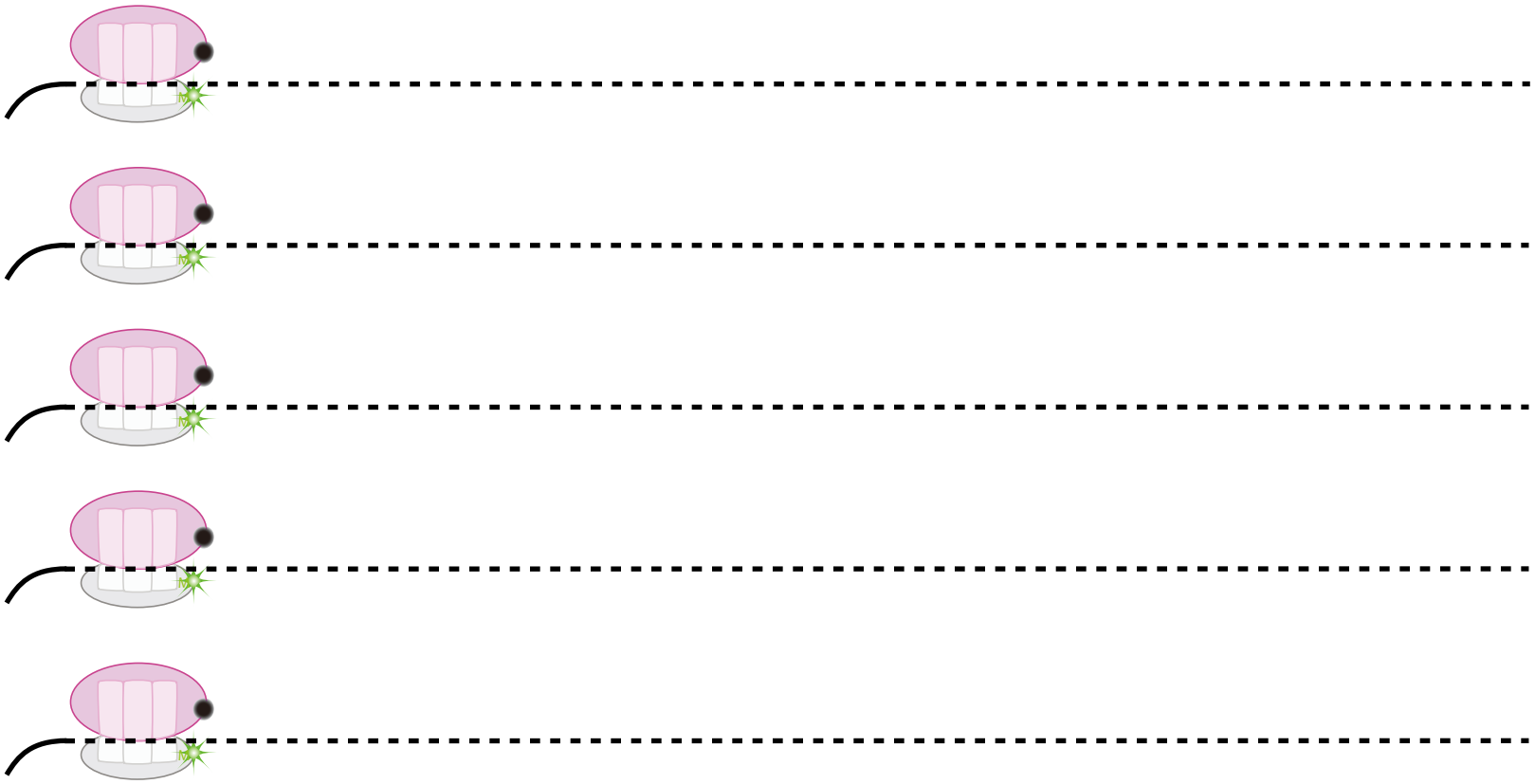




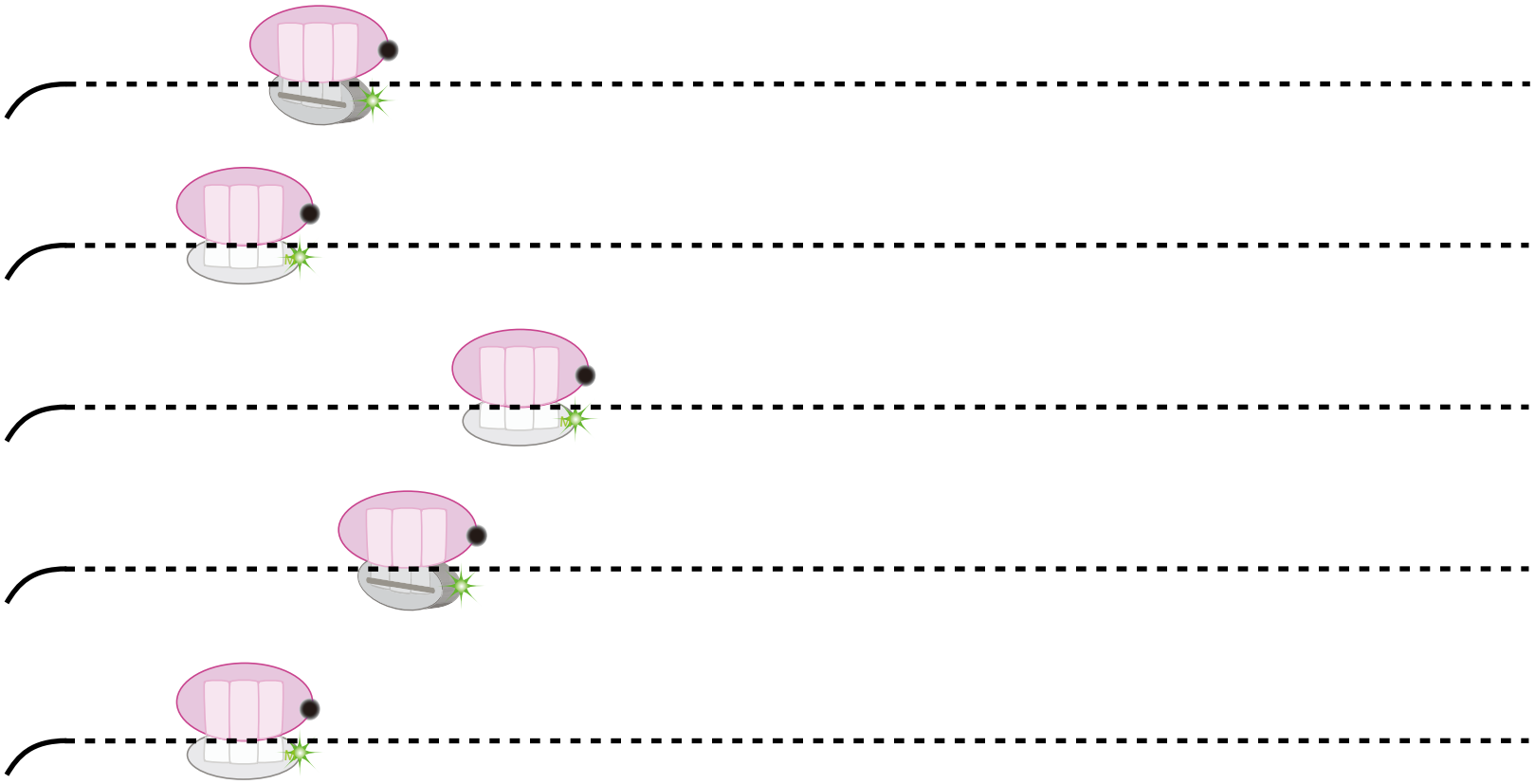
Solution Averaging Destroys Signal



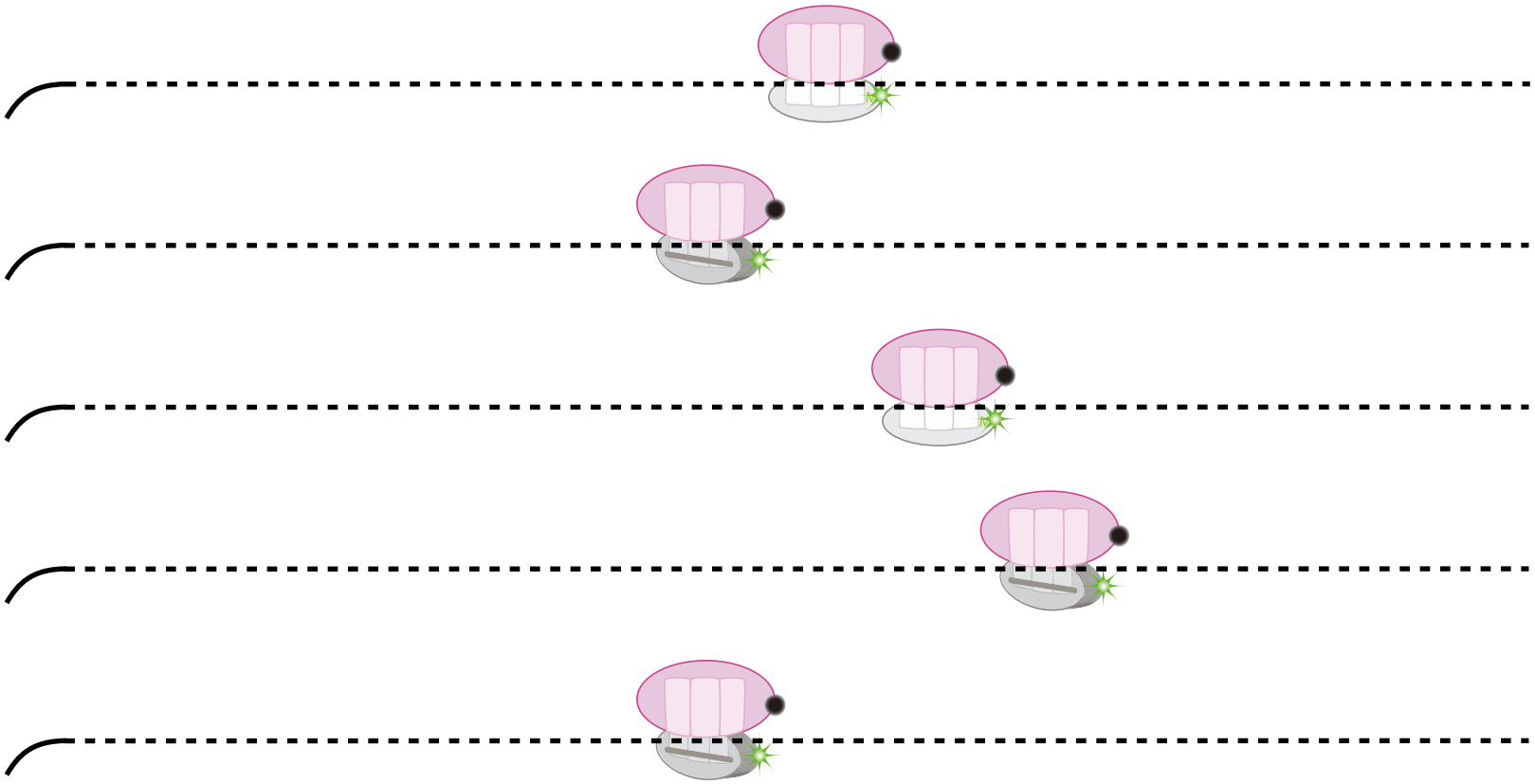
Ribosomes translating in bulk rapidly become heterogeneous



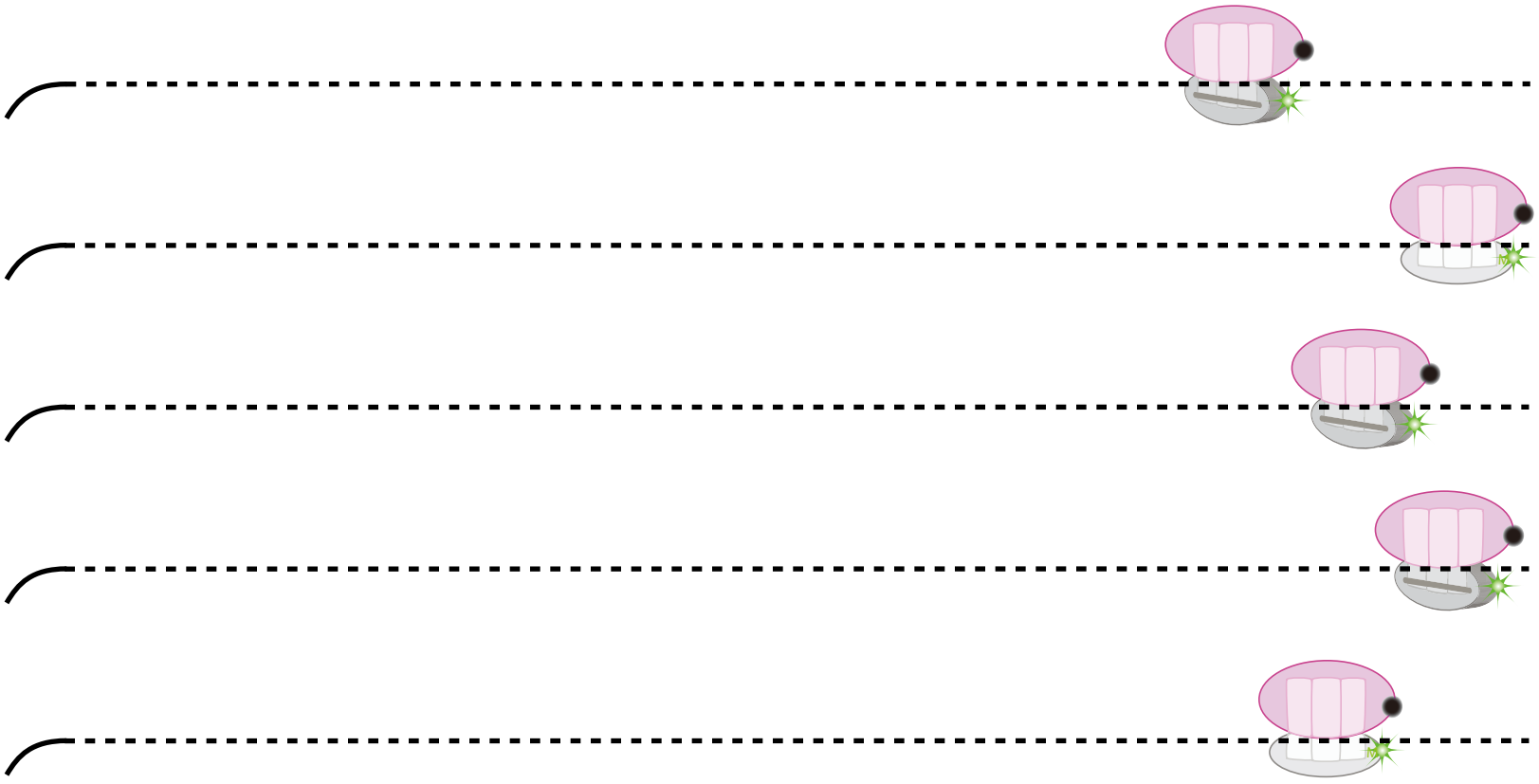
Ribosomes translating in bulk rapidly become heterogeneous



Ribosomes translating in bulk rapidly become heterogeneous

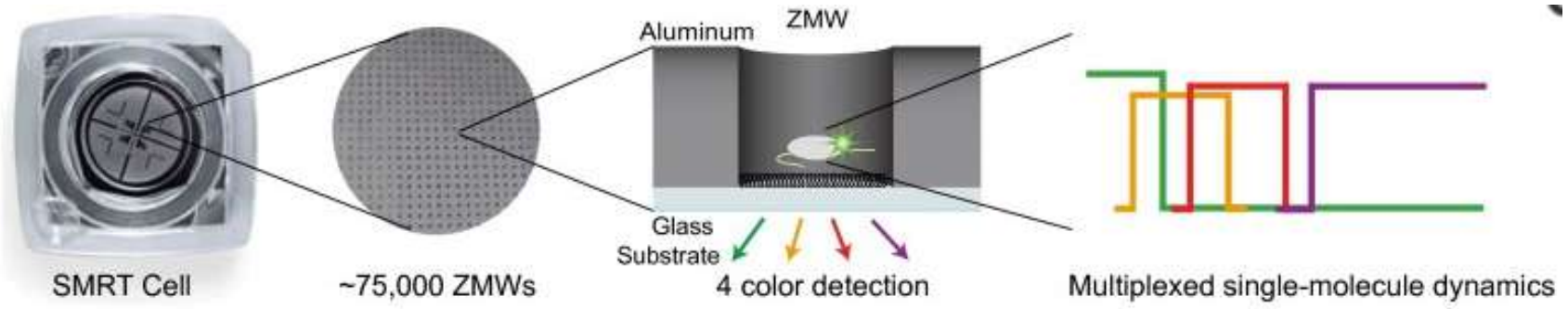


Ribosomes translating in bulk rapidly become heterogeneous

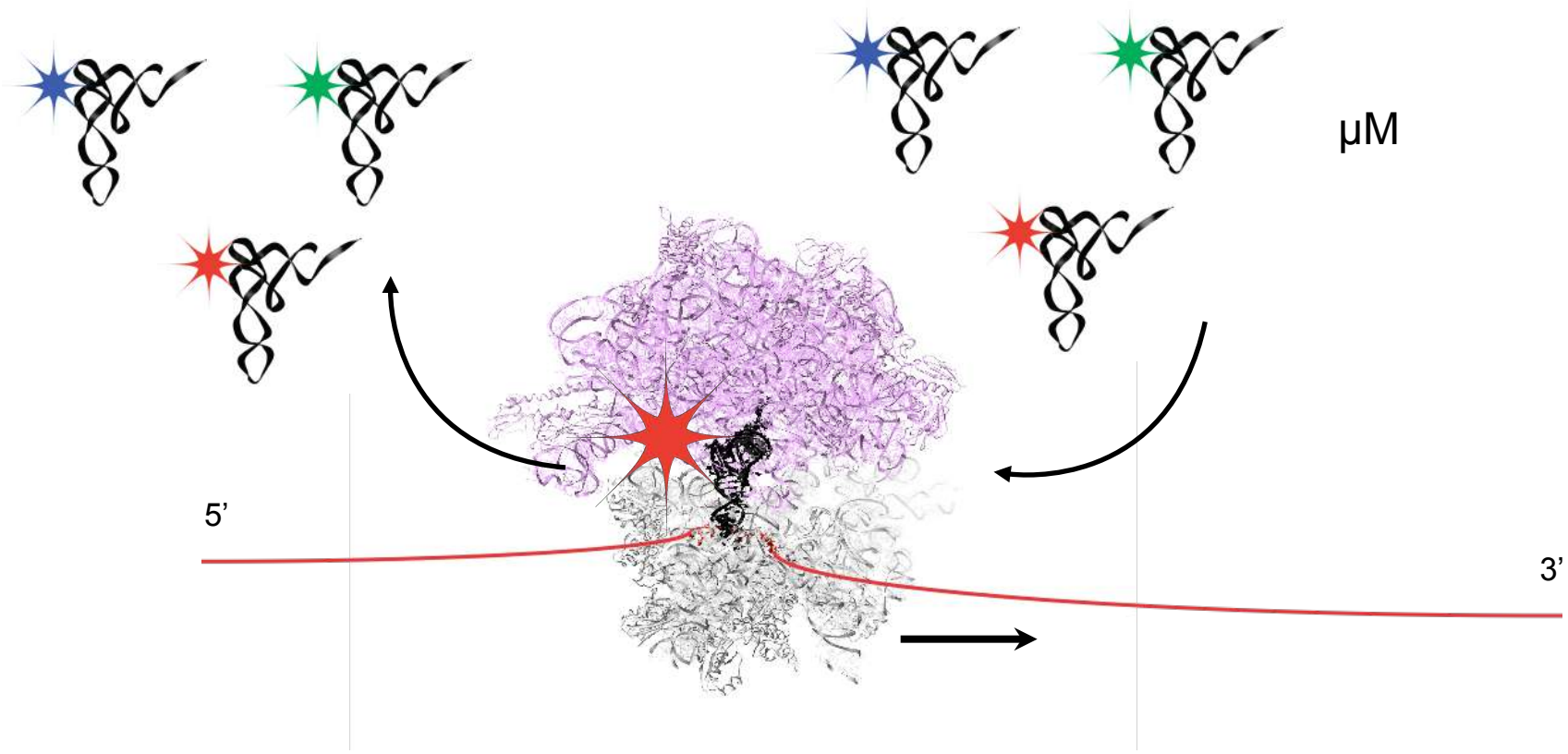


Single-molecule fluorescence to monitor translation in real time

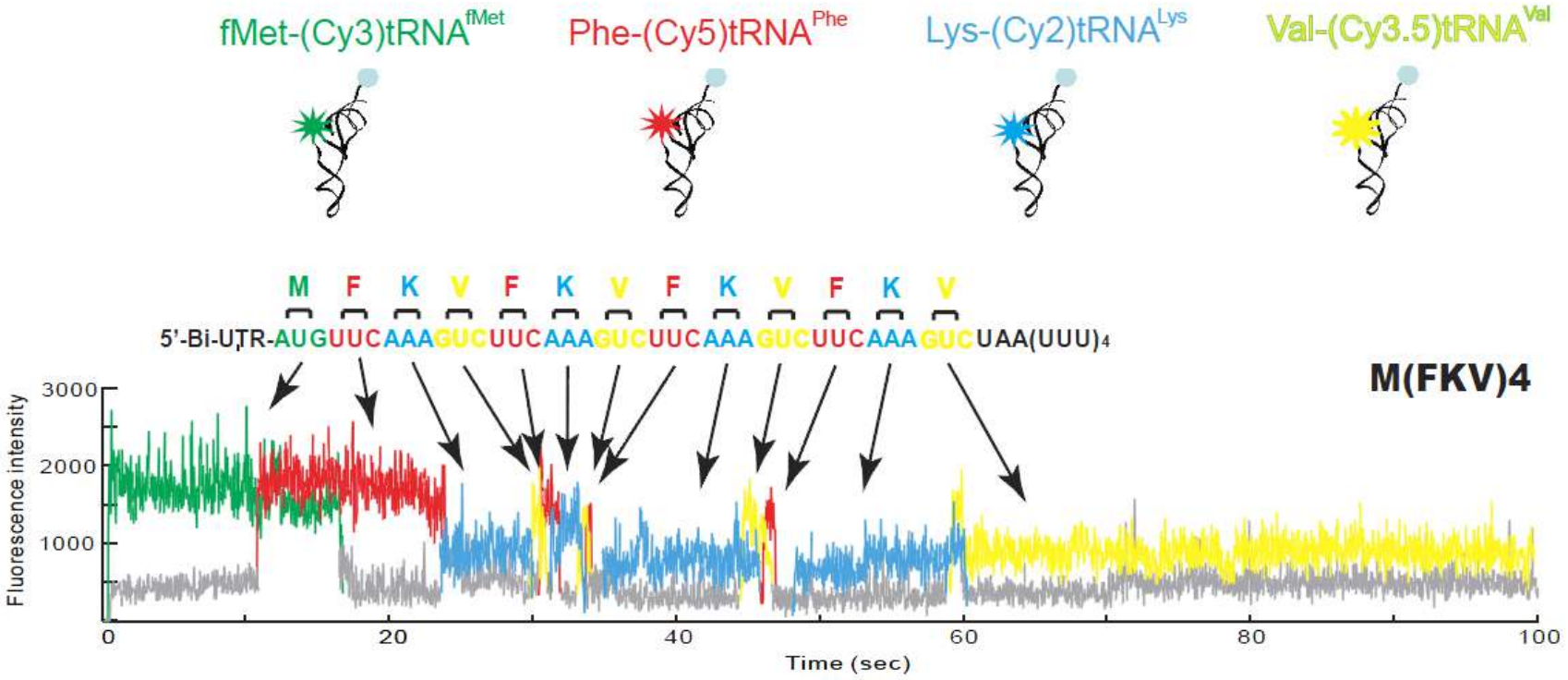
- Use up to 6 fluorescent labels
- Only several observable constraints (distances, composition)
- Reduce dynamics to >ms timescale
- Watch single-ribosomes translate in real time
- Score for translational output (pathway sorting)



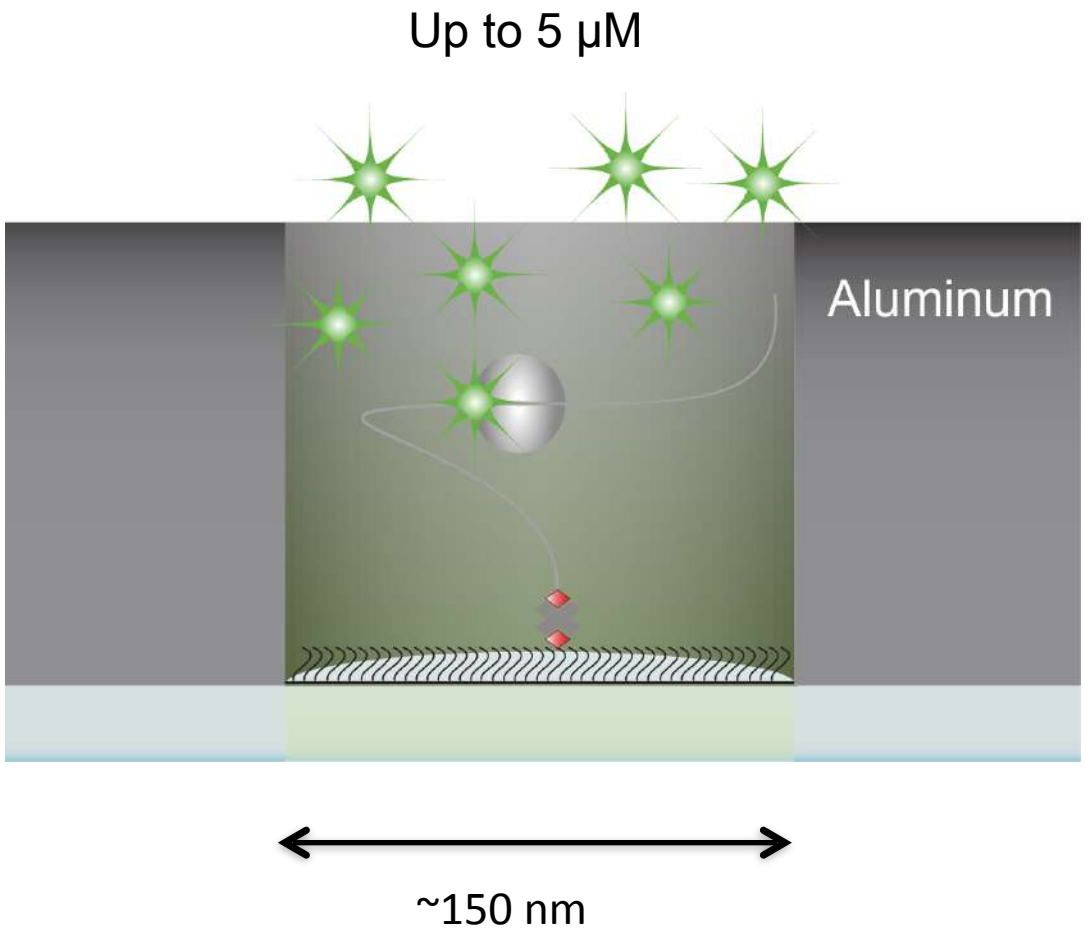
Monitor composition in real time with fluorescent ligands

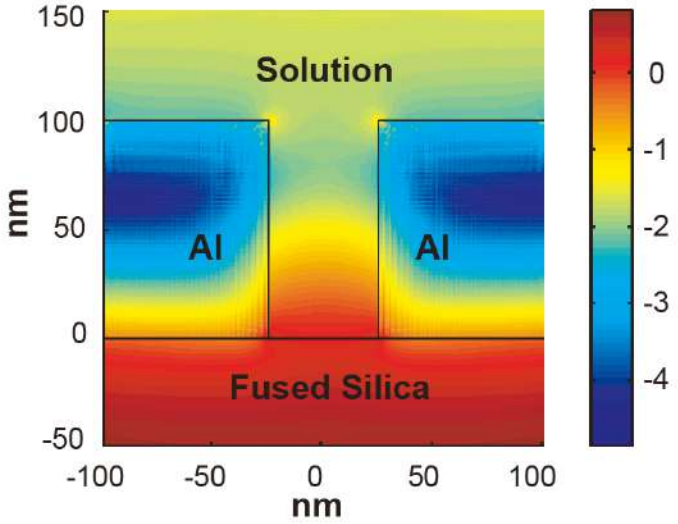


Compositional dynamics of translation in real time



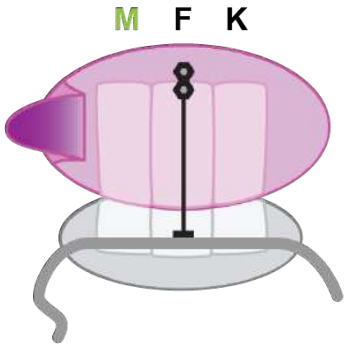
Overcoming the concentration barrier with the zero-mode waveguide (ZMW)



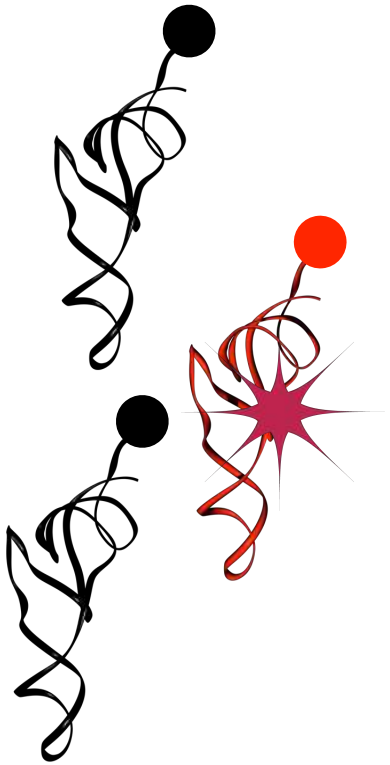


Chen, Jin, Dalal, Ravi, et al. ***PNAS*** 111.2 (2014)

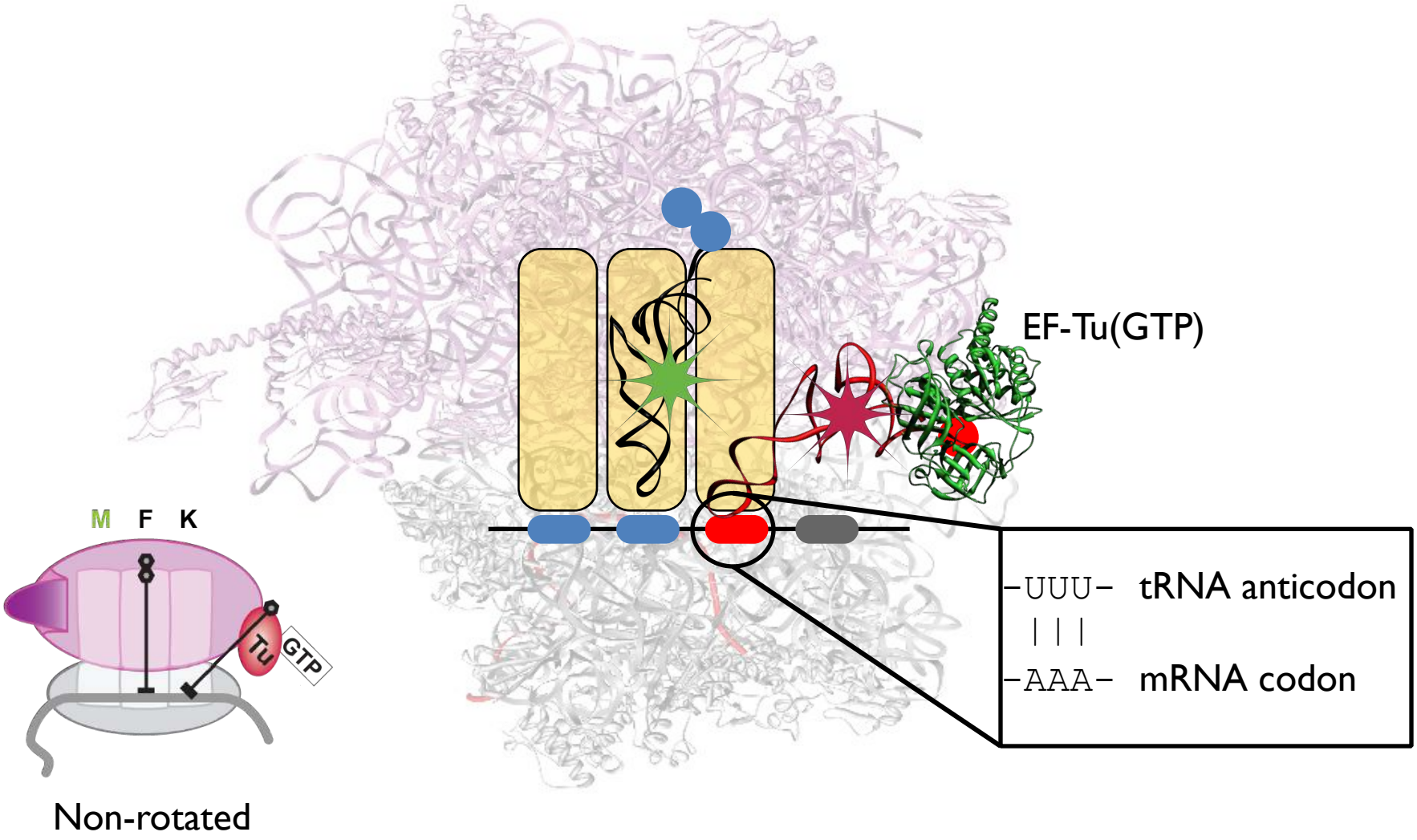
Elongation defined by smFRET



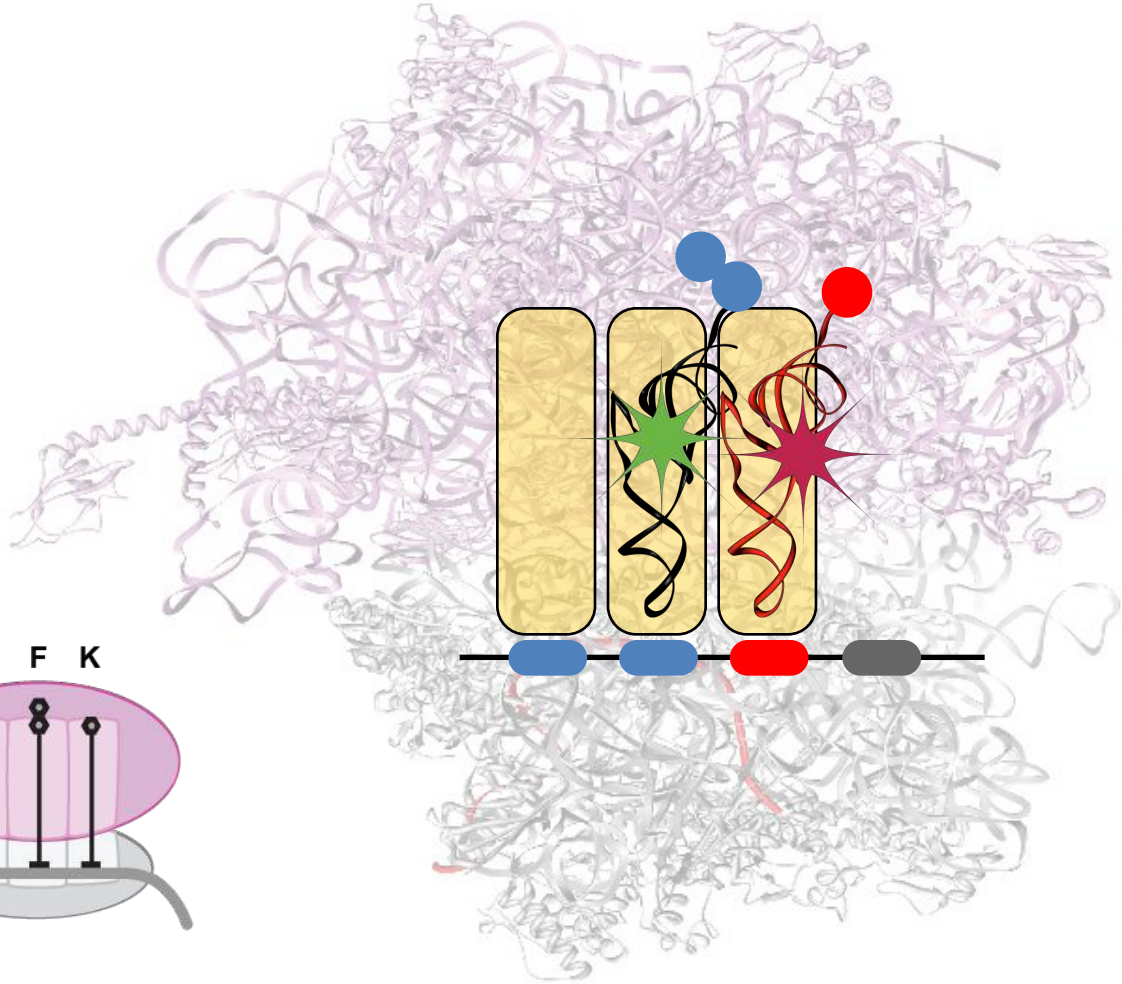
Non-rotated



tRNA selection

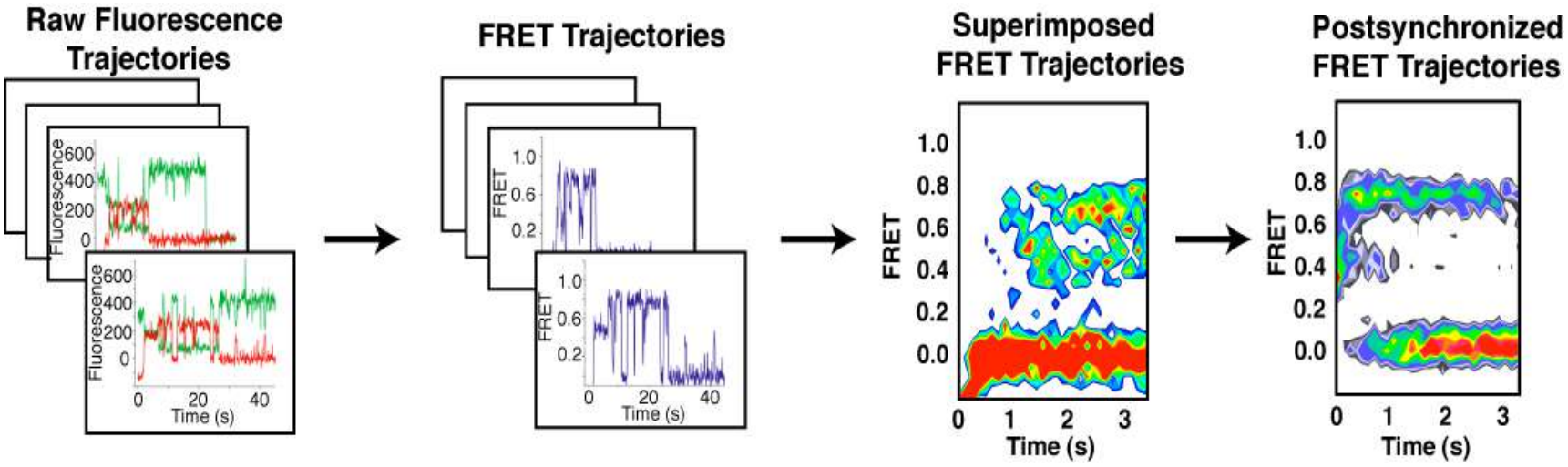


tRNA accommodation



Non-rotated

Pathway of tRNA selection and accommodation

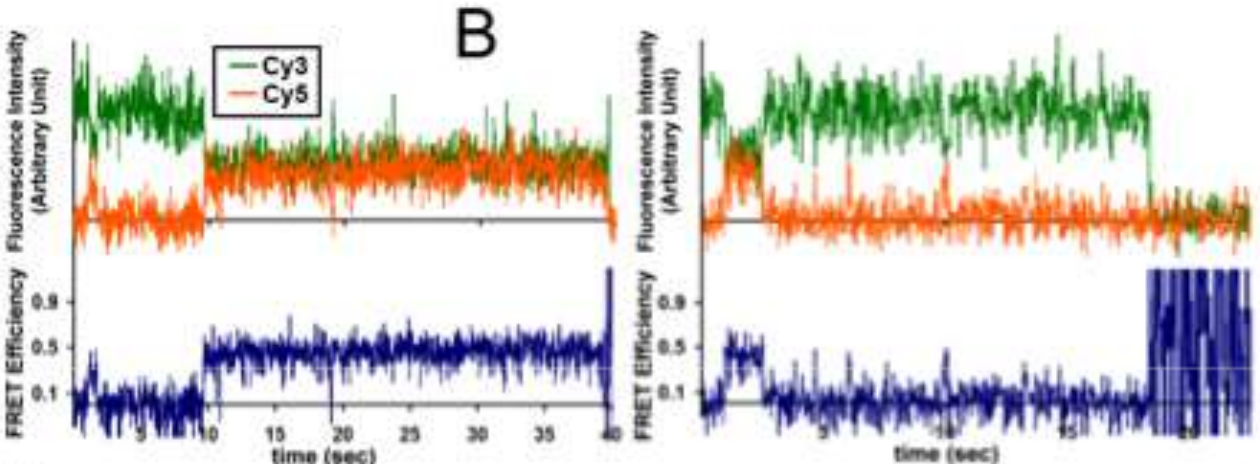


Blanchard et al. NSMB, 2004

GTP hydrolysis and tRNA identity change tRNA trajectories

+GDPNP
-UUU- codon

+GDPNP
-CUU- codon



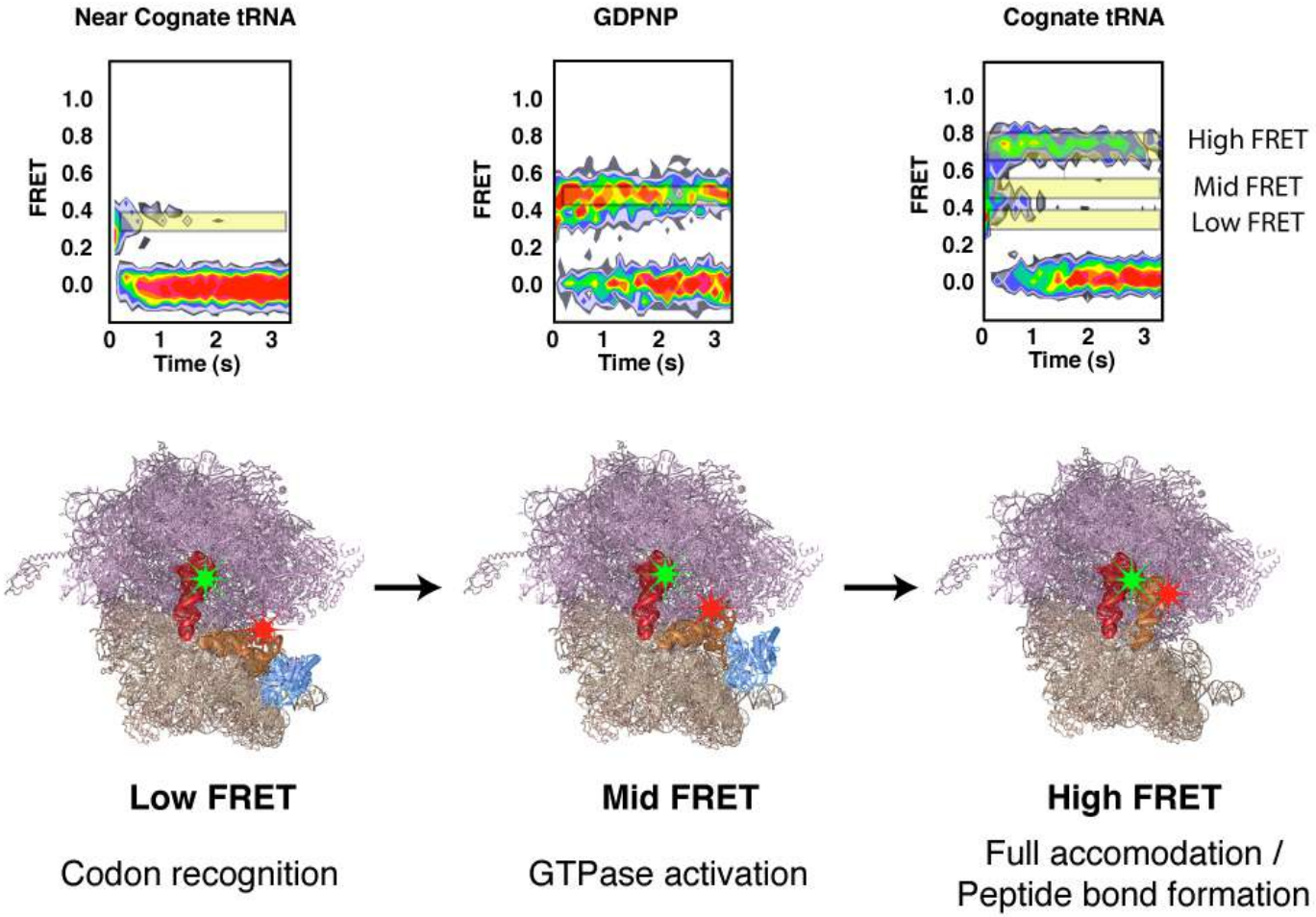
Cognate tRNA

Stable FRET

Near-cognate tRNA

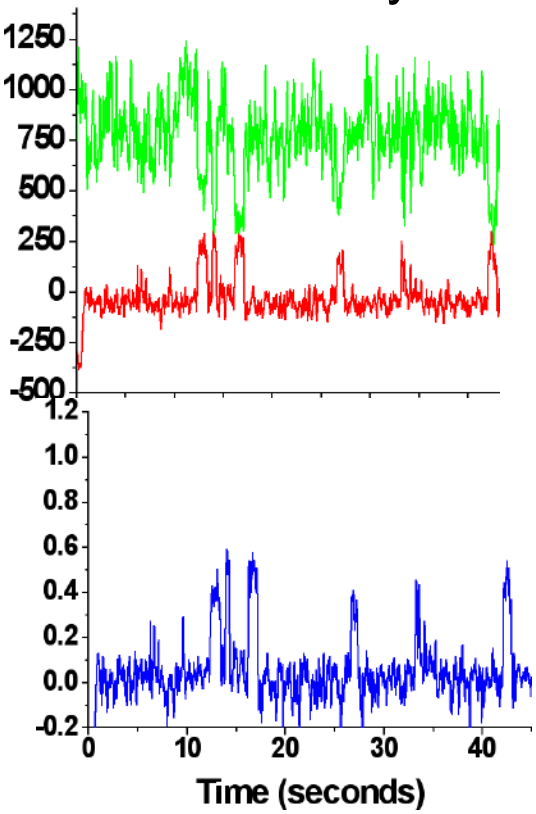
sampling

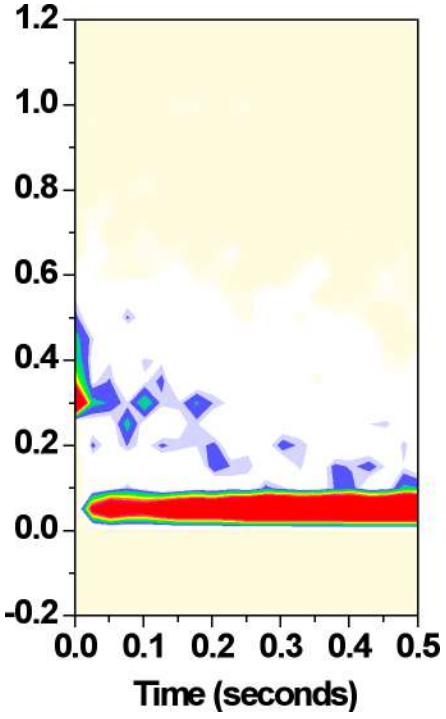
Pathway for tRNA selection by the ribosome

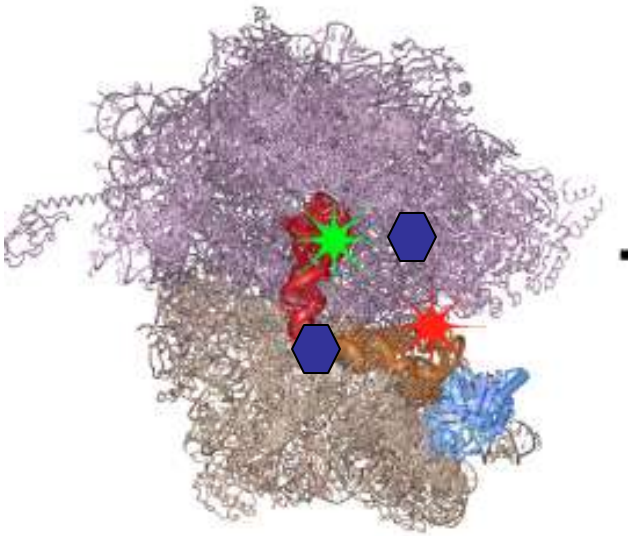


Certain antibiotics block tRNA trajectories through the ribosome

+ tetracycline or thiostrepton

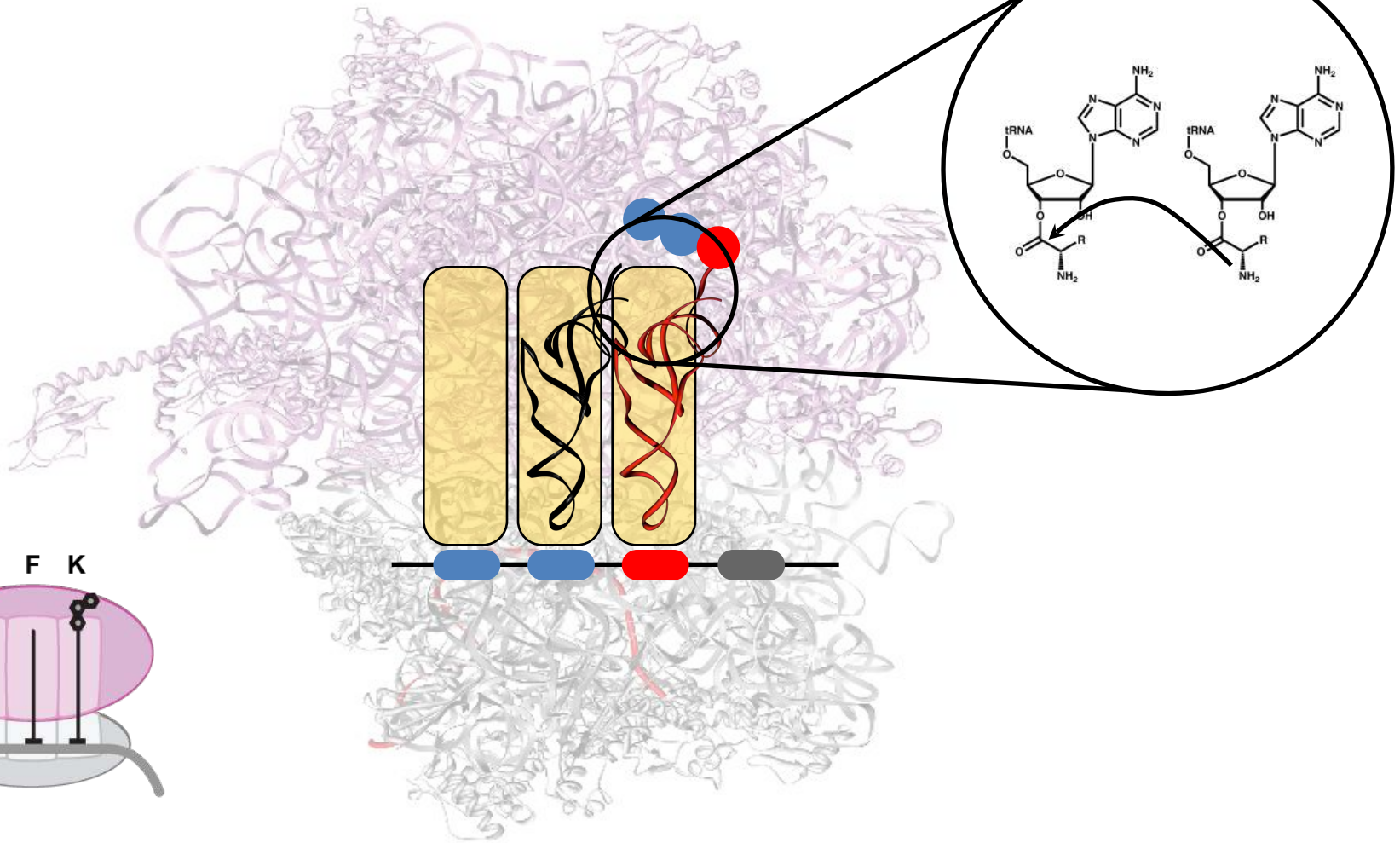






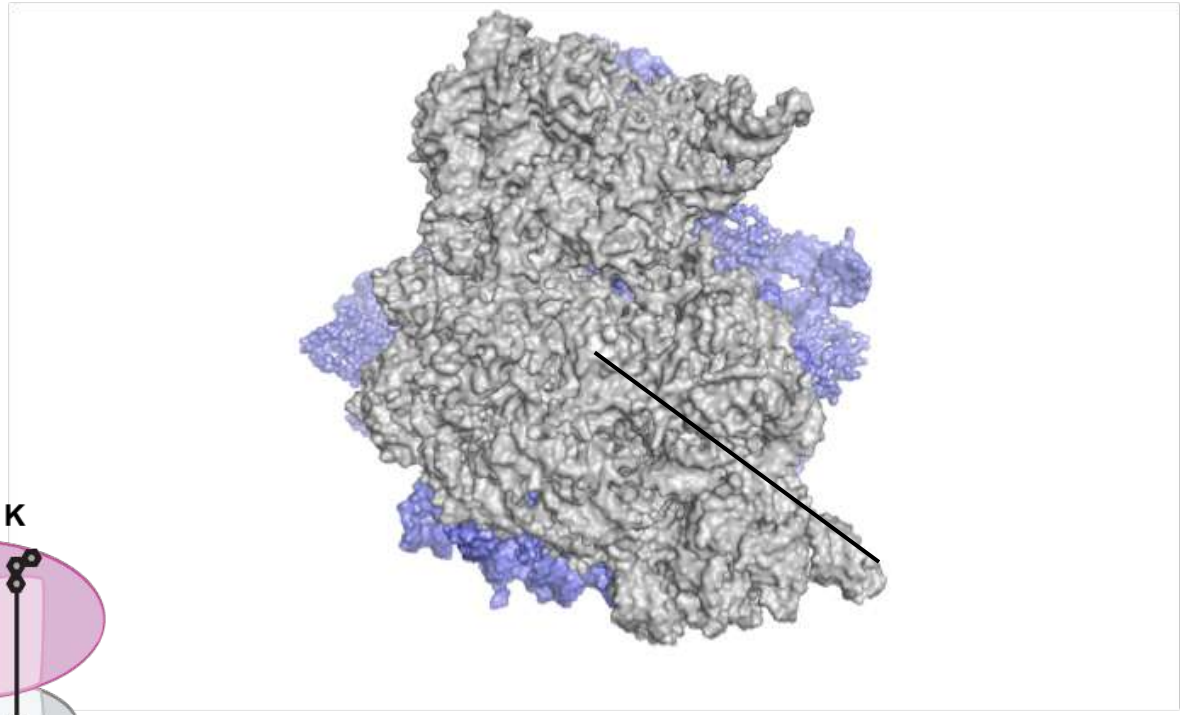
tRNAs ejected at initial selection step in presence of drug

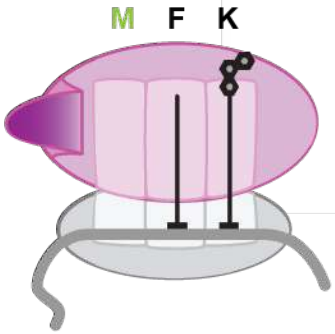
Peptide bond formation



Non-rotated

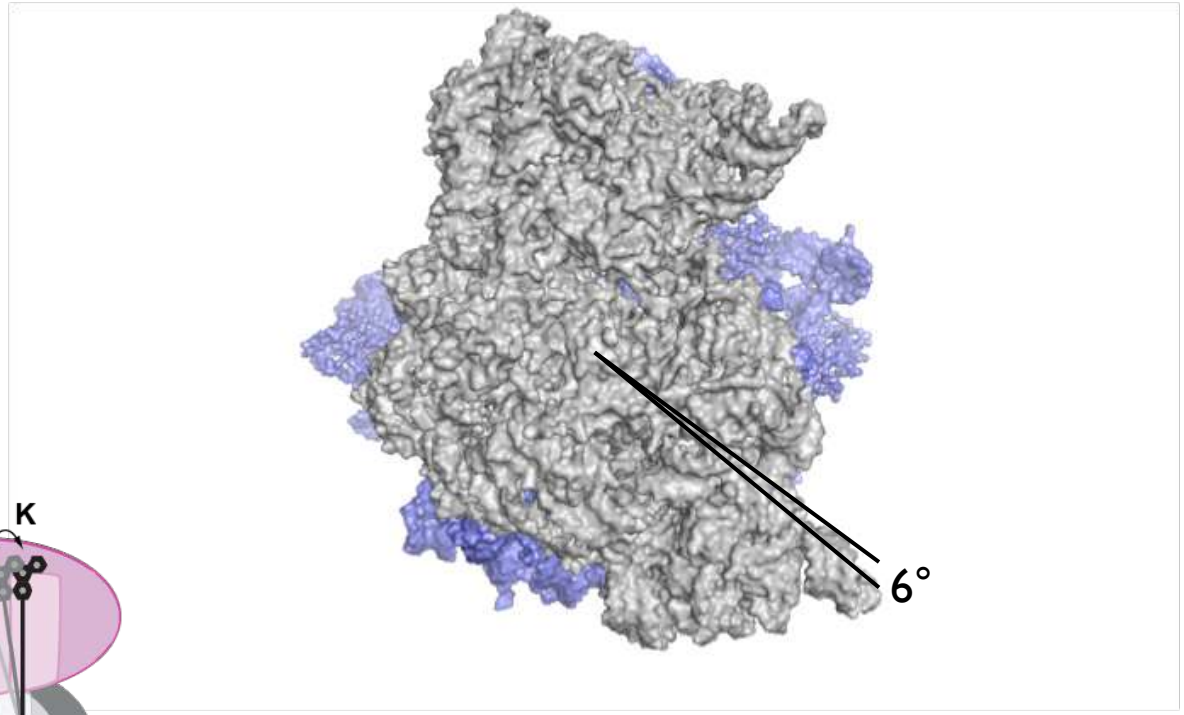
Subunit rotation

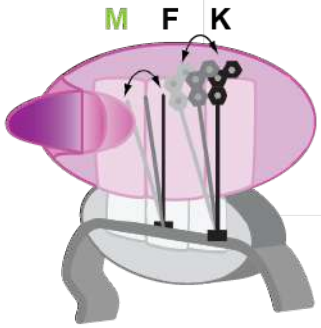




Non-rotated

Subunit rotation

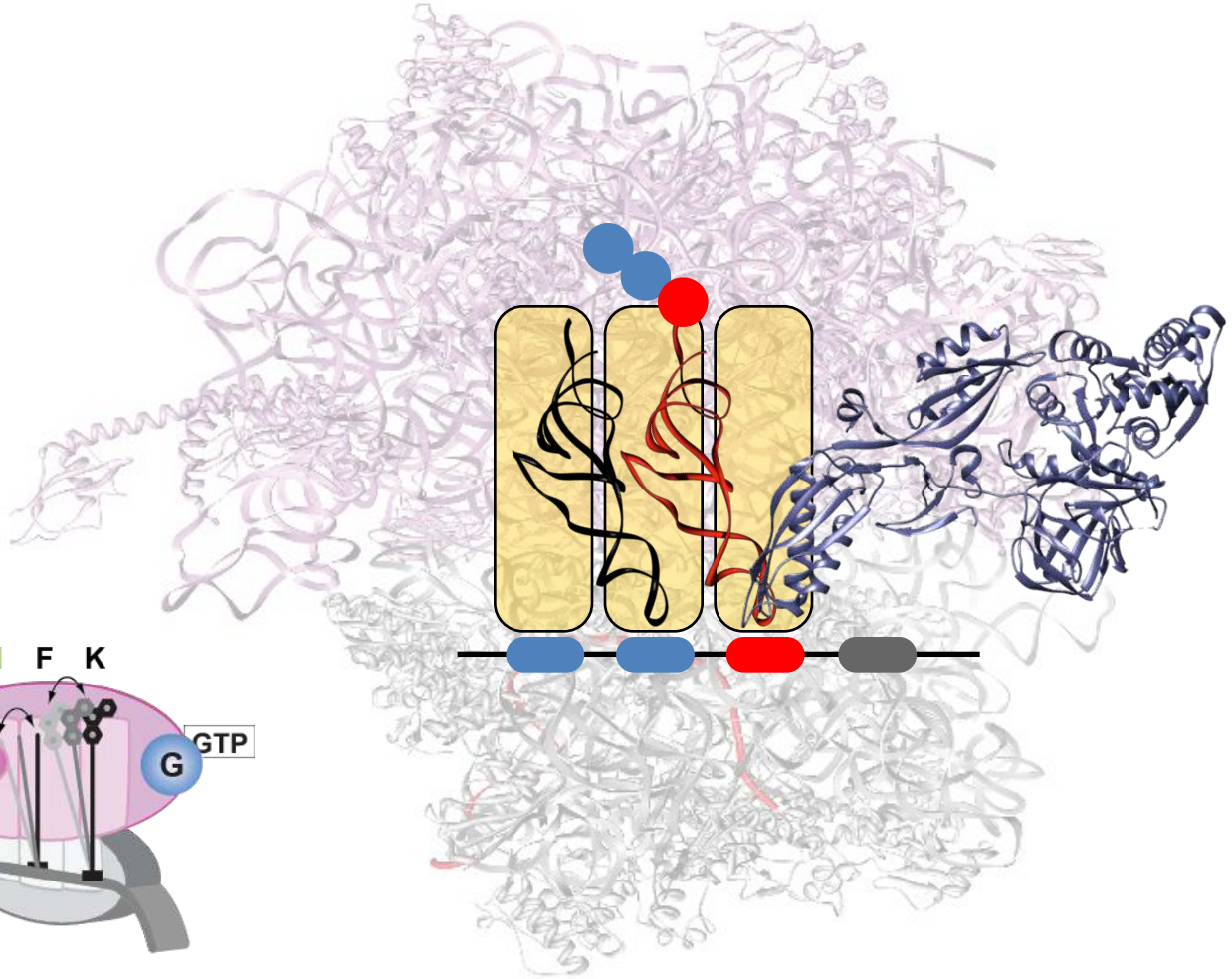




Rotated

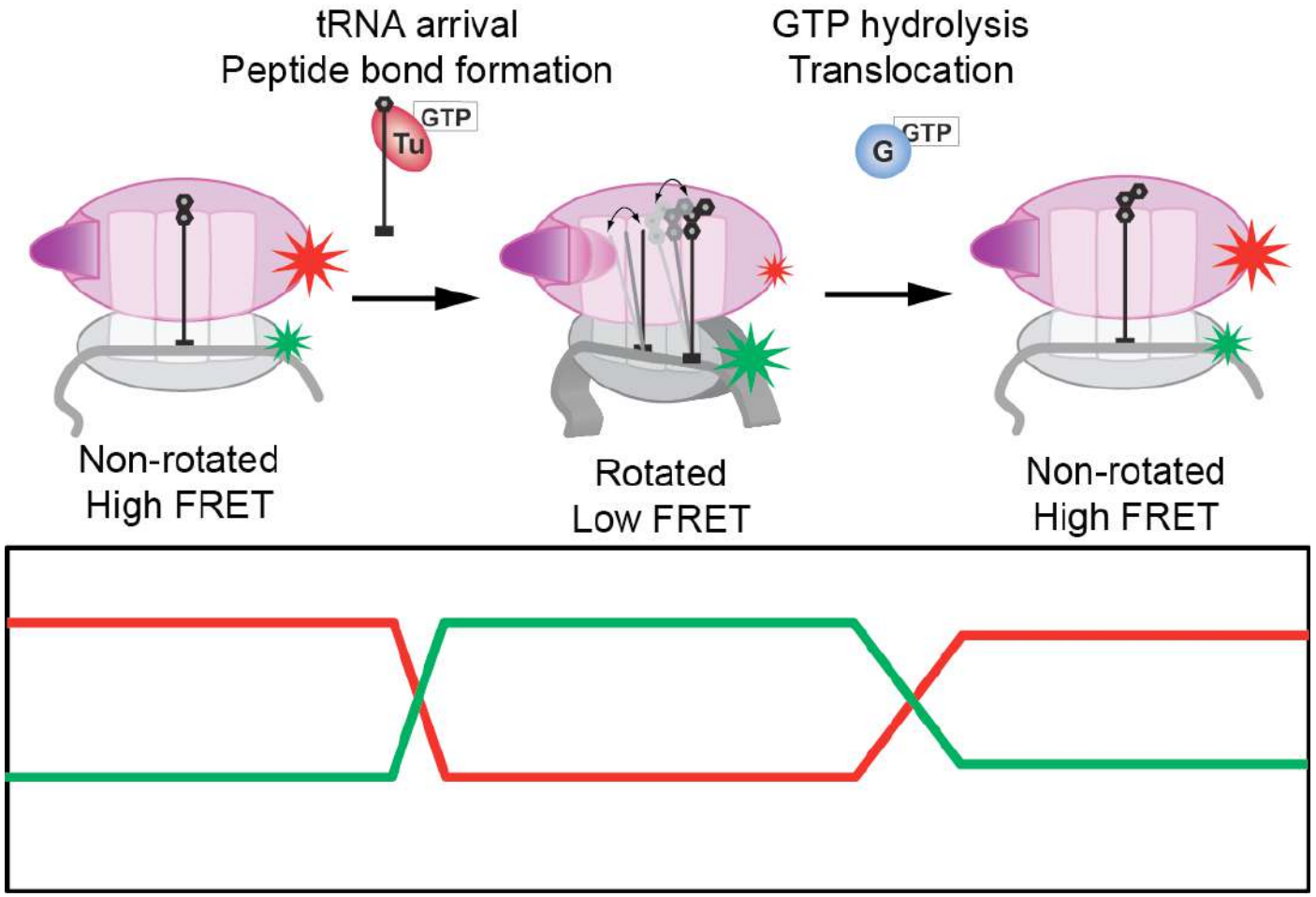
Frank and co-workers

Preparation for translocation

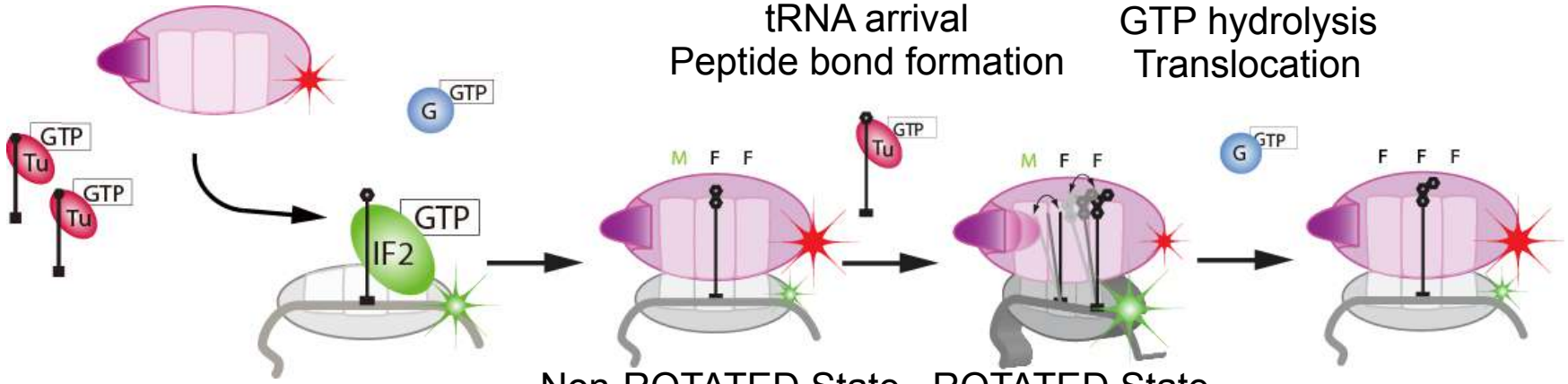


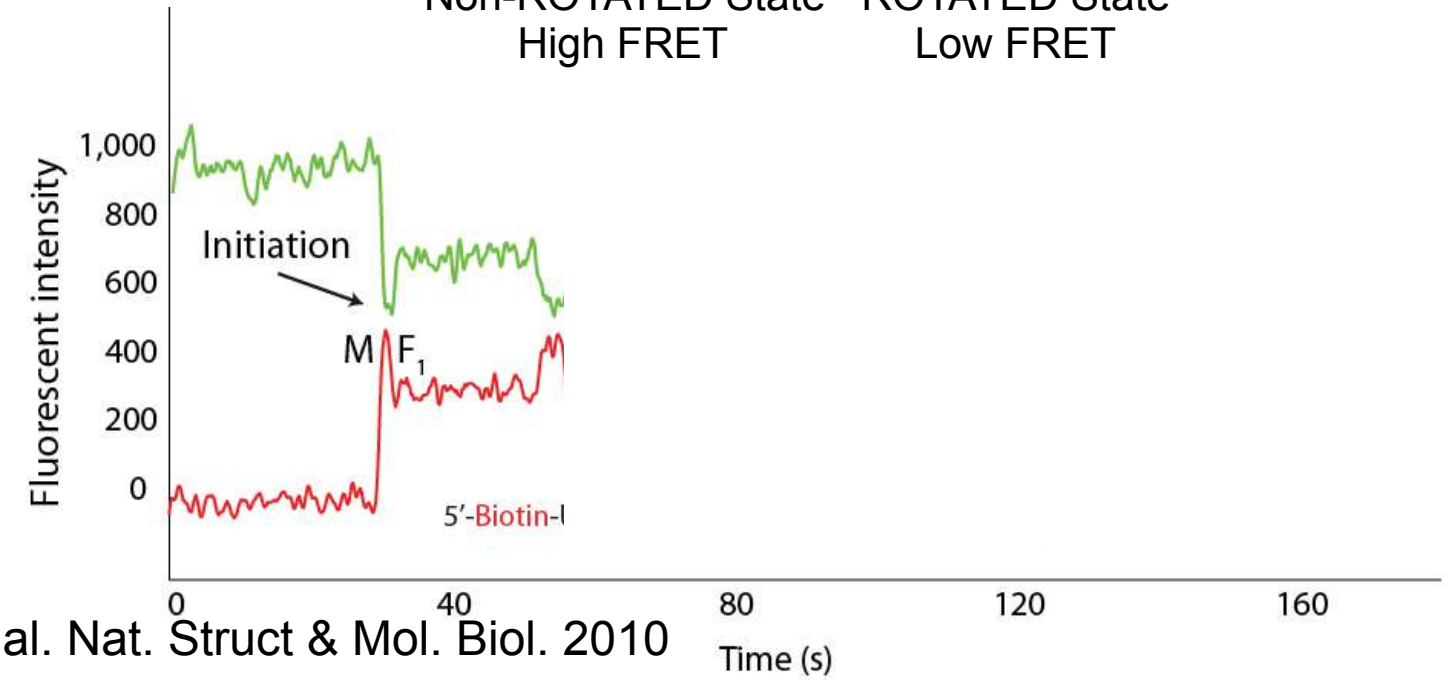
Rotated

Following the substeps of elongation



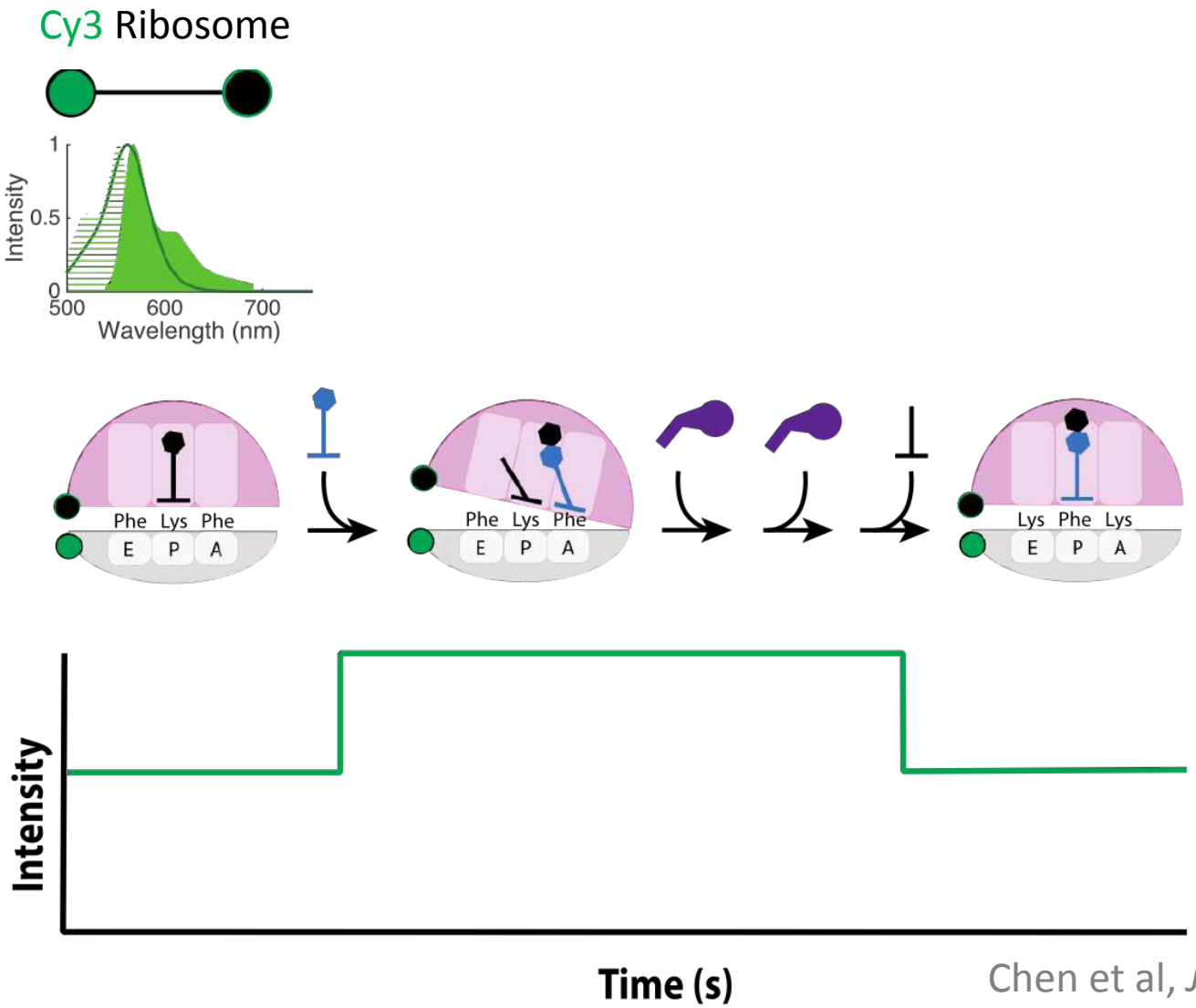
Following ribosome conformation directly





Aitken et al. Nat. Struct & Mol. Biol. 2010

Dye-quencher pair compresses FRET signal

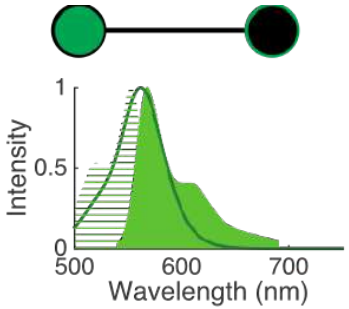


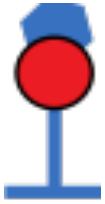
Chen et al, *JACS* 2013

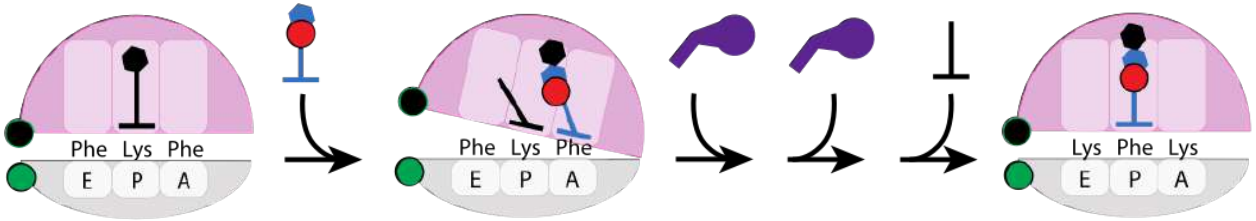
Tracking ribosome conformation and tRNA

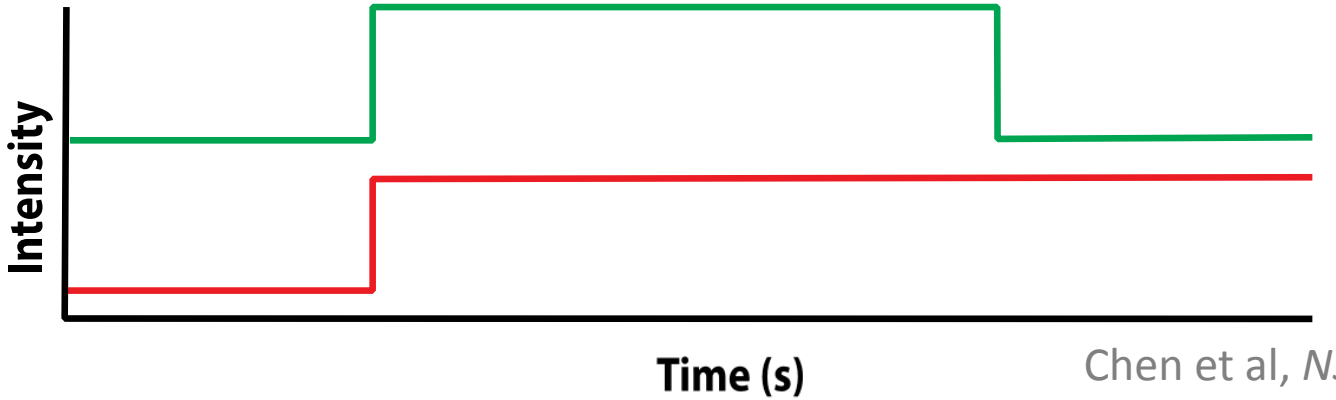
Cy3 Ribosome

Cy5-tRNA Transit (A-P site)



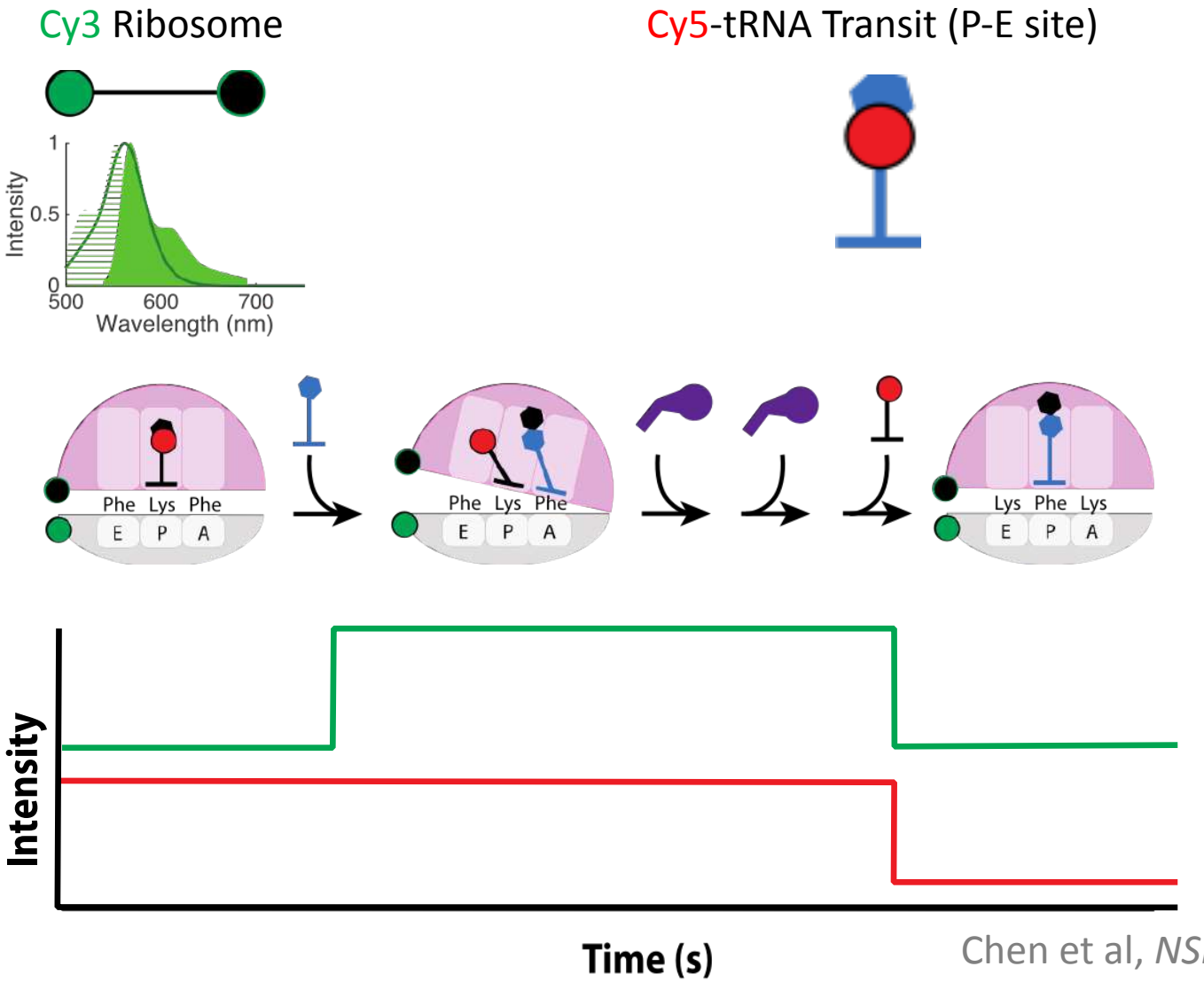






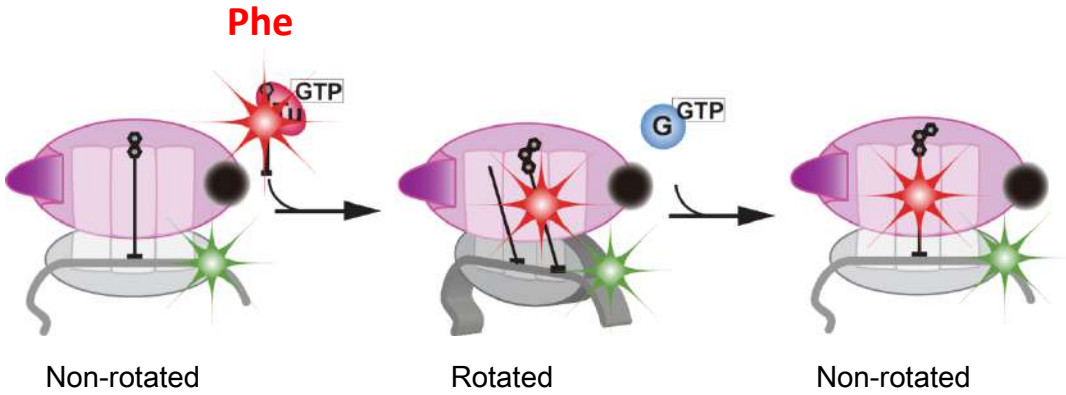
Chen et al, *NSMB* 2014

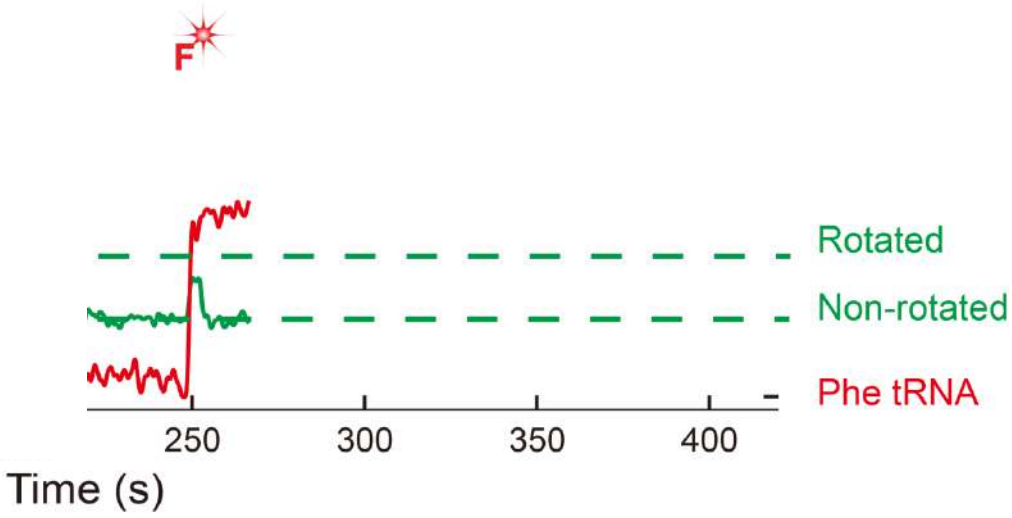
Tracking ribosome conformation and tRNA



Chen et al, *NSMB* 2014

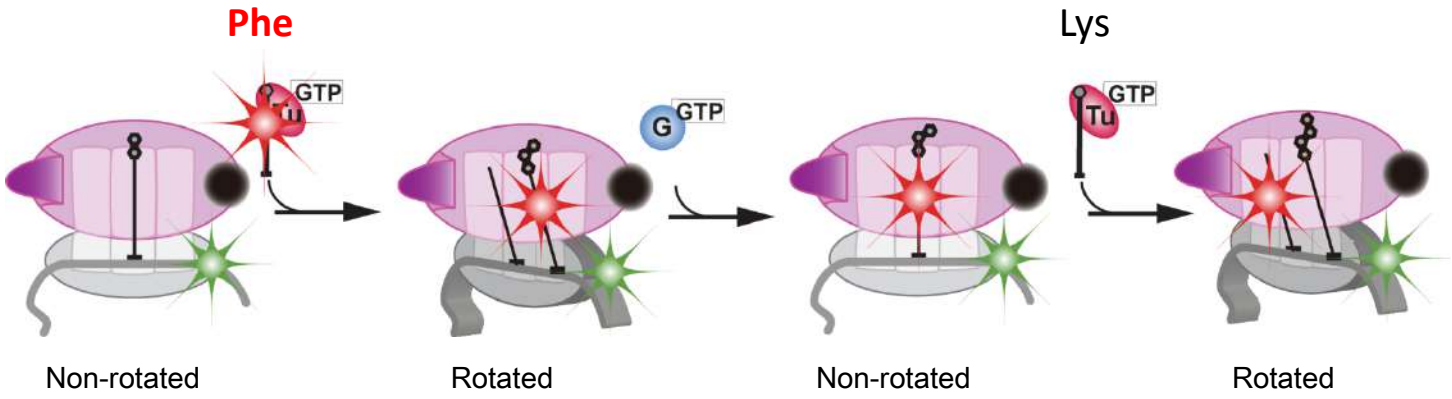
Correlating conformation and tRNA occupancy

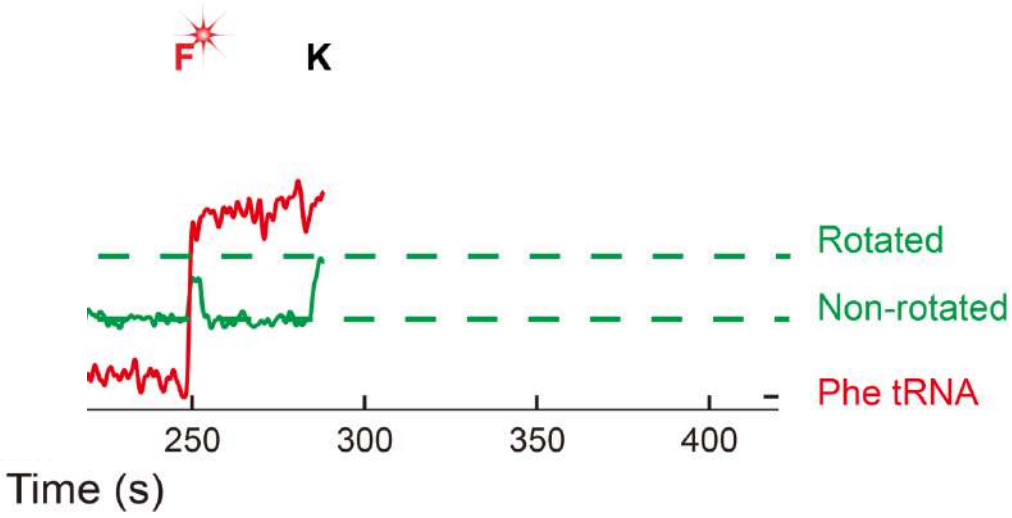




Chen, Jin, et al. ***Nature structural & molecular biology*** 20.6 (2013)

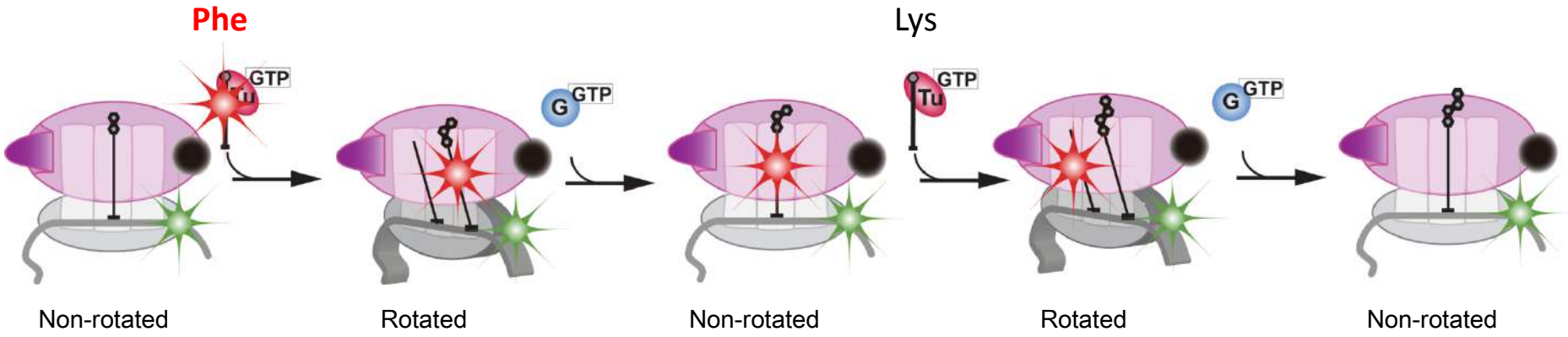
Correlating conformation and tRNA occupancy

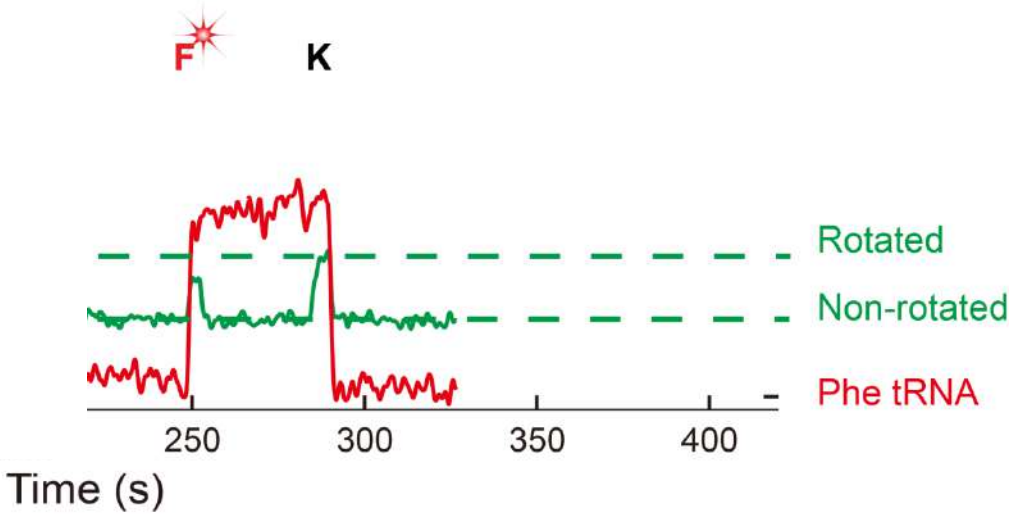




Chen, Jin, et al. ***Nature structural & molecular biology*** 20.6 (2013)

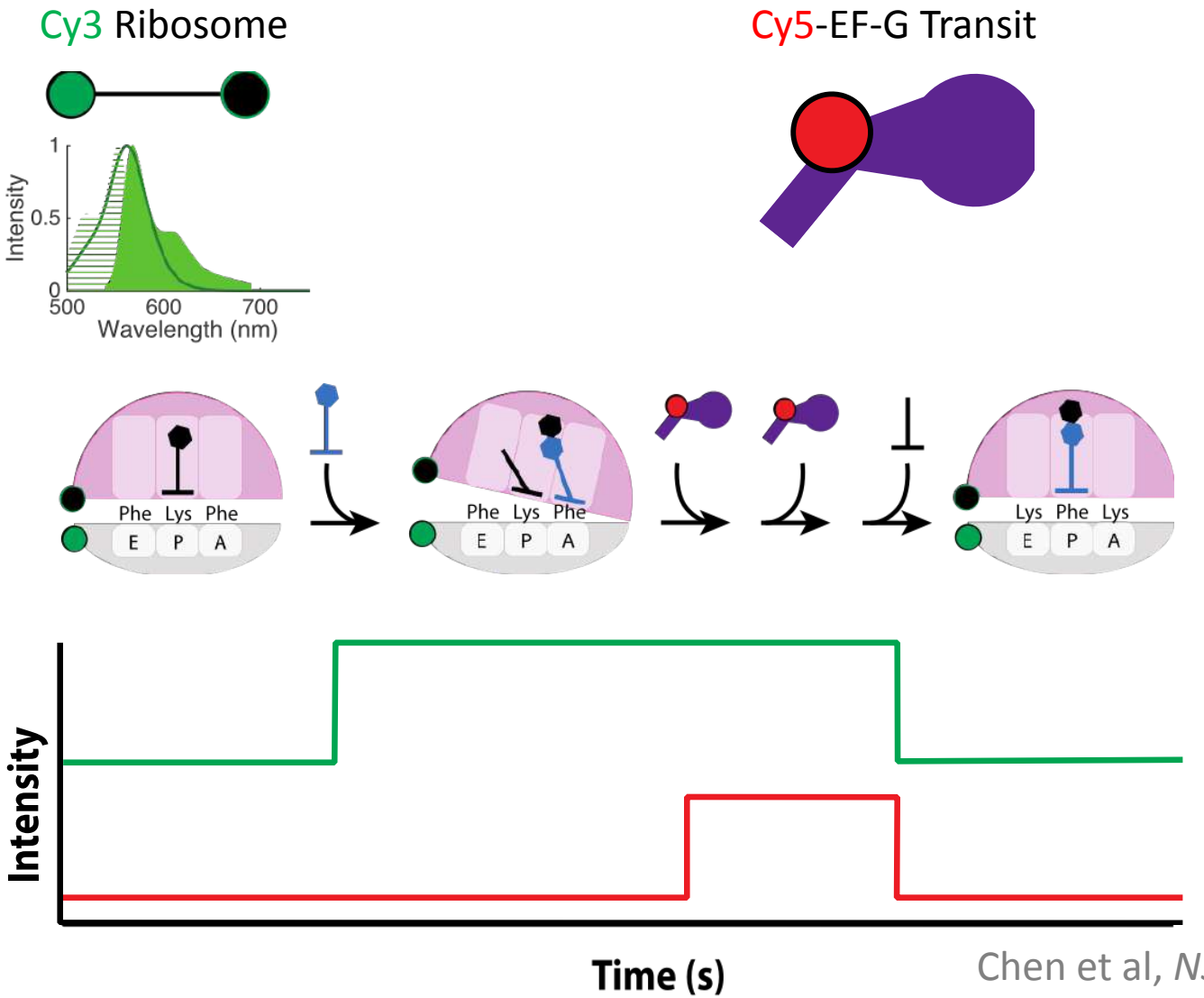
Correlating conformation and tRNA occupancy





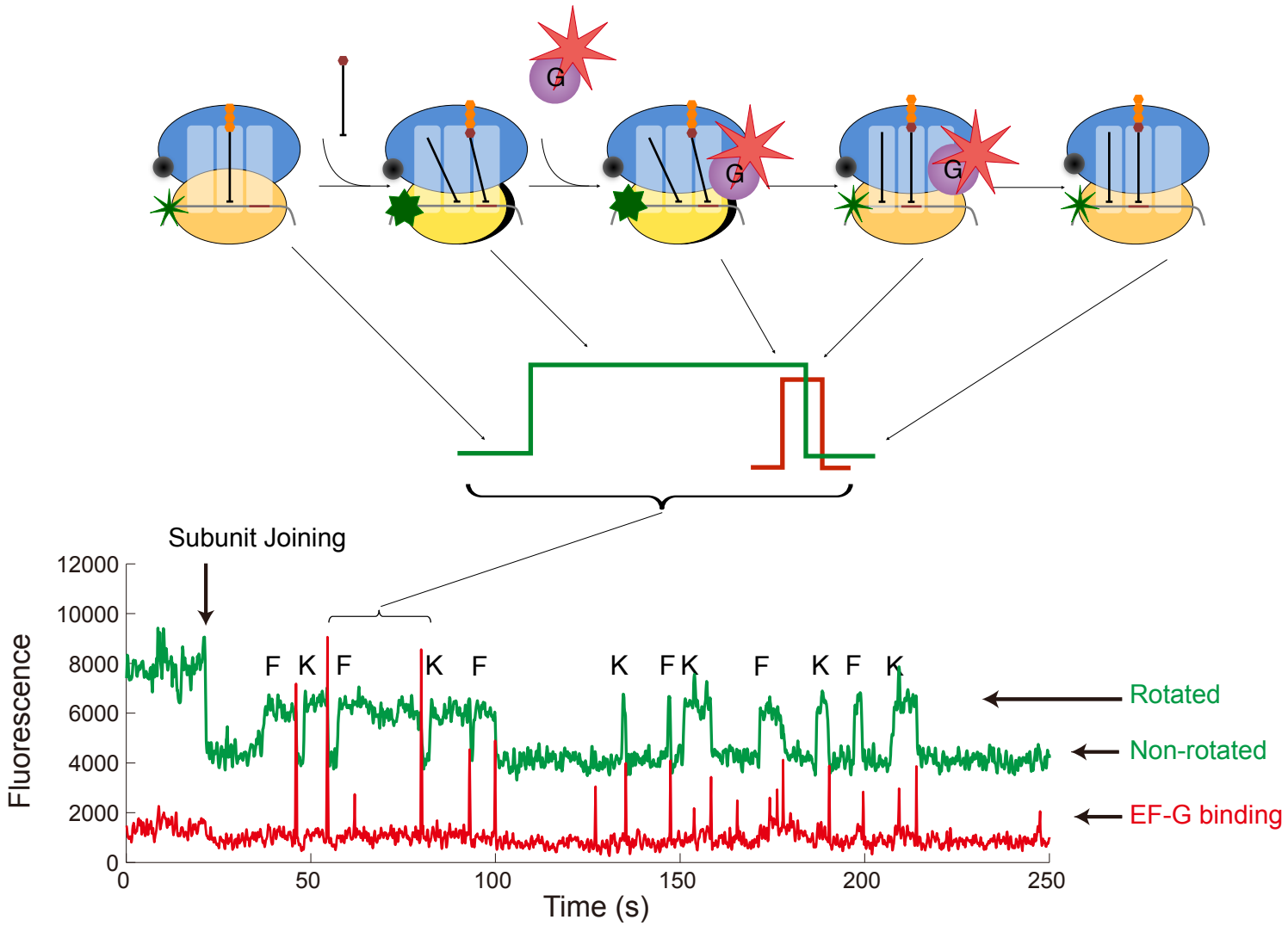
Chen, Jin, et al. ***Nature structural & molecular biology*** 20.6 (2013)

Tracking ribosome conformation and EF-G

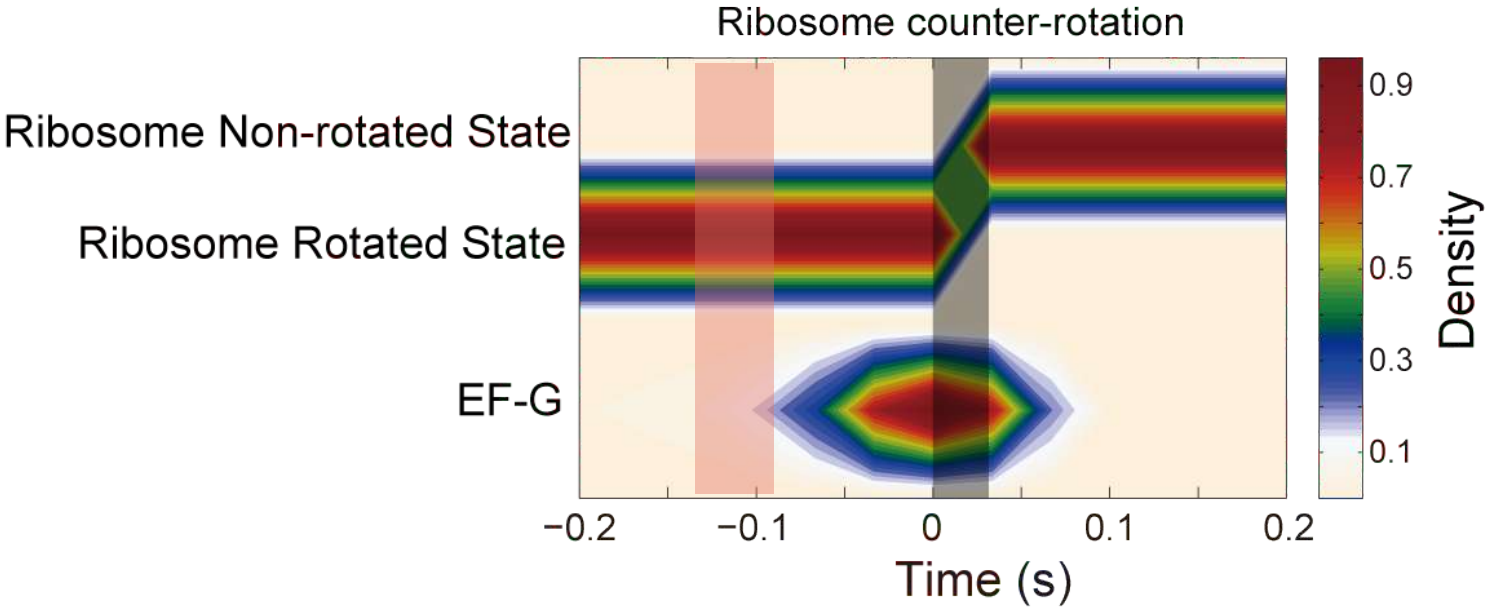


Chen et al, *NSMB* 2014

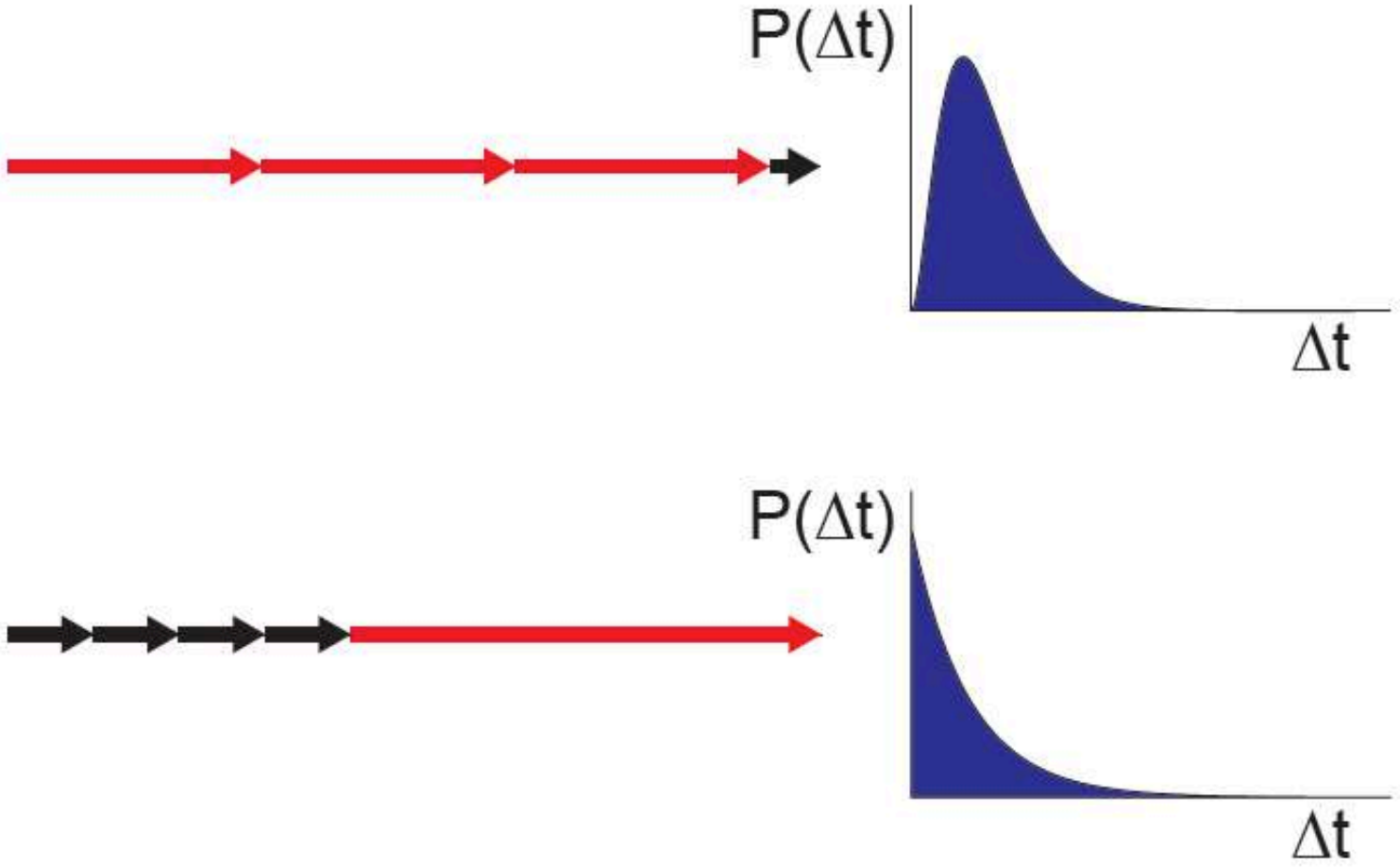
EF-G binding and GTP hydrolysis reset ribosome conformation



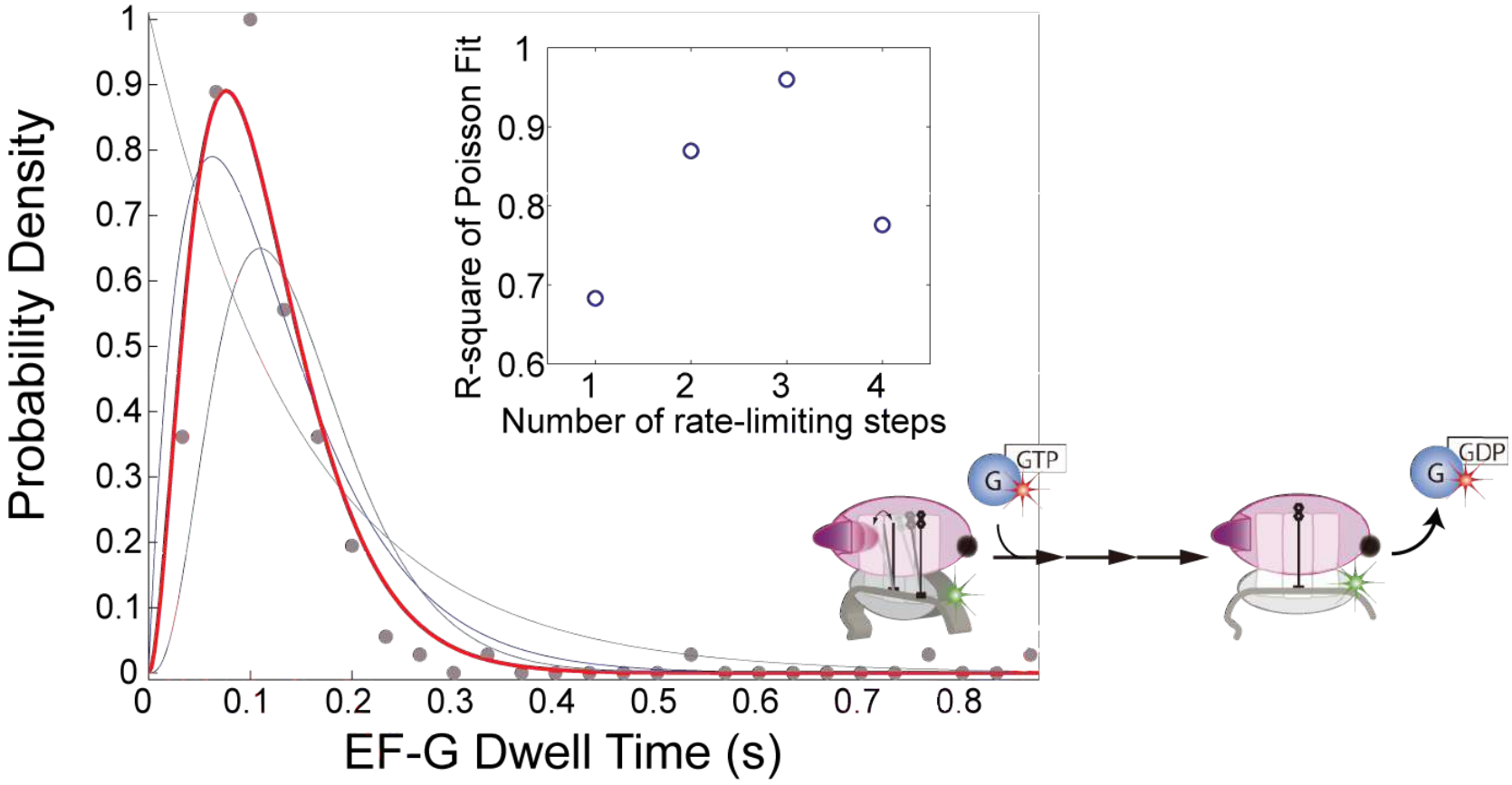
EF-G binding itself is NOT correlated to ribosomal counter rotation



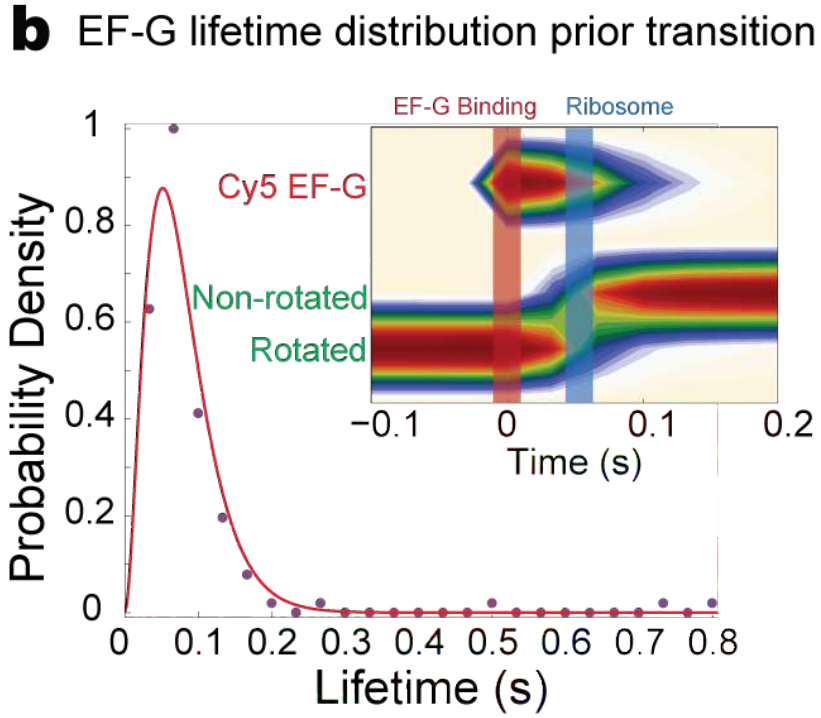
Multiple rate limiting steps give peaked, Poisson distributions

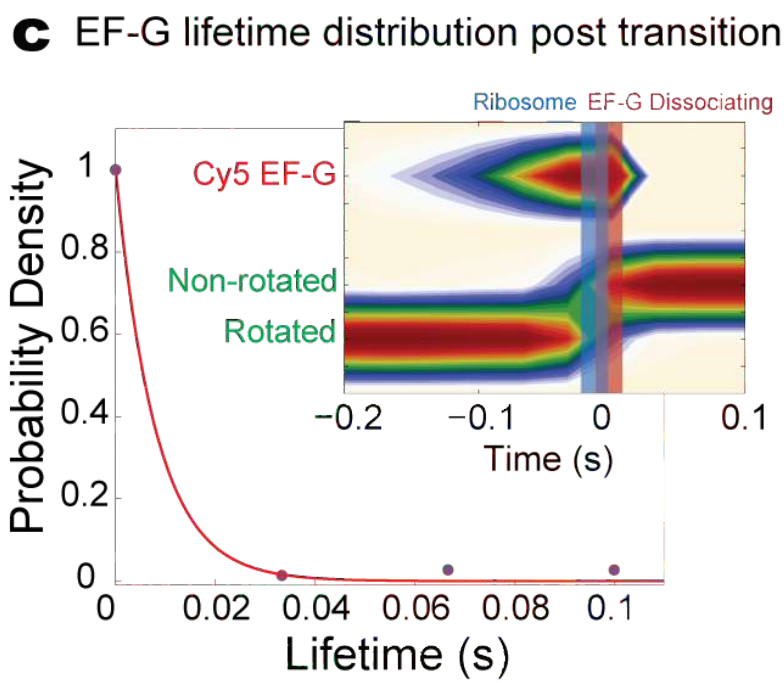


Multistep behavior for EF-G binding events that drive translocation



Multiple steps occur BEFORE ribosome rotation

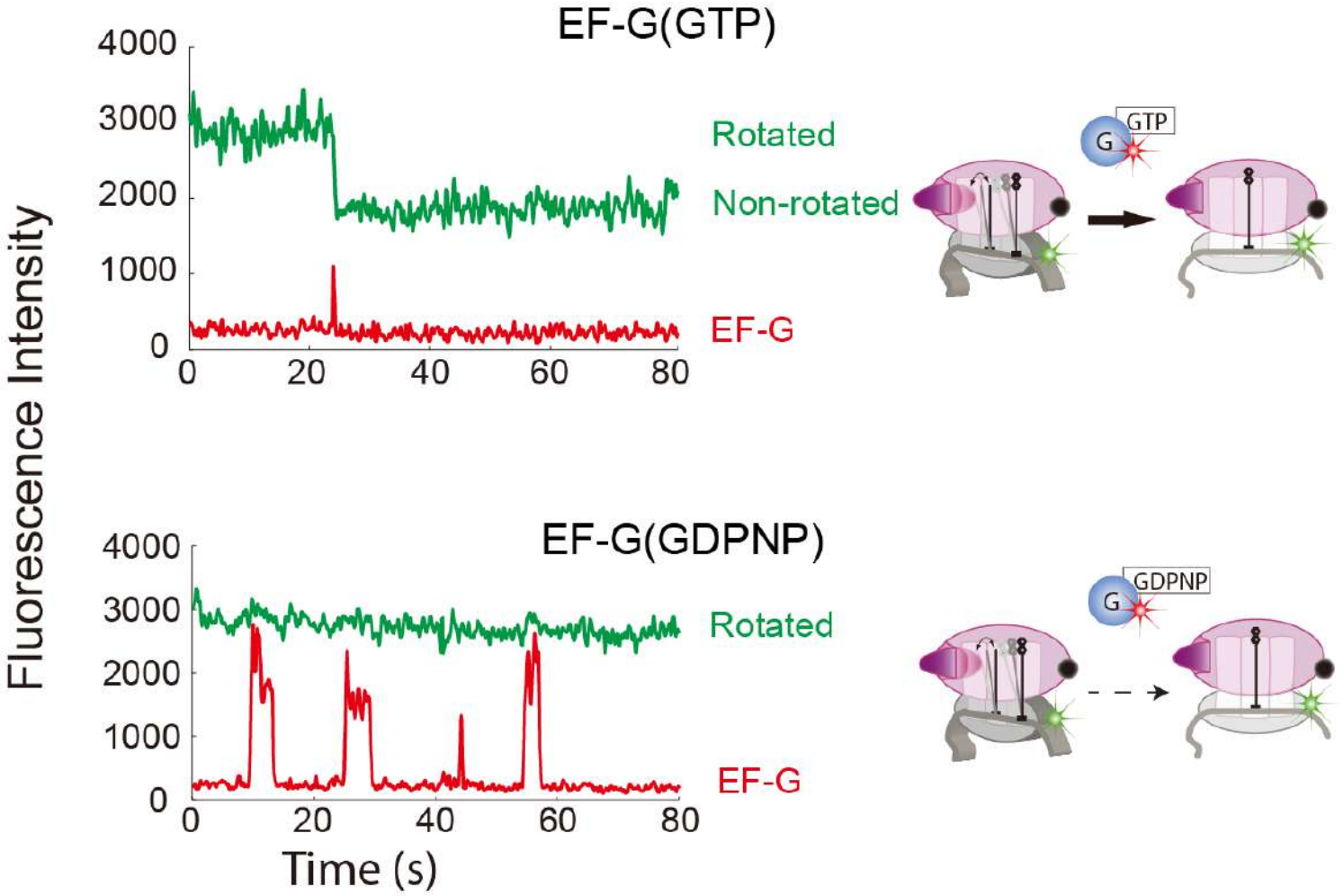




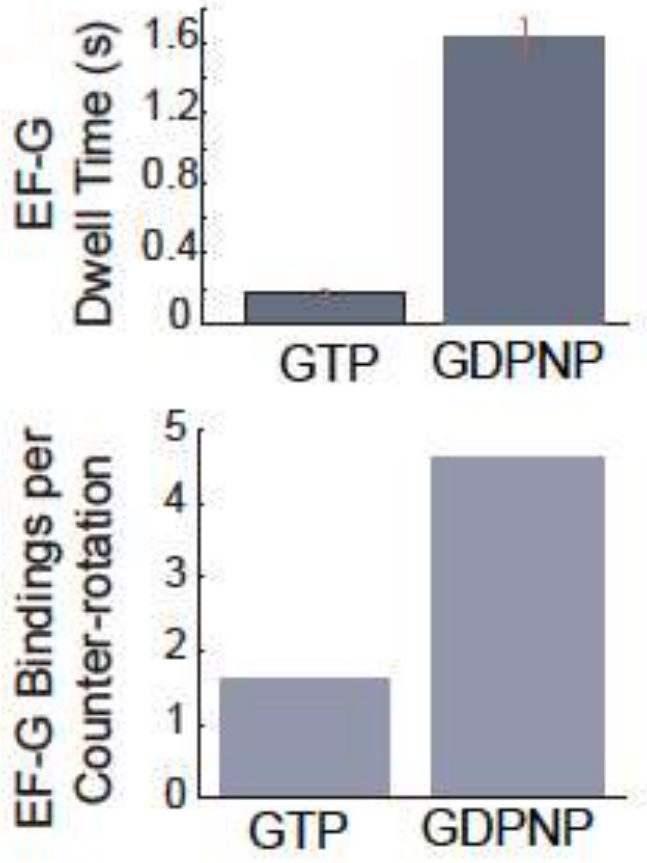
What is the role of GTP hydrolysis?

- active, power stroke?
- passive, controlling factor occupancy?

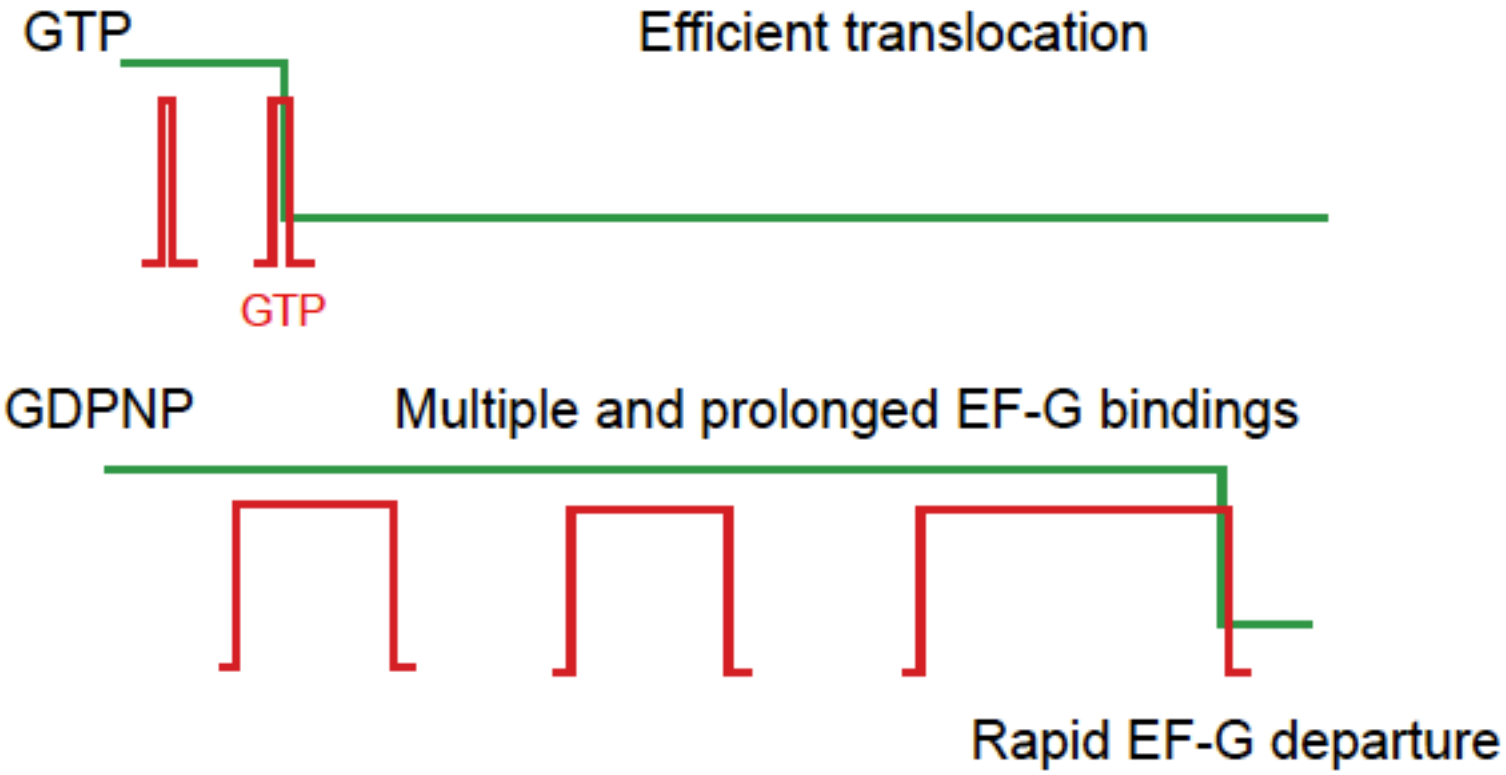
GTP hydrolysis is required for efficient translocation



Multiple, long-lived EF-G binding events are required to translocate without GTP hydrolysis



Multiple, long-lived EF-G binding events are required to translocate without GTP hydrolysis



Chemical reactions provide the free energy for ribosomal conformational rearrangements

<u>Peptide Bond Formation</u>

aa-tRNA + pep-tRNA ⟶ tRNA-OH + pep(n+1)-tRNA

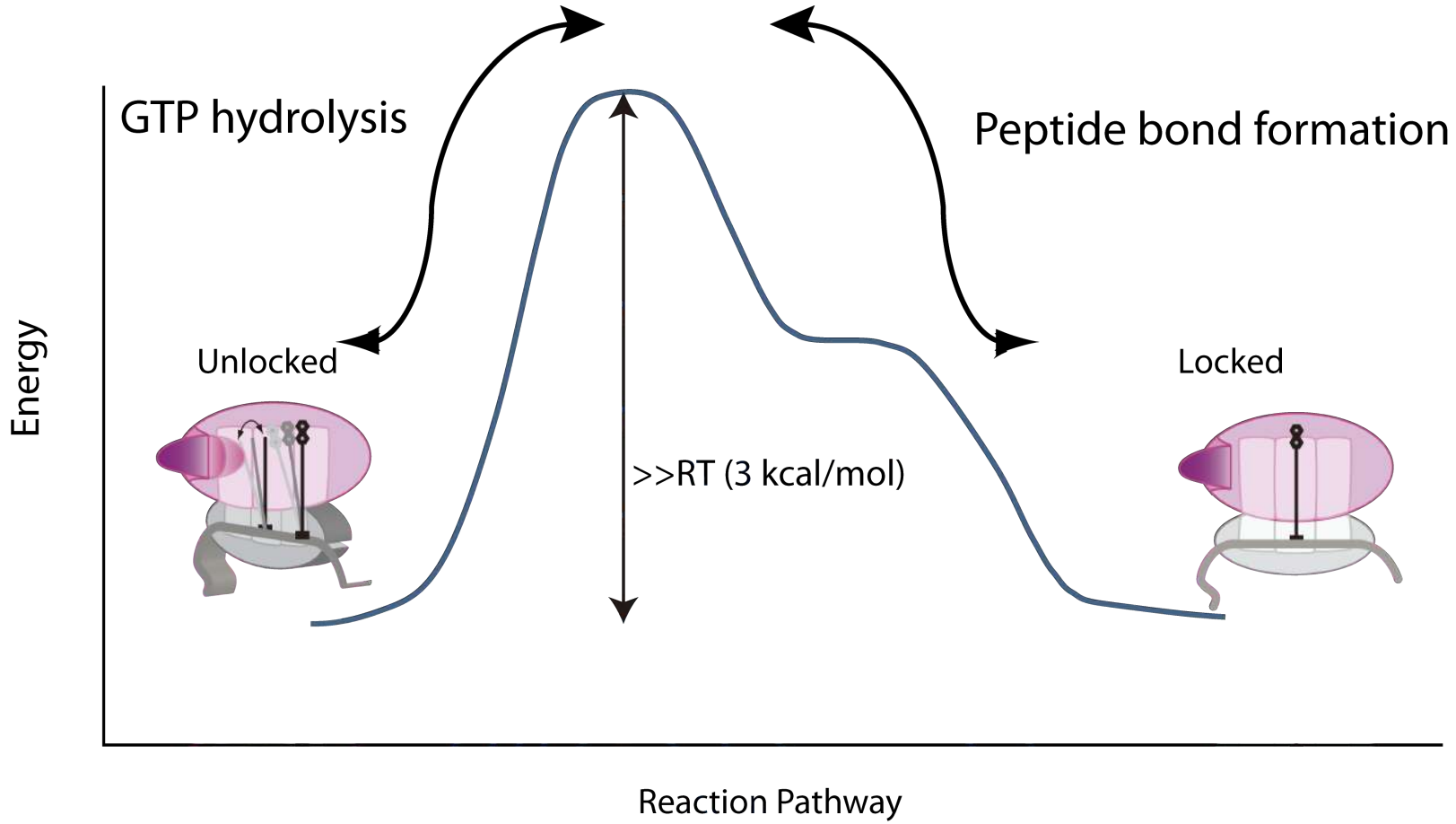
$\Delta G^{o} \approx -8\ kcal \cdot mol^{-1}$

<u>GTP Hydrolysis</u>

GTP ⟶ GDP + P_i

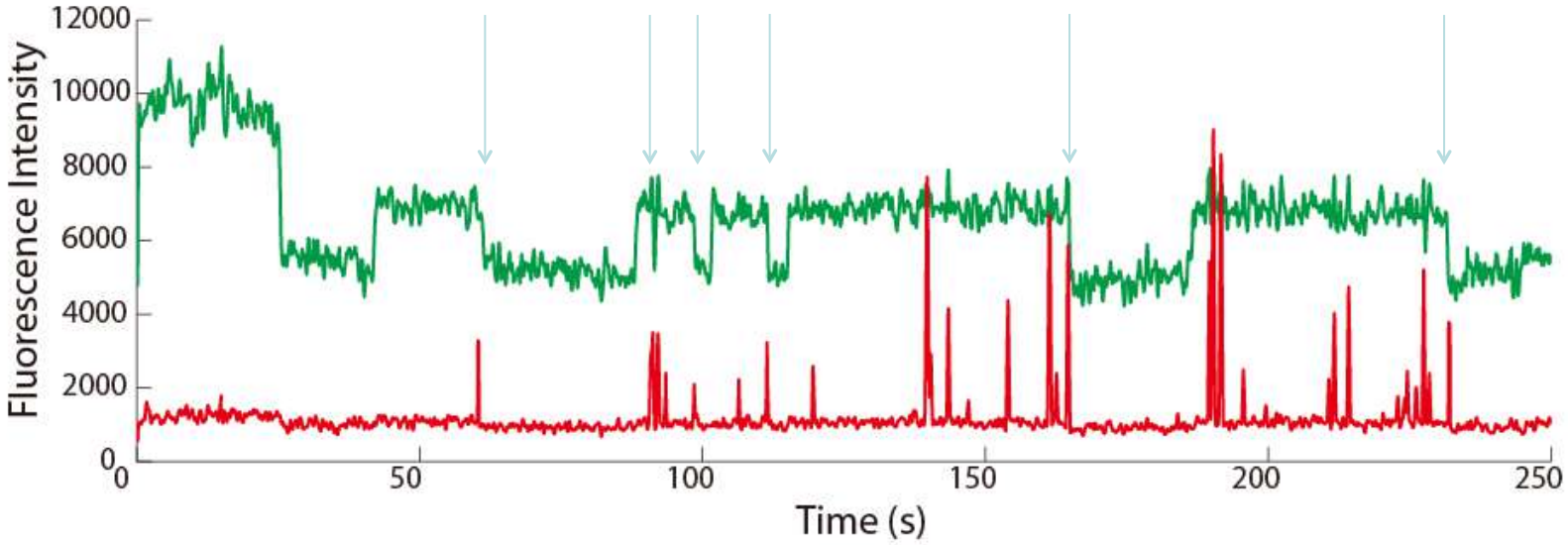
$\Delta G^{o} \approx -8\ kcal \cdot mol^{-1}$

Chemical reactions provide the free energy for ribosomal conformational rearrangements

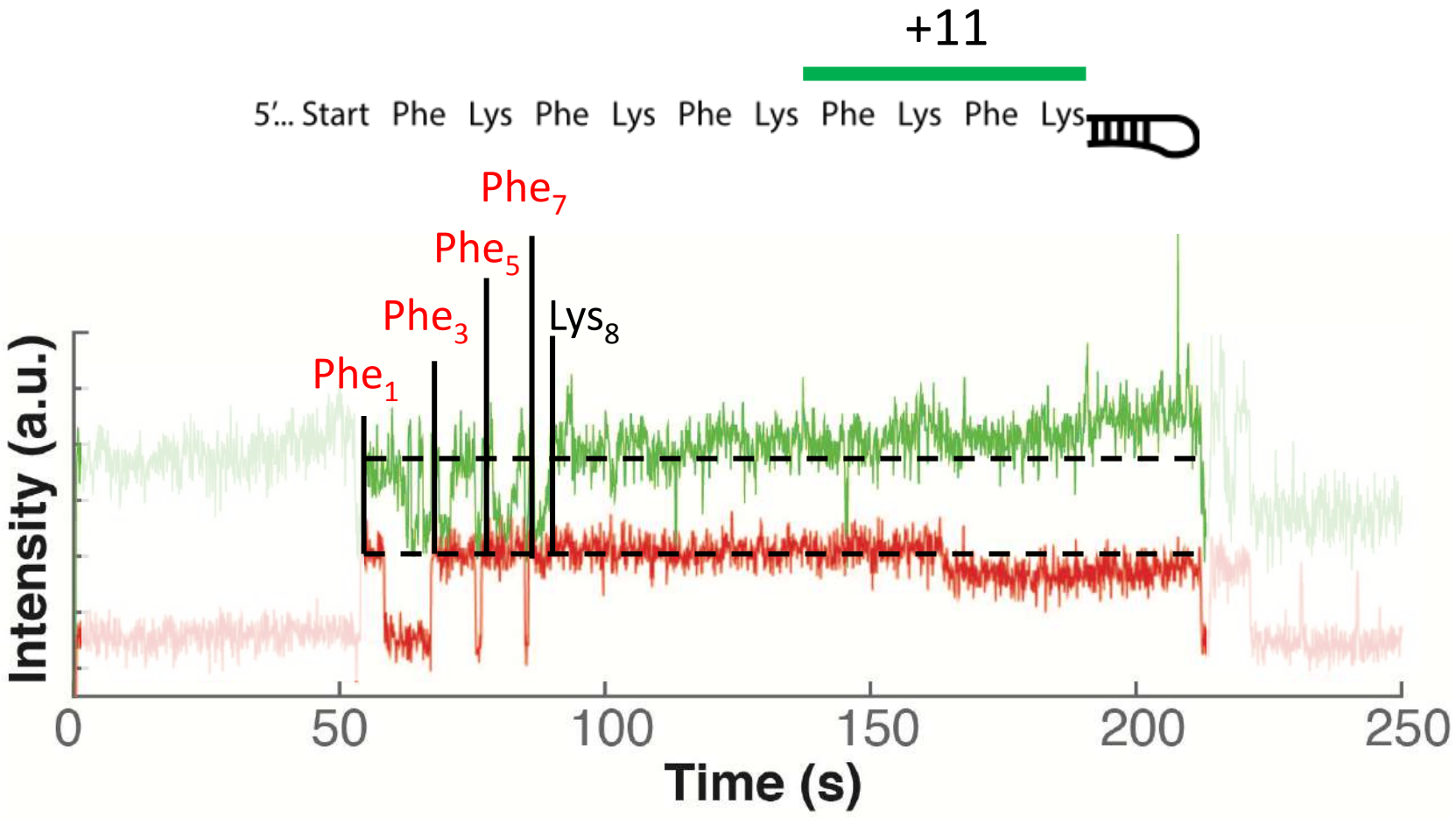


Drugs can disrupt the EF-G cycle

Increased EF-G sampling/futile cycles + spectinomycin



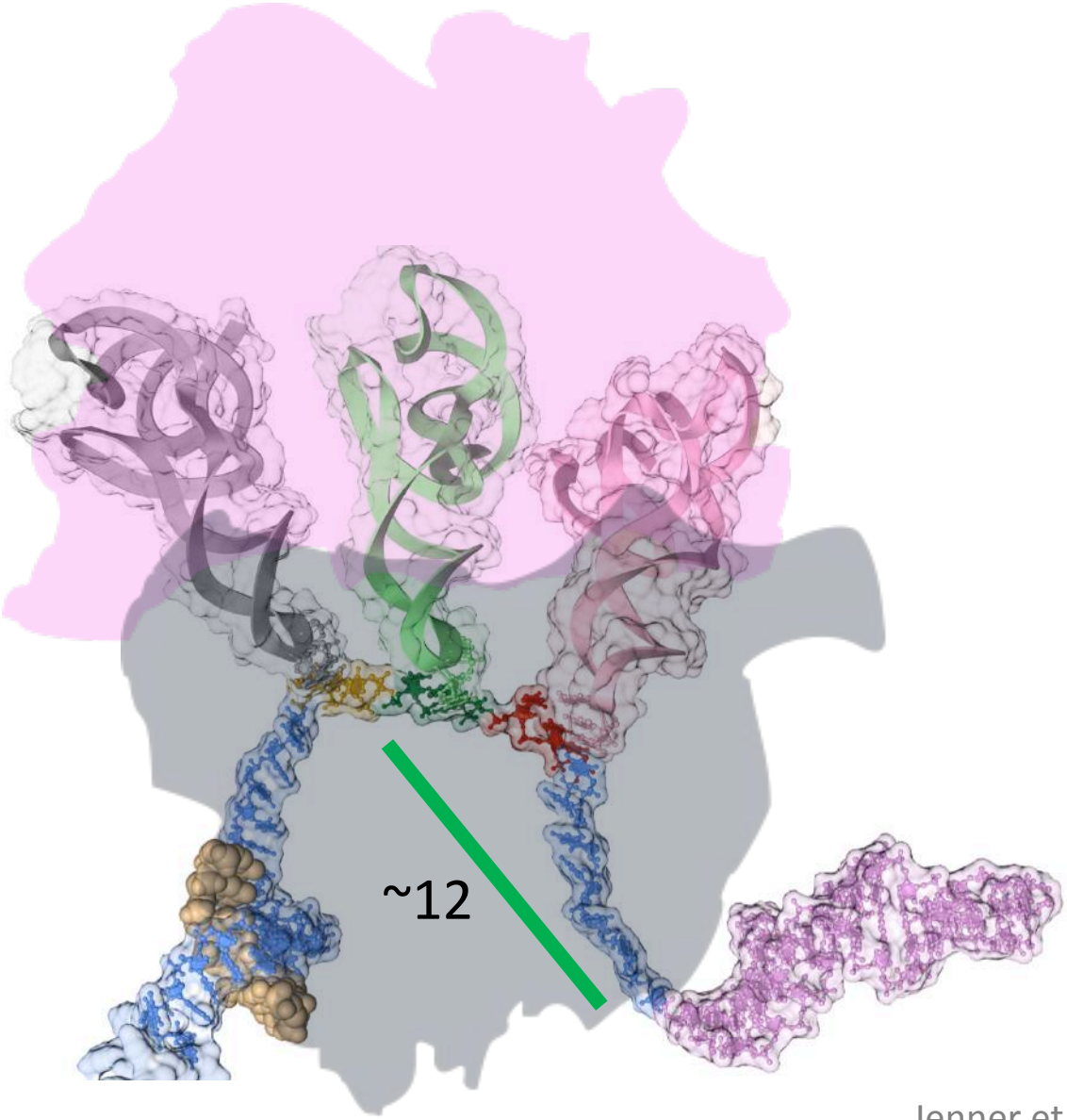
When does ribosome "feel" mRNA structure?



Summary of basic translation dynamics

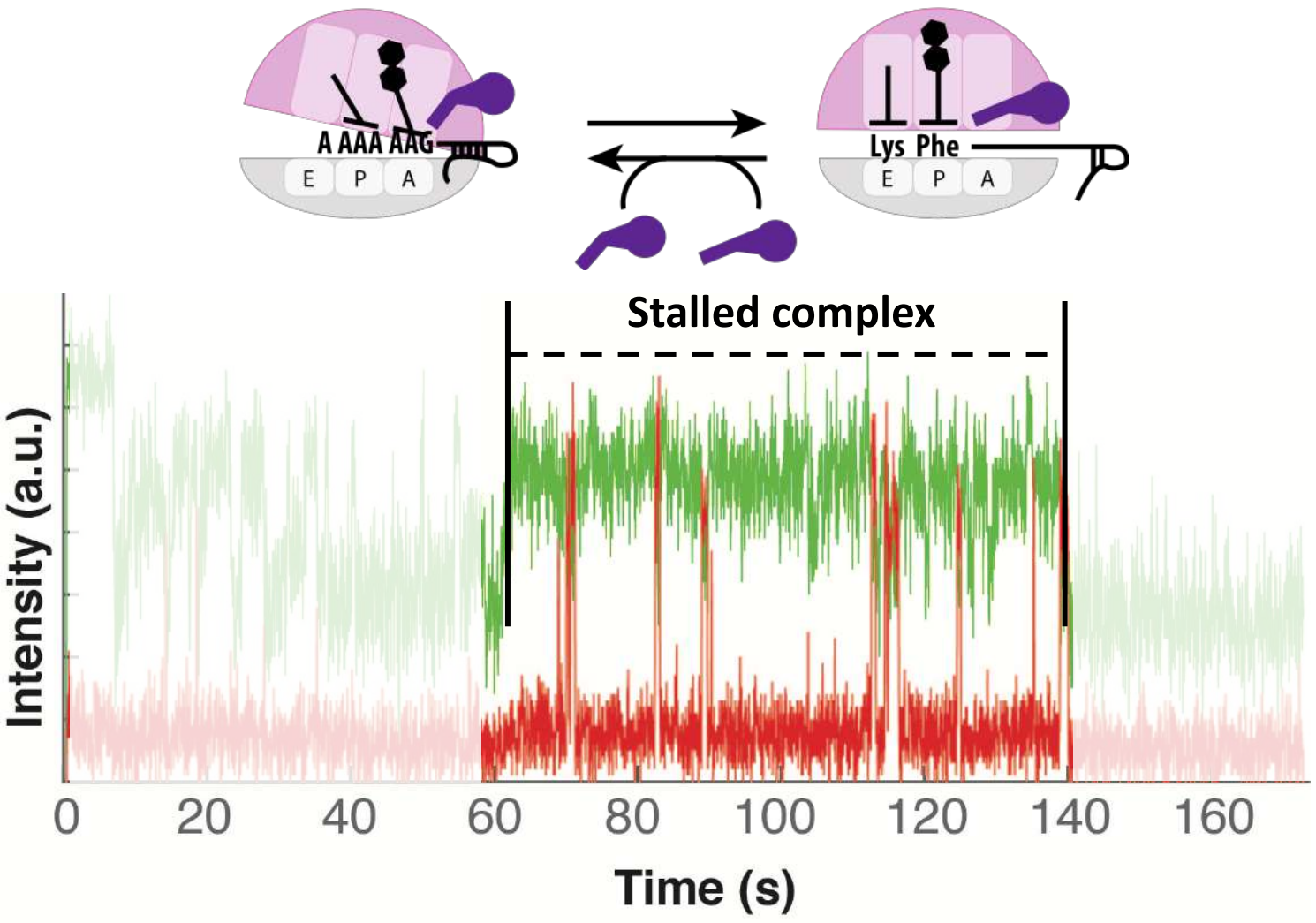
- tRNA is selected in the non-rotated state
- Selection involves multiple states, GTP hydrolysis and proofreading
- peptide bond formation changes ribosome and tRNA conformation
- EF-G uses GTP energy to move codon-anticodons and reset ribosome conformation
- Processes are rapid and efficient, little waste of energy or time
- Drugs and mRNA structure disrupt this efficiency

How does mRNA structure affect translation?



Jenner et al, *NSMB* **2010**

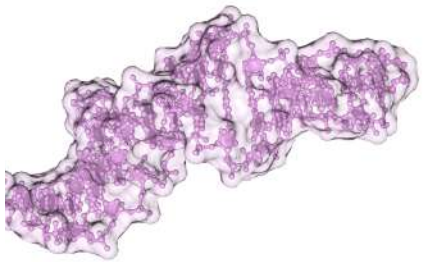
Multiple EF-G samples paused complex



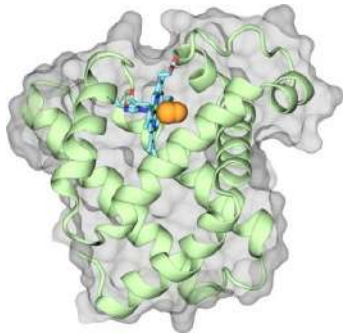
Does translation unfurl smoothly?

START | Ala | Thr | Lys | Ala | Lys | Lys | Ser | Glu | STOP

AUG --- GCA ACC AAA GCA AAA AAG AGU GAA --- UAA

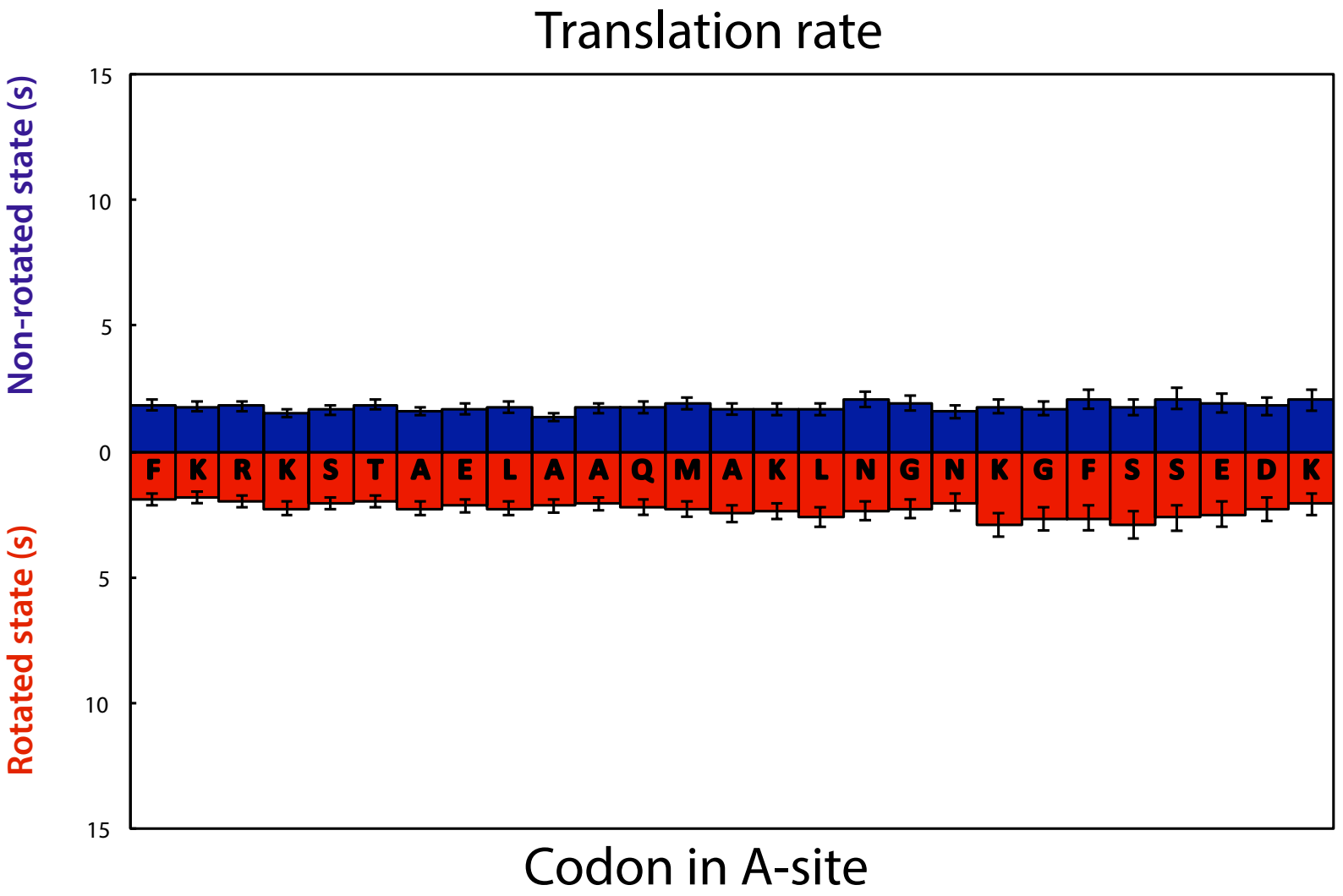


mRNA structure and folding

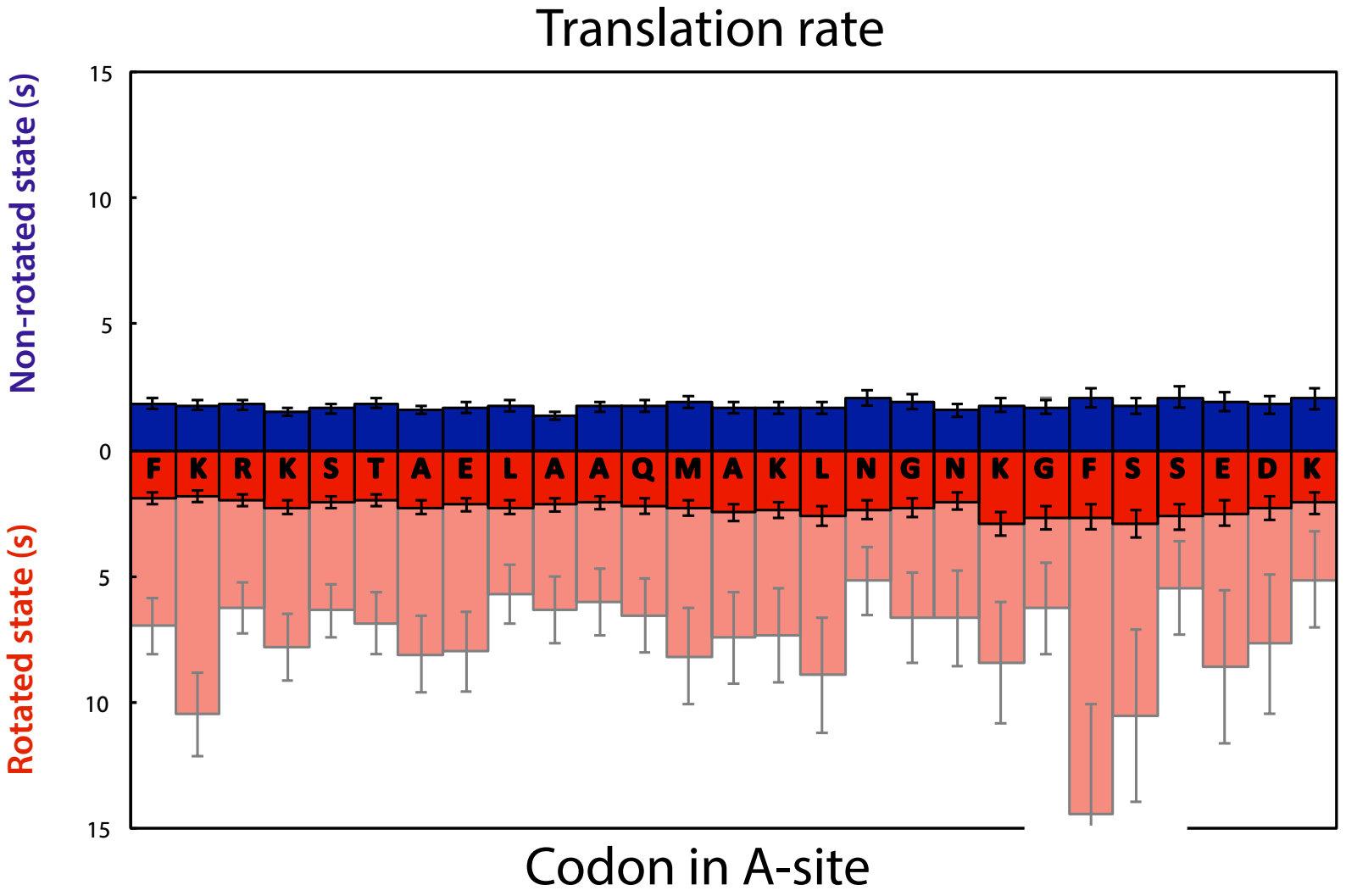


protein structure and folding

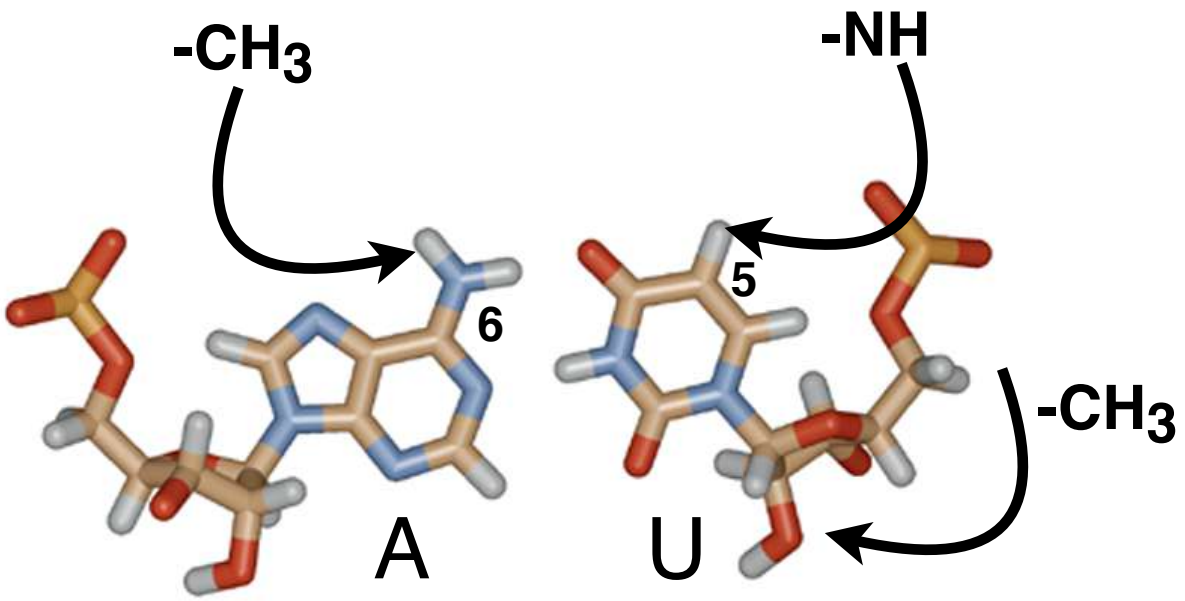
Translation rates at each codon in an mRNA



Spectinomycin causes a 3-4x decrease in translocation rate



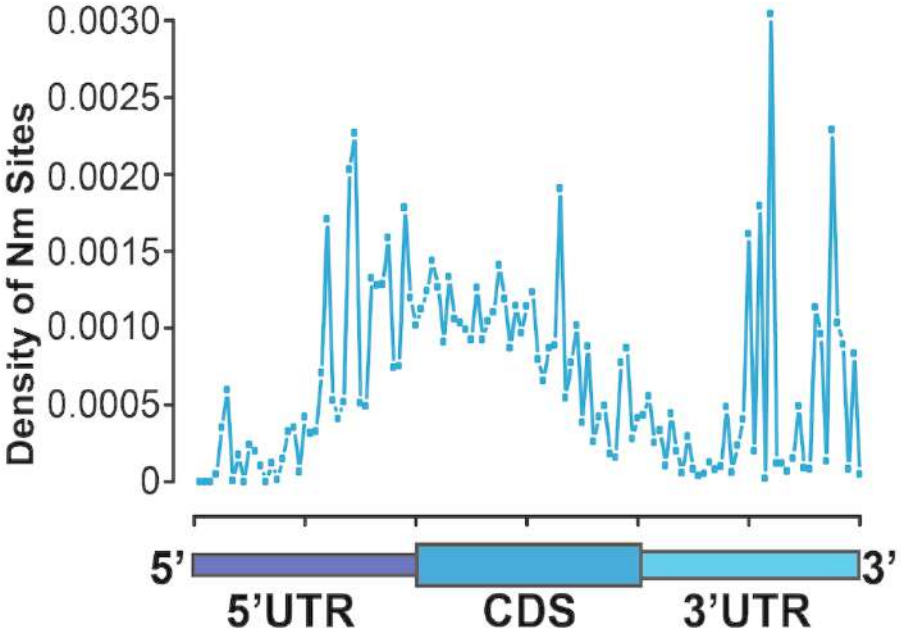
Chemical modification of mRNA: m6A, pseudouridine, 2'O methyl

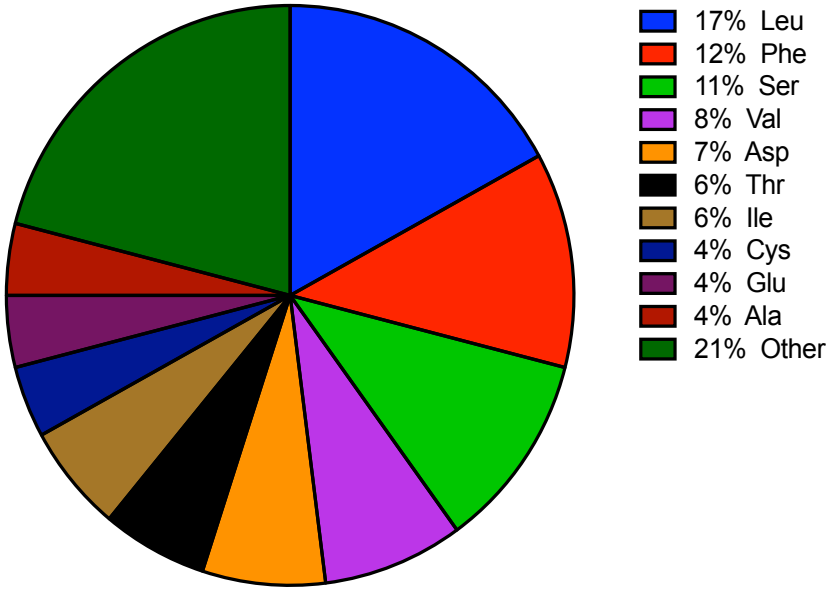


- modified nucleotides found in coding regions of mRNAs
- pseudouridine, 6-methyl-adenosine, 2'Omethyl

How do modifications affect translation?

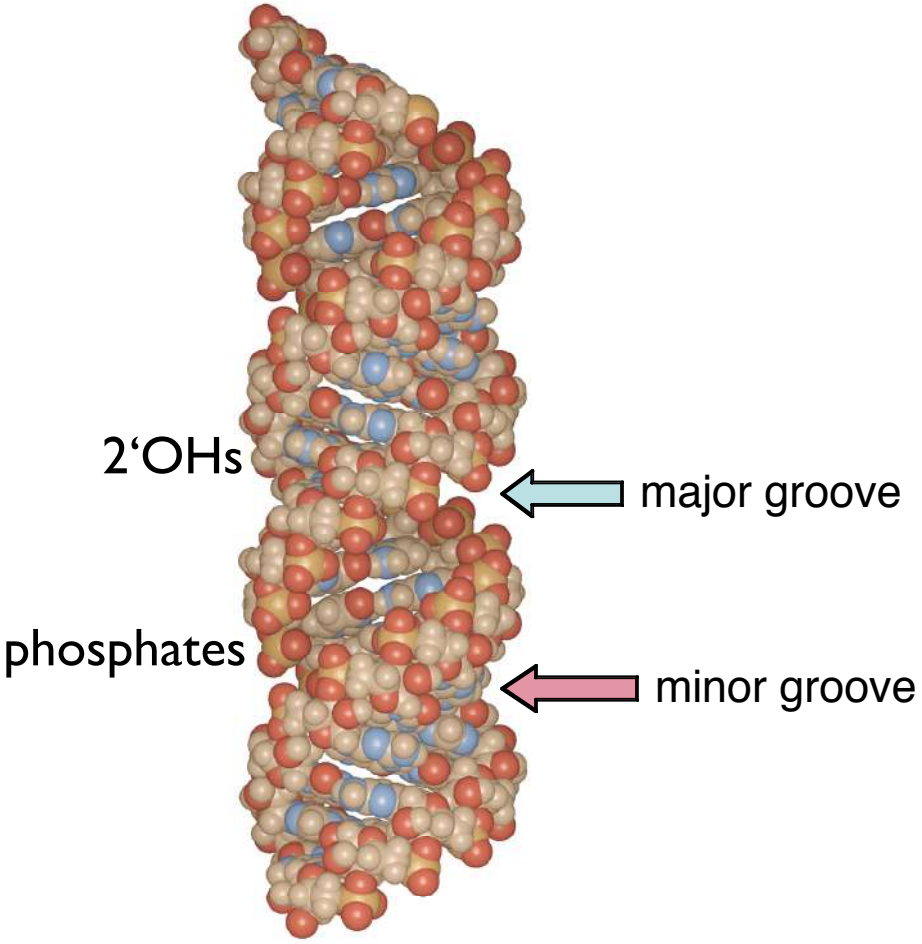
2’-O-methylation (Nm) within the coding region





Chuan He, U of Chicago, unpublished

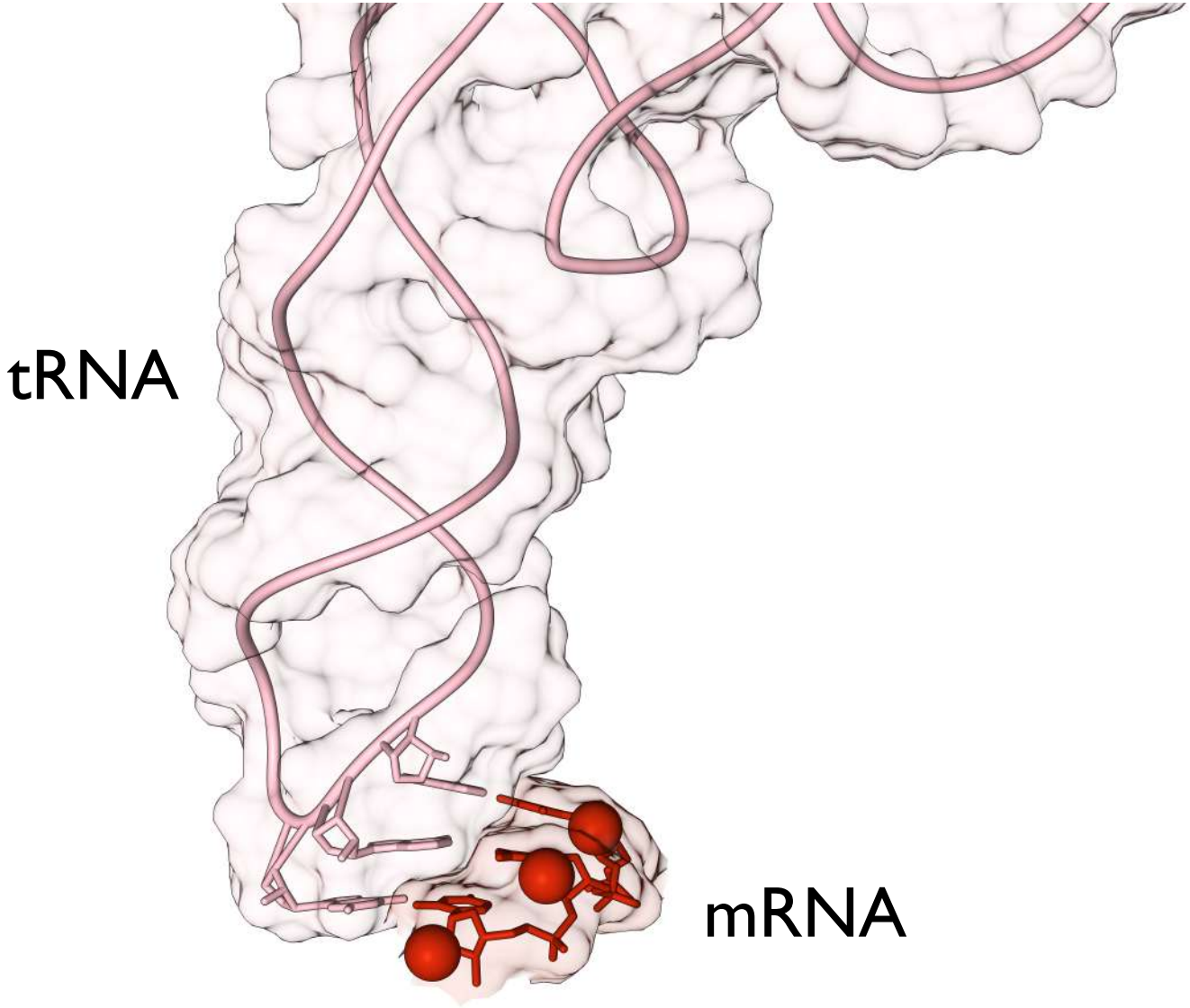
2'OH and 2'O-methyl in the minor groove of RNA



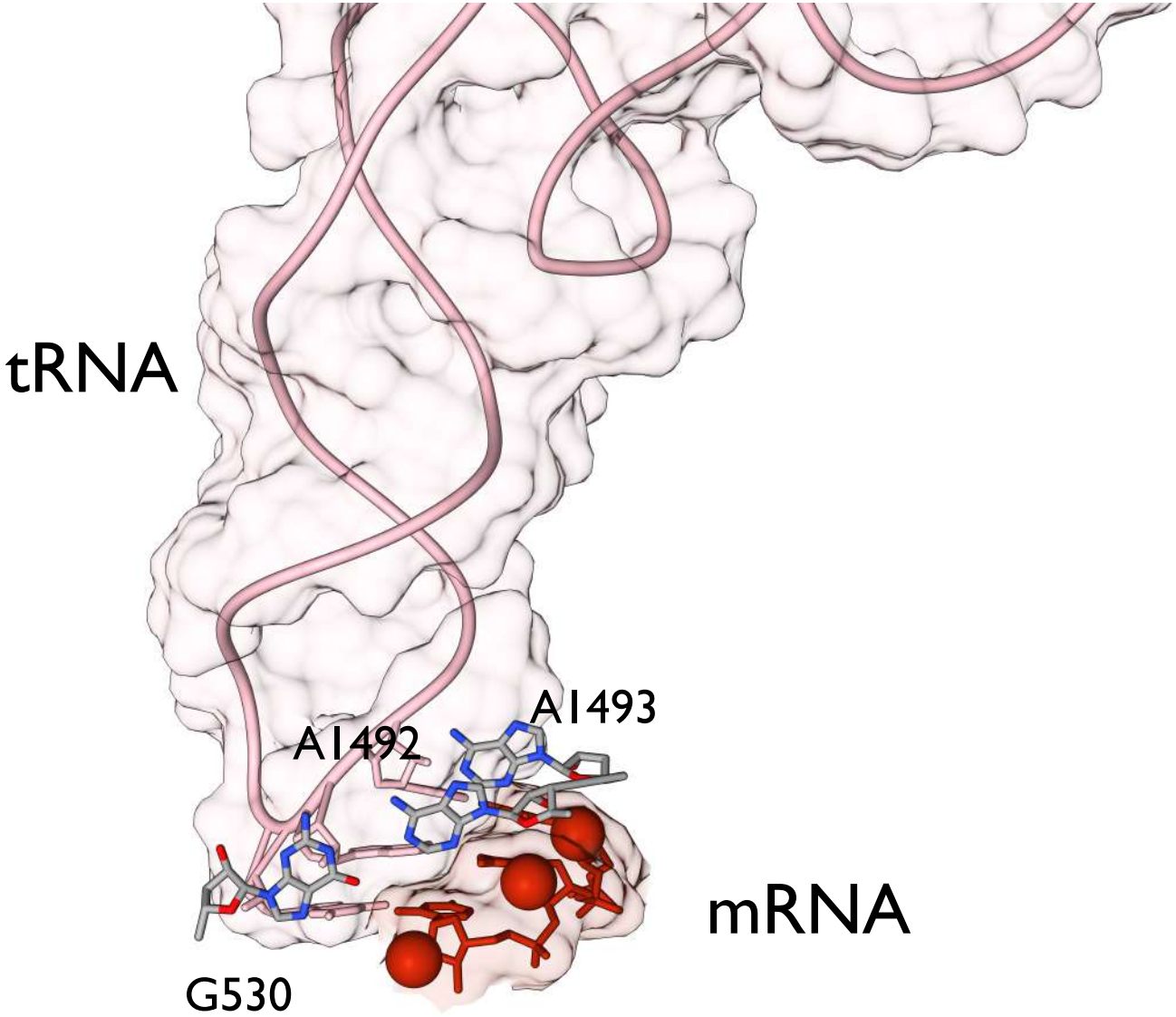
A-RNA

2'Omethyl stabilizes RNA helices

2'OH and 2'O-methyl in the minor groove of RNA



Ribosomal RNA recognizes the shape of the codon-anticodon helix in the A site



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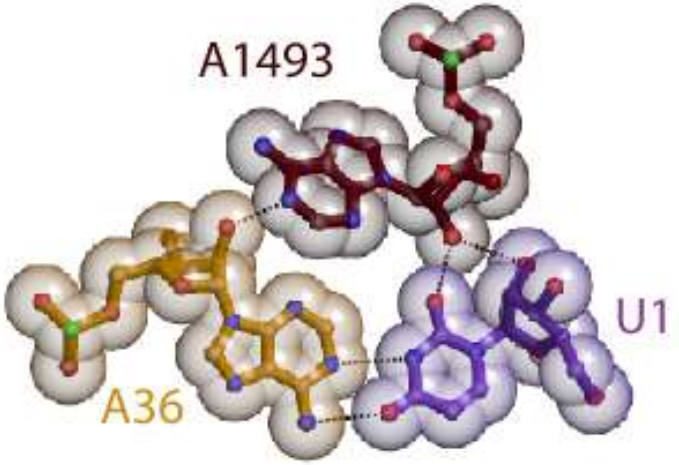
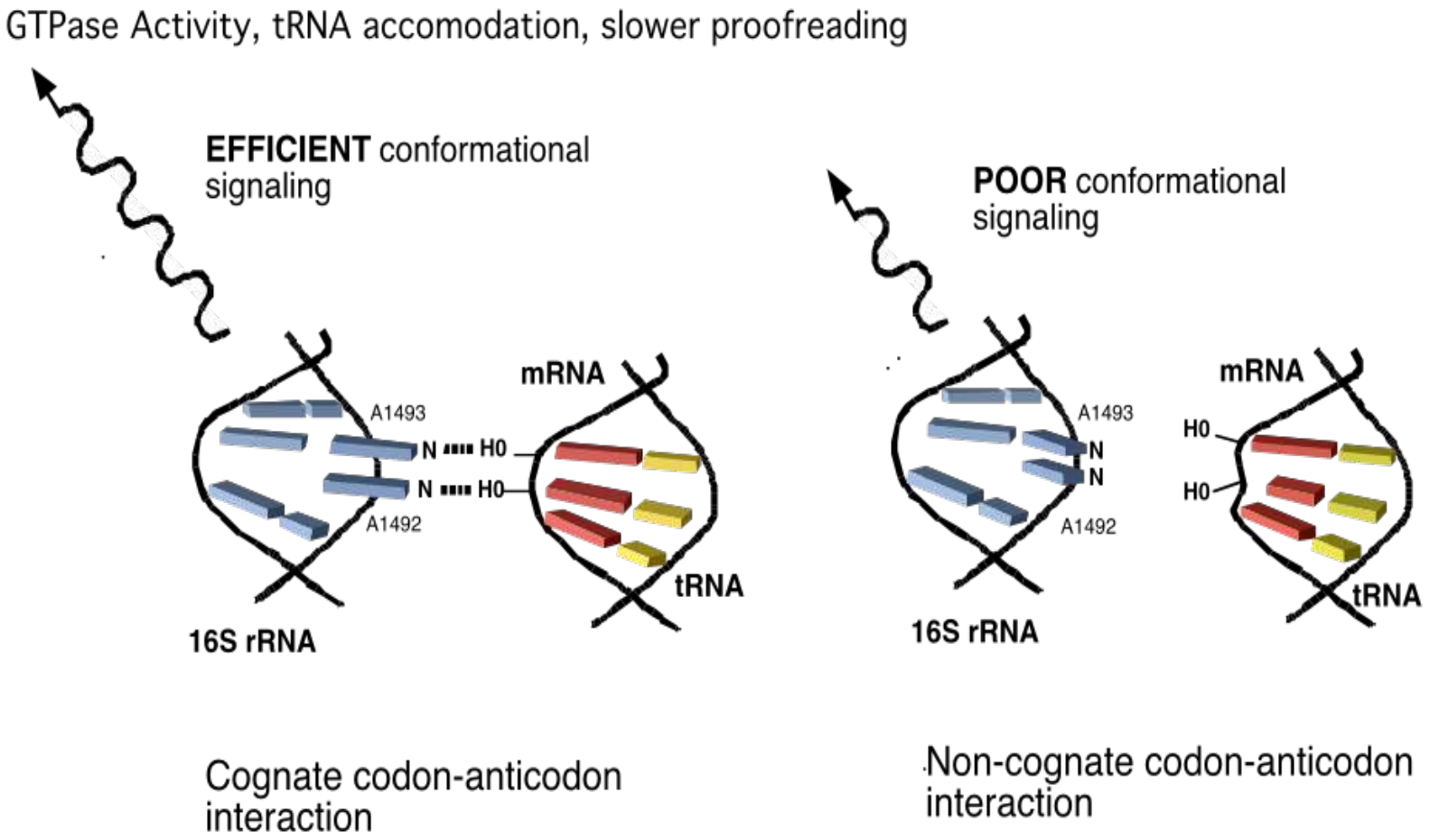
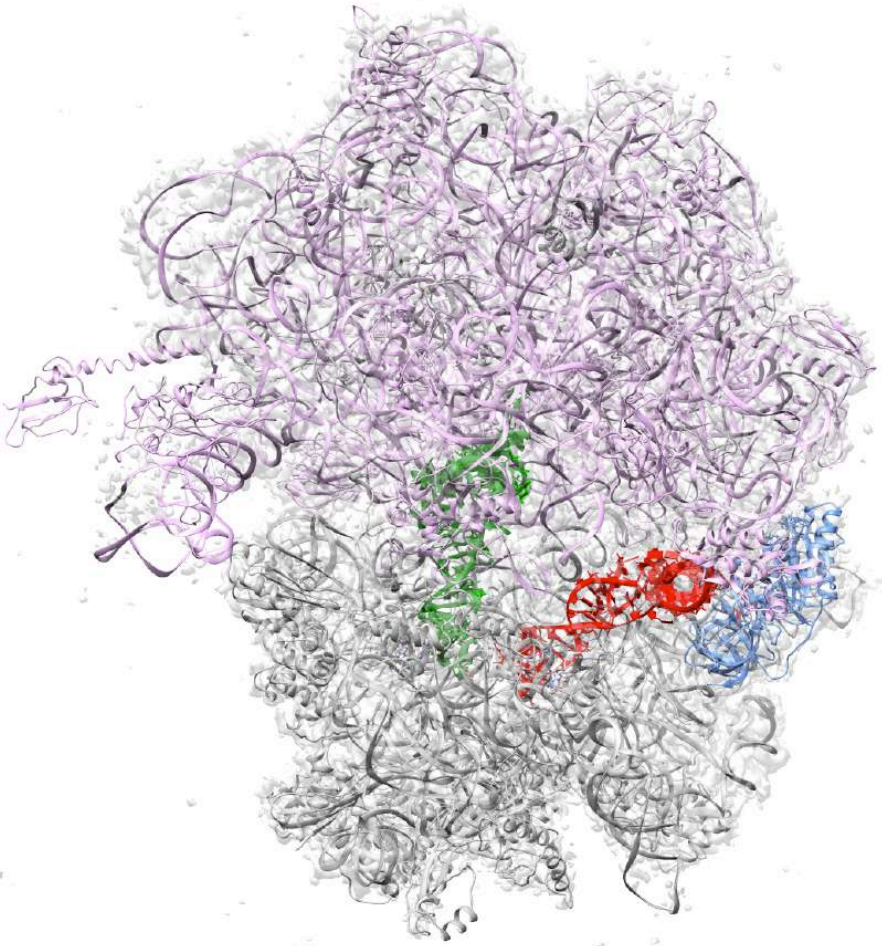


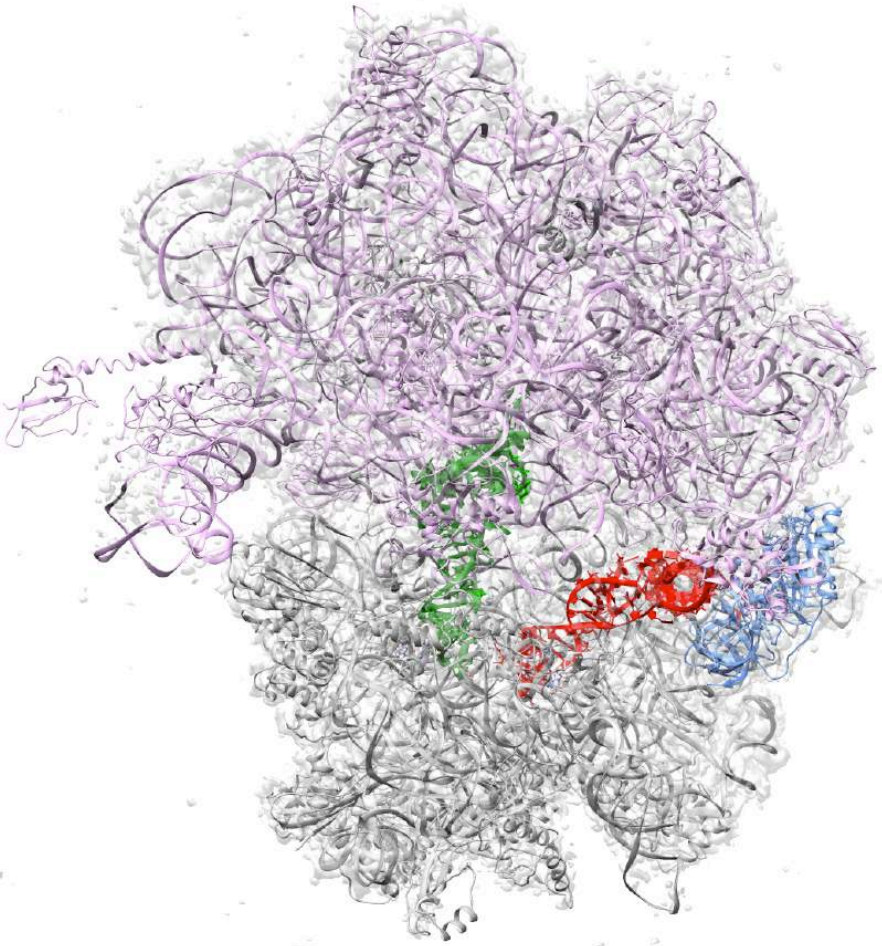
A1493 recognition of codon-anticodon helix

Ramakrishnan and co-workers

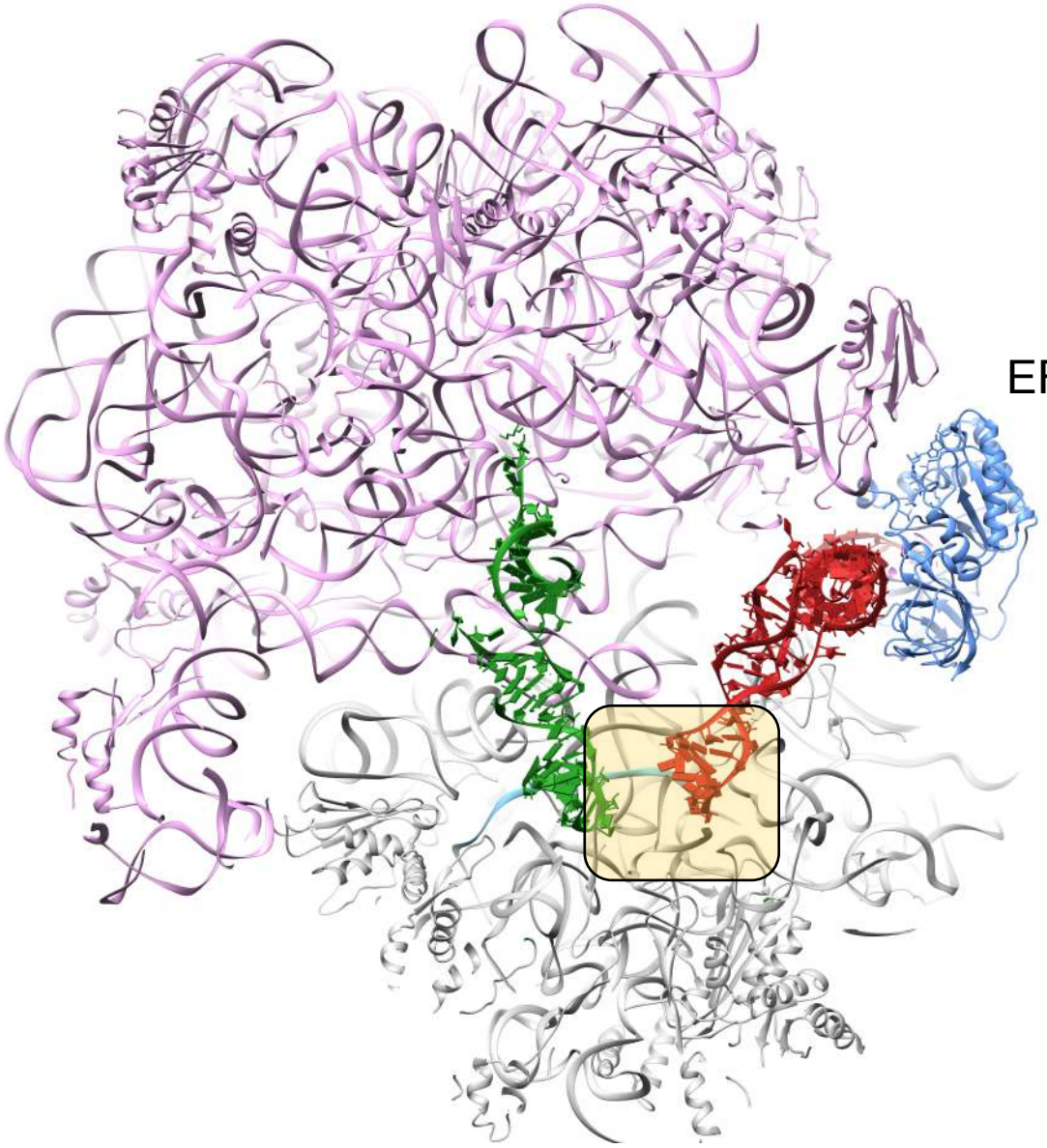
How do ribosomes discriminate right from wrong?





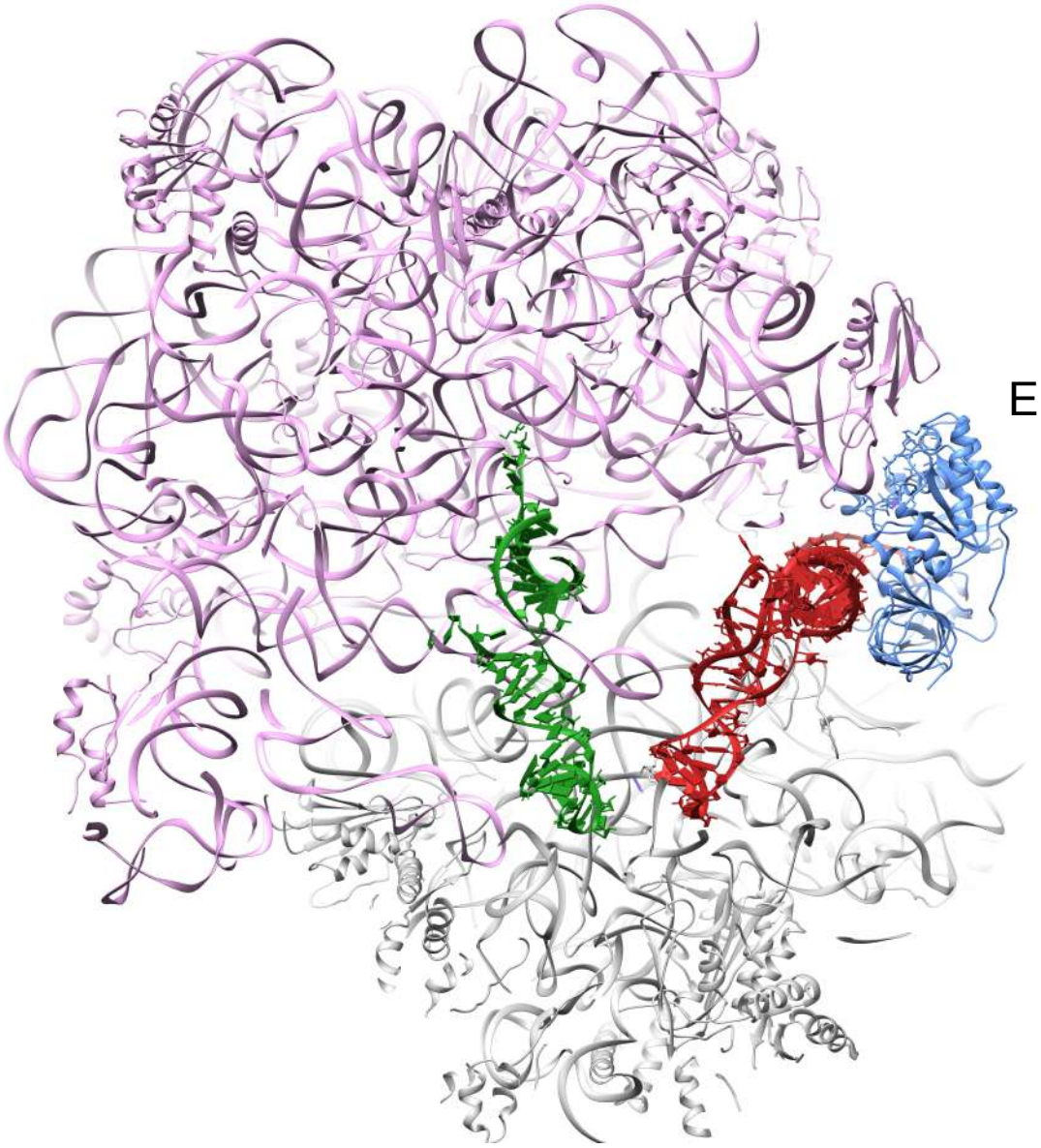


EF-Tu activation by the monitoring bases and domain closure



EF-Tu >8Å from SRL

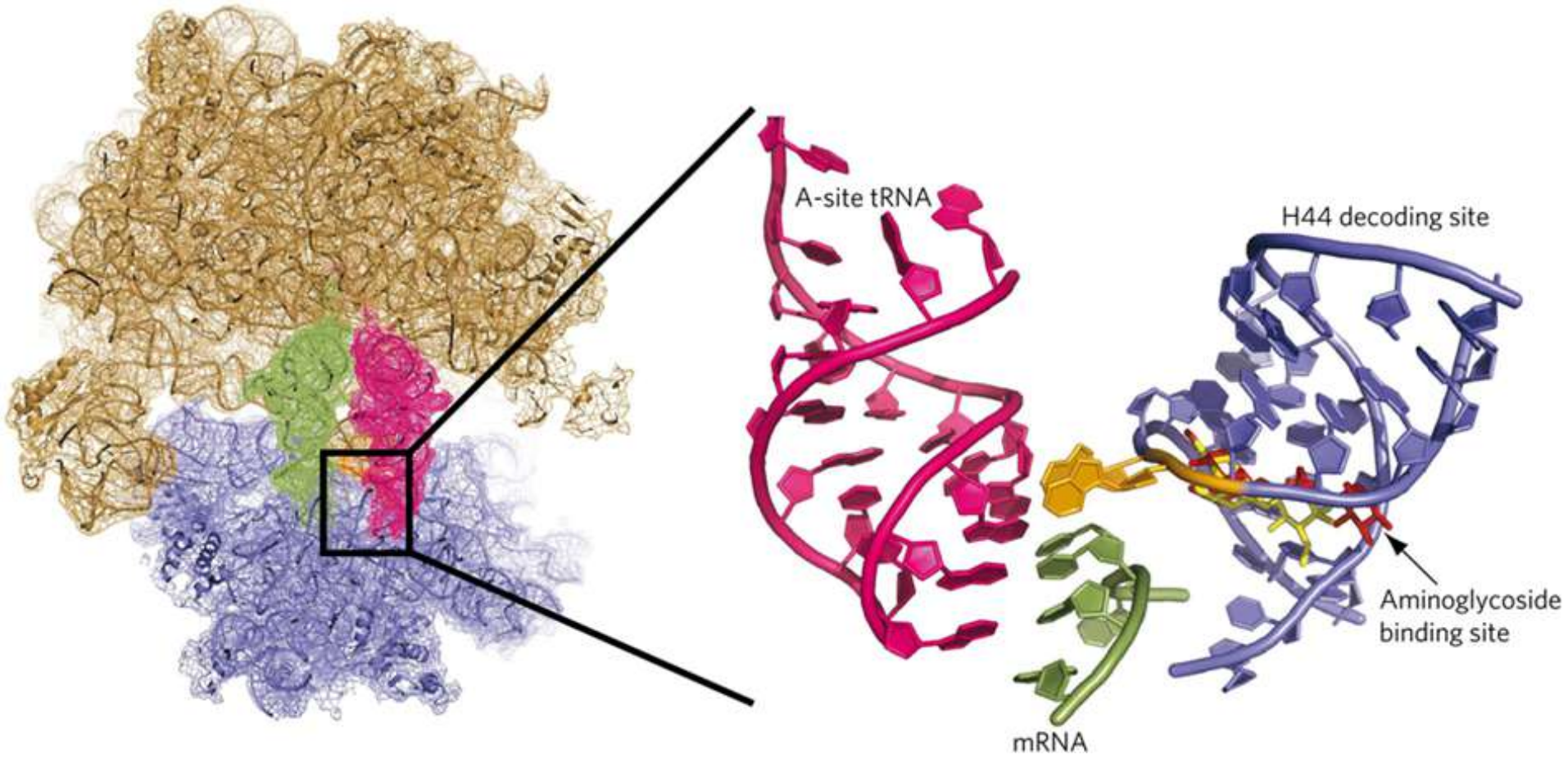
EF-Tu activation by the monitoring bases and domain closure



EF-Tu contacts SRL

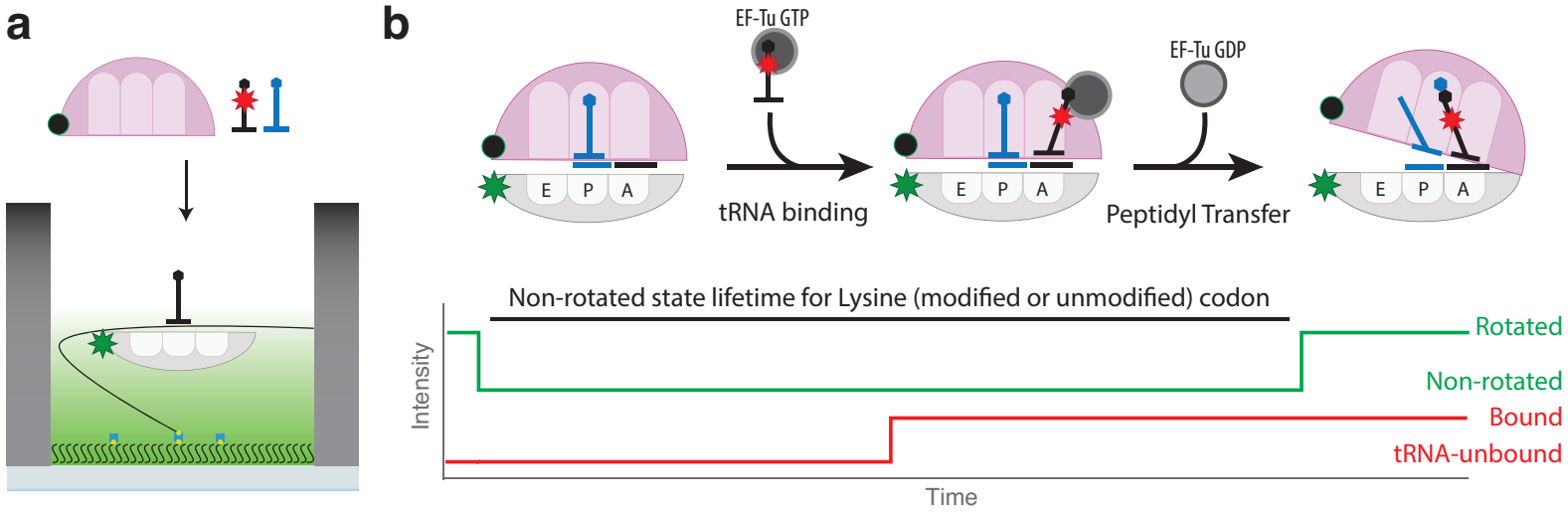
monitoring bases/
domain closure

Aminoglycoside antibiotics fool ribosomes during tRNA selection



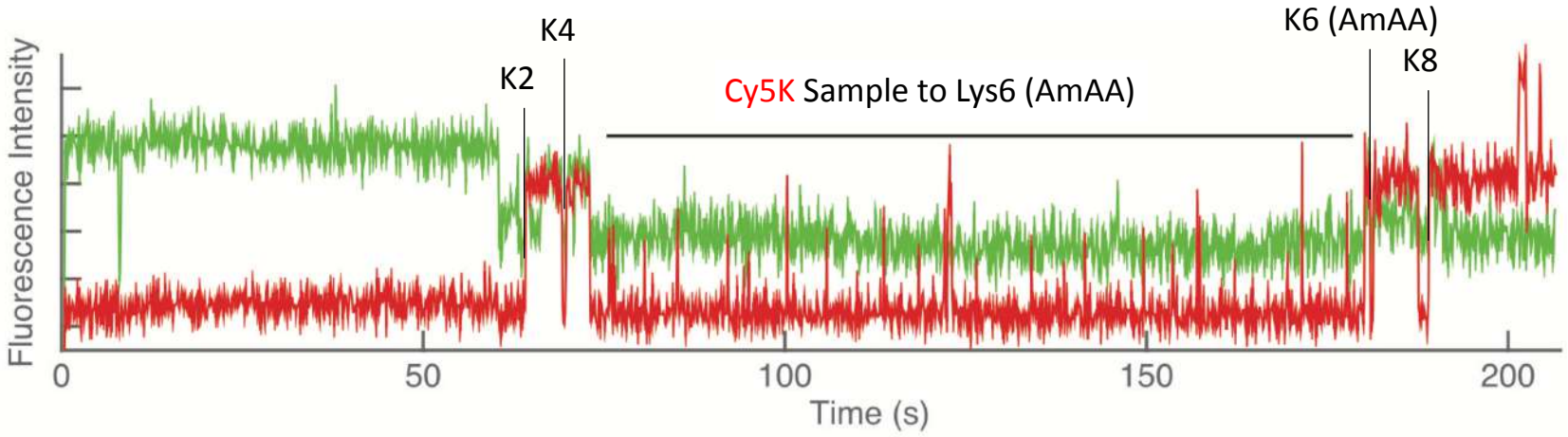
Feldman *et al.* (2010) *Nat. Chem. Biol.* **6**:54-62

Single-molecule experimental schematic



5′... Start - F1 - K2 - F3 - K4 - F5 - **K6 (AmAA)** - F7 - K8 - STOP *... 3′*

Nm causes a specific pause during translation

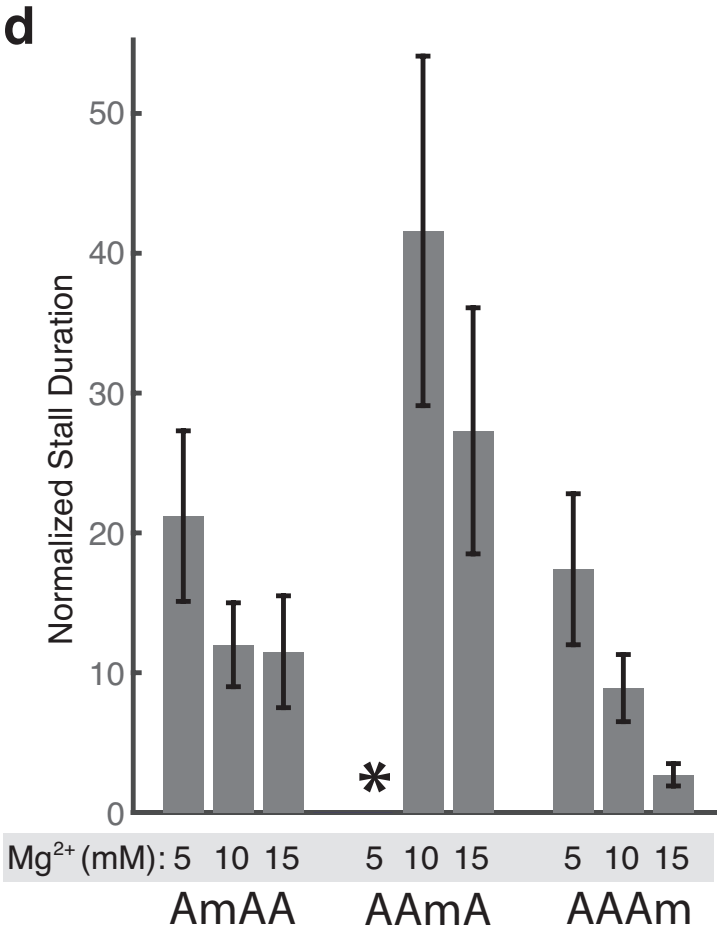


5′... Start - F1 - K2 - F3 - K4 - F5 - **K6 (AmAA)** - F7 - K8 - STOP *... 3′*

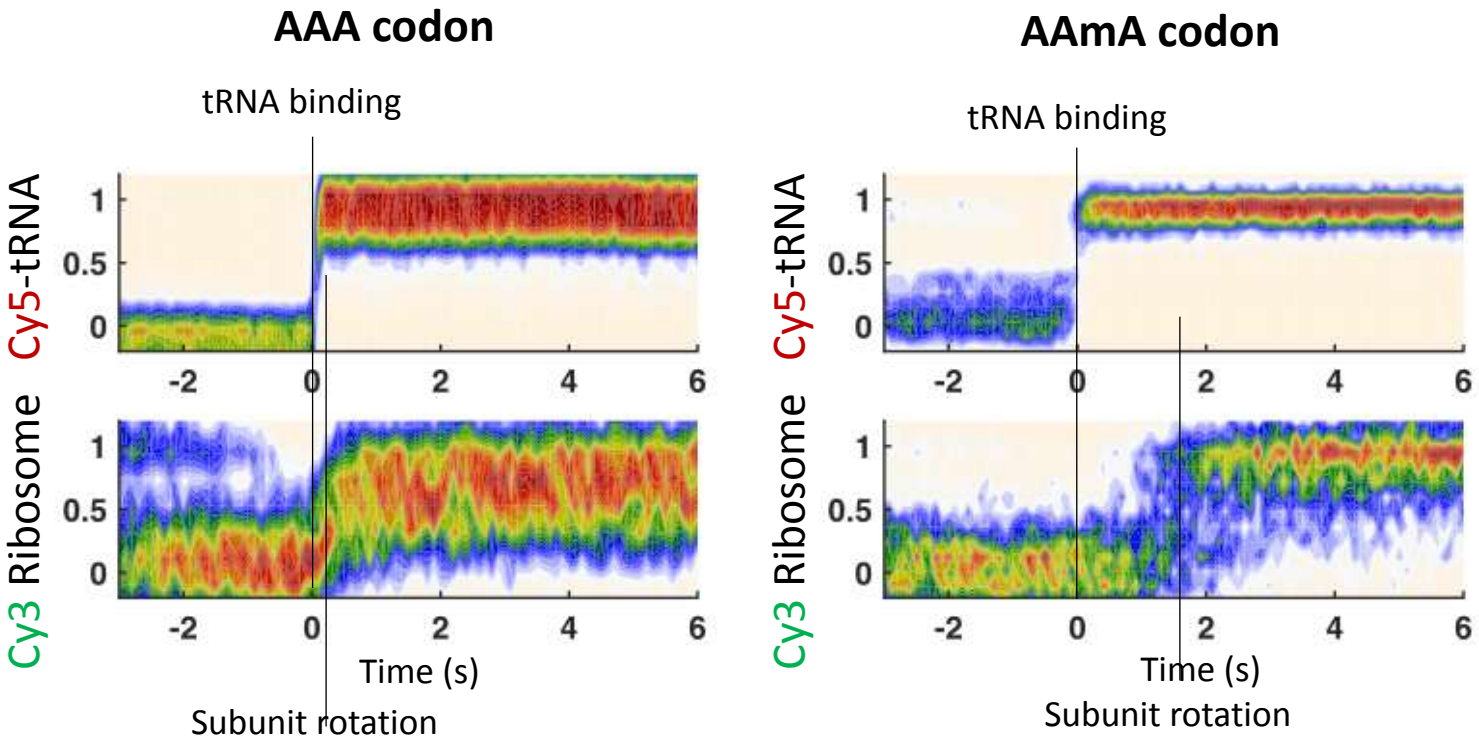
Nm induces cognate tRNA rejection

Fold-increase in non-rotated state lifetime =
Fold-increase in the number of tRNA rejection (tRNA rejection factor)

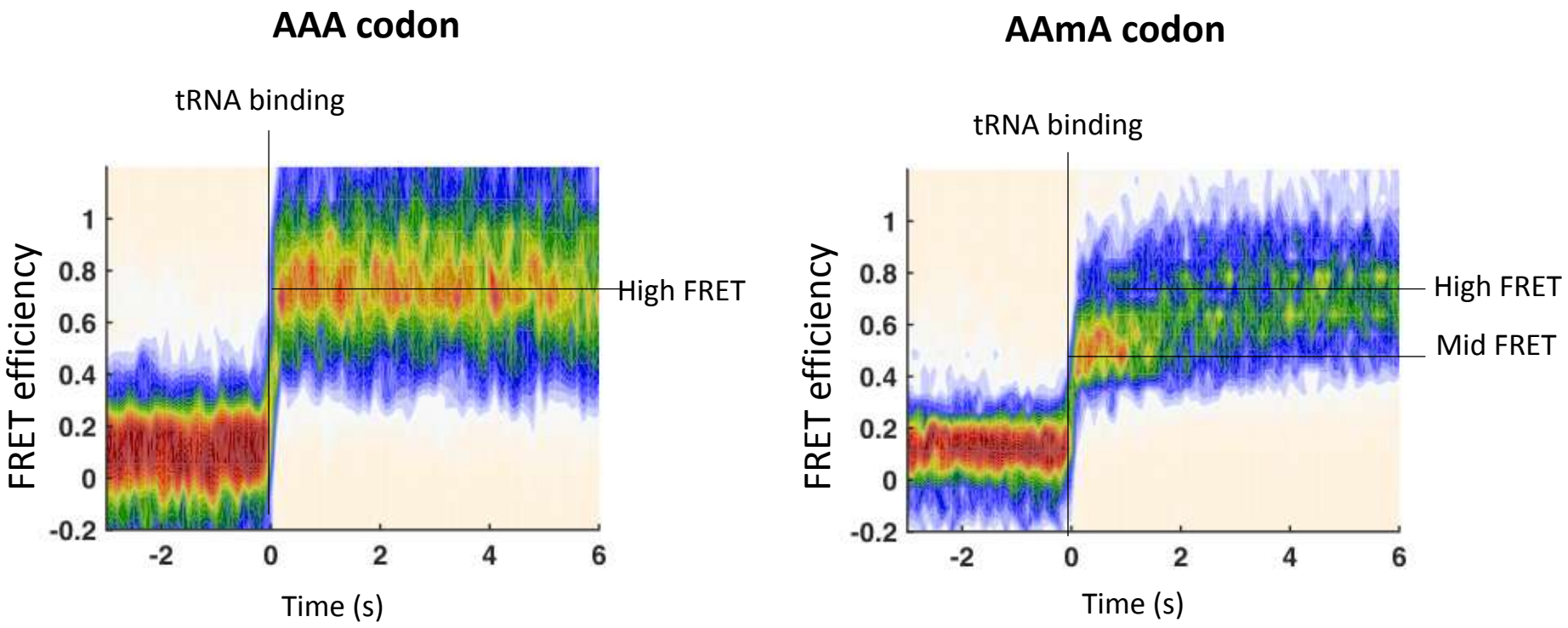
The duration of the Nm-induced stall depends on codon position and Mg^{2+}



Nm causes a lag between tRNA binding and accommodation



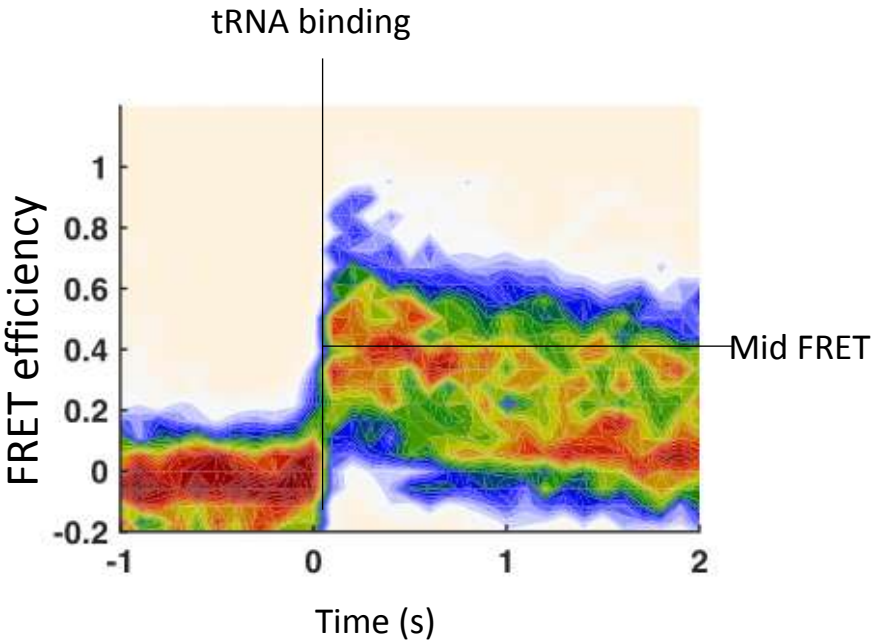
Nm stalls tRNA-EF-Tu at the GTPase activation state



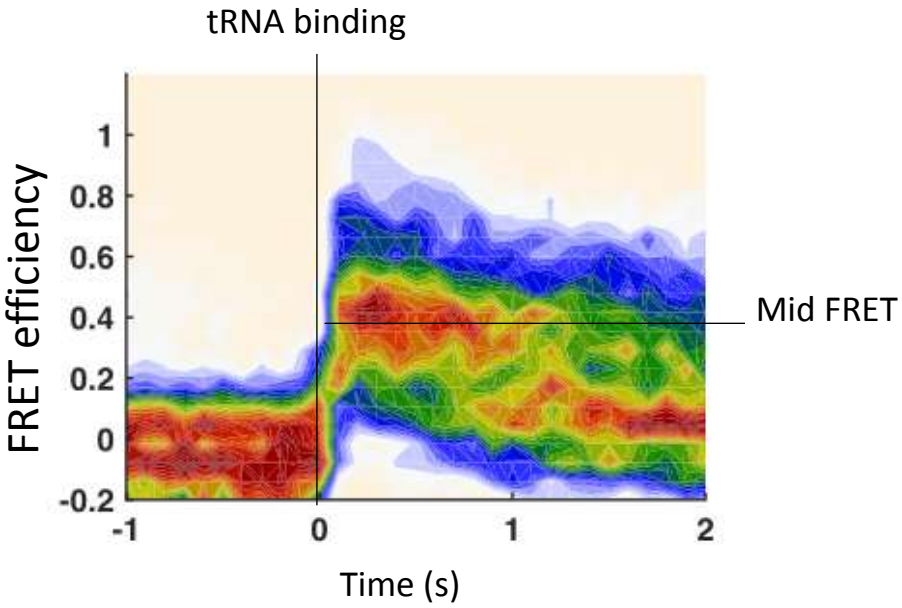
tRNA-tRNA FRET

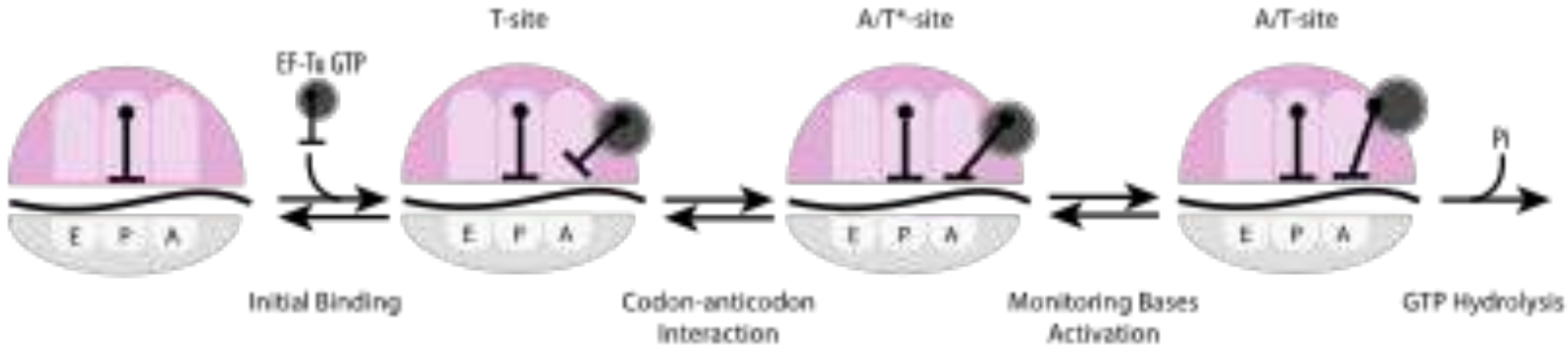
Non-hydrolyzable GTP eliminates the effect of Nm

AAA codon / GDPNP



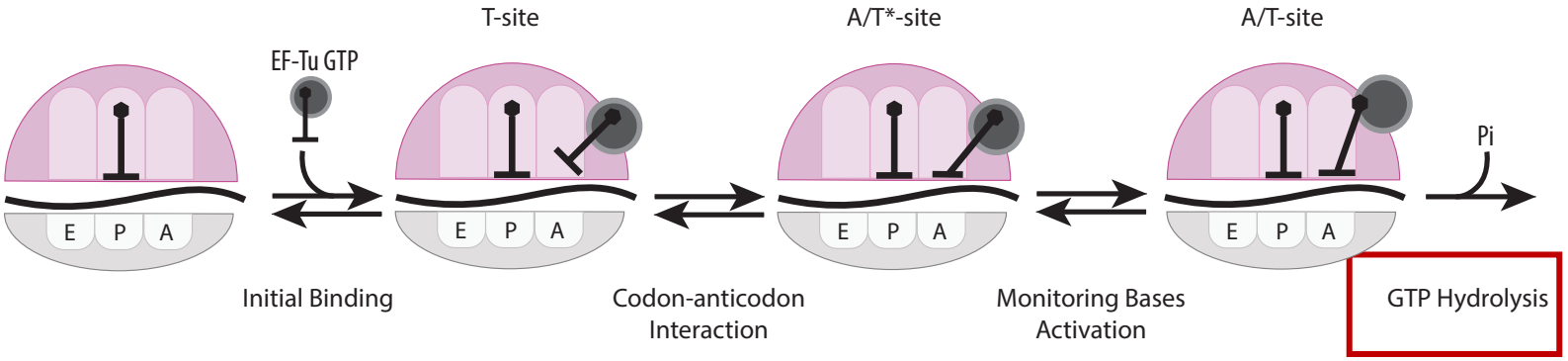
AAmA codon / GDPNP



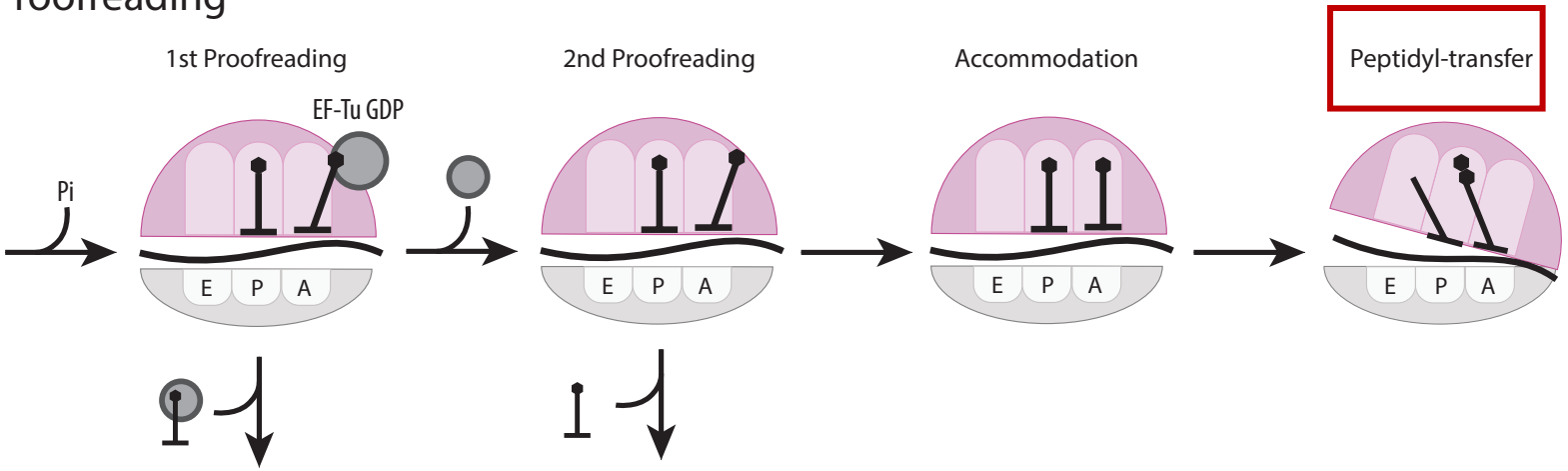


Kinetics of sub-steps

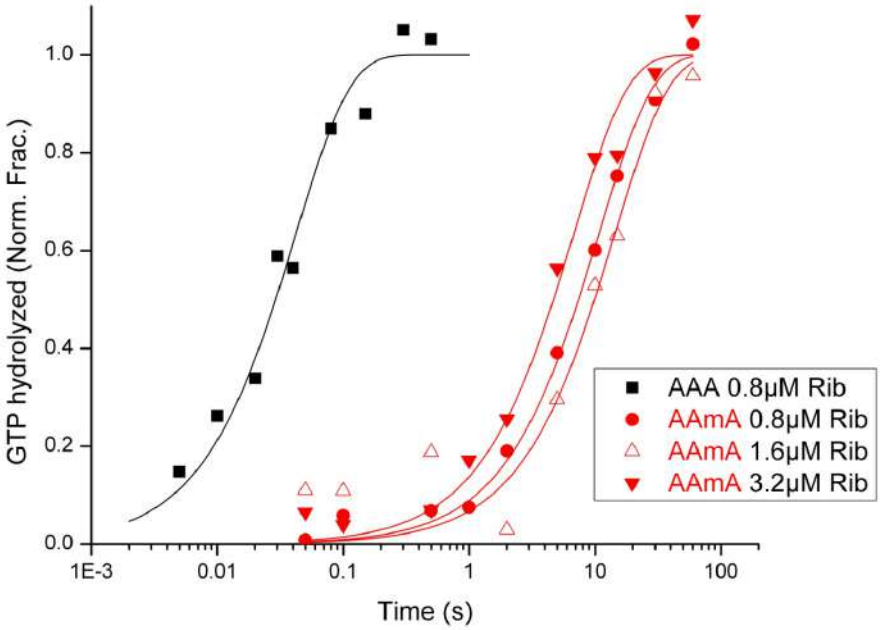
(i) Initial Selection



(ii) Proofreading



Nm leads to a greatly decreased GTP hydrolysis rate by EF-Tu

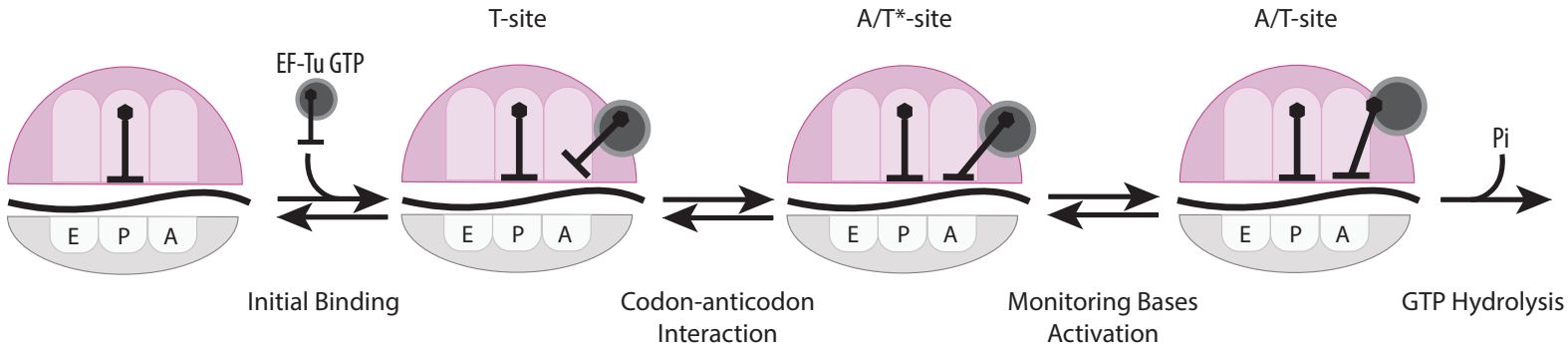


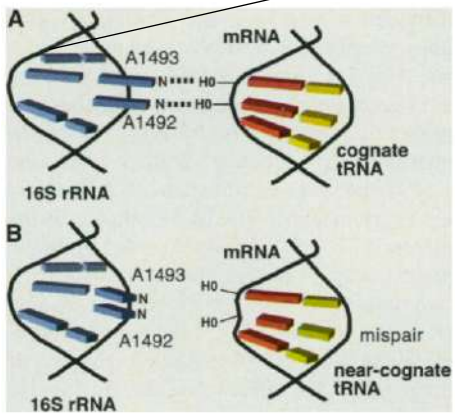
mRNA	Ribosome (μM)	k_{GTP} (s^{-1})	k_{cat} (s^{-1})
AAA	0.8	24 ± 3.6	25.5 ± 2.7
	1.6	26 ± 2.7	
	3.2	31 ± 2.4	
AAmA	0.8	0.094 ± 0.0094	0.097 ± 0.008
	1.6	0.070 ± 0.022	
	3.2	0.15 ±0.025	

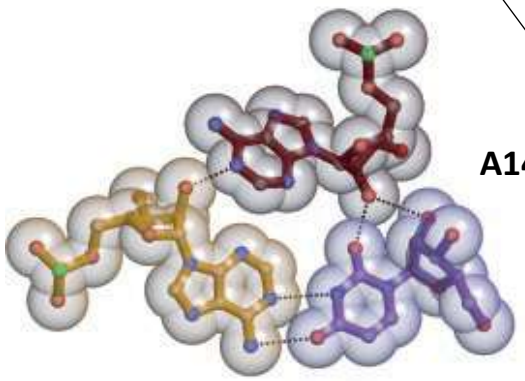
260-fold slow down of GTP-hydrolysis on Nm-modified codon
4.5x increased proofreading (not shown)

Can aminoglycosides compensate for the effect of Nm?

(i) Initial Selection





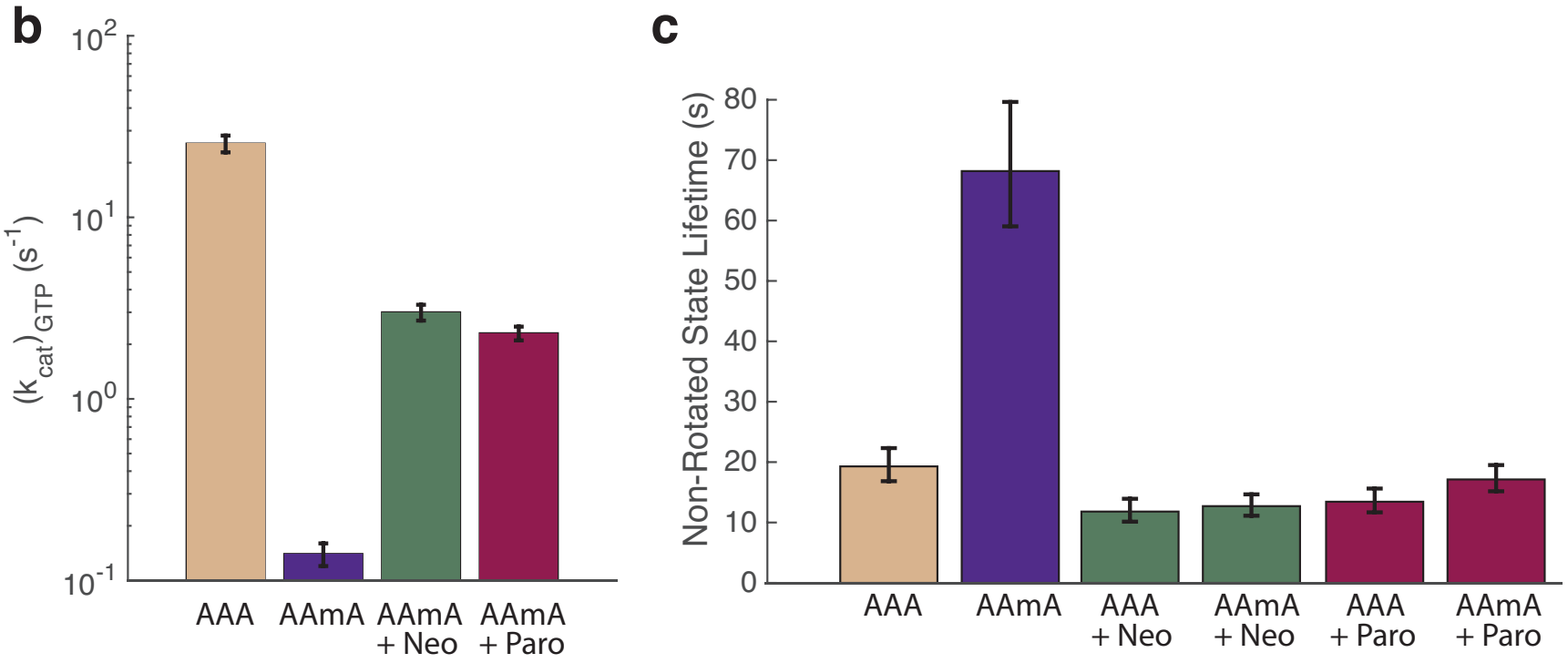


A1493 (rRNA)

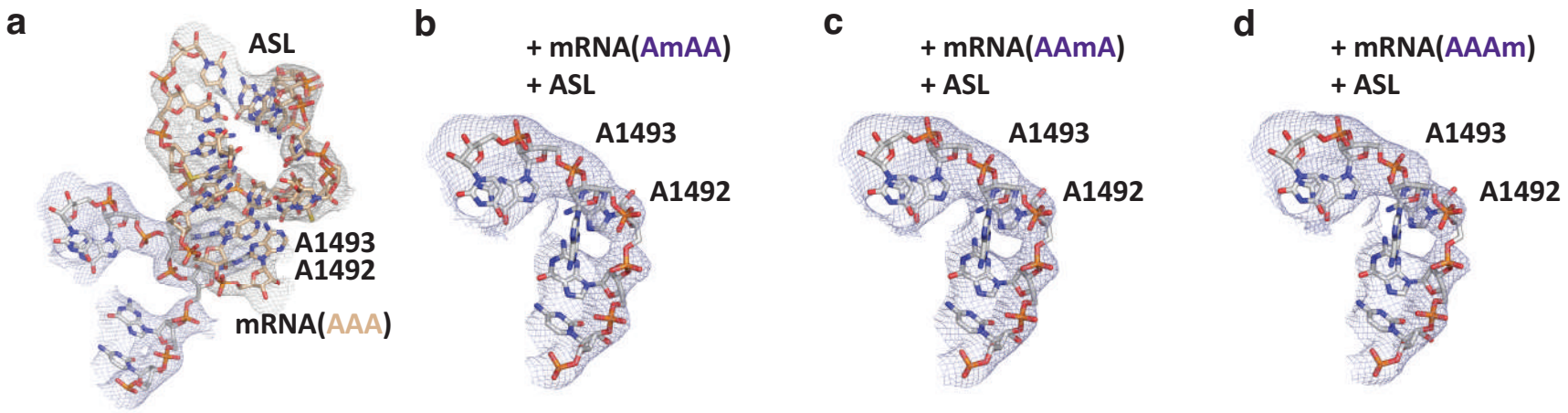
A36 (tRNA) **U1 (mRNA)**

Fourmy et al. 1996, Ogle *et al.* **Science** 2001

Aminoglycosides rescue the effect of Nm on decoding

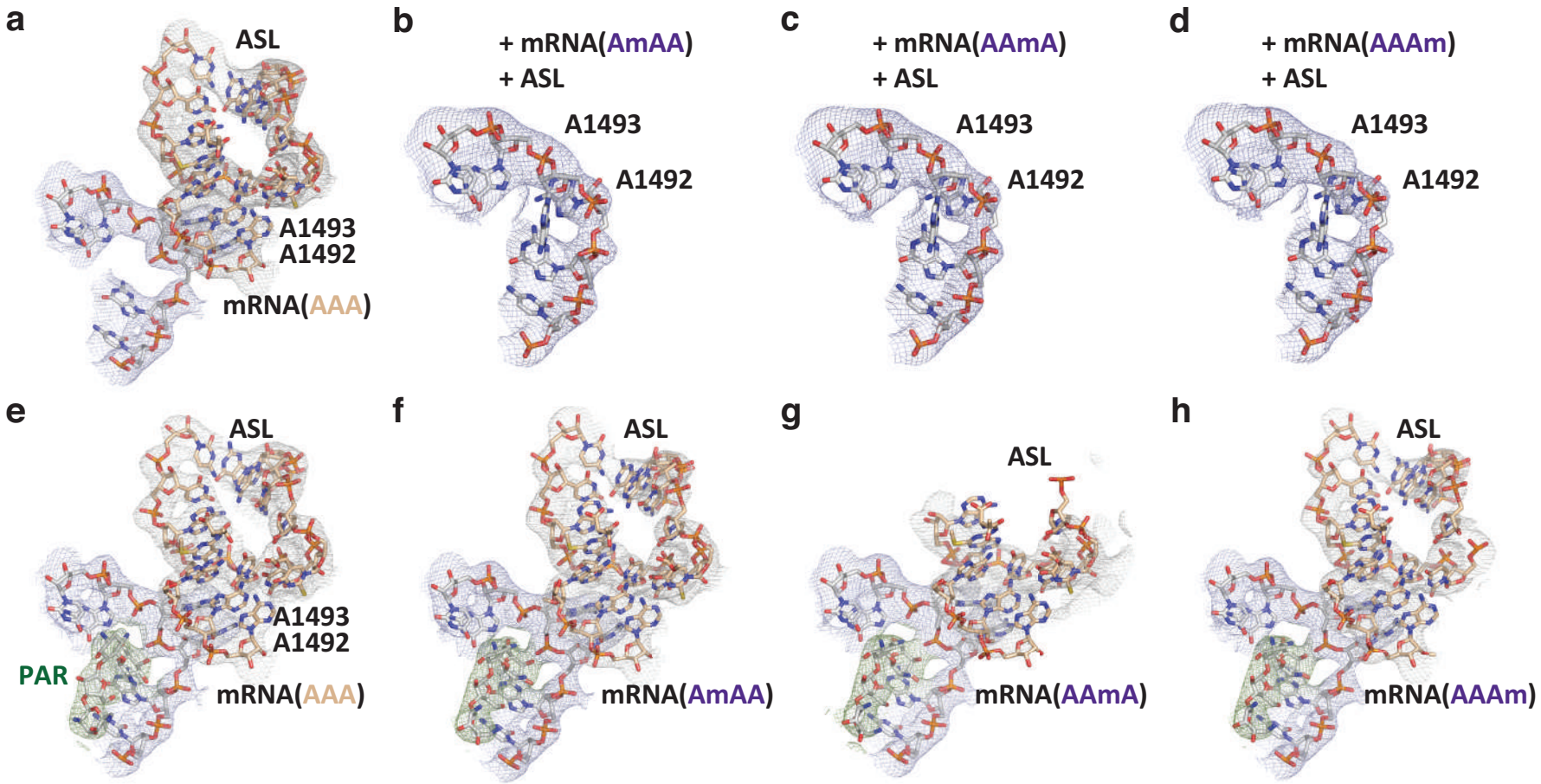


Structures of mRNA-ribosome-tRNA complexes: (AAA, AmAA, AAmA and AAAm, - paromomycin)



No density observed for tRNA and mRNA with Nm

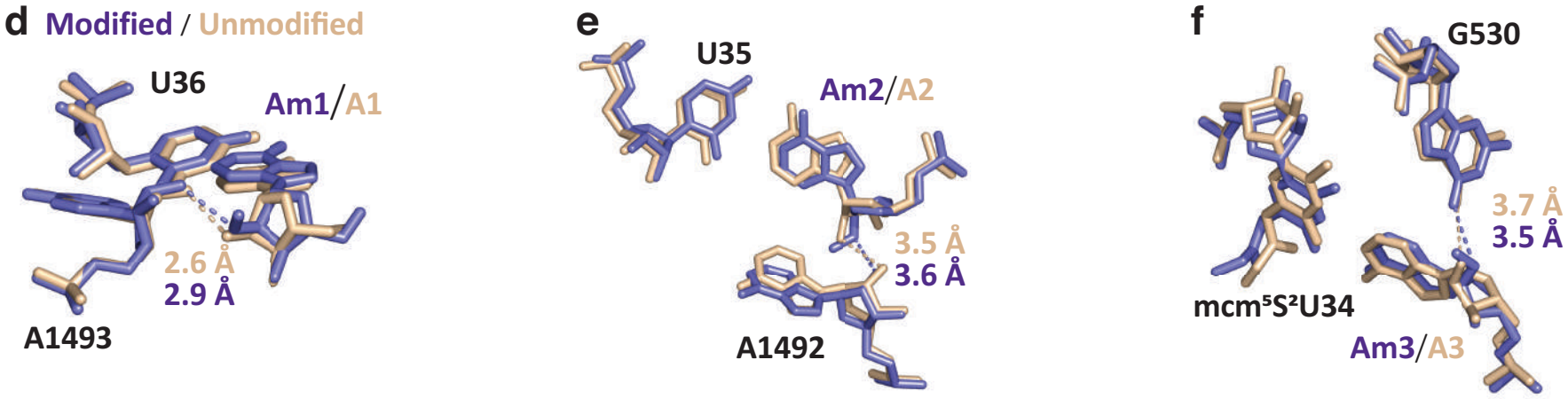
Density for tRNA-mRNA observed in presence of paromomycin



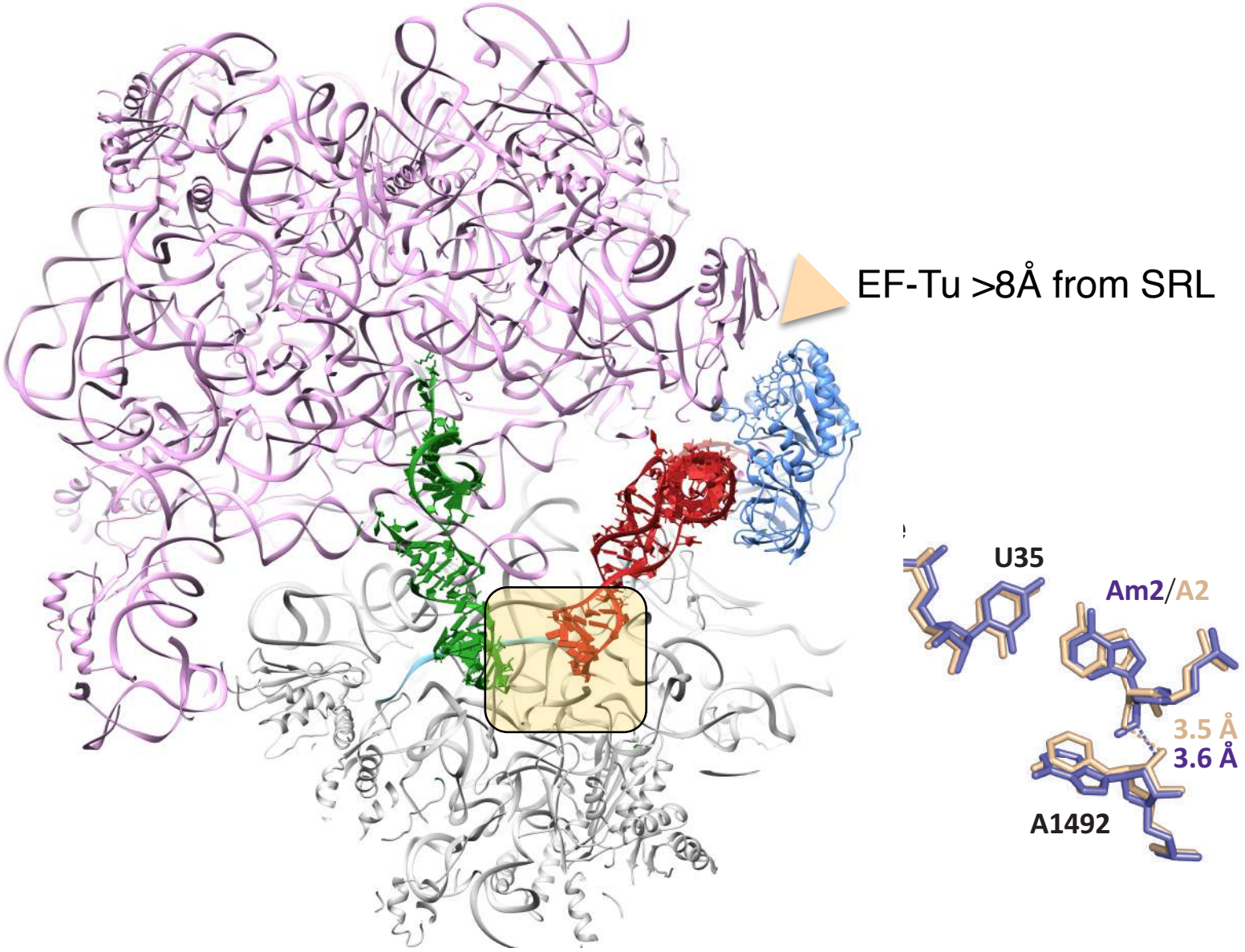
3.4-3.5Å resolution

Hasan Demirci, SLAC

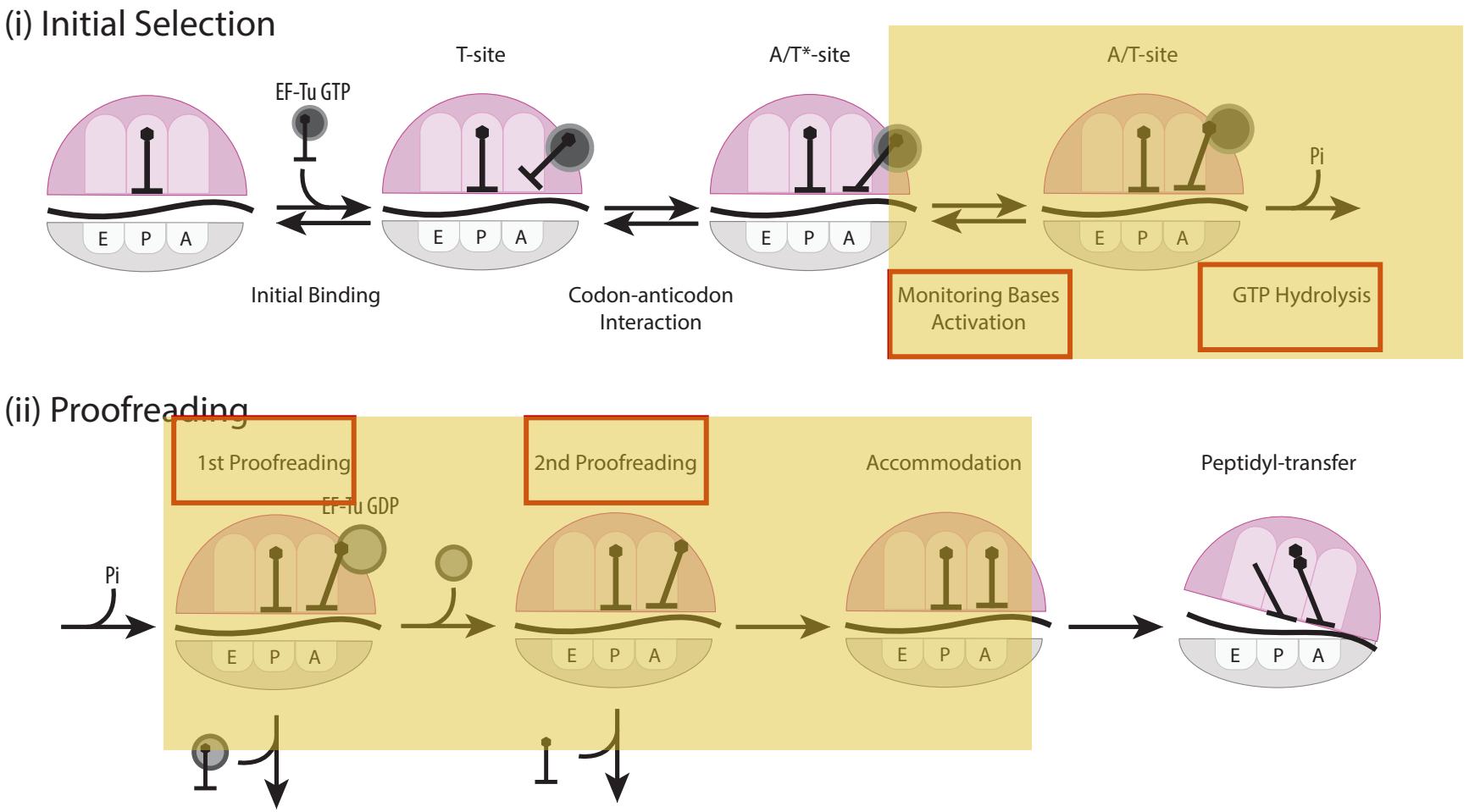
Nm leads to perturbed structures of the monitoring bases



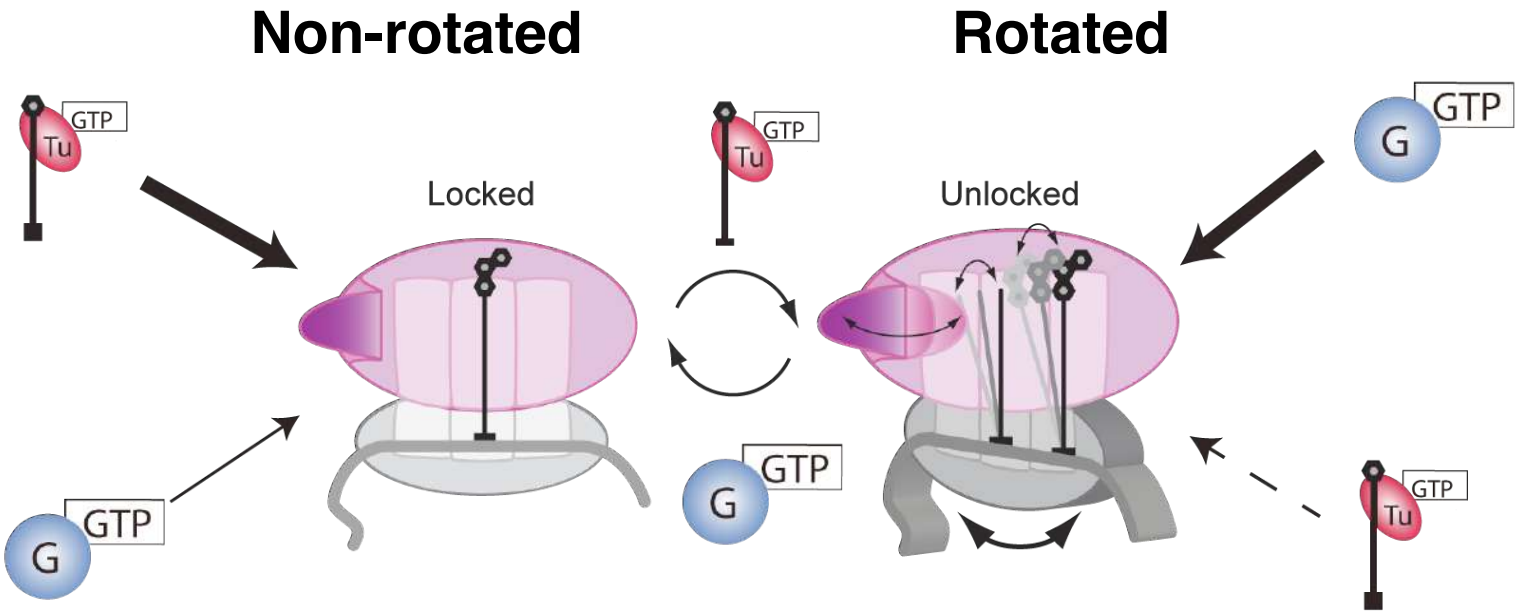
Nm disrupts long range allostery during selection



2'Omethyl mRNA disrupts tRNA selection



Pauses in translation are not the same



- rare codons
- modified codons
- nascent chain interactions

protein folding or processing

- secondary structures
- ribosomal interactions
- nascent chain interactions
  barriers to movement

recoding

Junhong Choi
Alexey Petrov
Albert Tsai
Guy Kornberg
Alex Johnson
Sean O'Leary
Rosslyn Grosely
Arjun Prabhakar

Hasan Demirci (SLAC)
Mike Soltis (SLAC)

NIGMS GM51266

Sweden
Mans Ehrenberg
Kaweng Leong
Gunnar von Heinje
Magnus Johansson

Israel
Gideon Rechavi
Dan Dominissini

Chicago
Chuan He

Ireland
John Atkins
Arthur Coakley
Michelle O'Connor

Suitcases and Bridges

Current Projects

Translation termination in bacteria and humans

Arjun Prabhakar
Michael Lawson

Mechanisms of translation initiation and its regulation

Jinfan Wang
Chris Lapointe
Alex Johnson

Role of RNA structure in translational control

Junhong Choi
Mark Capece

Human translation and disease

Rosslyn Grossly

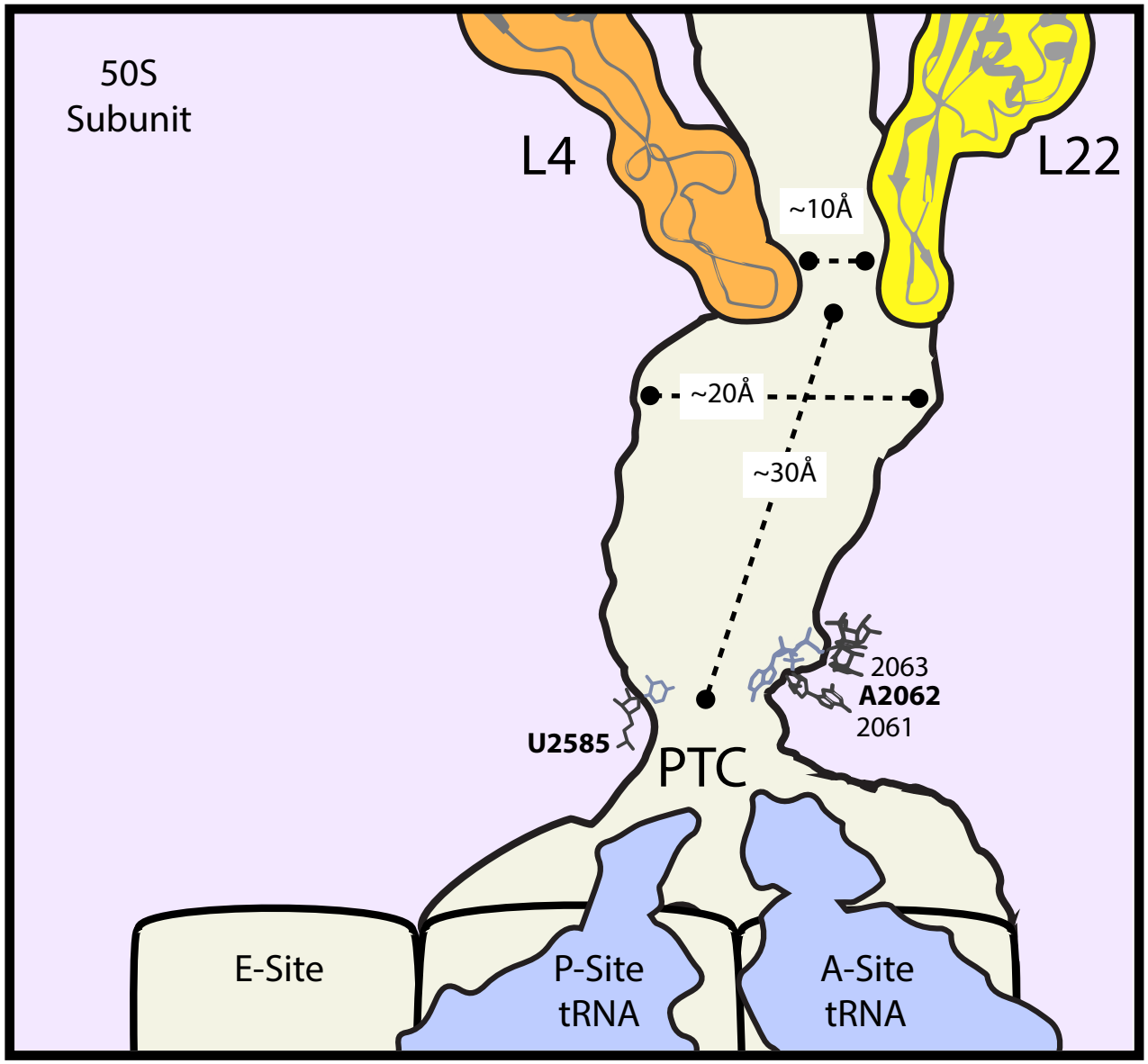
Early stages of HIV replication

Kevin Larsen
Aaron Coey
Junhong Choi

Factors affecting local translational dynamics

Nascent peptide sequence

Is the exit-tunnel a passive conduit?



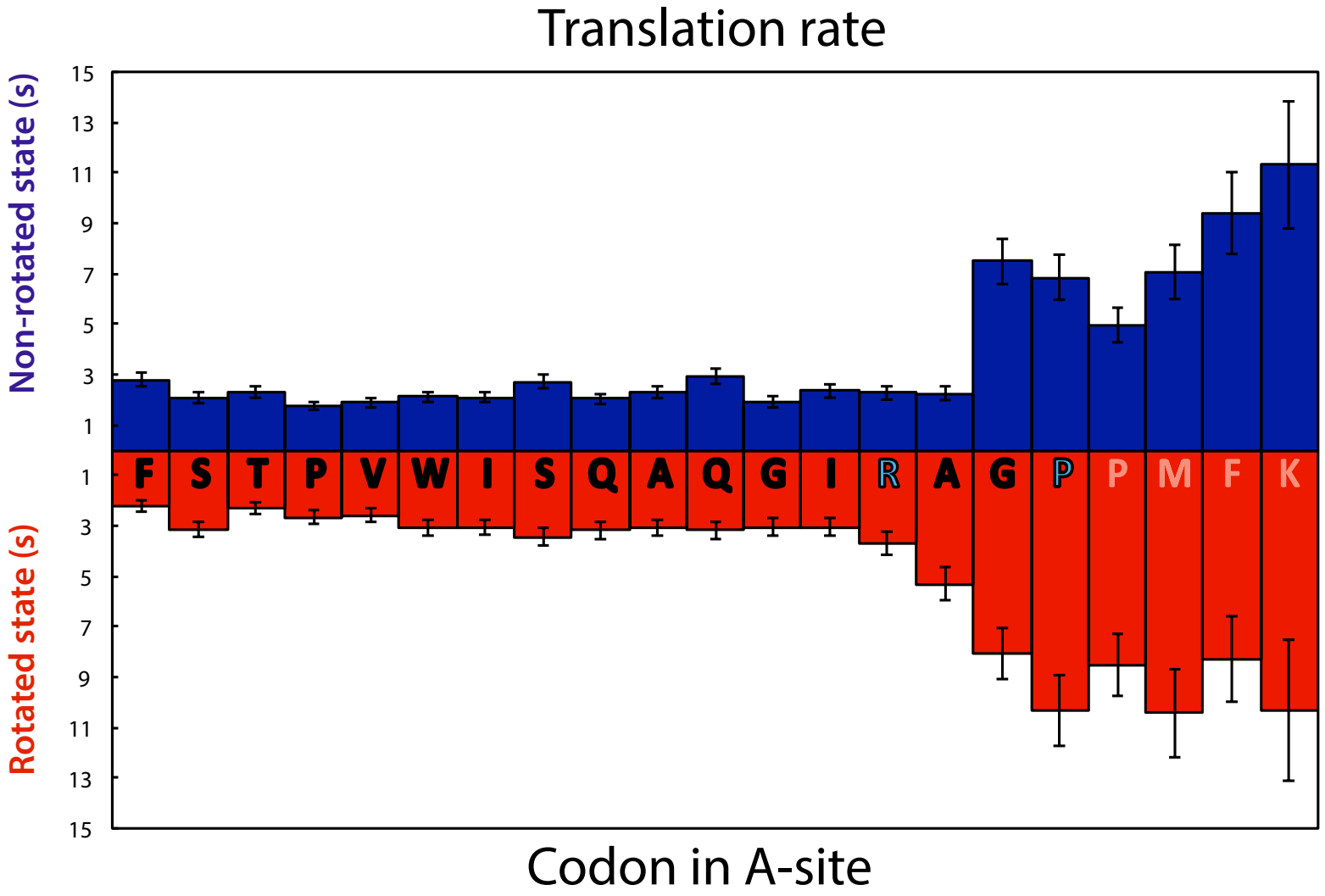
Nascent Chain Stalling Sequences

K. Ito et al. / Biochemical and Biophysical Research Communications 393 (2010) 1–5

---- *L22/L4 constriction* -------- ---------- *PTC* -----

	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	P	A	
Inducible class																						
1								Erythromycin +				**M**	**x**	**x**	**x**	**x**	**I**	**F**	**V**	**I**	*s*	ErmCL
2					Tryptophan +			**W**	**x**	**x**	**x**	**D**	**x**	**x**	**I**	**x**	**x**	**x**	**x**	**P**	*	TnaC *Ec*
3					Tryptophan +			**W**	**x**	**x**	**x**	**D**	**x**	**x**	**L**	**x**	**x**	**x**	**x**	**P**	*k*	TnaC *Pv*
Intrinsic class																						
4					**F**	**x**	**x**	**x**	**x**	**W**	**I**	**x**	**x**	**x**	**x**	**G**	**I**	**R**	**x**	**G**	**P**	SecM *Ec*
5					**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**P**	***P***	**I**	**R**	**x**	**G**	**P**	SecM mut
6				**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**H**	**A**	***P***	**I**	**R**	**G**	**S**	**P**	SecM *Ms*
7												**F**	**x**	**x**	**Y**	**x**	**I**	**W**	**P**	**P**	**P**	Evolved
8	**R**	**I**	**x**	**x**	**W**	**I**	**x**	**x**	**x**	**x**	**x**	**M**	**N**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	MifM *Bs*

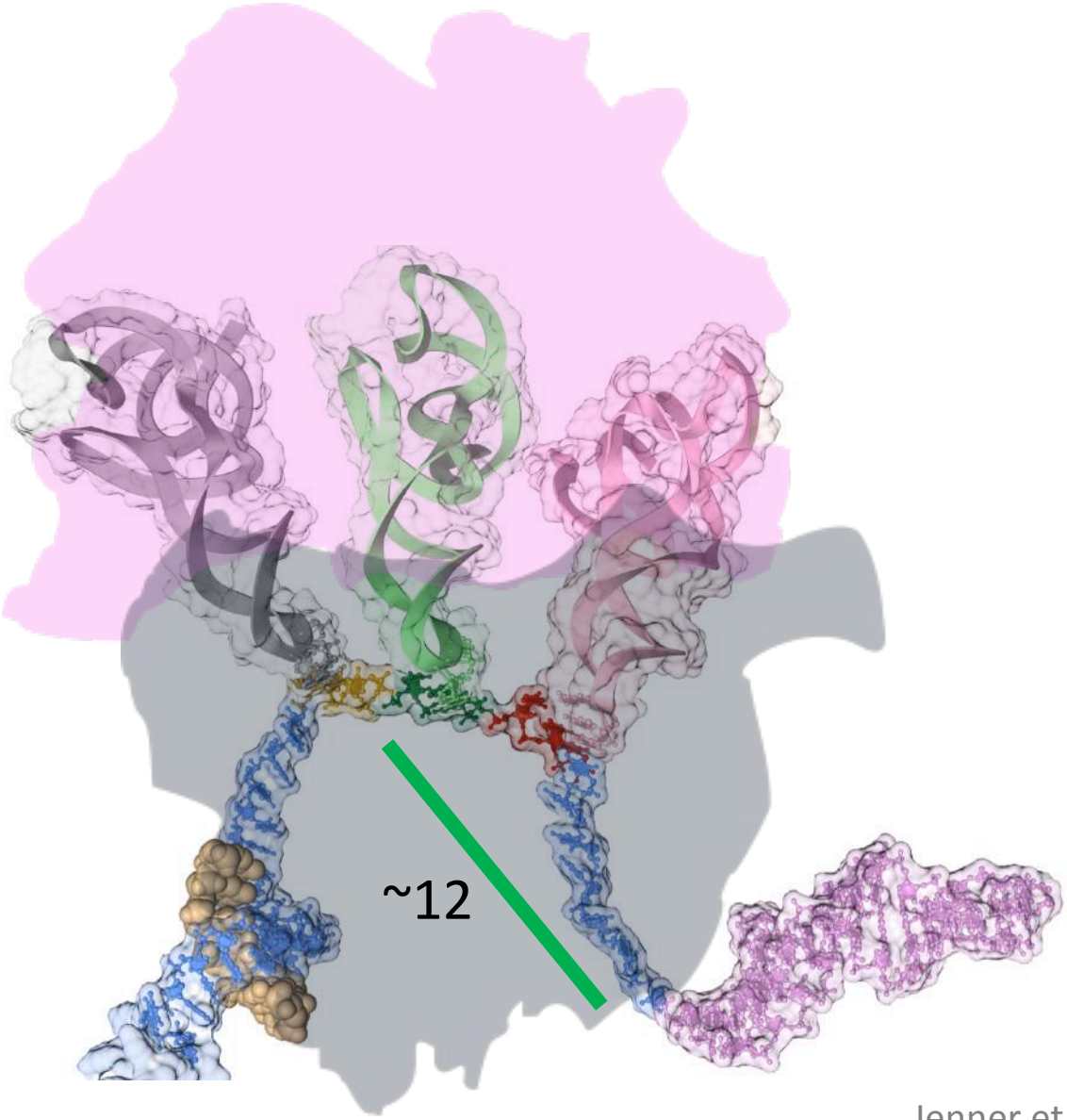
SecM nascent chain interactions cause complex translational pausing



Factors affecting local translational dynamics

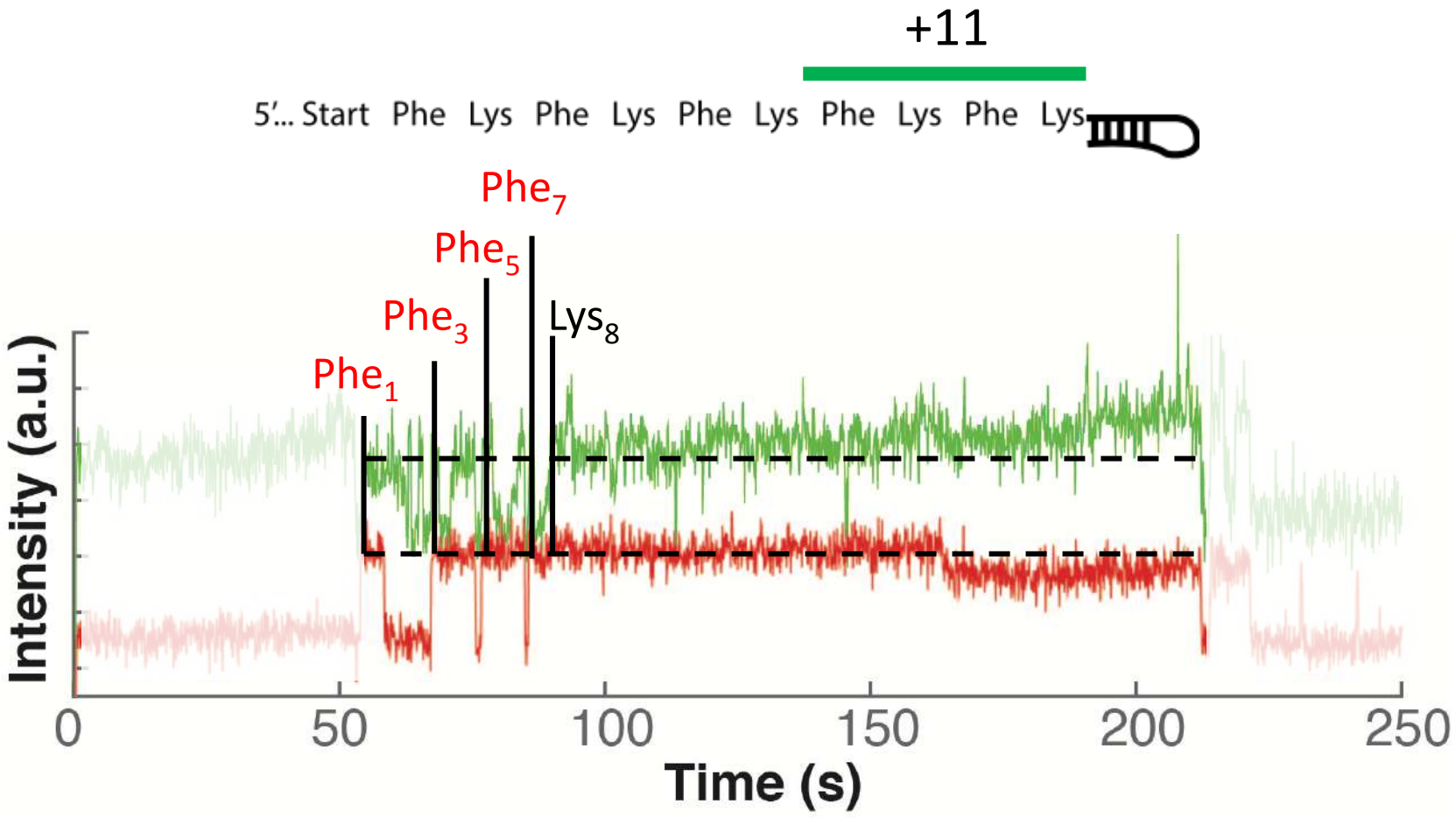
mRNA structure

How does mRNA structure affect translation?

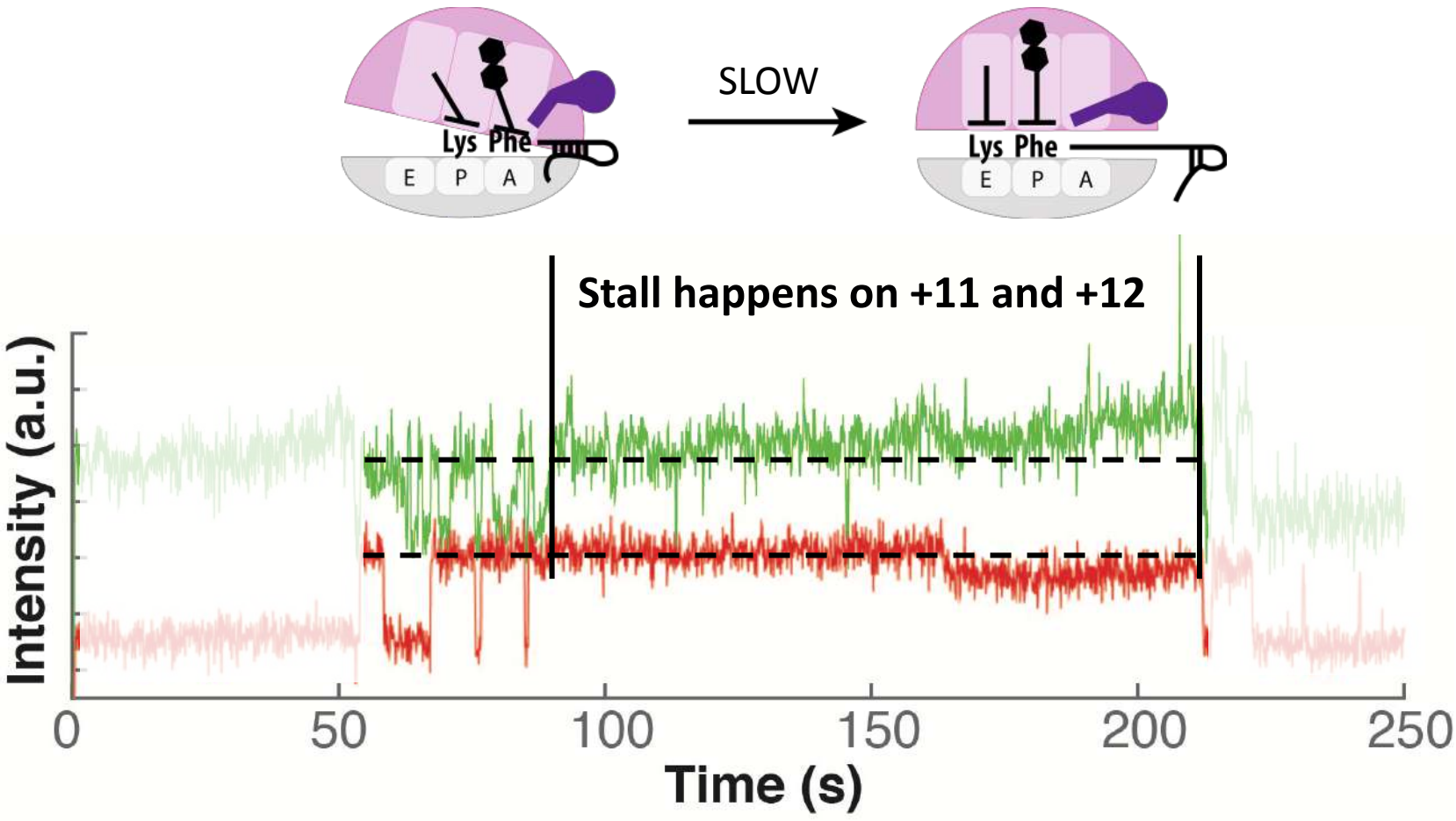


Jenner et al, *NSMB* **2010**

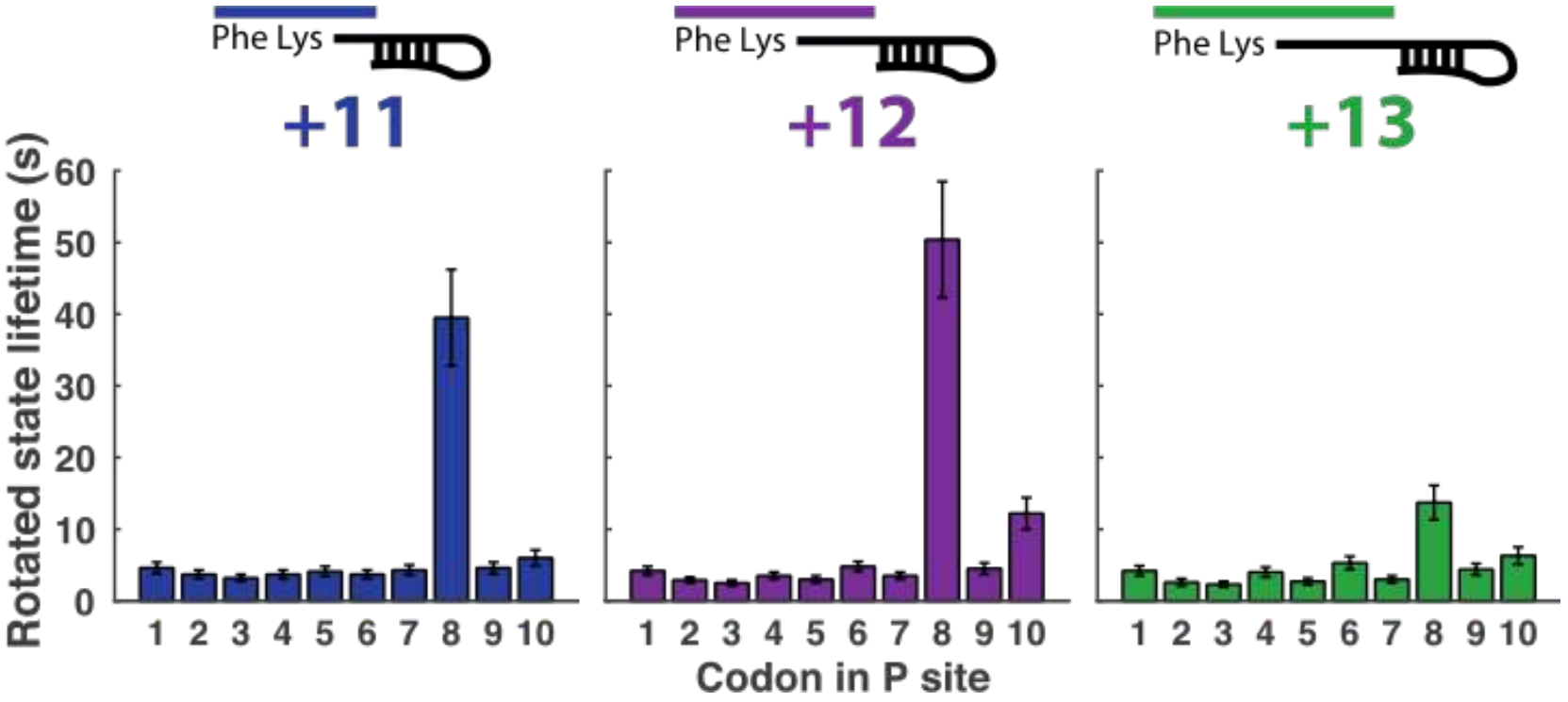
When does ribosome "feel" mRNA structure?



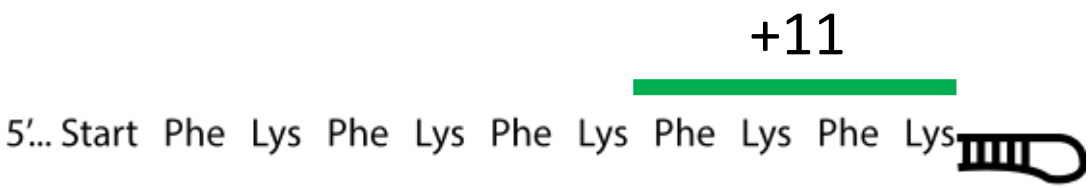
When does ribosome "feel" mRNA structure?

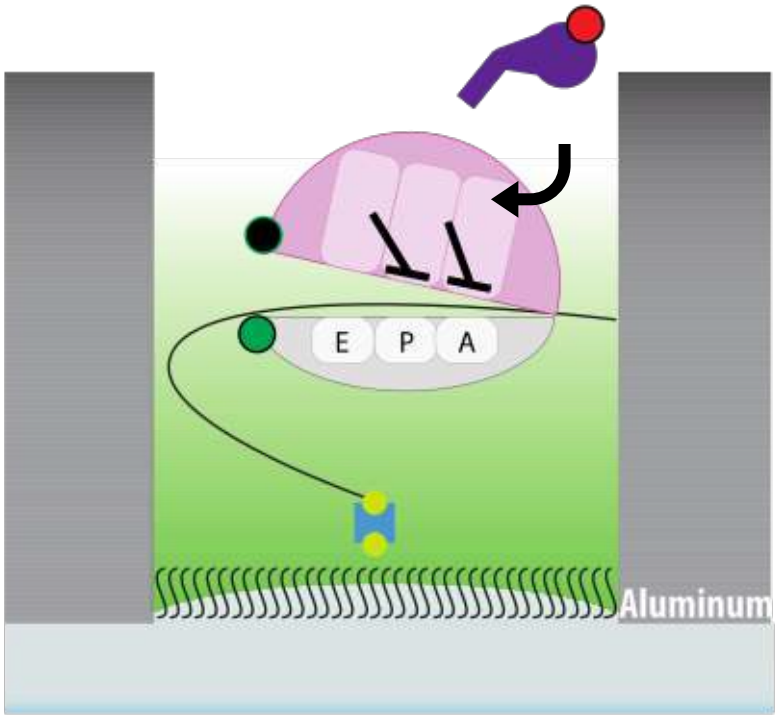


Hairpins cause pausing in a position-dependent manner

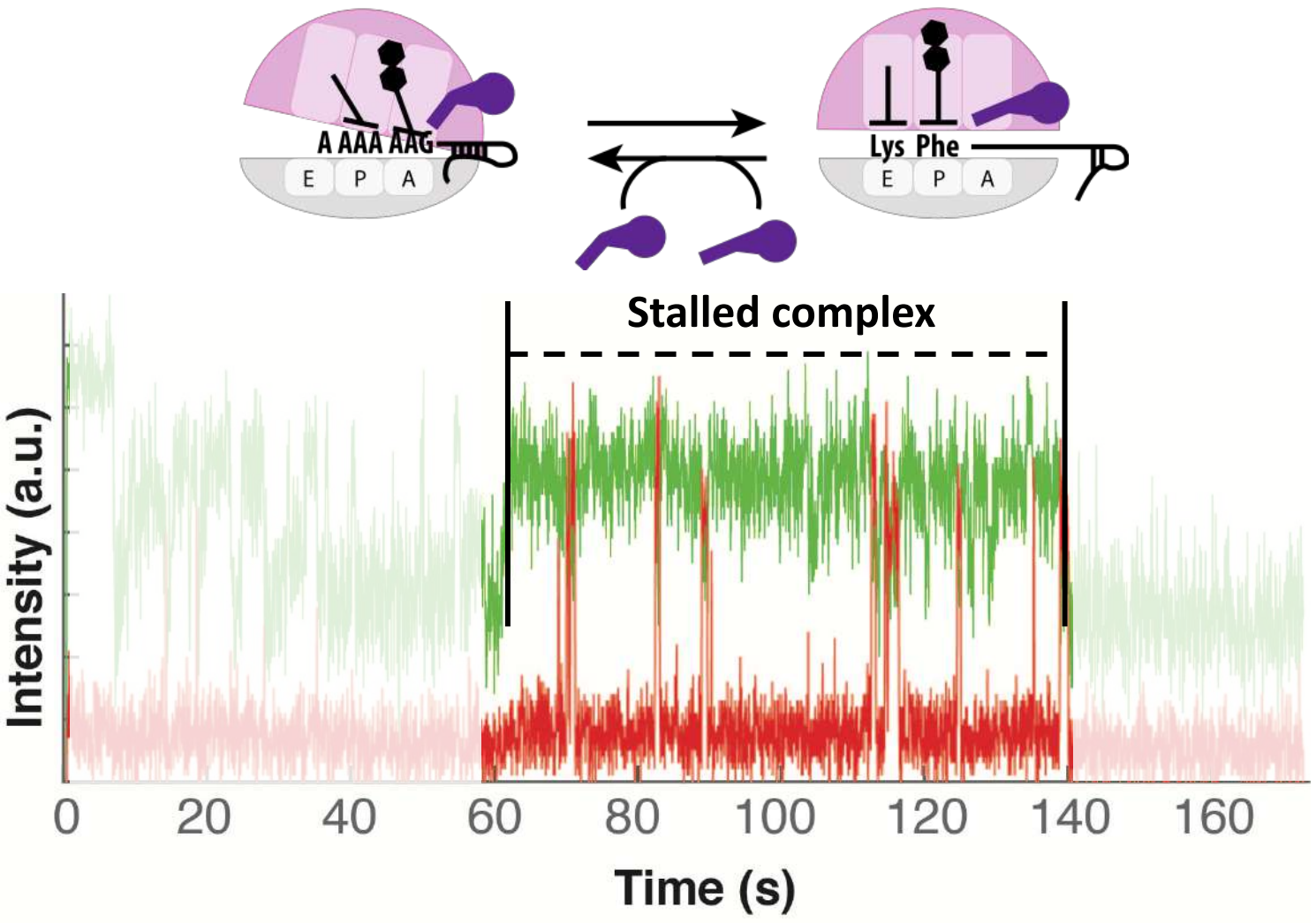


What happens during pause?

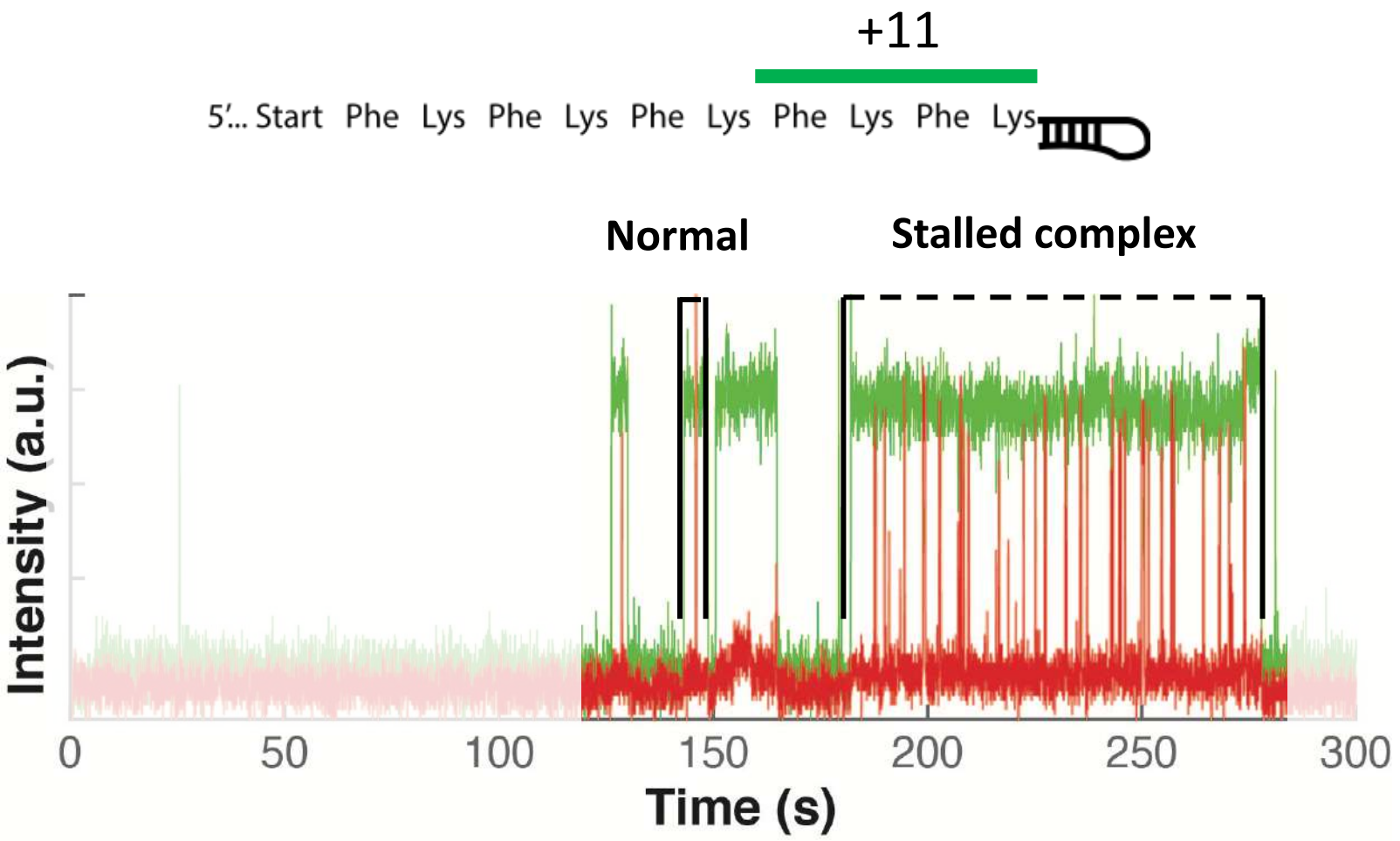




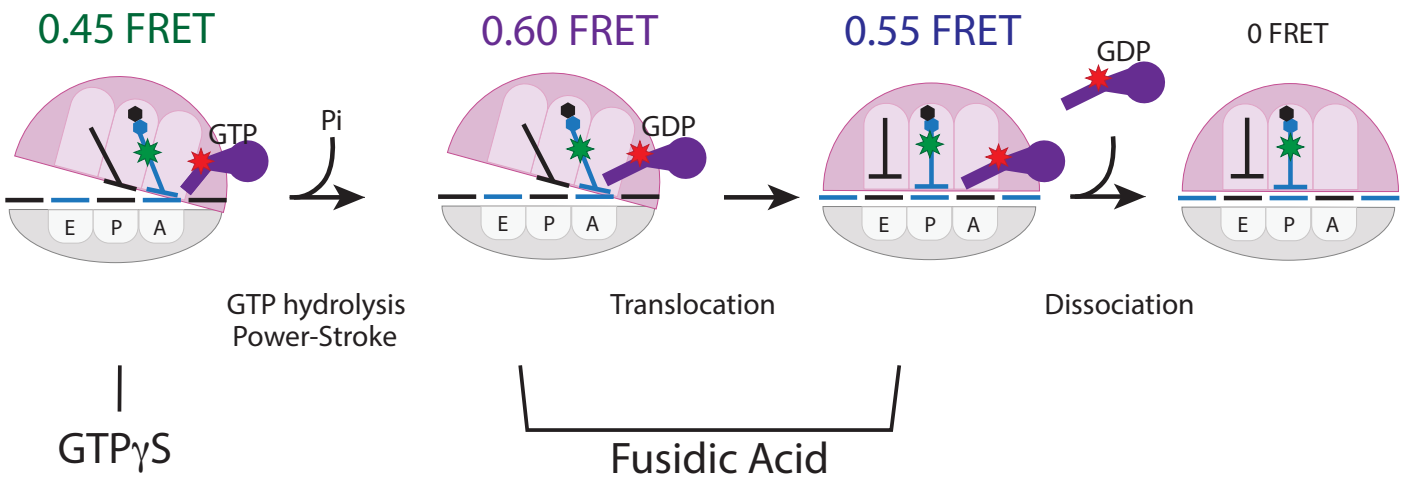
Multiple EF-G samples paused complex

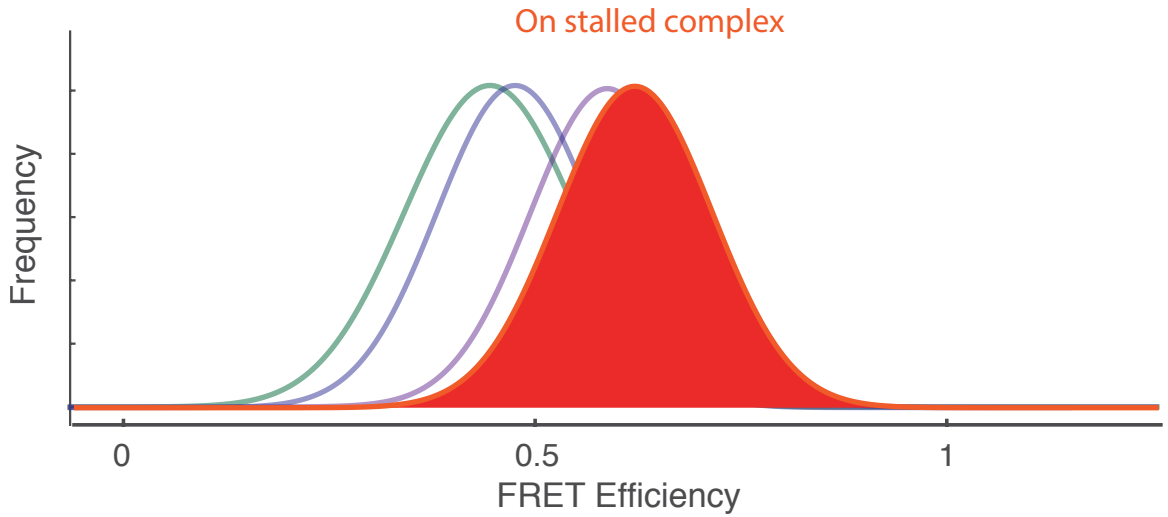


tRNA-EF-G FRET monitors EF-G mechanism



Hairpins cause futile cycles of EF-G GTP hydrolysis and power stroke

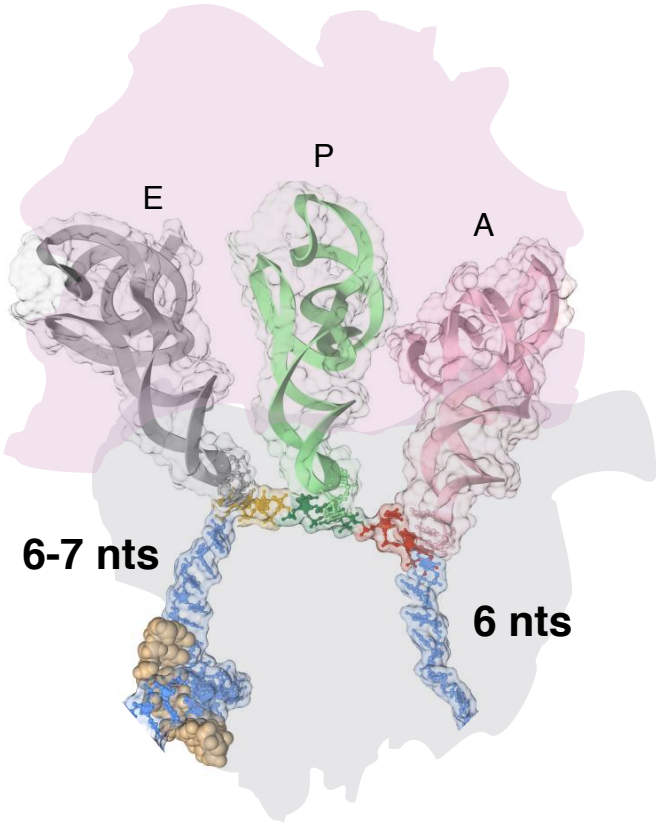


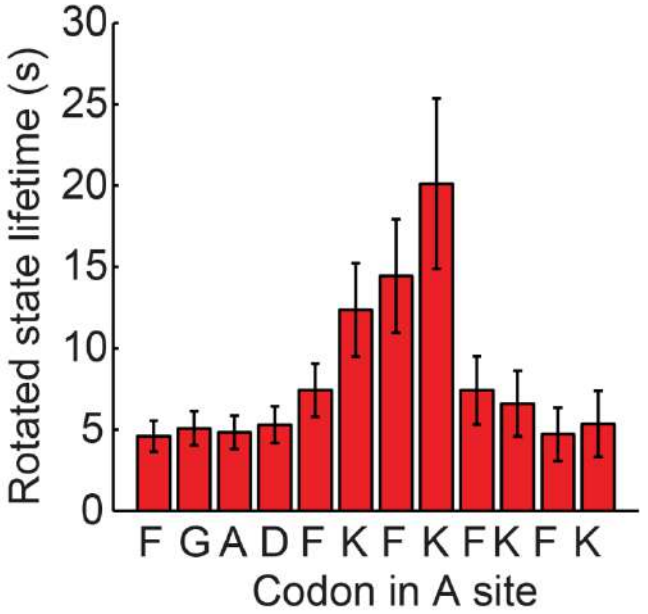


RNA structures are barriers to movement

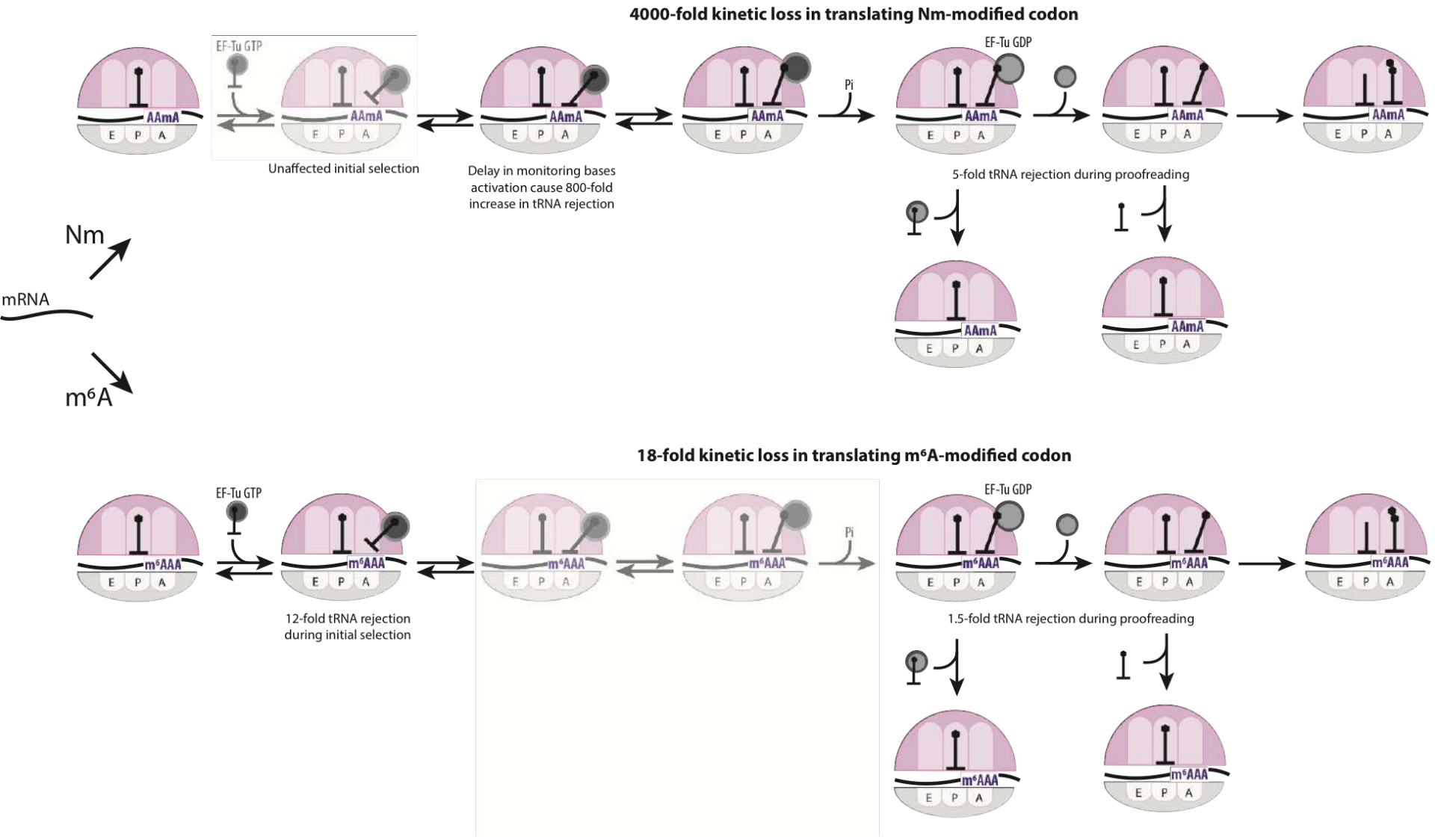
- hairpins cause ribosomal pausing, but only at the base of a short structure
- ribosome is a processive helicase
- pauses occur in the pretranslocation rotated state (increased energetic barrier to movement)
- EF-G uses futile cycles of GTP hydrolysis during the pause (loss of efficient coupling of energy to movement)
- These paused rotated states are preludes to translational recoding

mRNA sequences (Shine-Dalgarno) slow translocation





mRNA modifications modulate translation in different ways

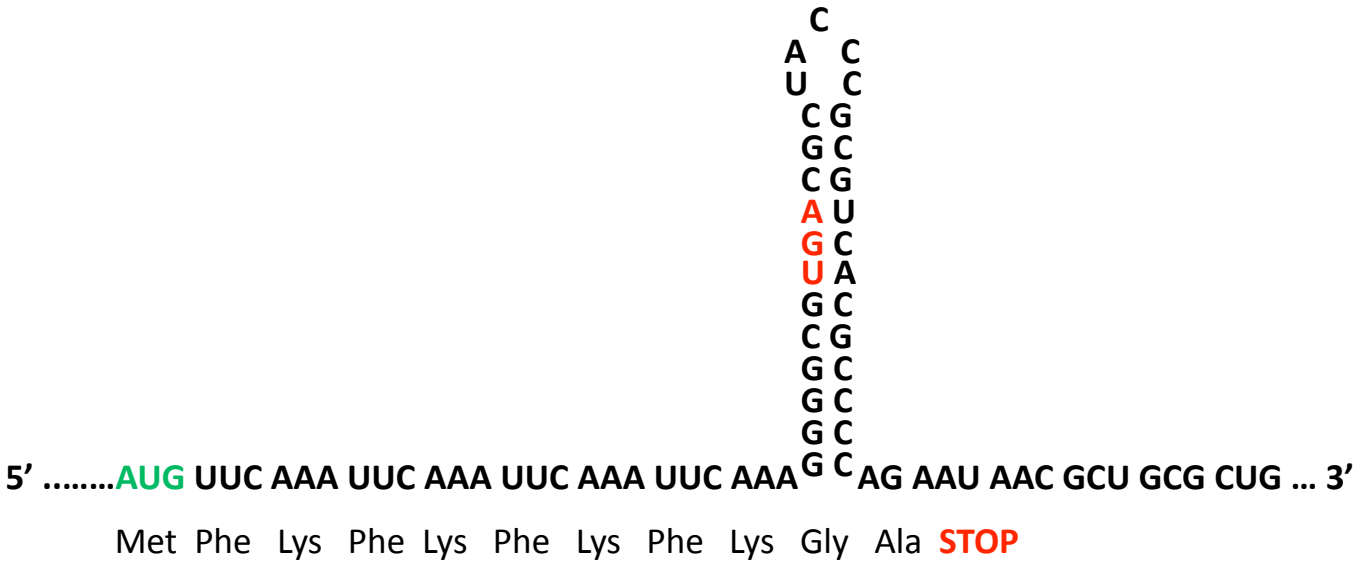


Factors affecting local translational dynamics

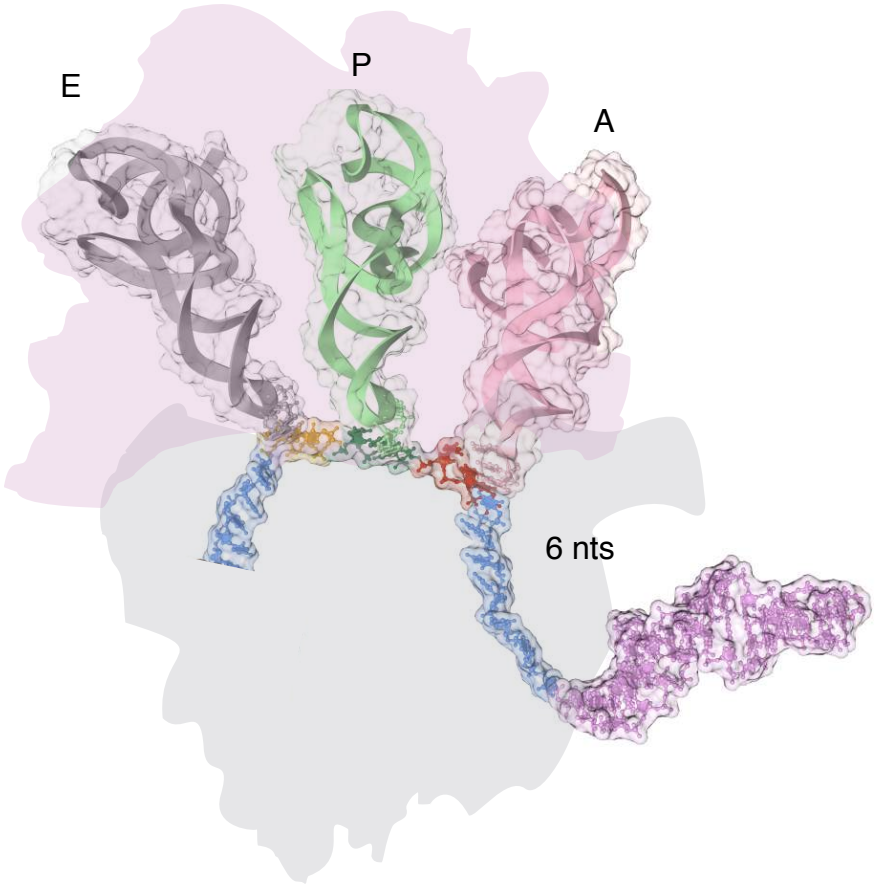
2. mRNA structure

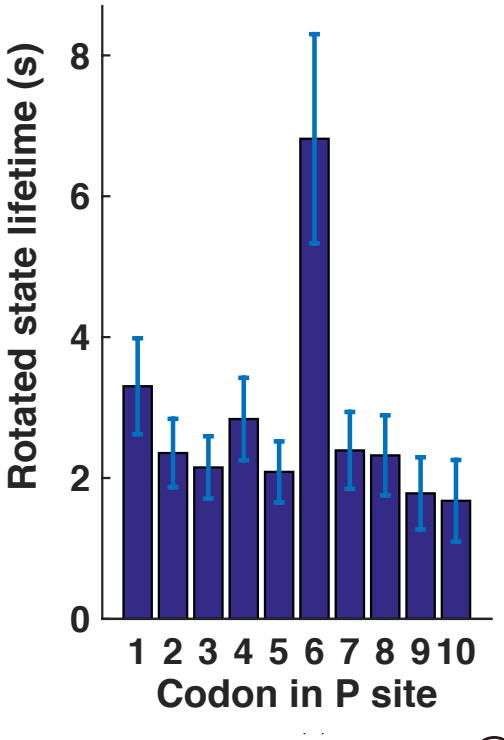
Modulation of translation by mRNA secondary structure

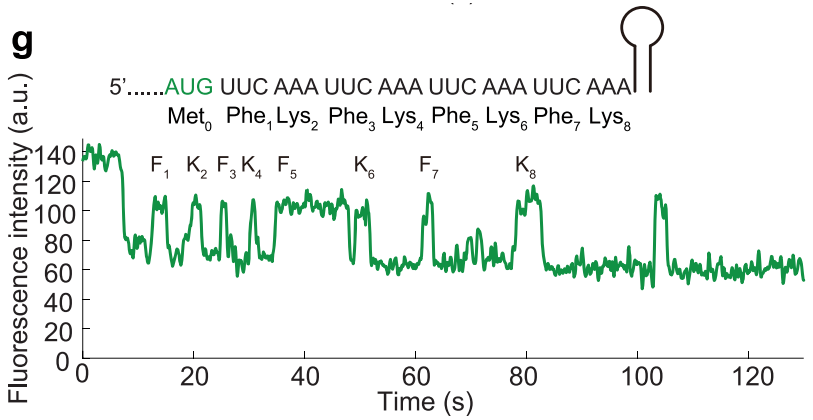
FK sequence with hairpin



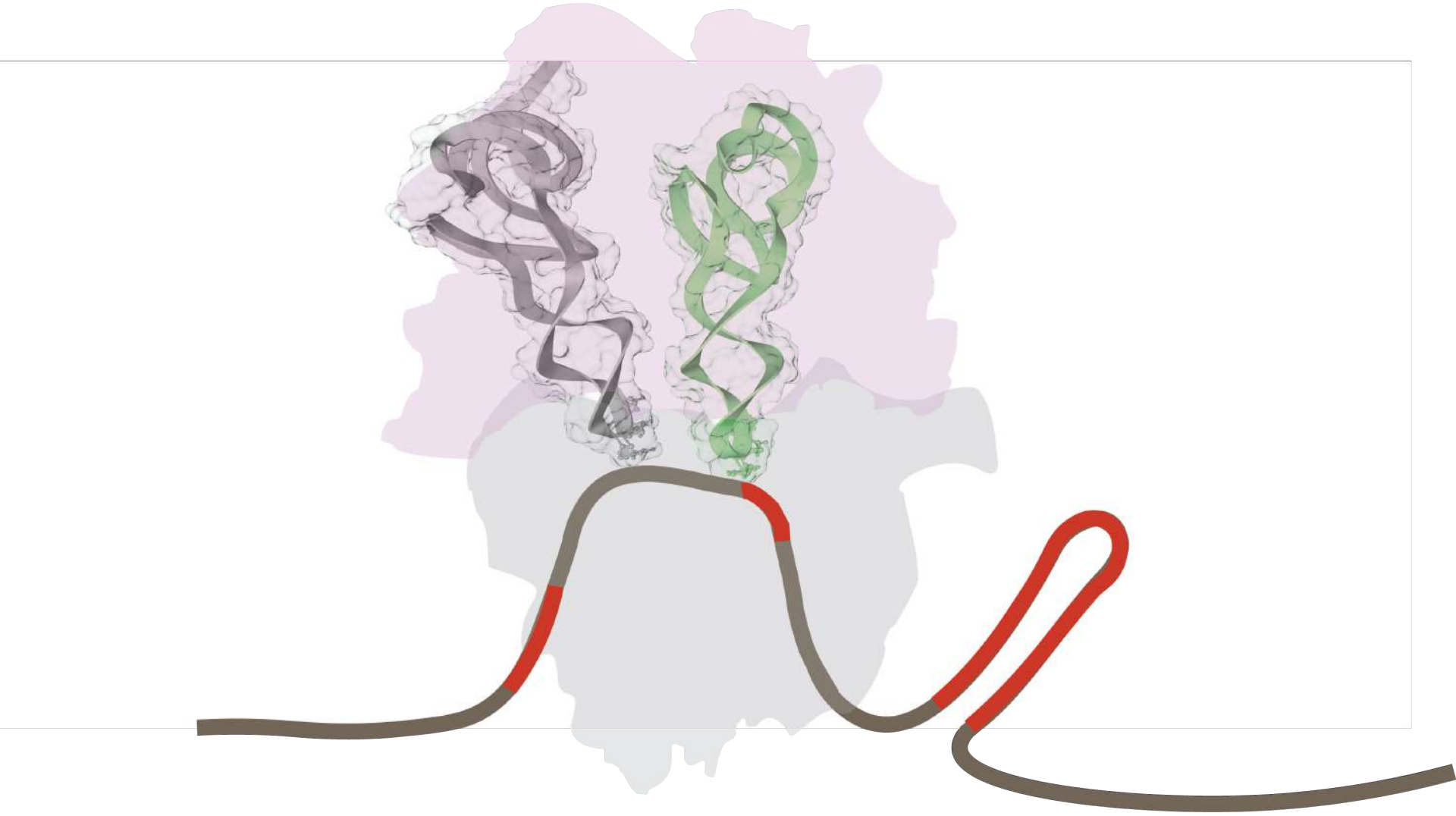
Hairpins are barriers to translocation…..



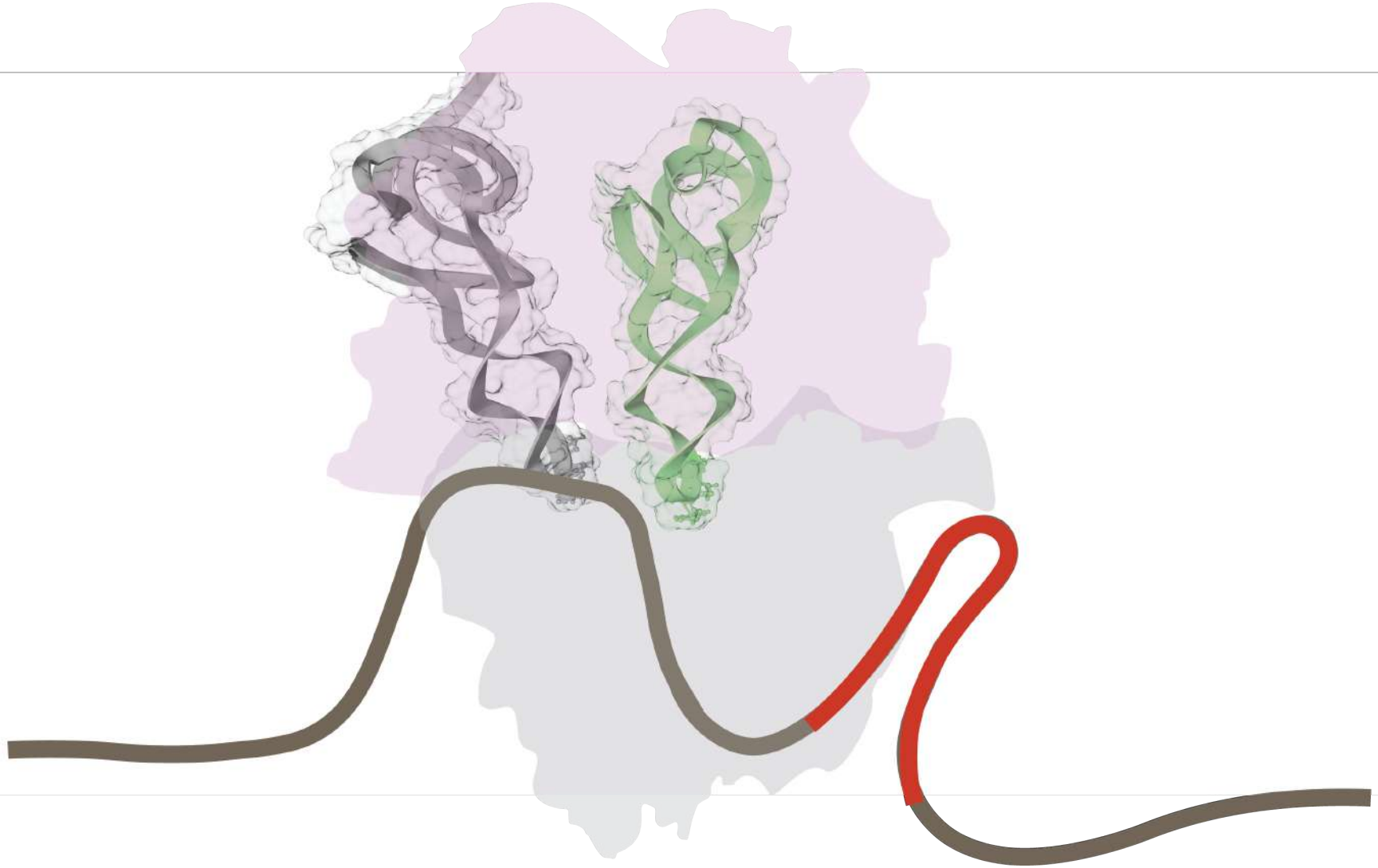




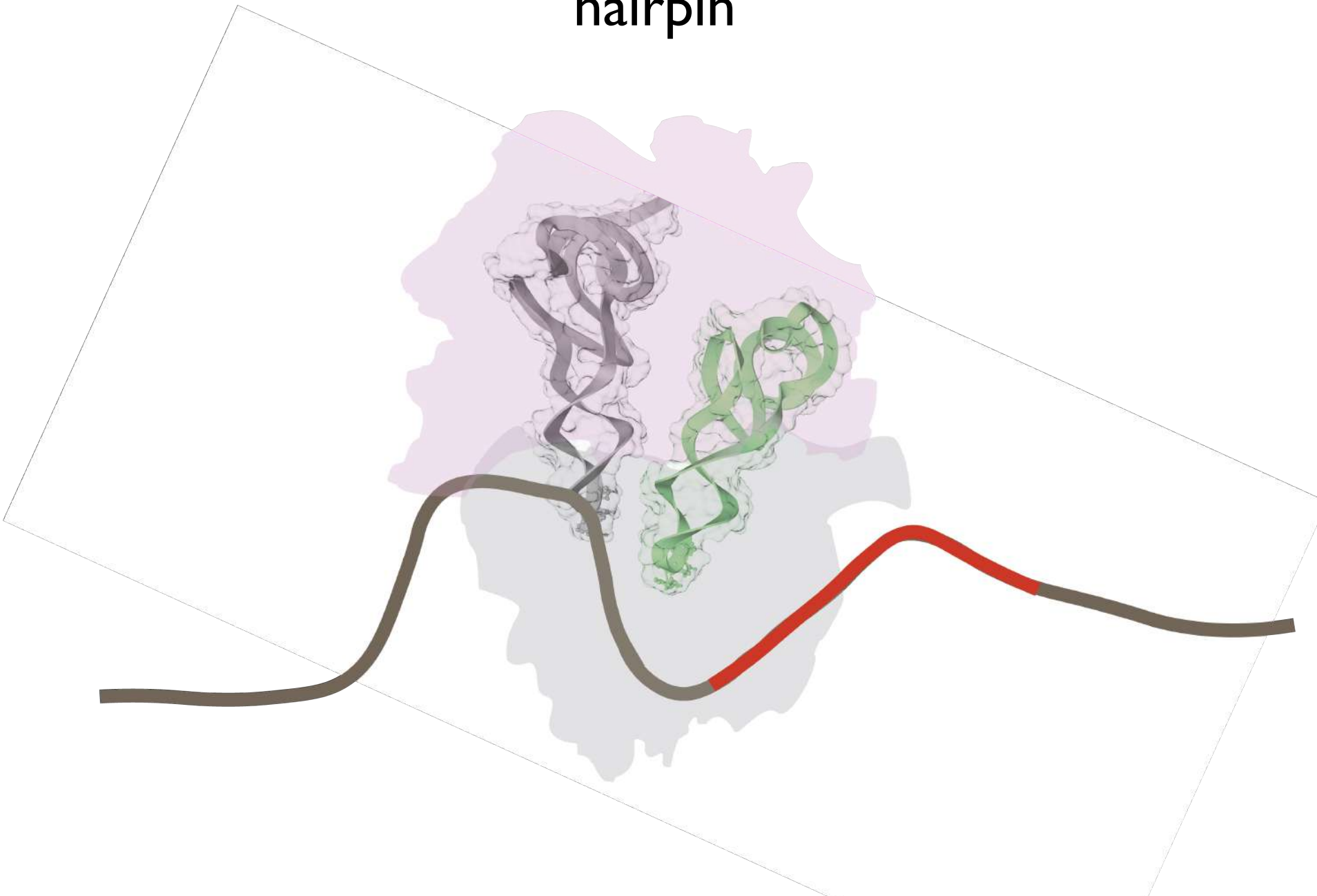
Ribosomes pause only at the base of the hairpin



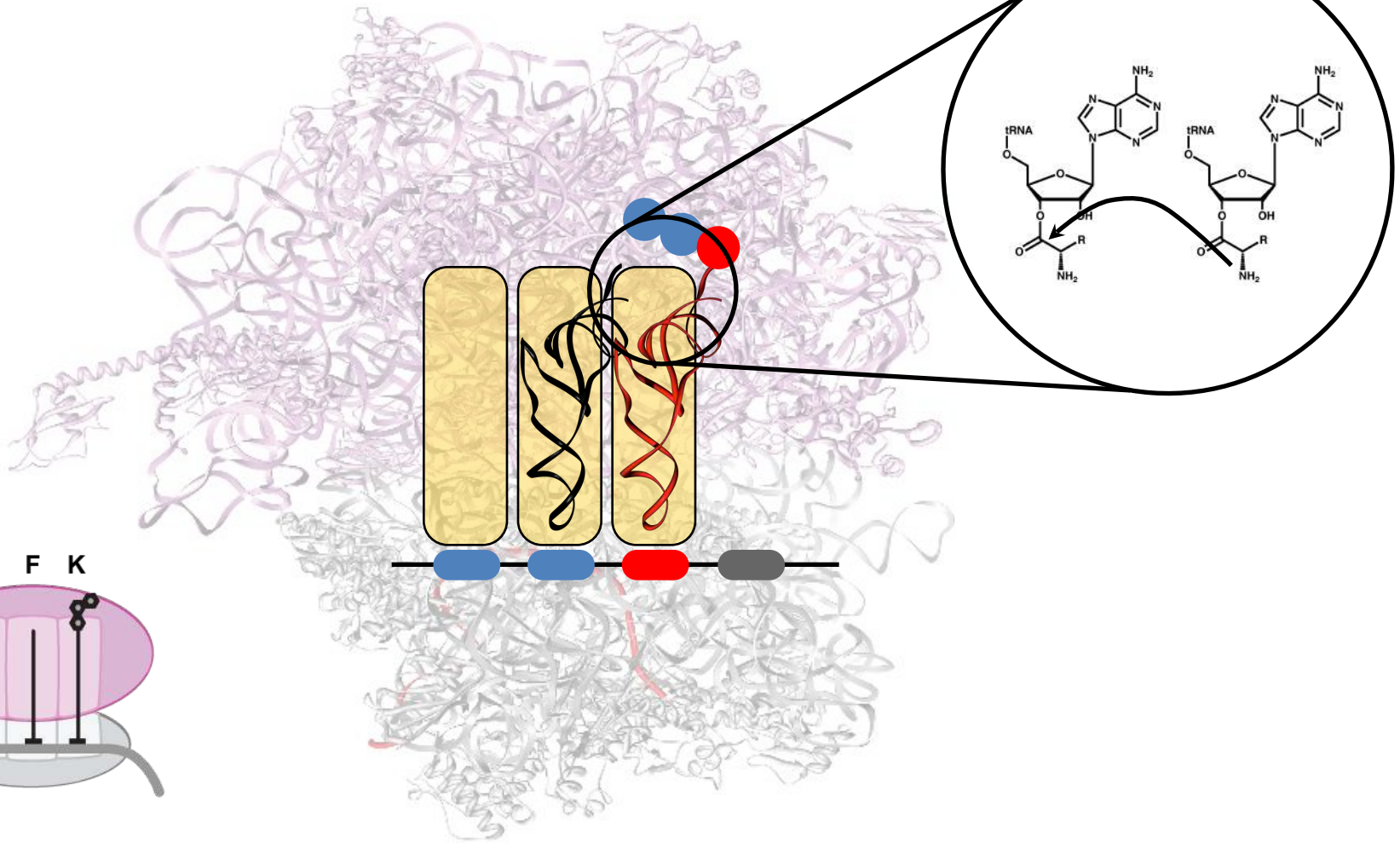
Ribosomes pause only at the base of the hairpin



Ribosomes pause only at the base of the hairpin

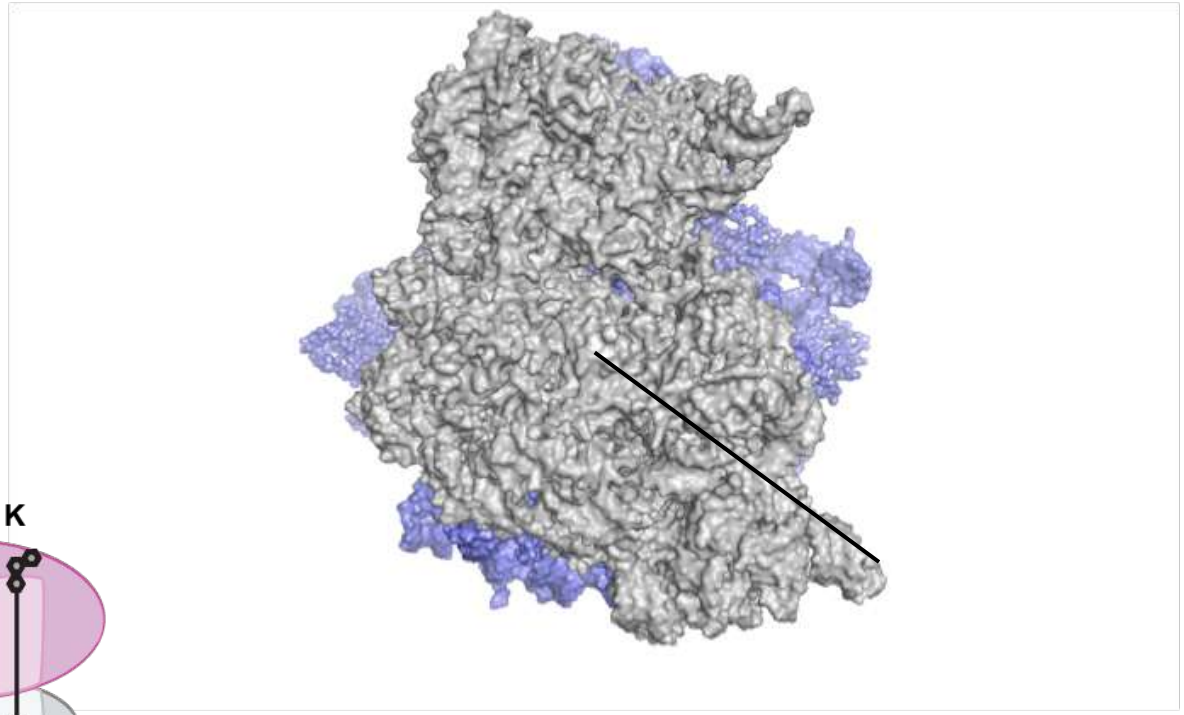


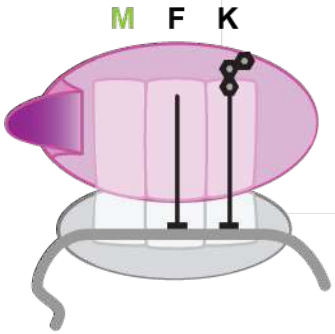
Peptide bond formation



Non-rotated

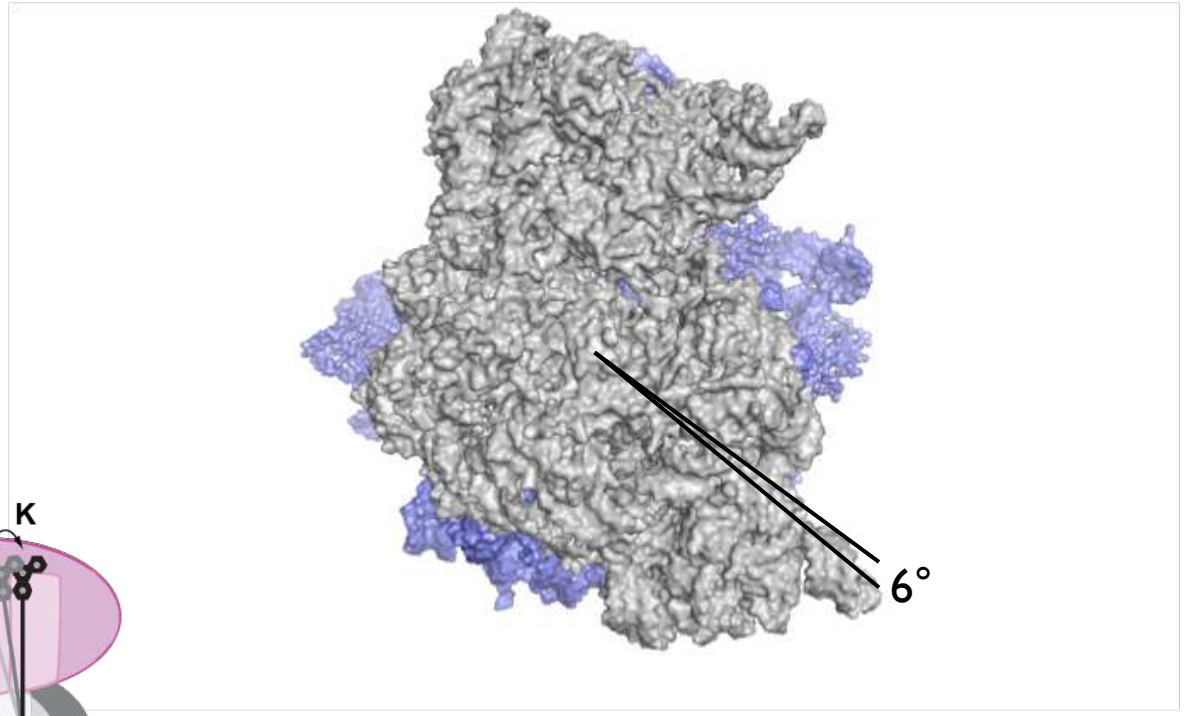
Subunit rotation

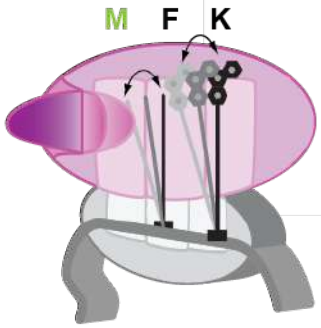




Non-rotated

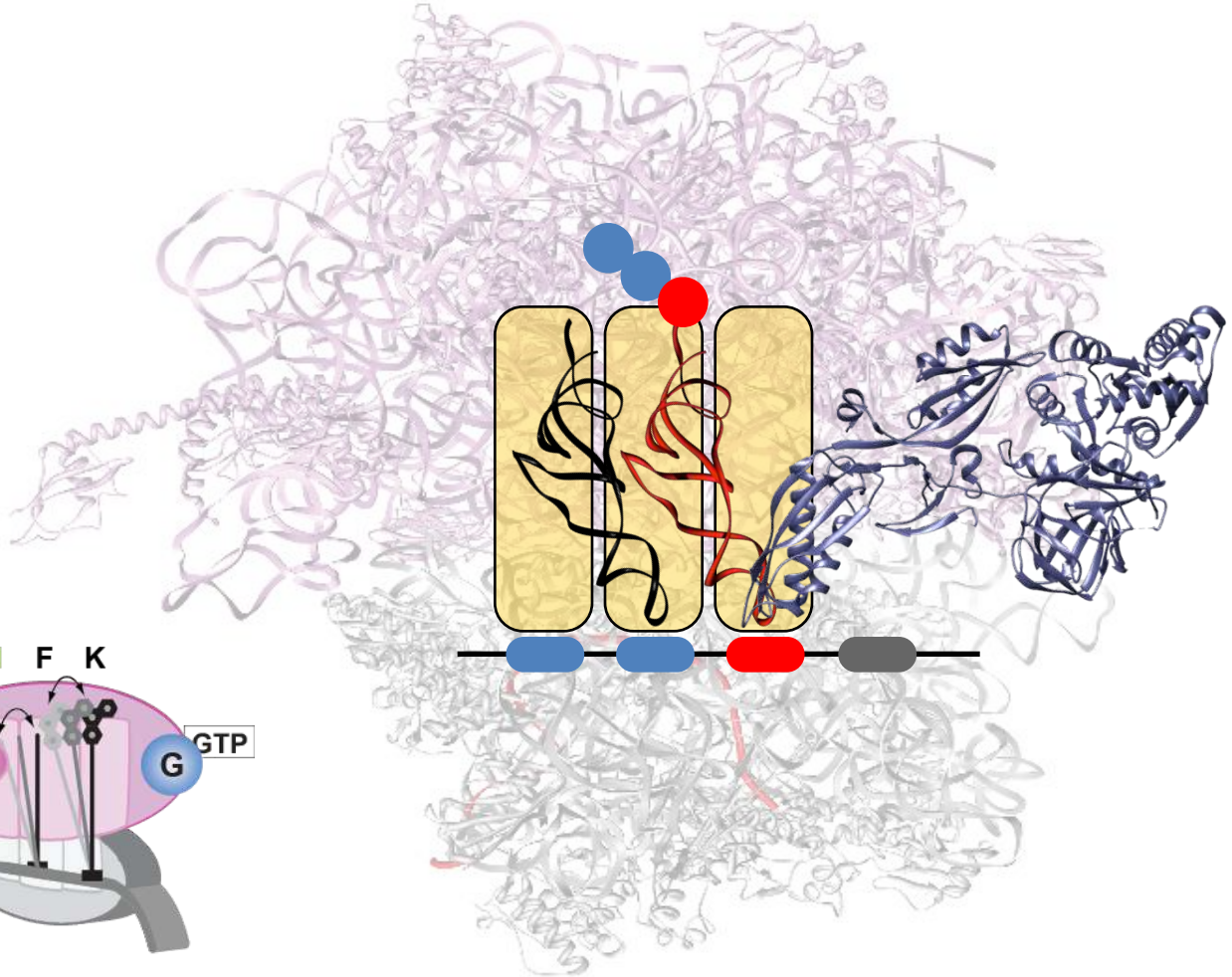
Subunit rotation





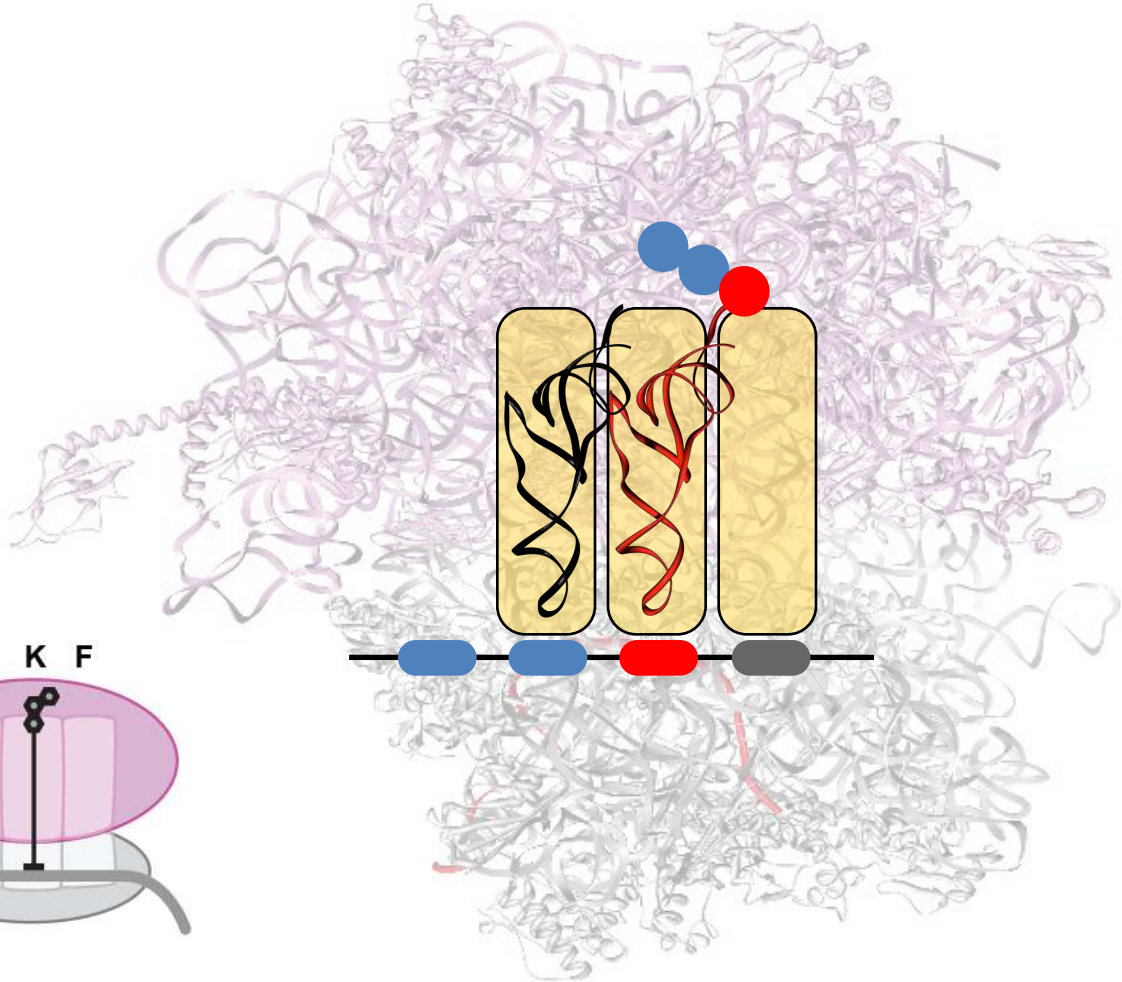
Rotated

Preparation for translocation



Rotated

Translocation

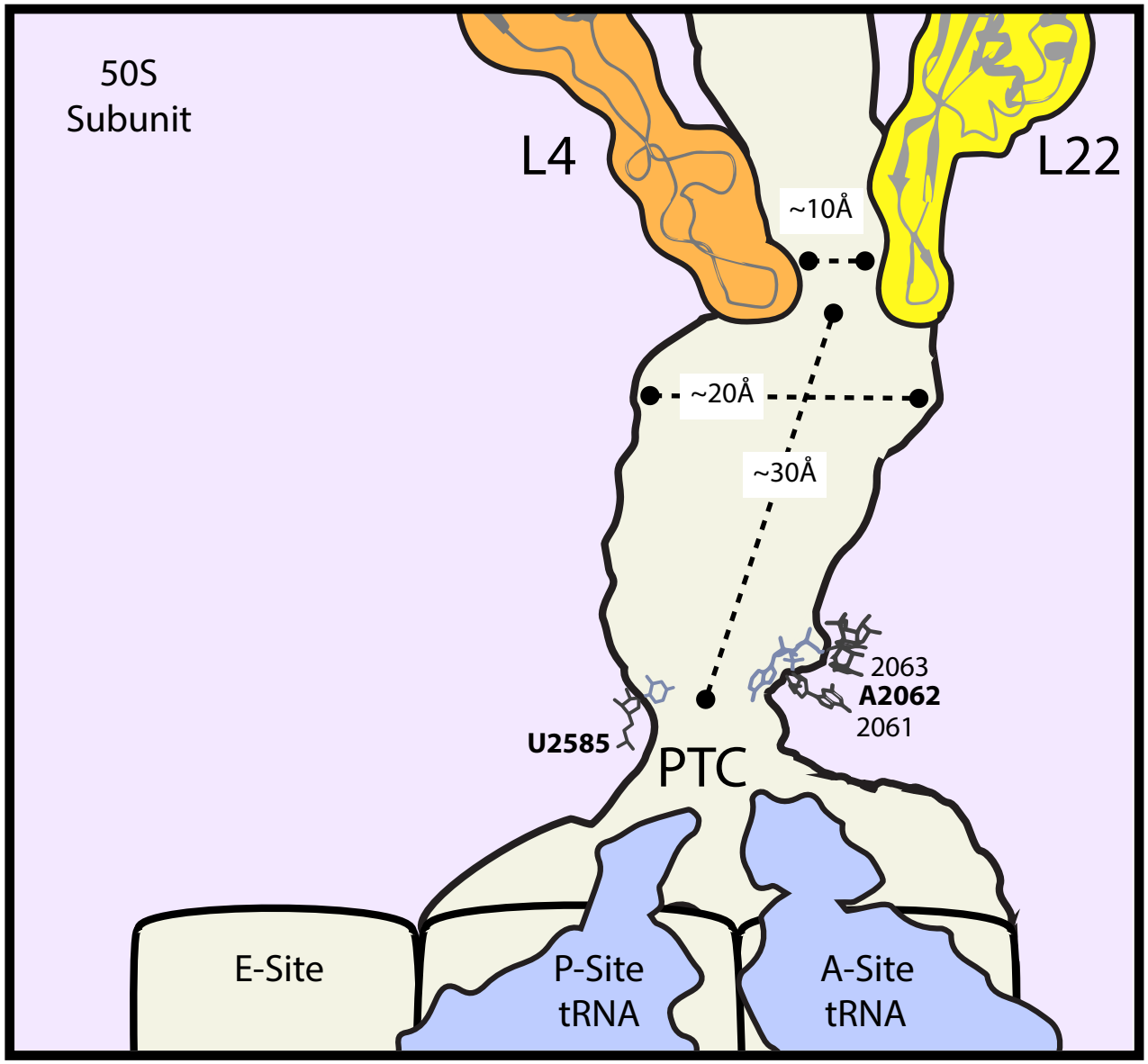


Non-rotated

Factors affecting local translational dynamics

3. Nascent peptide sequence

Is the exit-tunnel a passive conduit?



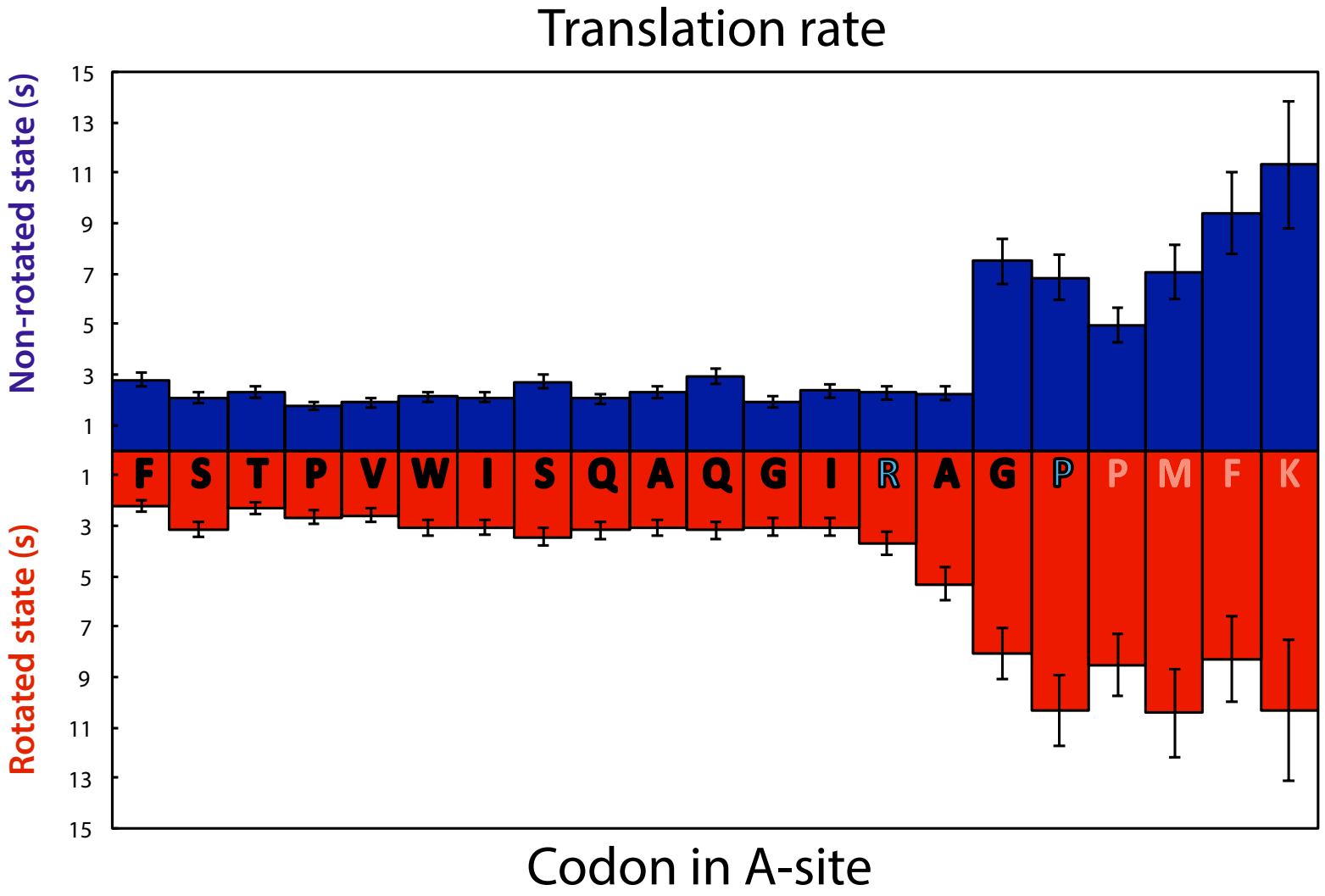
Nascent Chain Stalling Sequences

K. Ito et al. / Biochemical and Biophysical Research Communications 393 (2010) 1–5

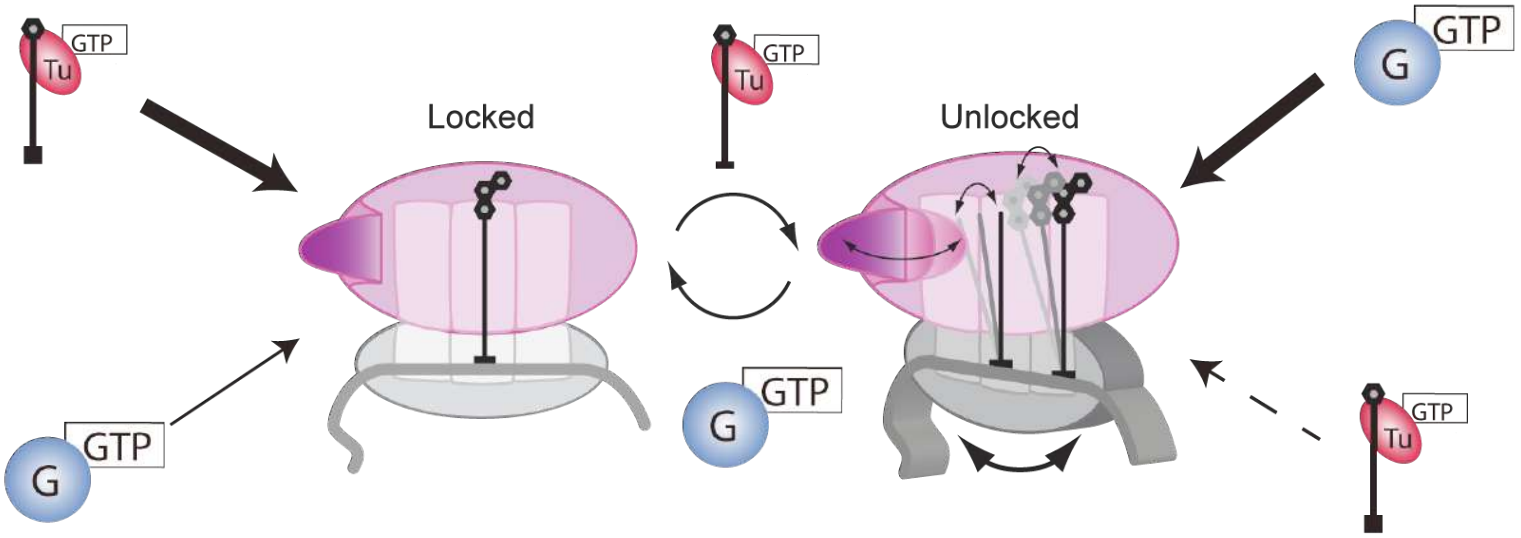
---- *L22/L4 constriction* -------- ---------- *PTC* -----

	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	P	A	
Inducible class																						
1								Erythromycin +				**M**	**x**	**x**	**x**	**x**	**I**	**F**	**V**	**I**	*s*	ErmCL
2					Tryptophan +			**W**	**x**	**x**	**x**	**D**	**x**	**x**	**I**	**x**	**x**	**x**	**x**	**P**	*	TnaC *Ec*
3					Tryptophan +			**W**	**x**	**x**	**x**	**D**	**x**	**x**	**L**	**x**	**x**	**x**	**x**	**P**	*k*	TnaC *Pv*
Intrinsic class																						
4					**F**	**x**	**x**	**x**	**x**	**W**	**I**	**x**	**x**	**x**	**x**	**G**	**I**	**R**	**x**	**G**	**P**	SecM *Ec*
5					**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**P**	***P***	**I**	**R**	**x**	**G**	**P**	SecM mut
6				**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**H**	**A**	***P***	**I**	**R**	**G**	**S**	**P**	SecM *Ms*
7												**F**	**x**	**x**	**Y**	**x**	**I**	**W**	**P**	**P**	**P**	Evolved
8	**R**	**I**	**x**	**x**	**W**	**I**	**x**	**x**	**x**	**x**	**x**	**M**	**N**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	MifM *Bs*

SecM nascent chain interactions cause complex translational pausing

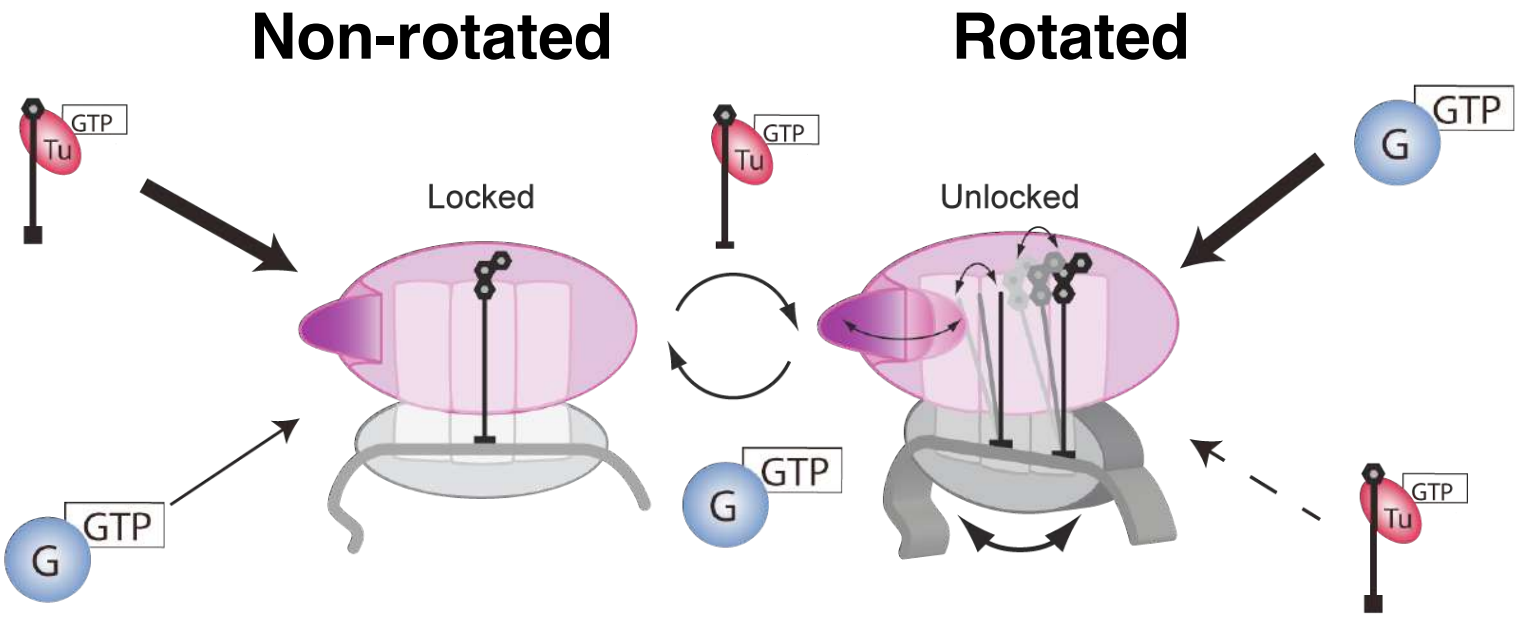


Are all pauses the same?



can different ribosomal conformations shunt to different translational pathways?

Two states, different properties

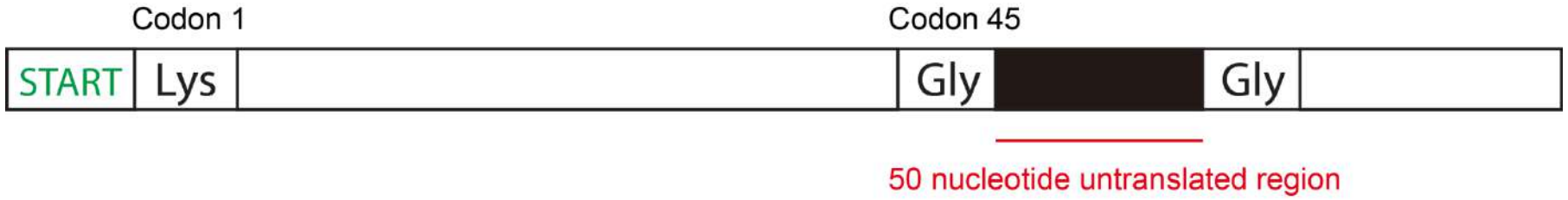


High affinity for tRNAs
High affinity for mRNA

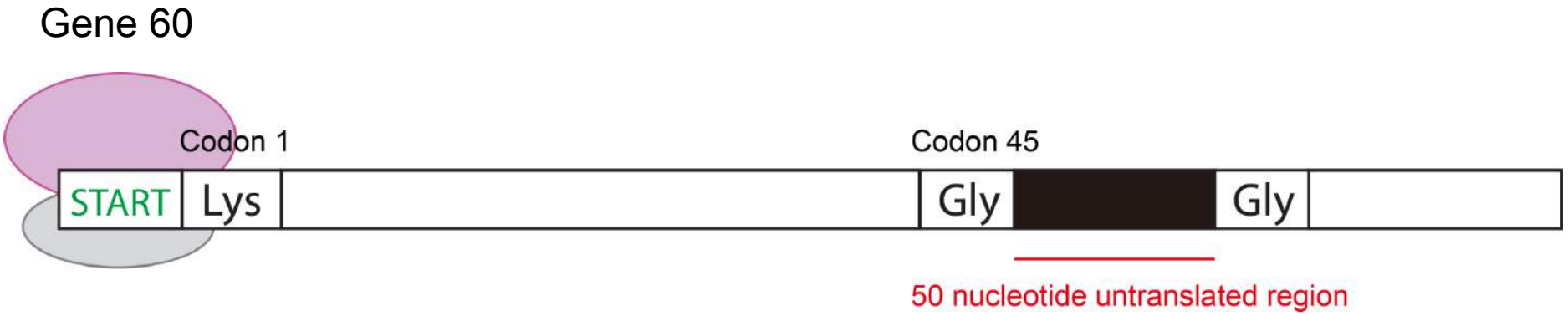
Lower affinity for tRNAs
Lower affinity for mRNA

Gene 60 consists of a 50 nucleotide untranslated region the ribosome bypasses

Gene 60



Gene 60 consists of a 50 nucleotide untranslated region the ribosome bypasses



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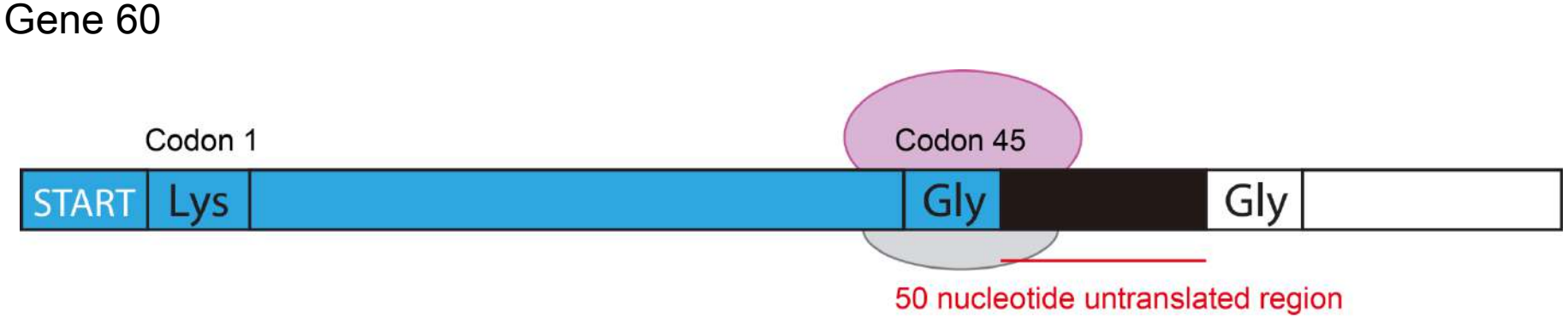

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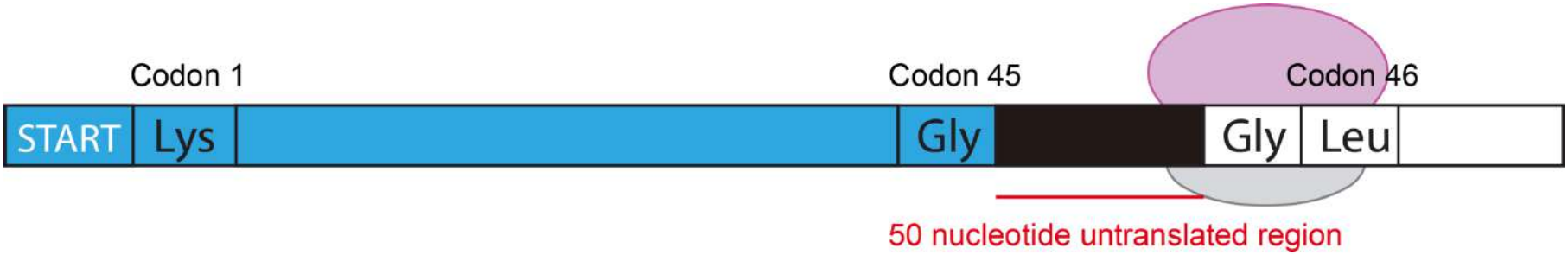

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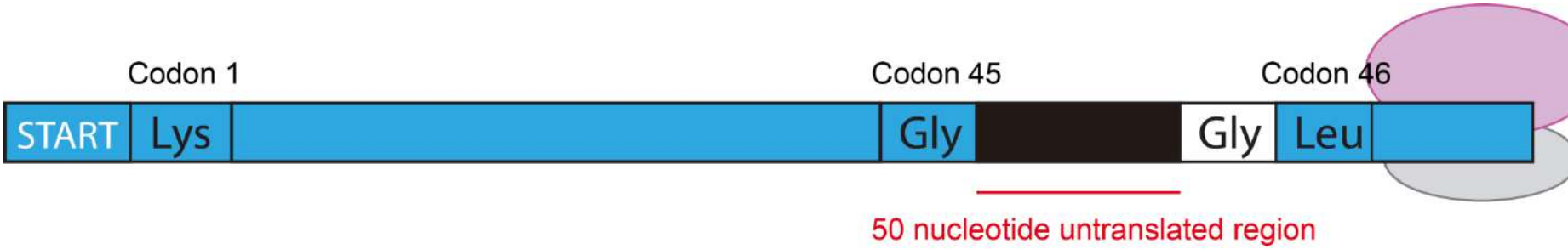
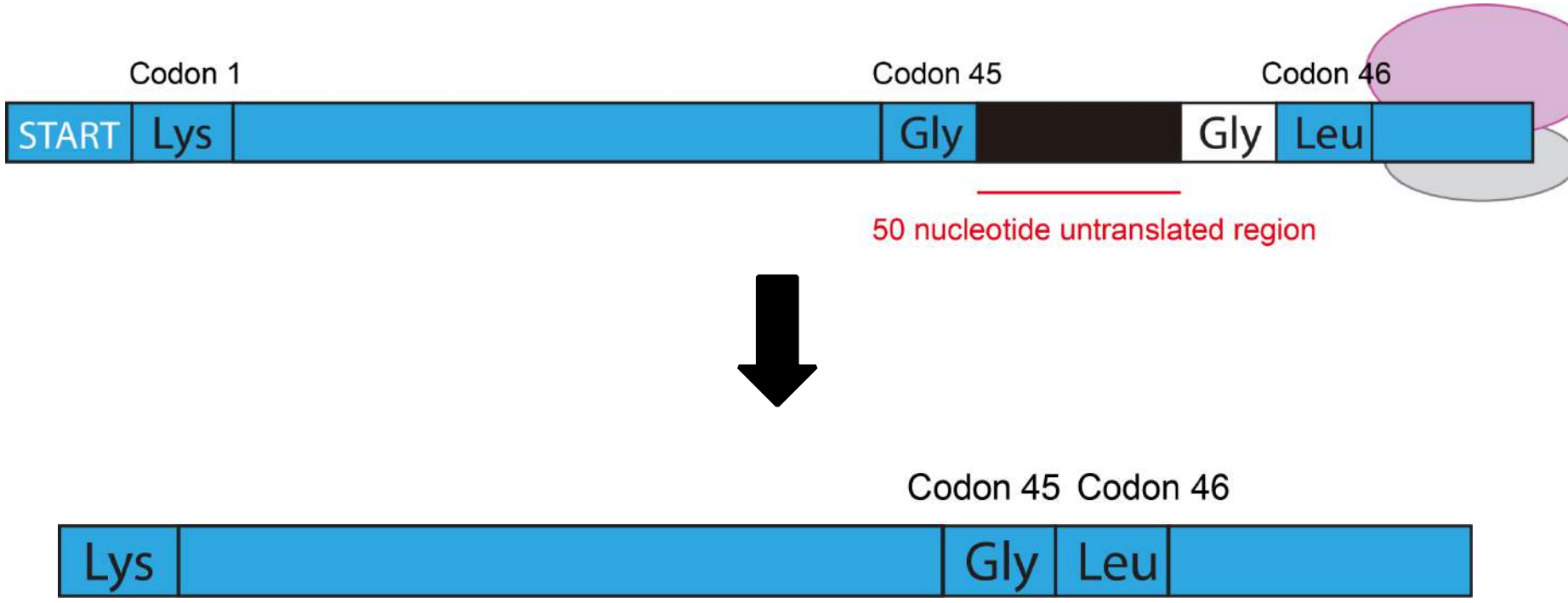
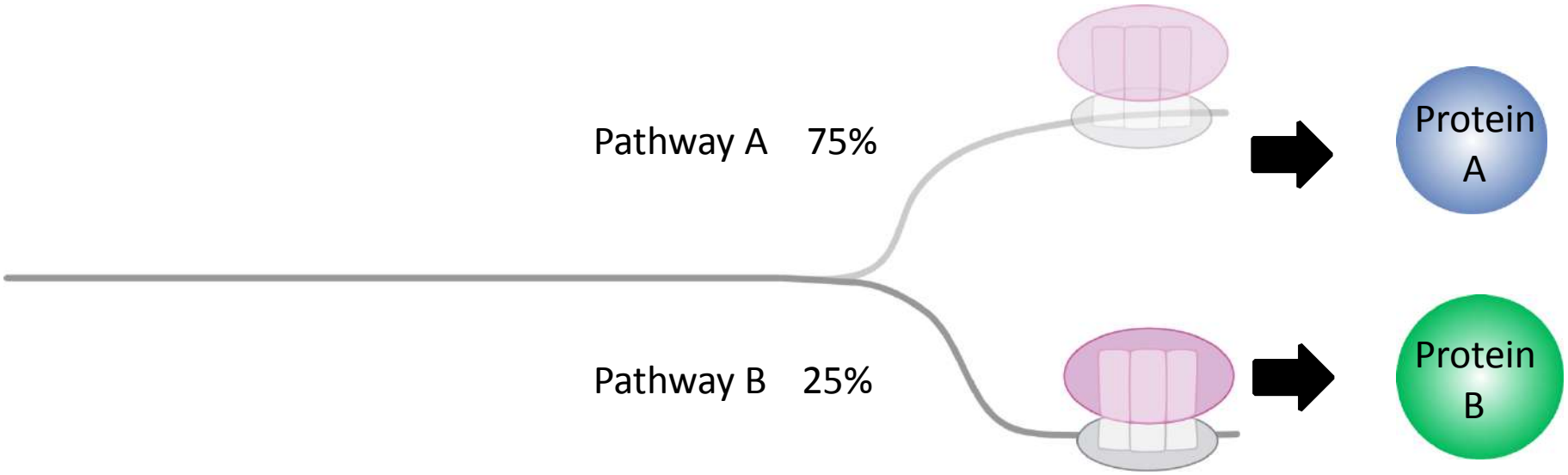


Gene 60 consists of a 50 nucleotide untranslated region the ribosome bypasses

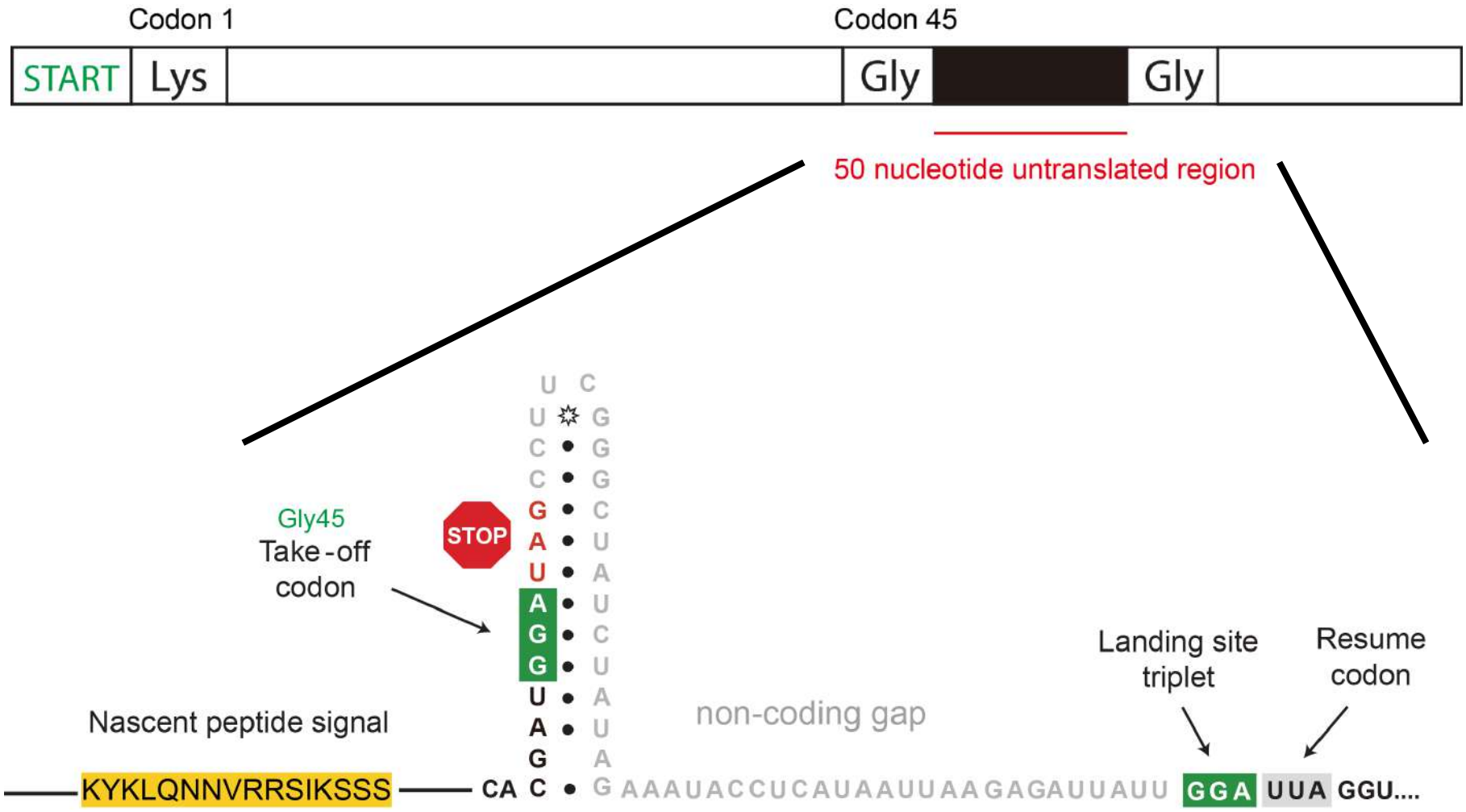


Recoding involves multiple pathways

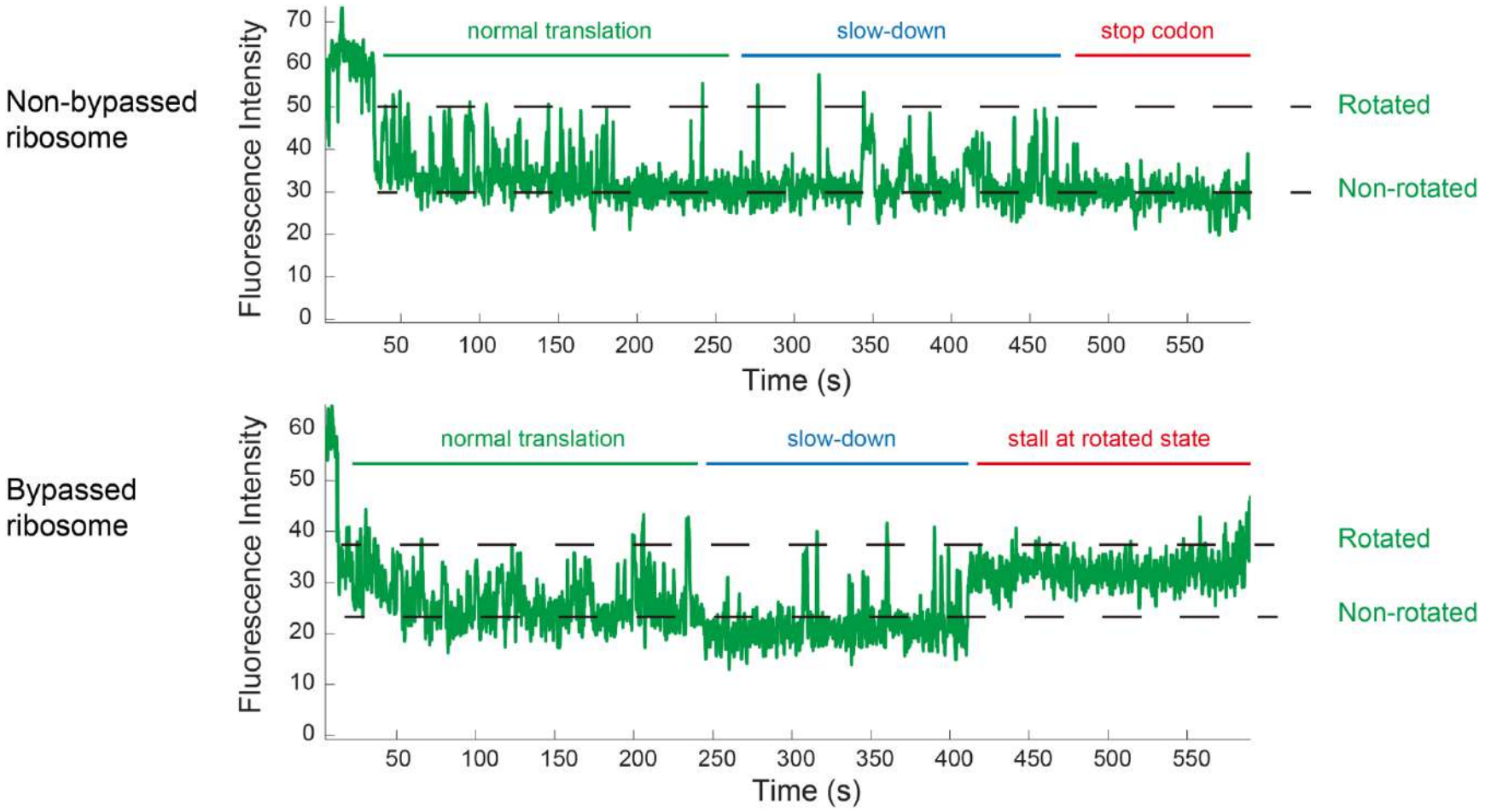


Multiple pathways

Stimulatory elements of gene 60 bypassing



Translation of the gene 60 mRNA reveals a slowdown and long rotated state pause



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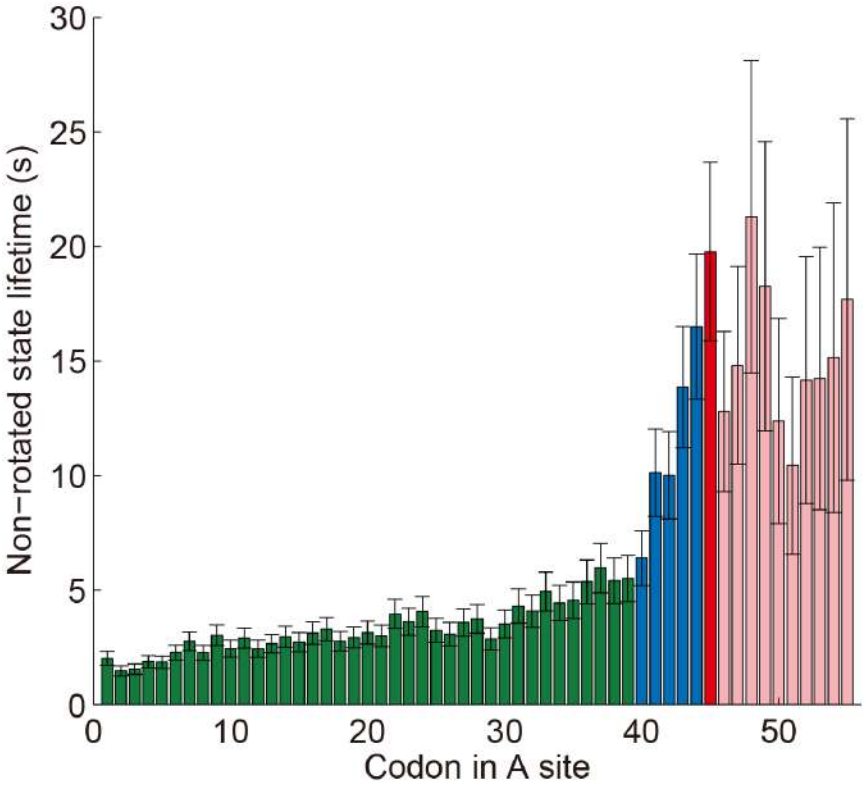
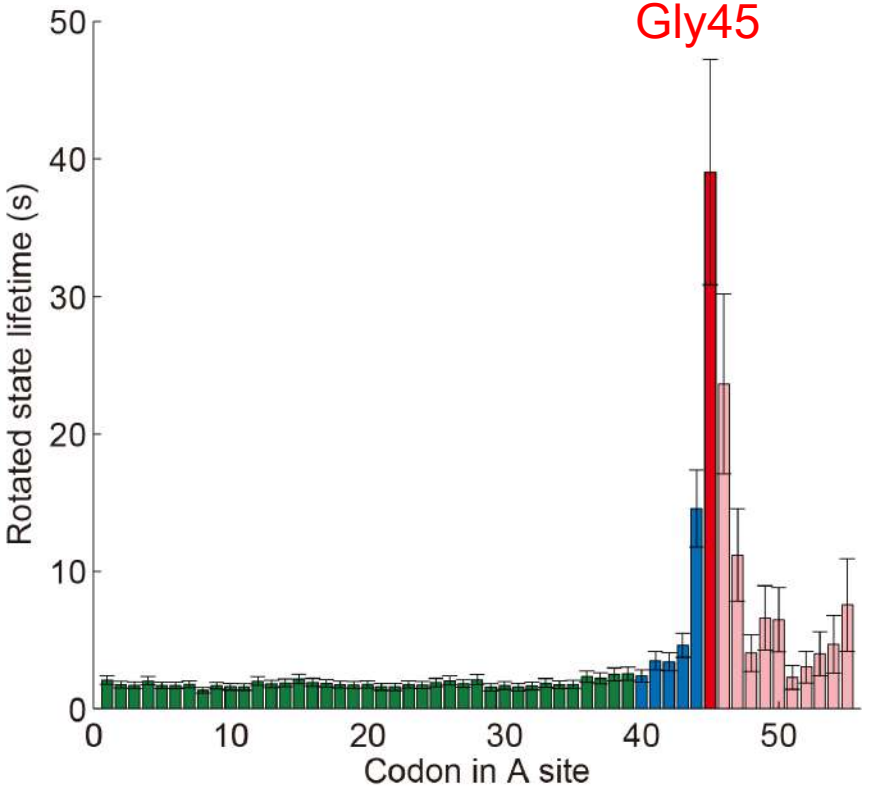
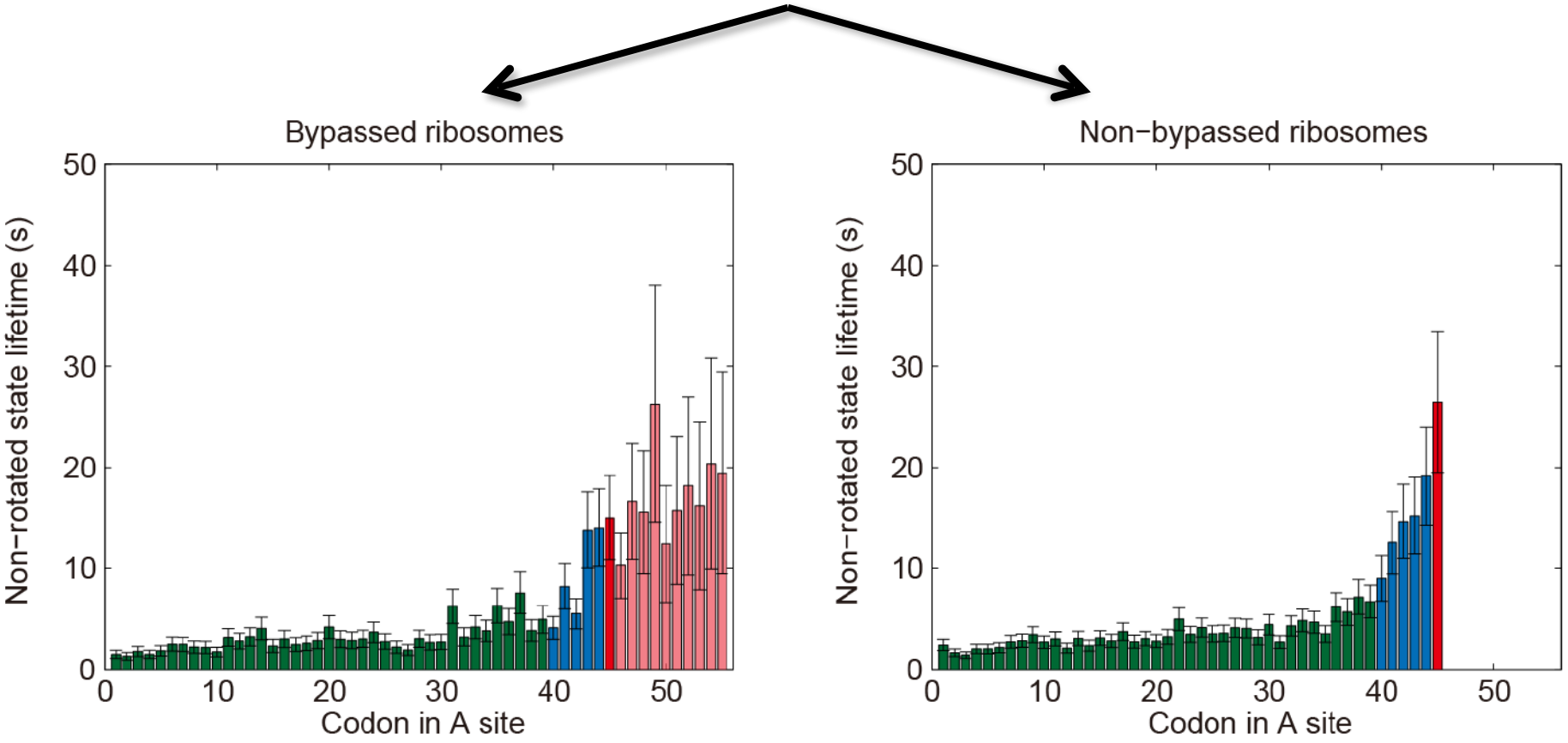




The long rotated state pause is characteristic of bypassed ribosomes

Bypassing efficiency = 35%



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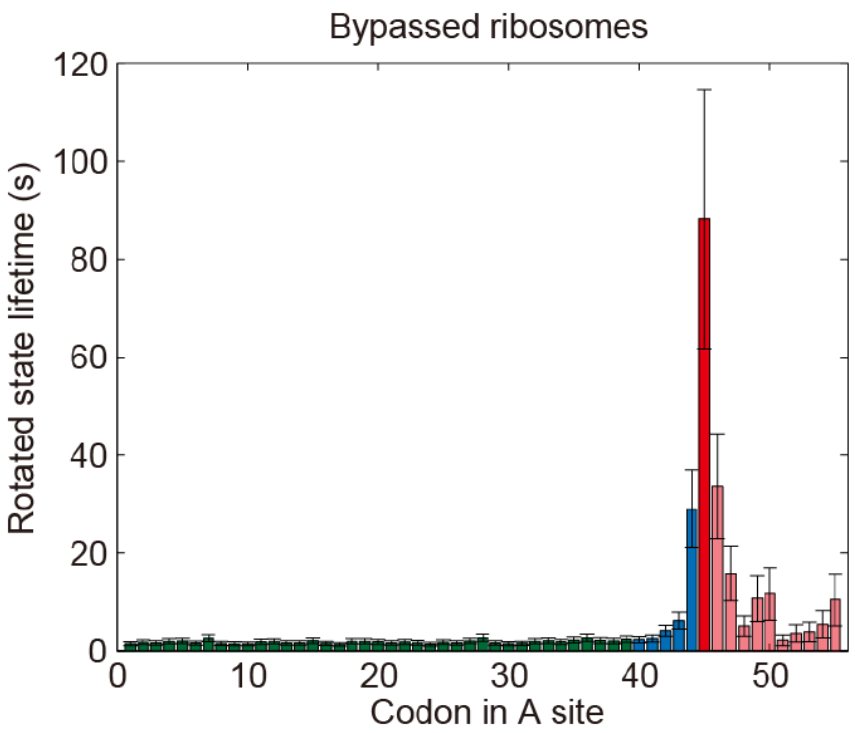
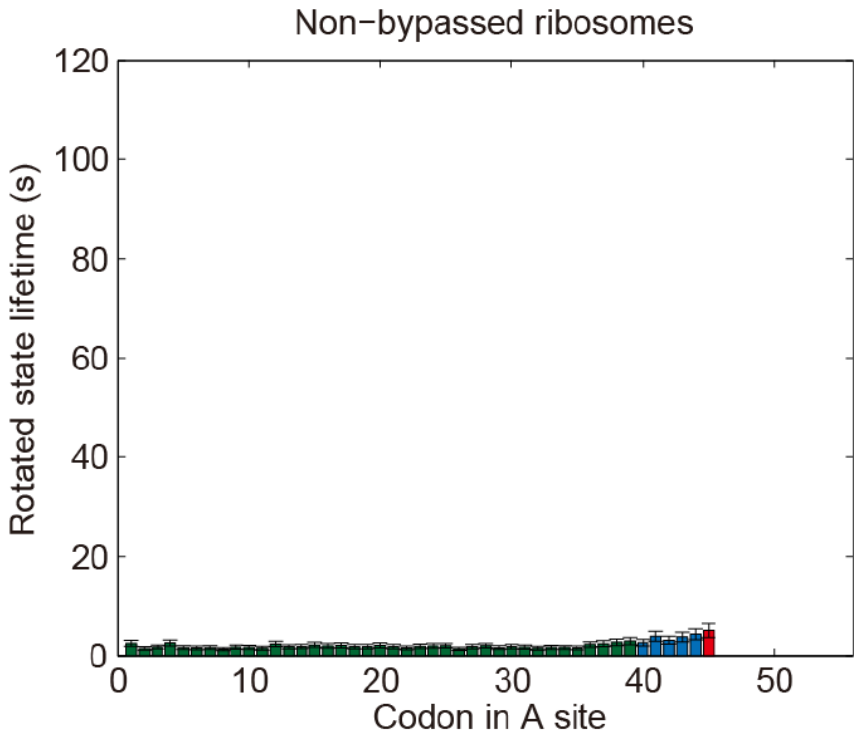
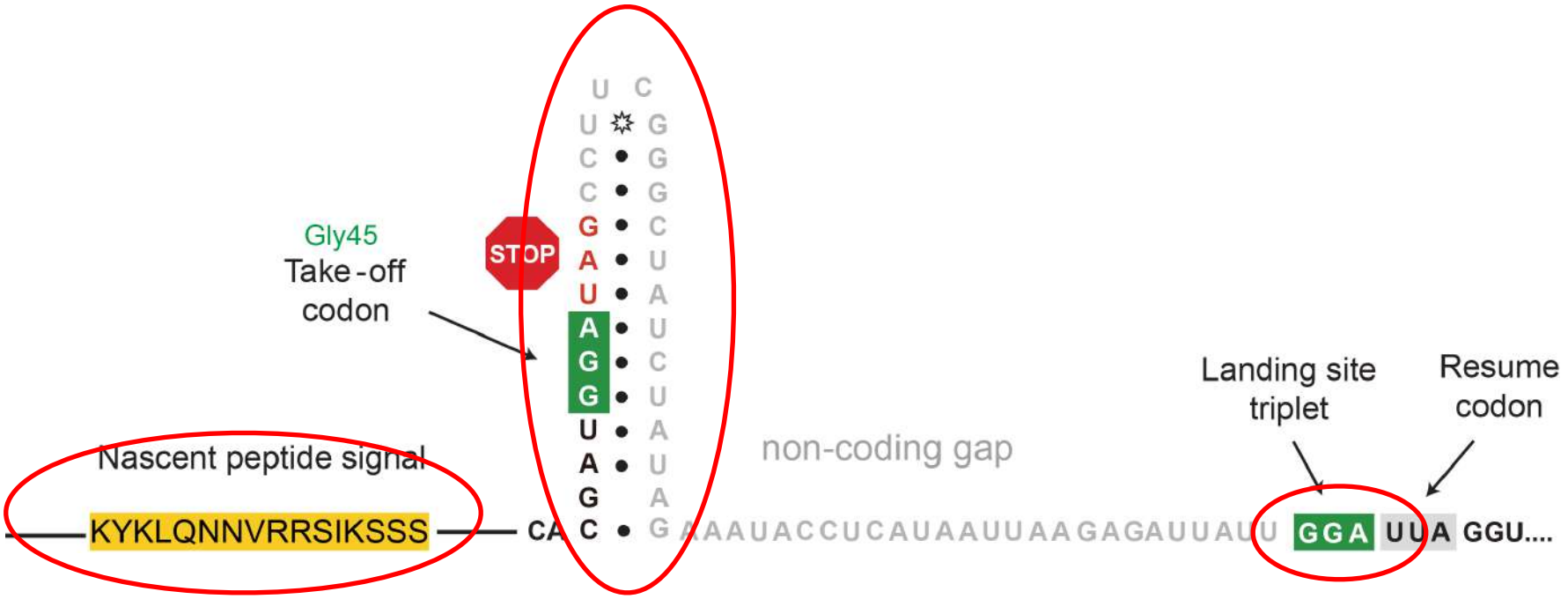
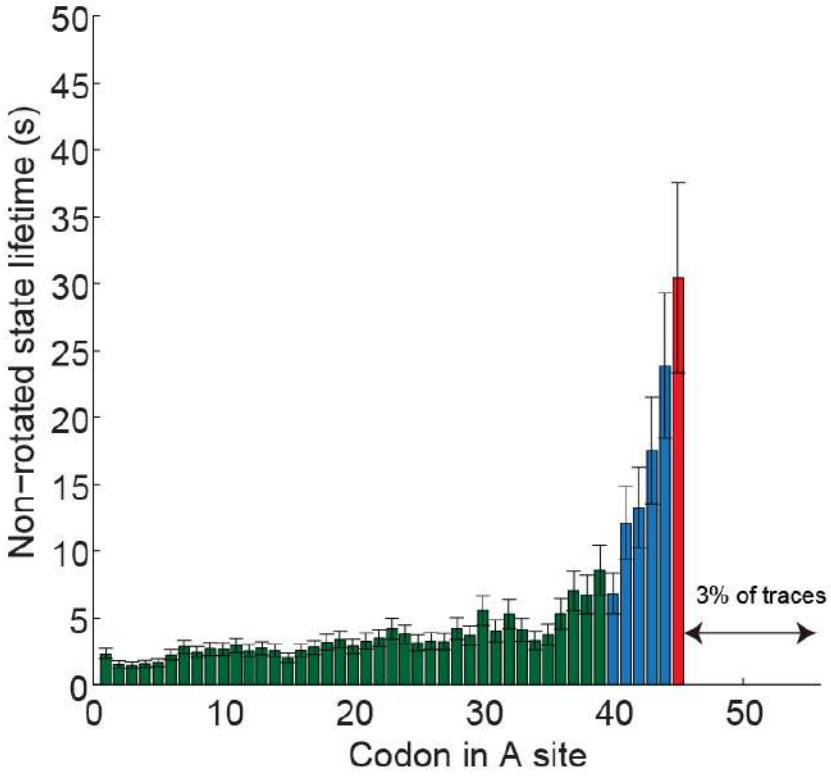


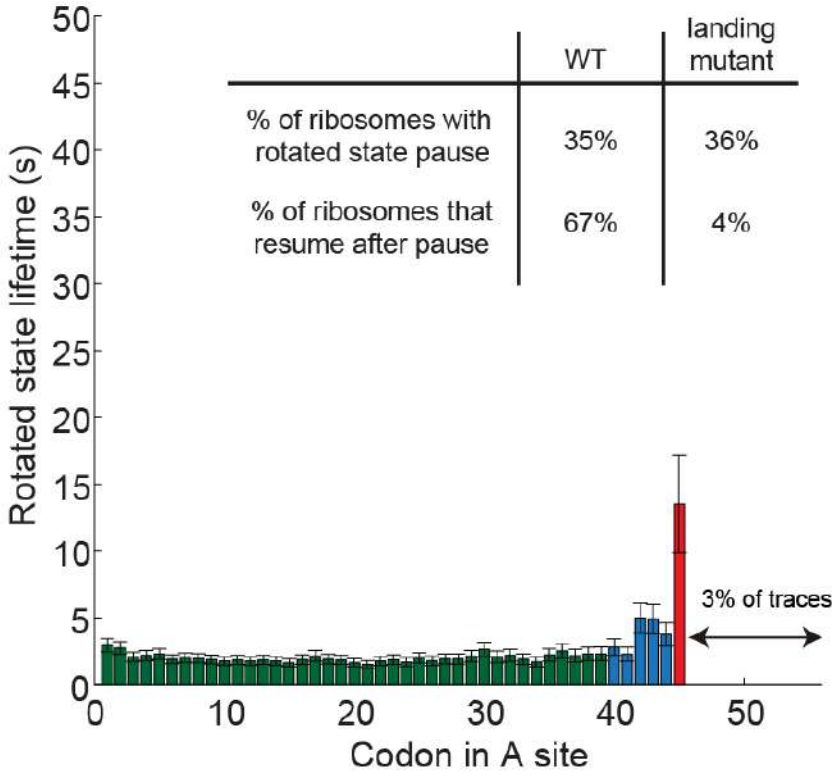


Stimulatory elements of gene 60 bypassing



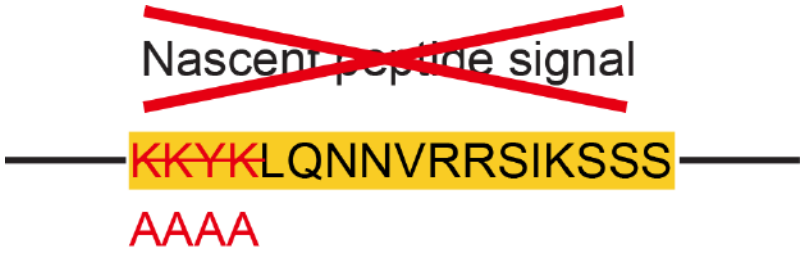
Mutating the landing codon prevents ribosome from successfully resuming translation

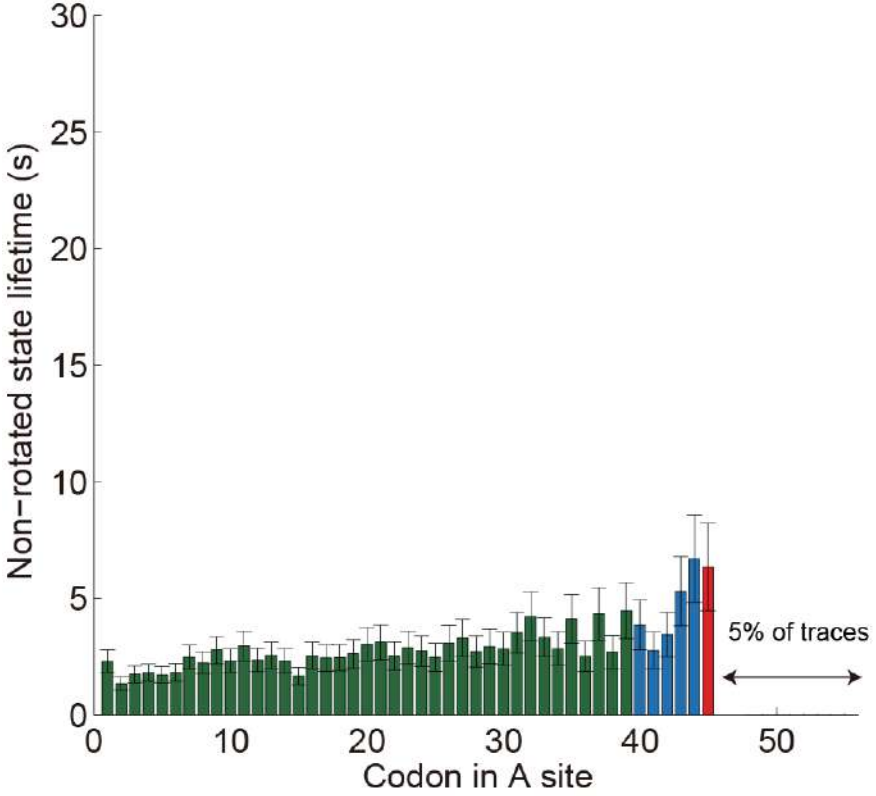


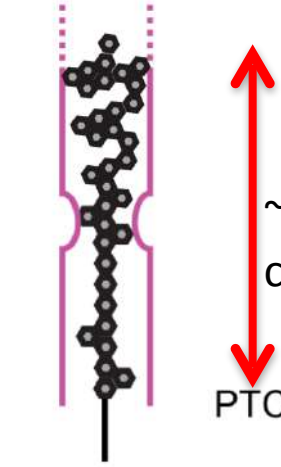


	WT	landing mutant
% of ribosomes with rotated state pause	35%	36%
% of ribosomes that resume after pause	67%	4%

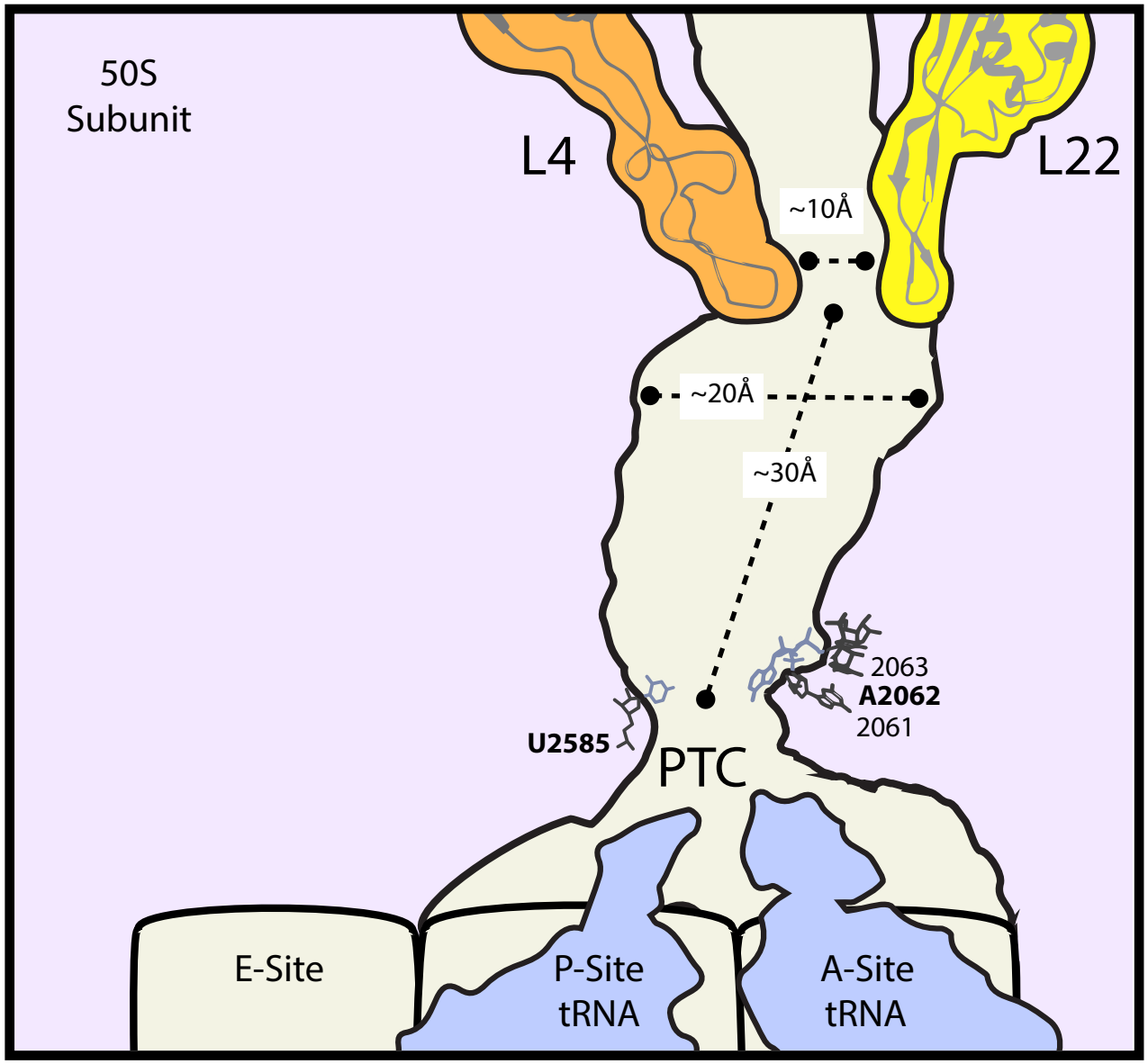
Mutating the nascent peptide abolishes the slow-down and rotated state pause



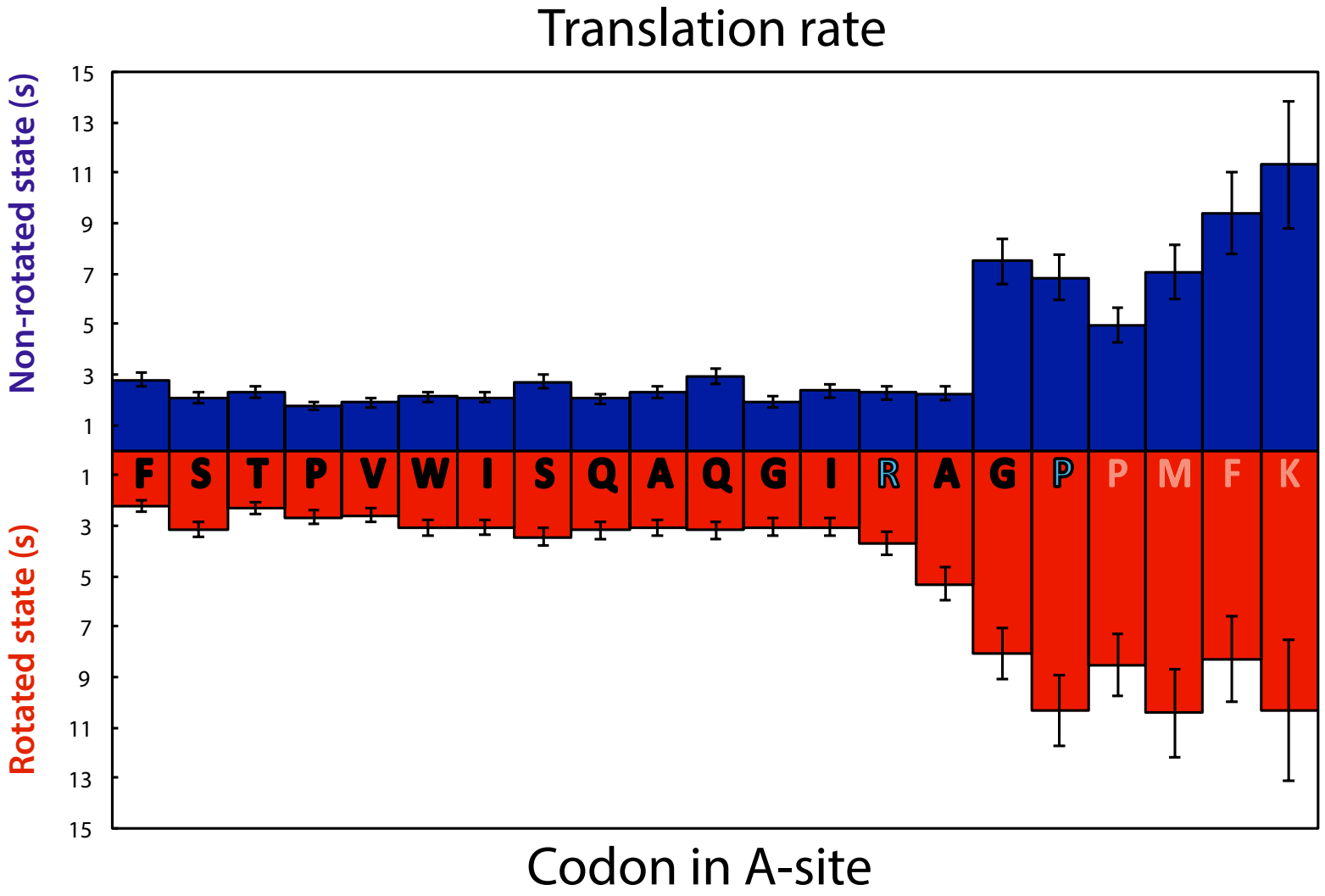




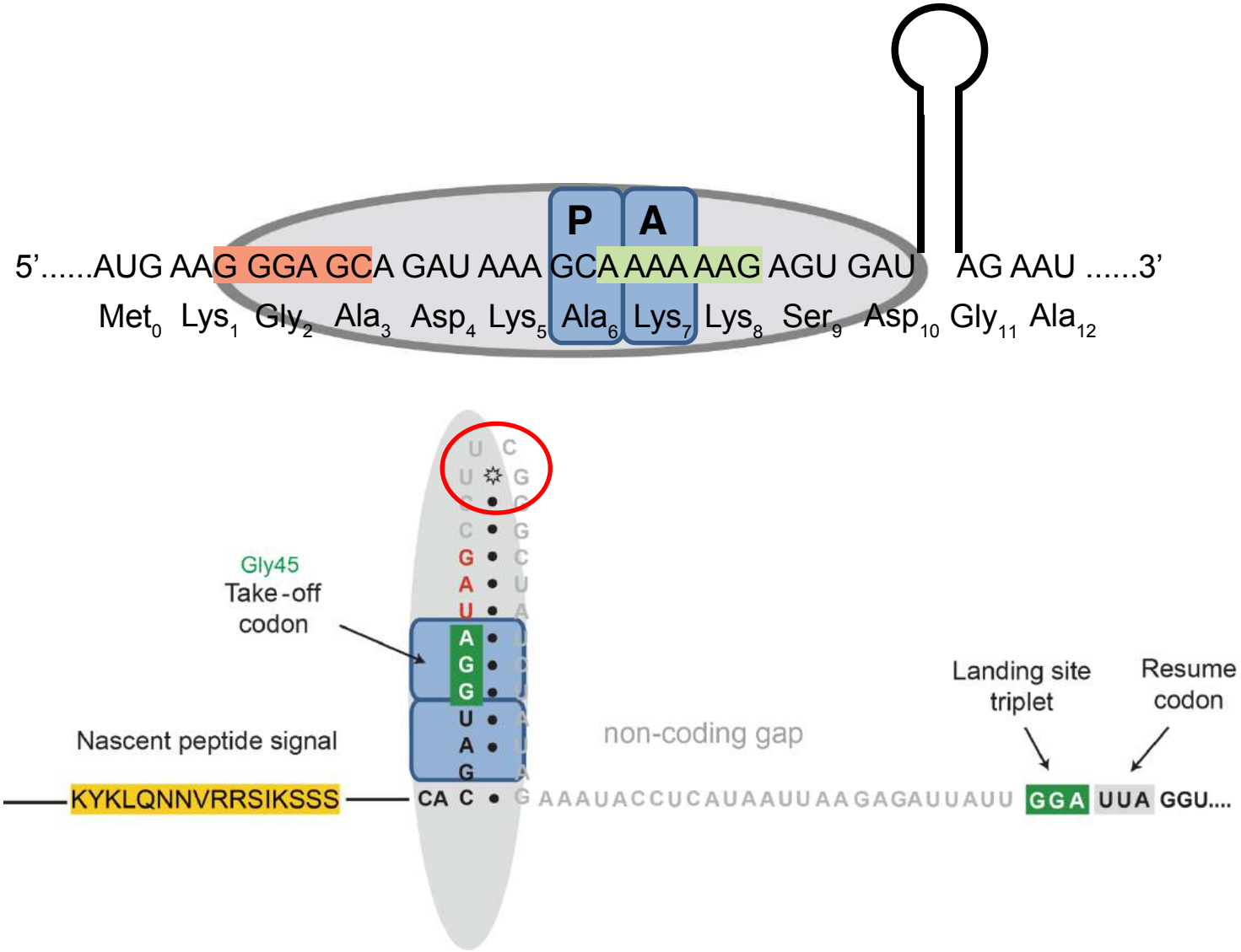
Is the exit-tunnel a passive conduit?



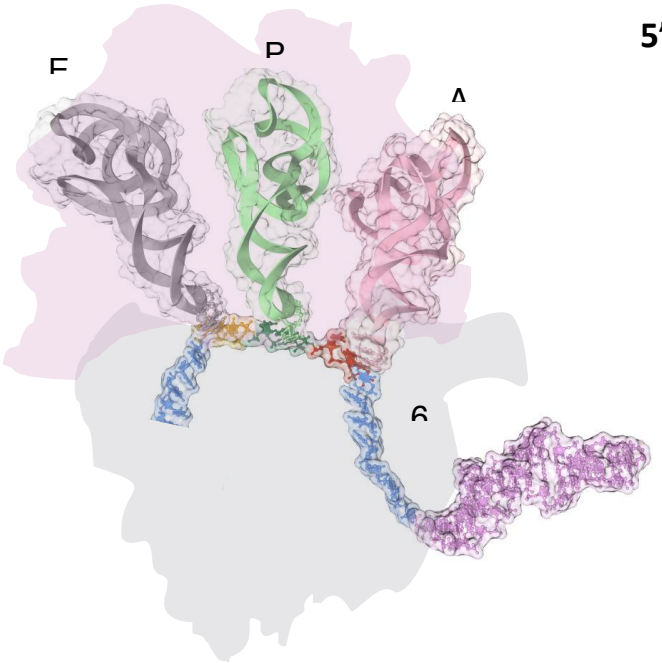
SecM nascent chain interactions cause complex translational pausing



The role of the hairpin?



Hairpins are barriers to translocation…..



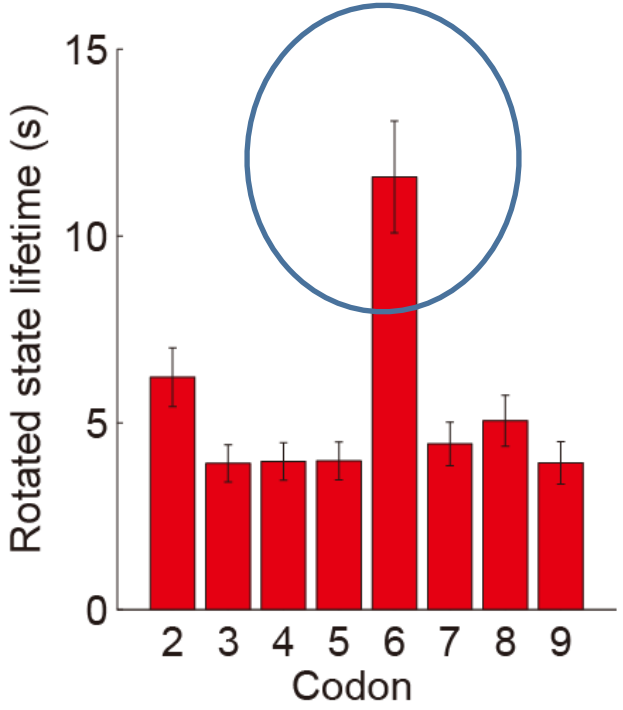
4(FK)-hairpin

1 2 3 4 5 6 7 8 9

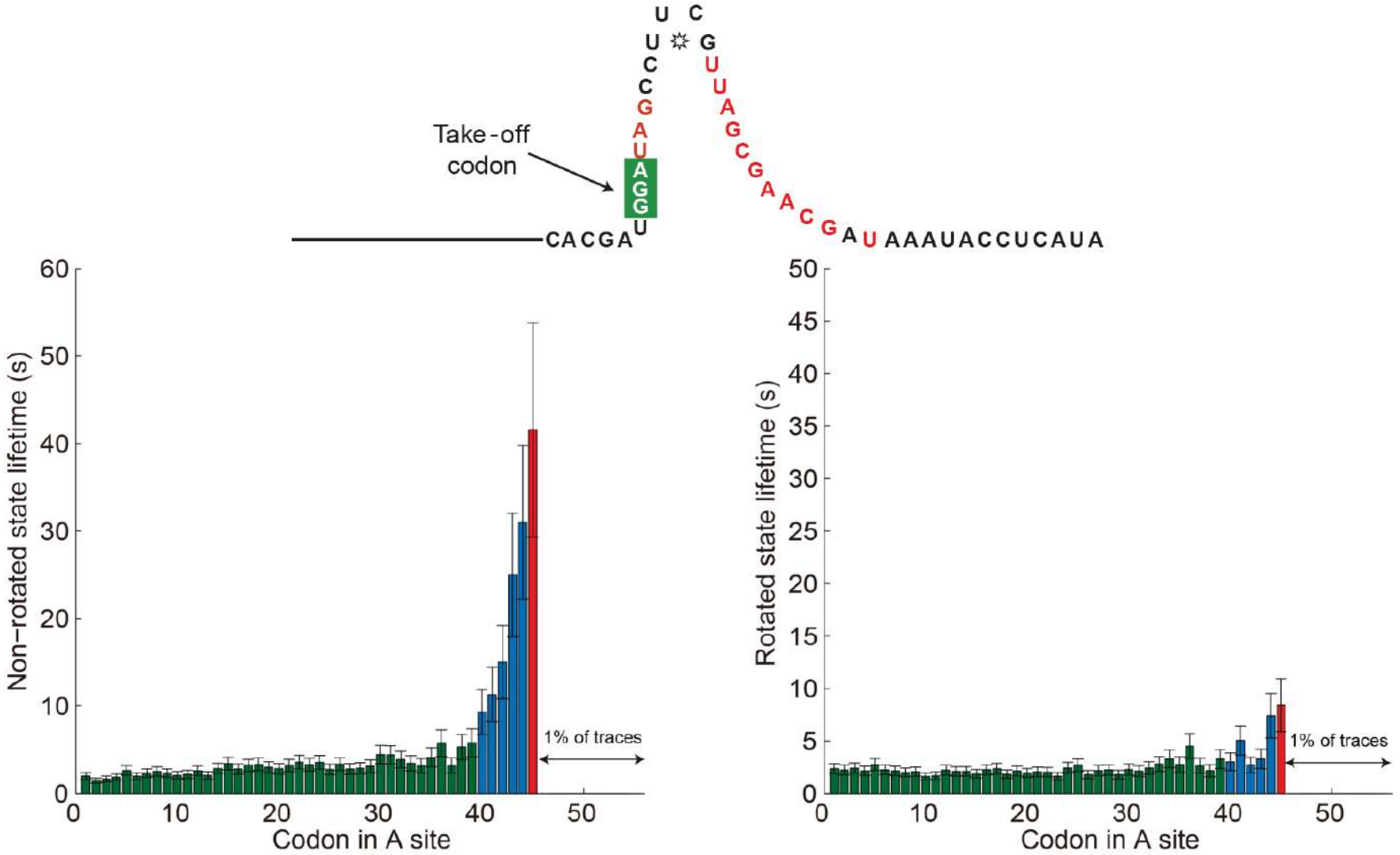
5’ ……AUG UUC AAA UUC AAA UUC AAA UUC AAA … 3’

Met Phe Lys Phe Lys Phe Lys Phe Lys Gly Ala STOP

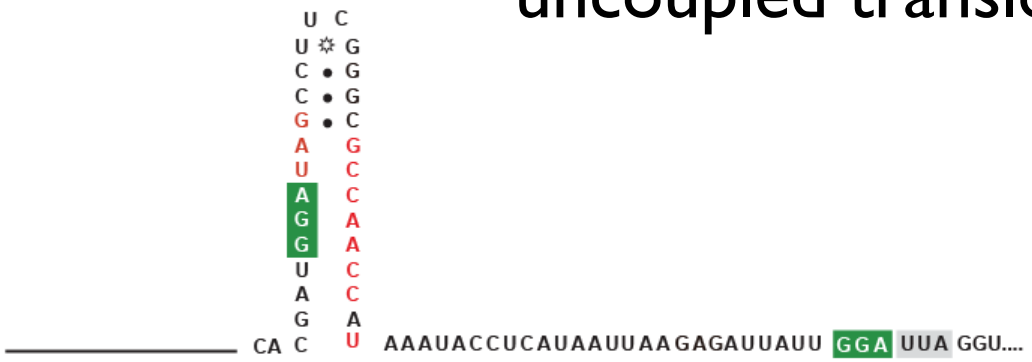
10 11



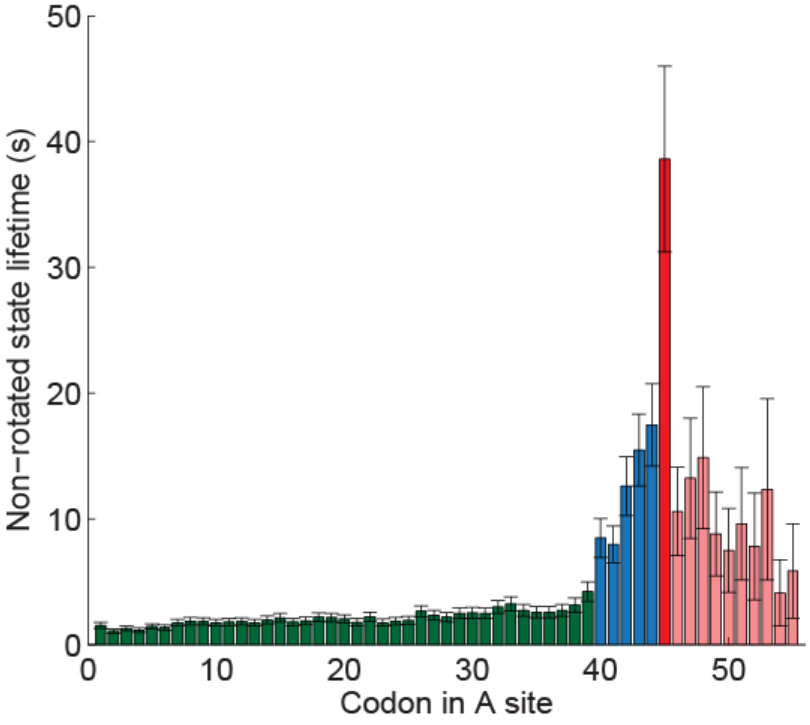
Destabilizing the hairpin abolishes the rotated state pause

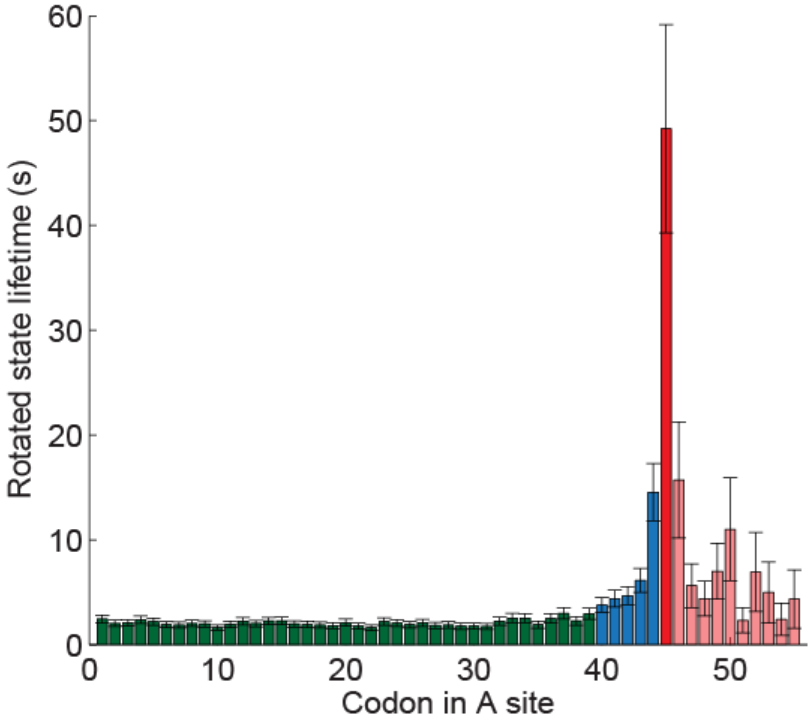


The UUCG tetraloop has high tendency to refold – uncoupled translocation

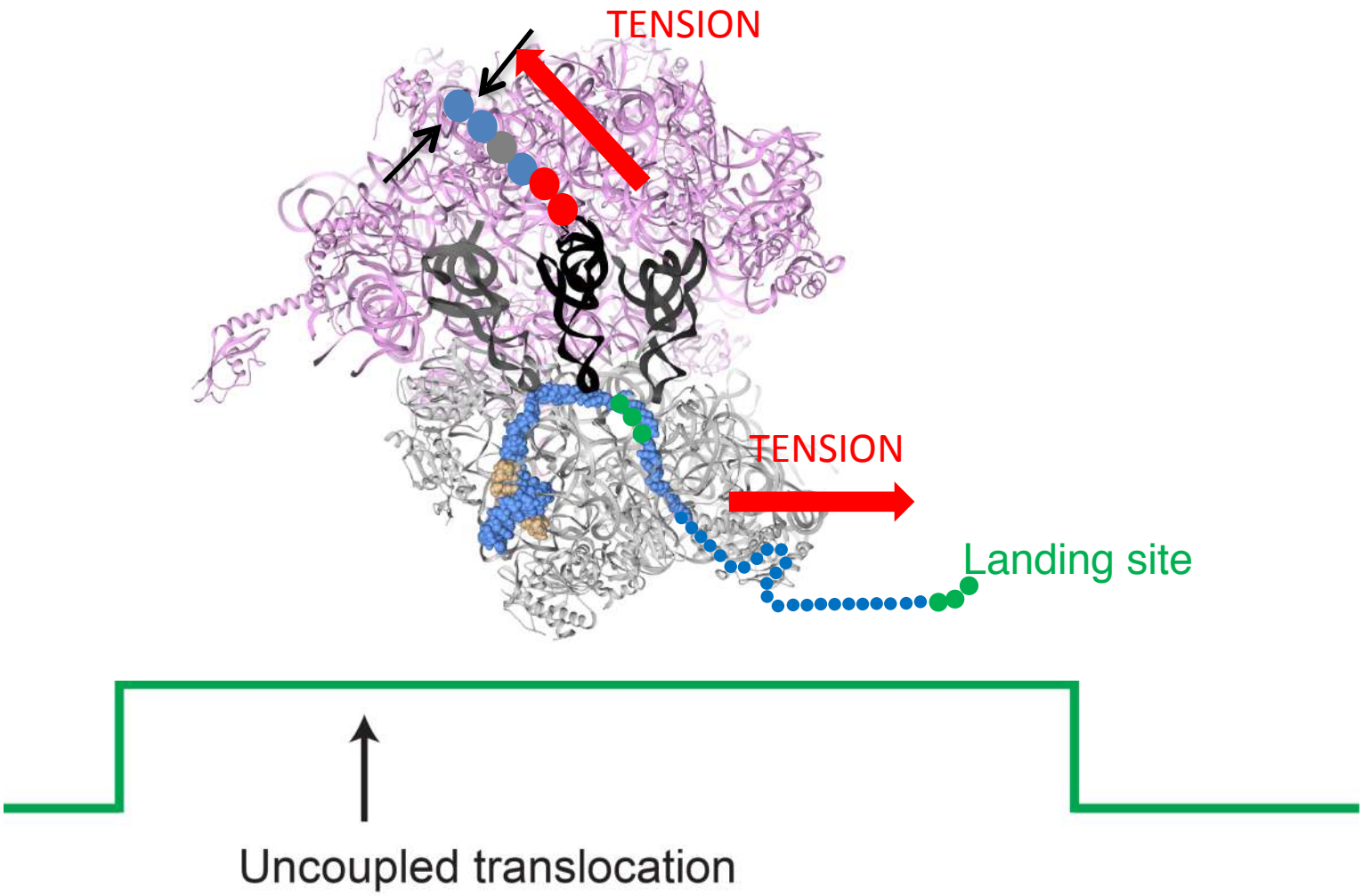


Bypass efficiency = 36%

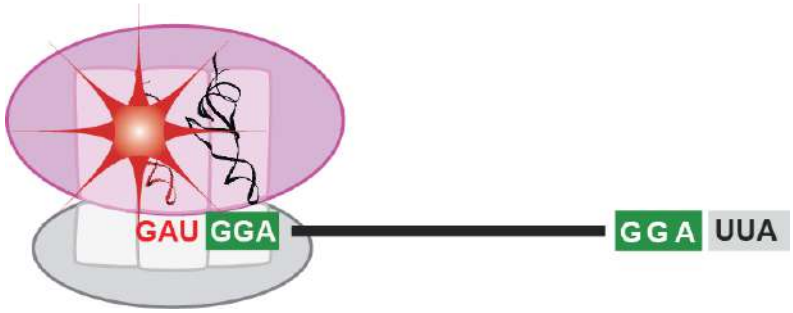


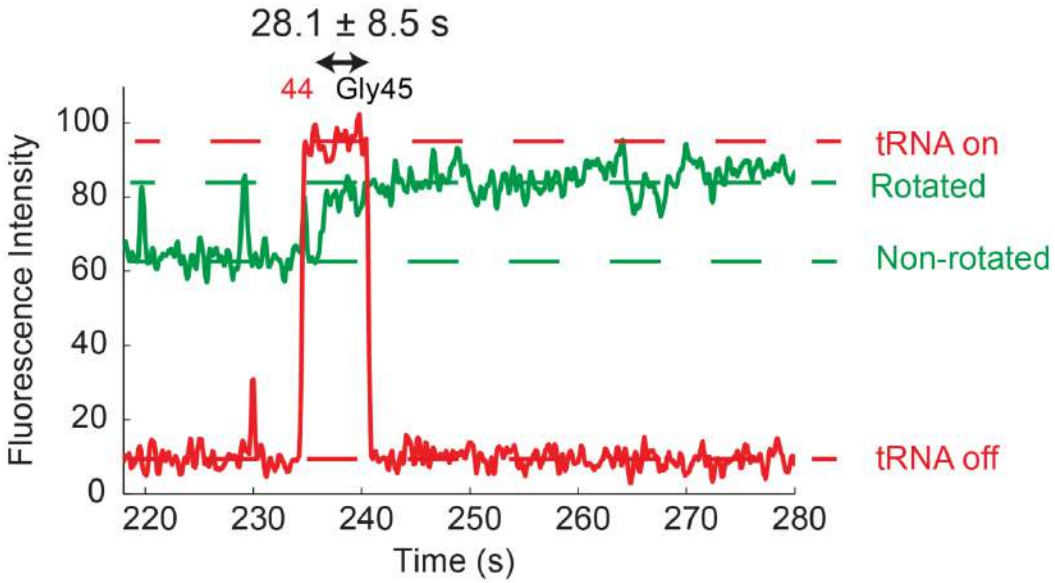


Is uncoupled translocation also involved like in frameshifting?

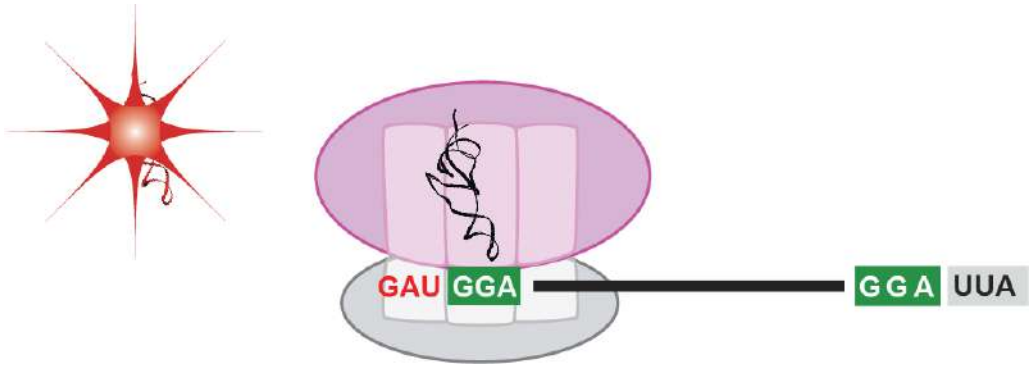


Uncoupled translocation occurs during the rotated state pause

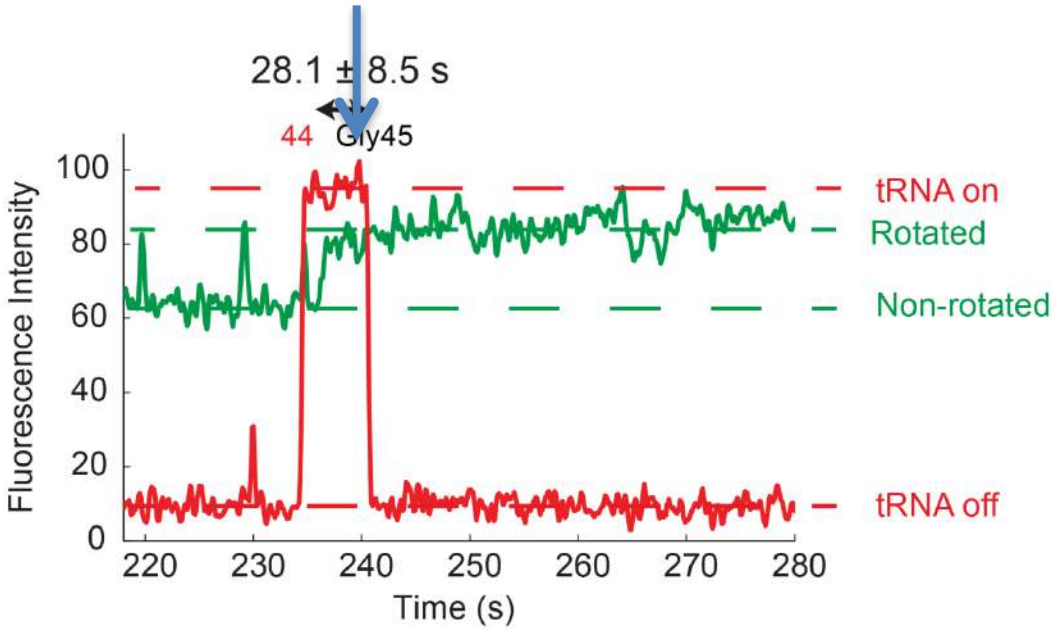




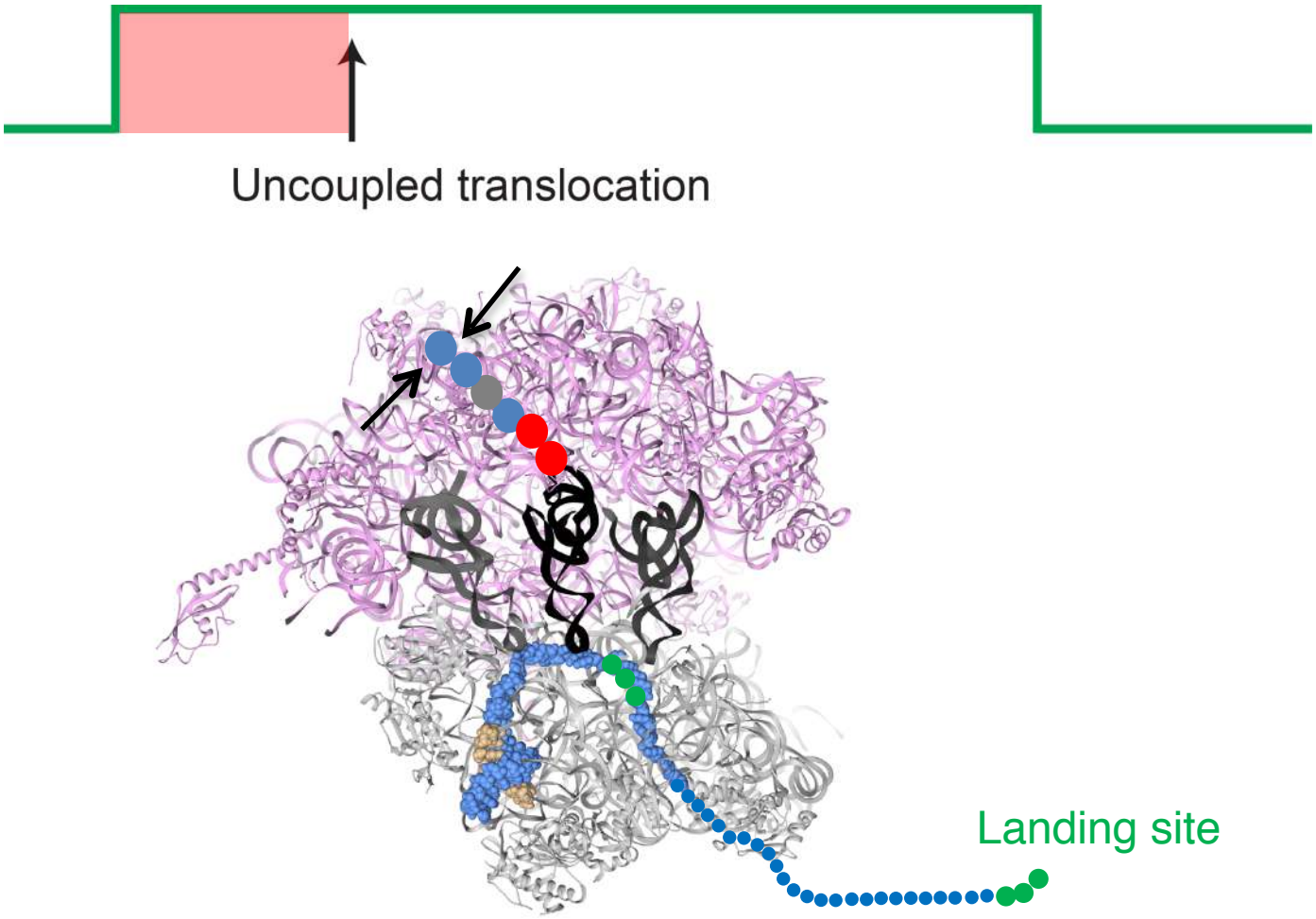
Uncoupled translocation occurs during the rotated state pause



Uncoupled translocation



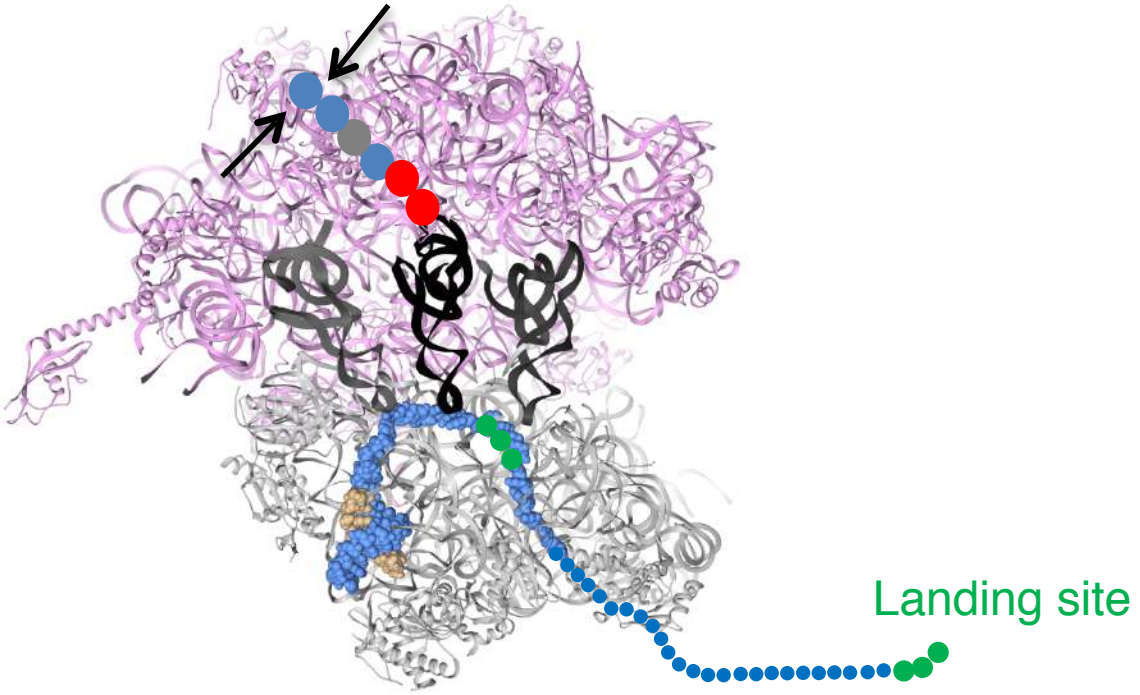
Uncoupled translocation occurs during the rotated state pause



mRNA rearrangements drive bypassing

Model 1: Scanning

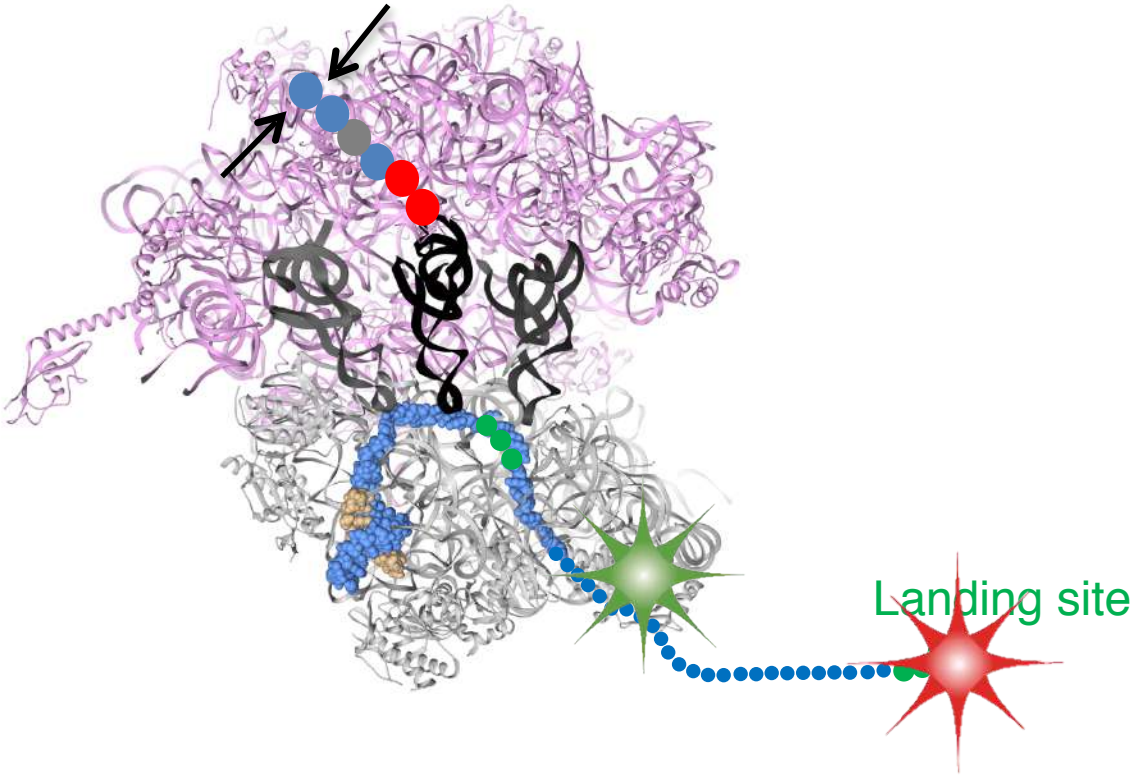
Model 2: mRNA rearrangement facilitated “jump”



mRNA rearrangements drive bypassing

Model 1: Scanning

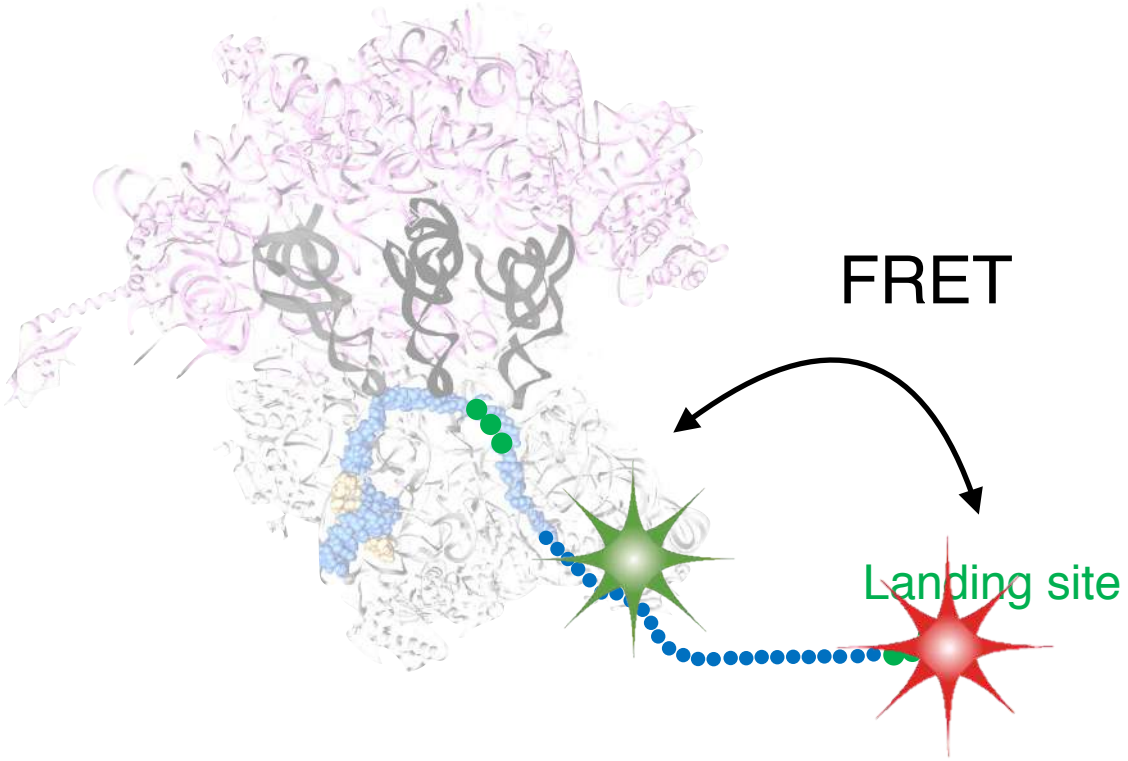
Model 2: mRNA rearrangement facilitated “jump”



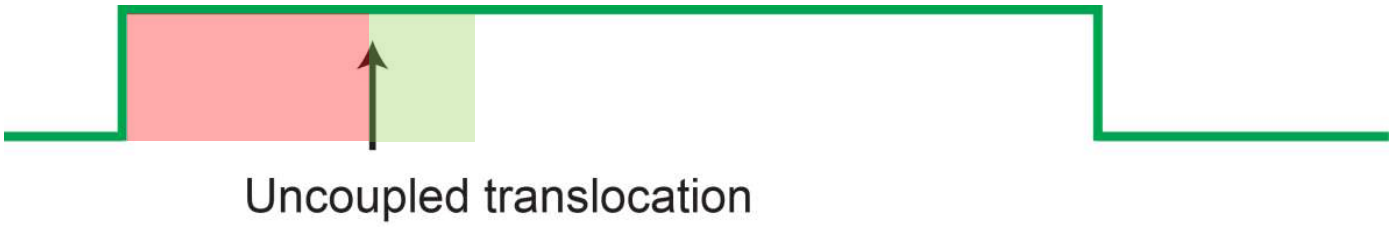
mRNA rearrangements drive bypassing

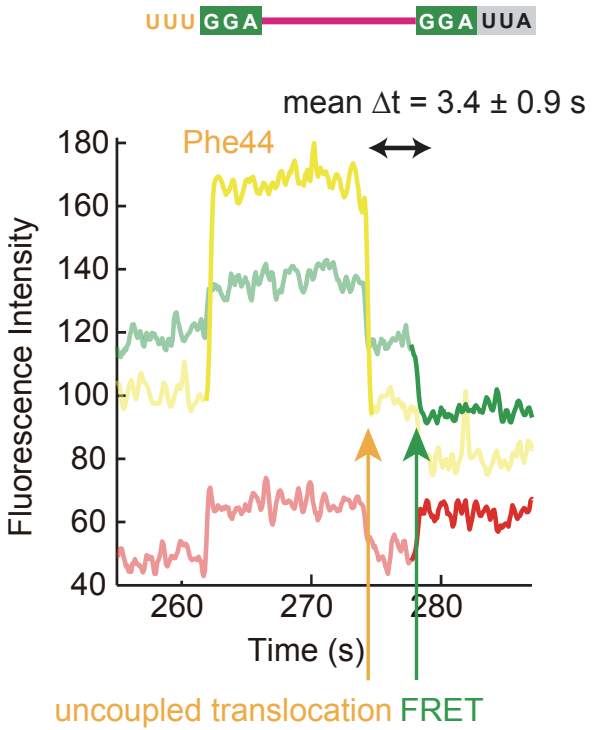
Model 1: Scanning

Model 2: mRNA rearrangement facilitated “jump”

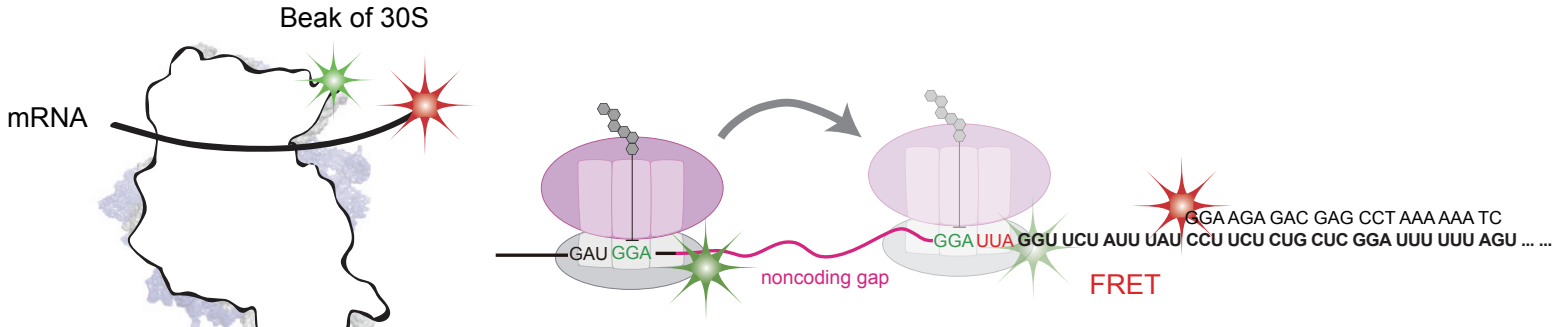


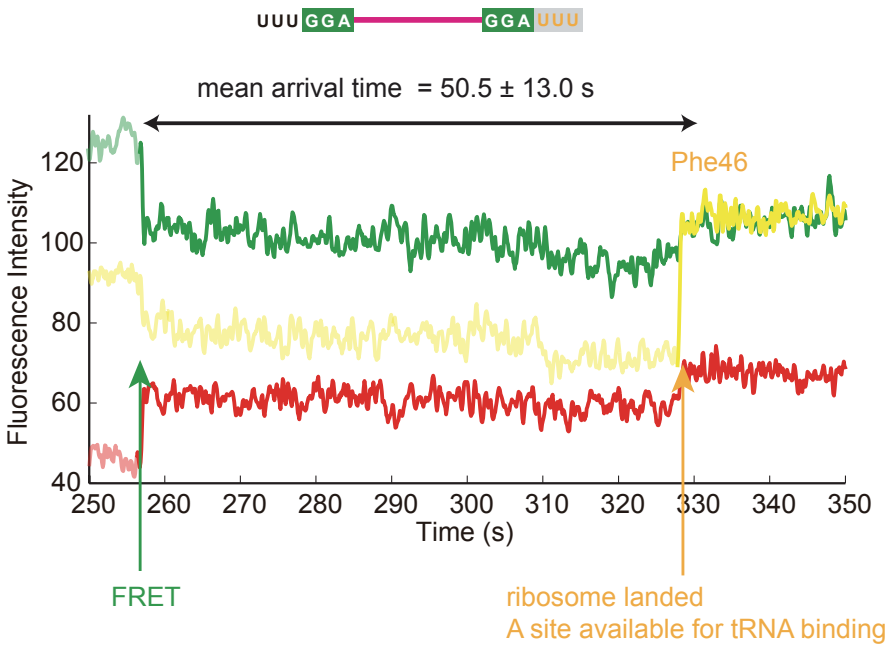
The hop occurs shortly after (or during) uncoupled translocation



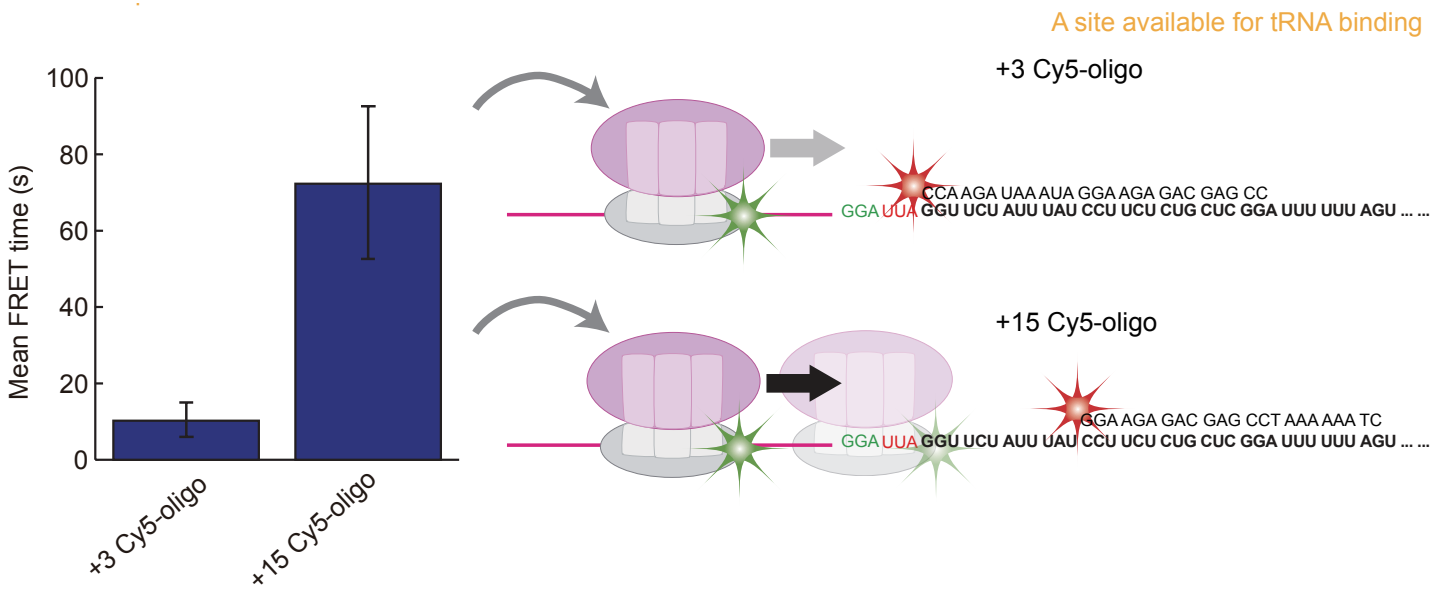


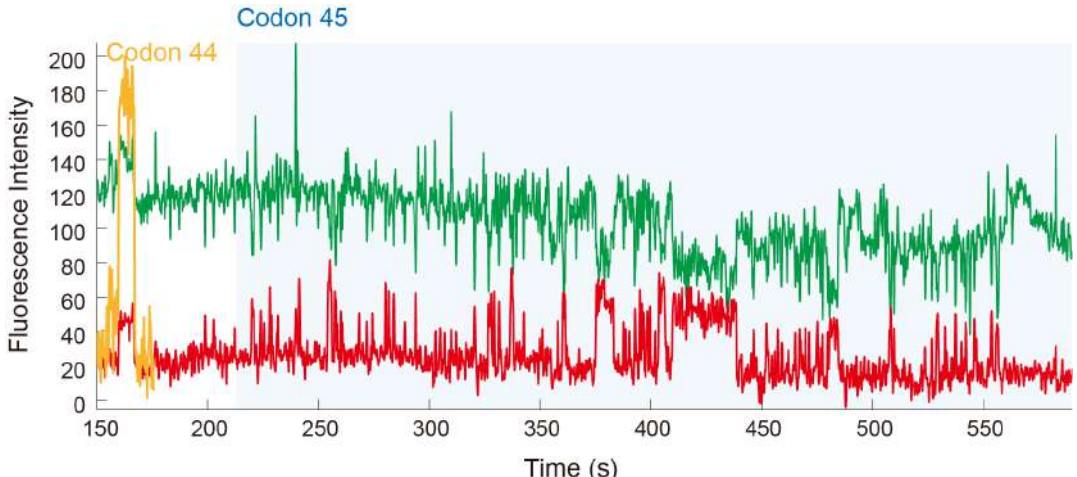
translation resumes after the mRNA rearrangement



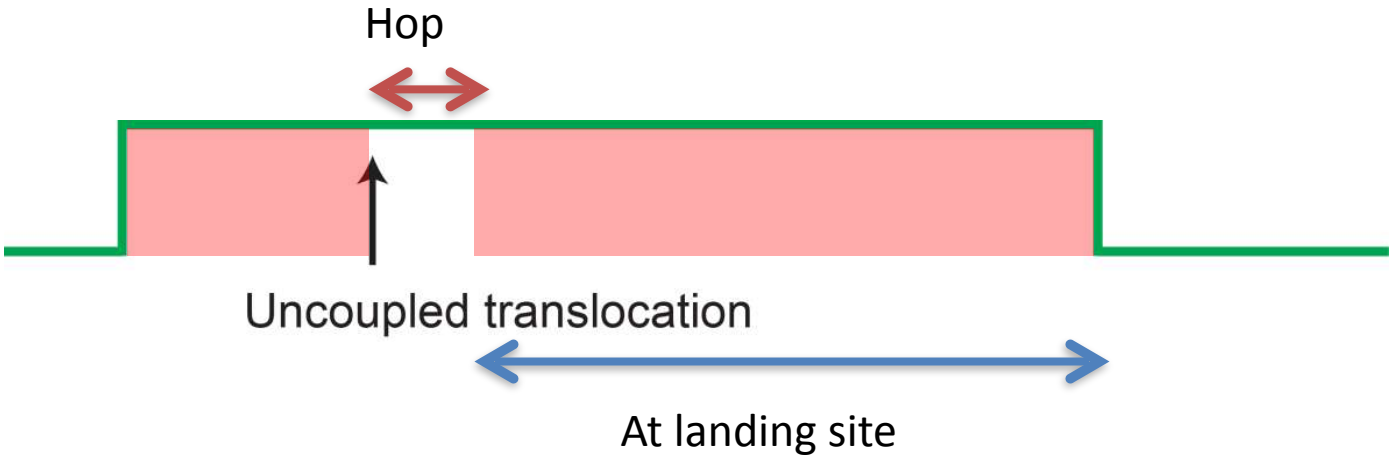


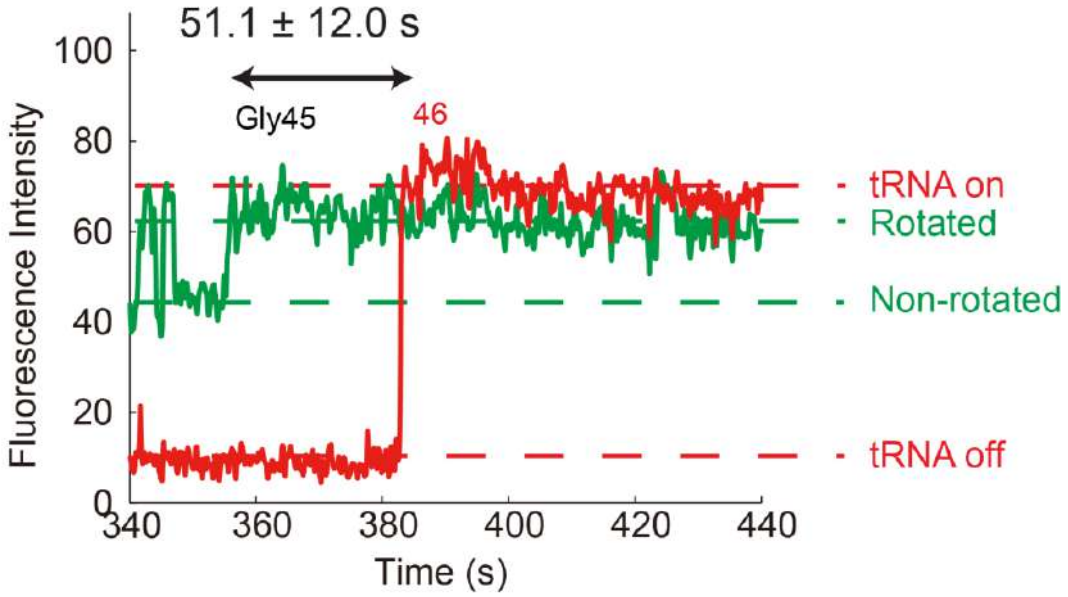
Stability of rearrangement requires a proper landing site



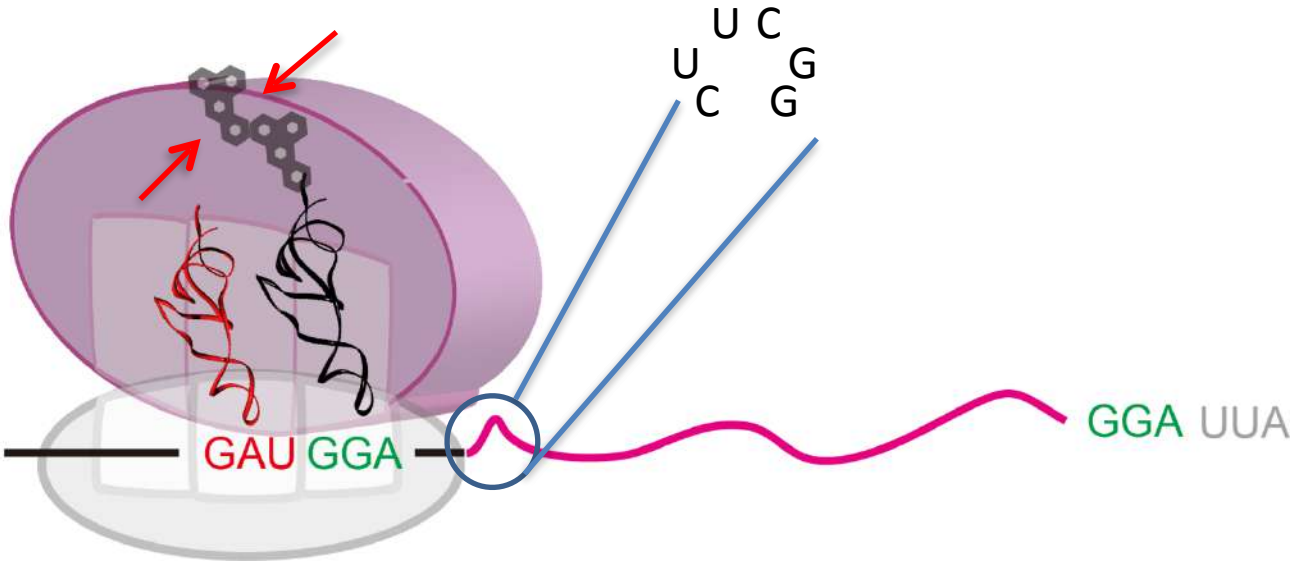


tRNA binding to the non-canonical rotated state to resume translation



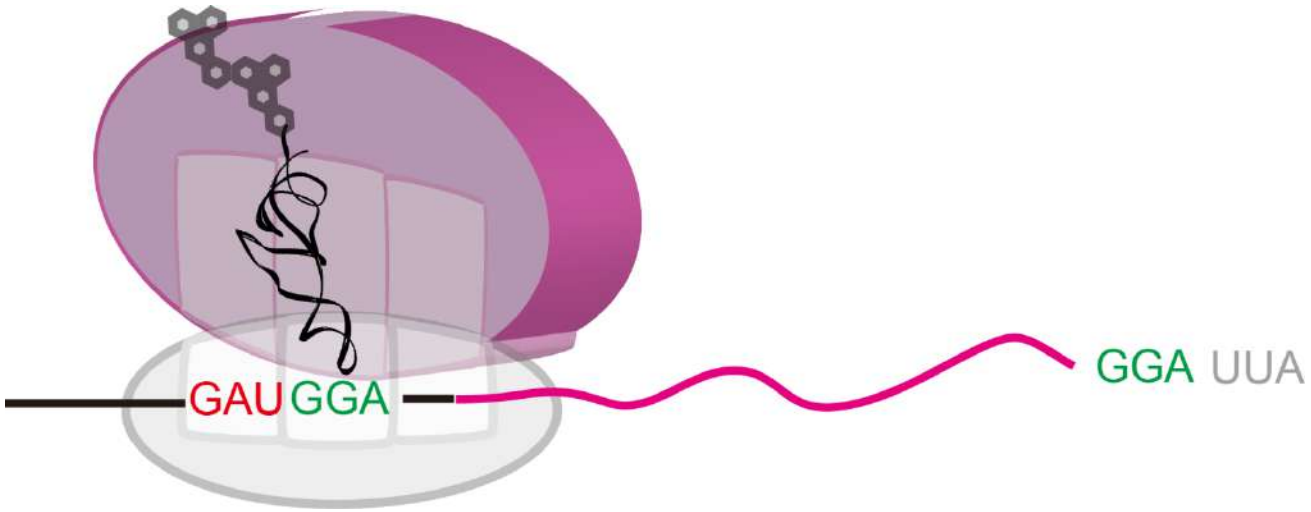


Bypassing induced by mRNA rearrangements and nascent peptide interactions

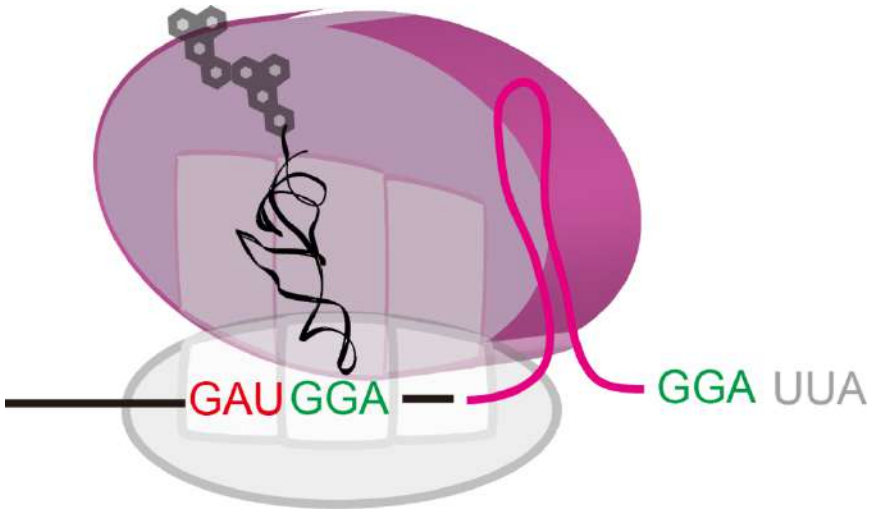


Bypassing induced by mRNA rearrangements and nascent peptide interactions

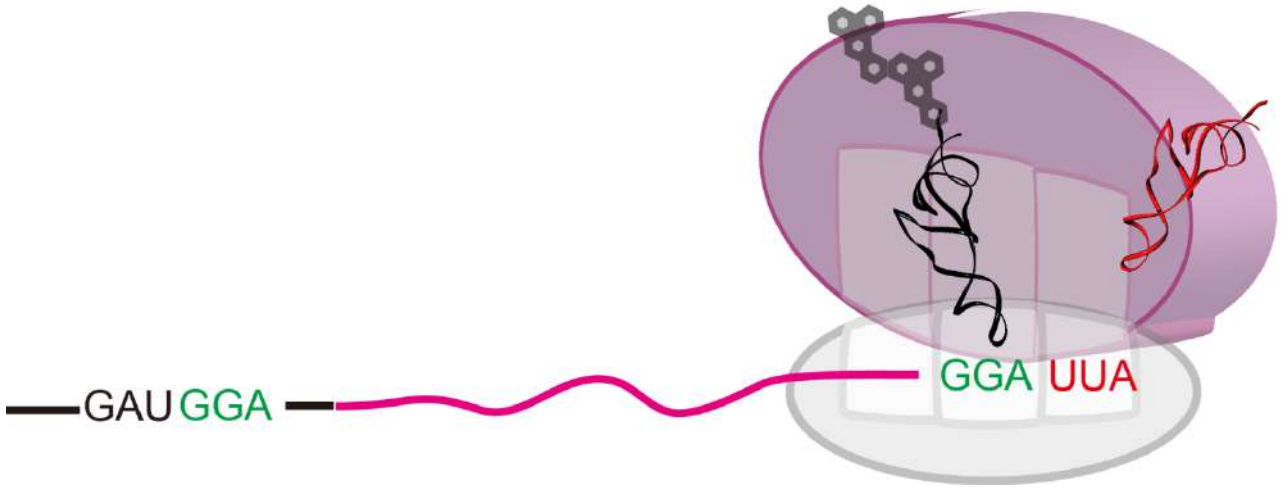
Uncoupled translocation



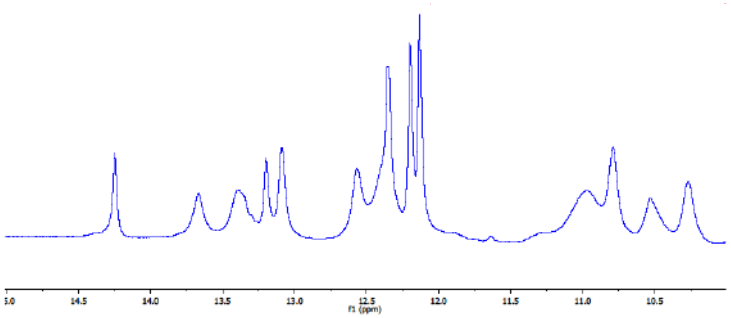
Bypassing induced by mRNA rearrangements and nascent peptide interactions

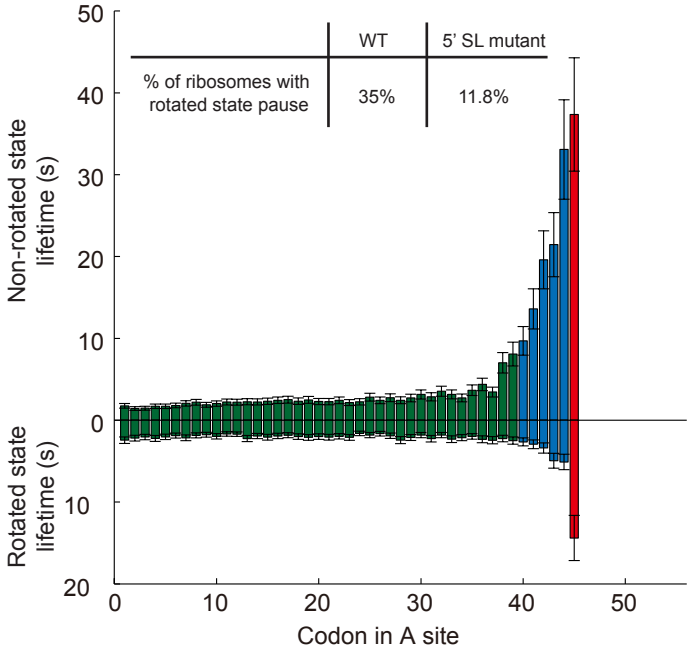


Bypassing induced by mRNA rearrangements and nascent peptide interactions

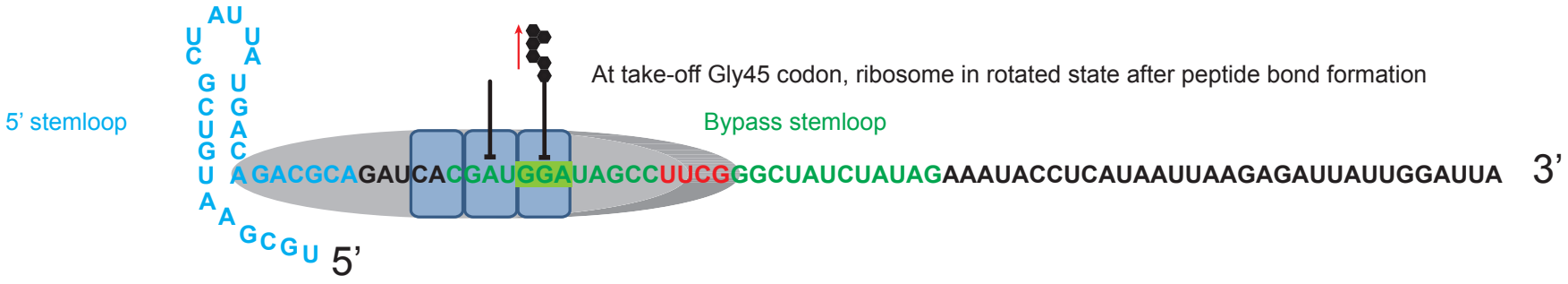


A 5’ hairpin structure is required for bypassing

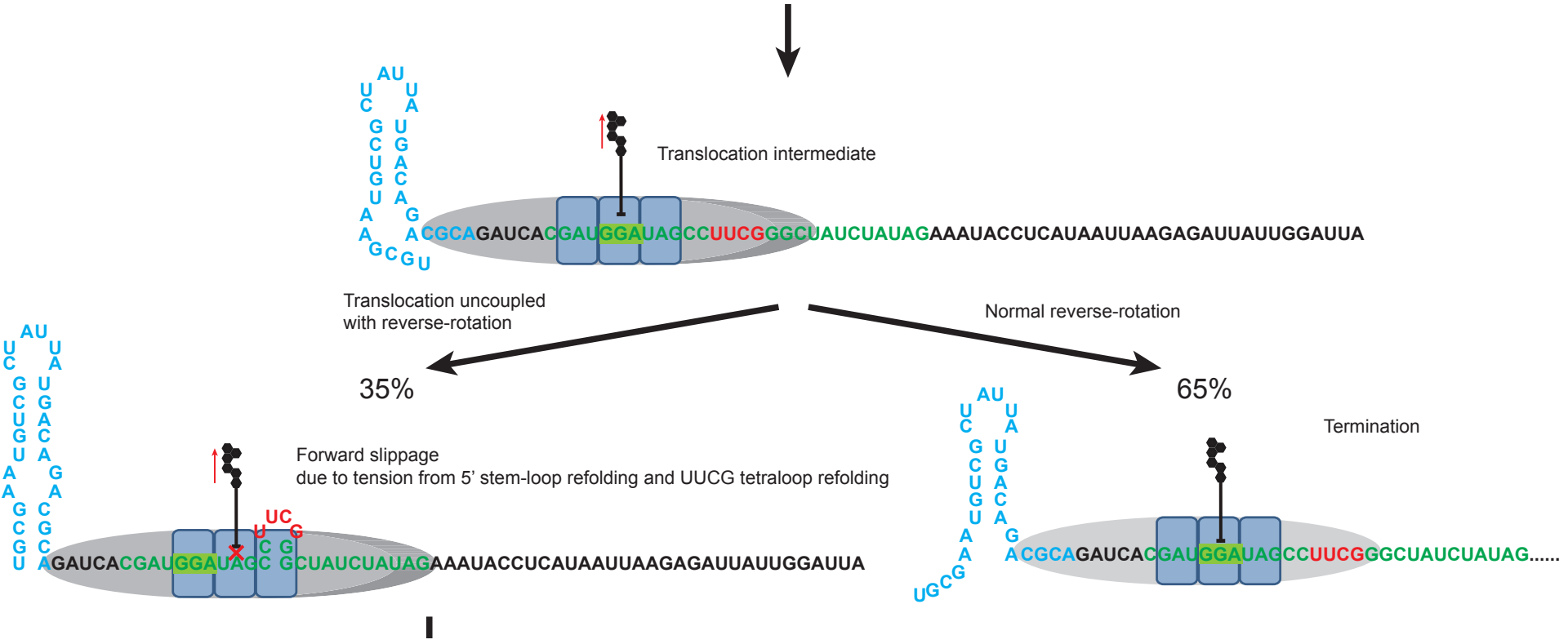




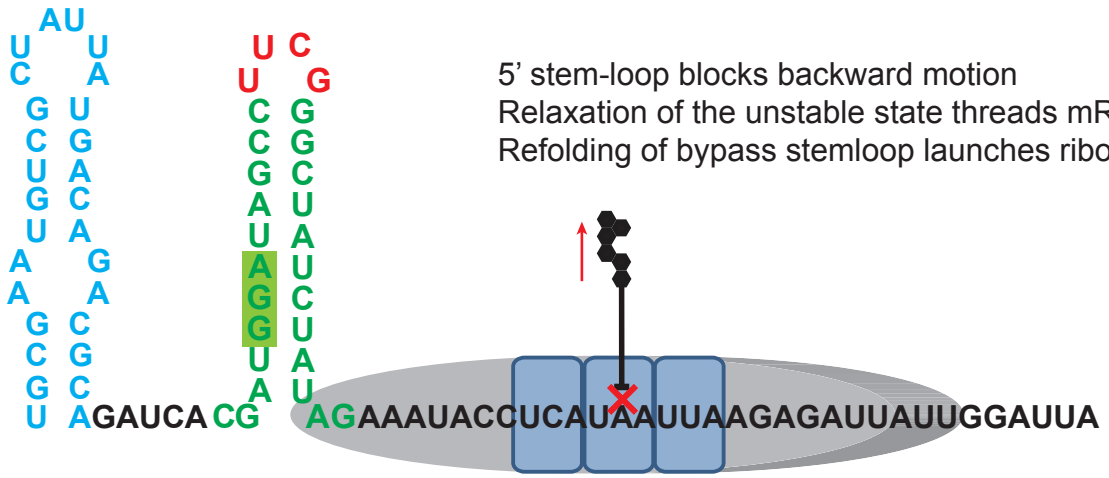
Coupled mRNA rearrangements lead to bypassing



Coupled mRNA rearrangements lead to bypassing

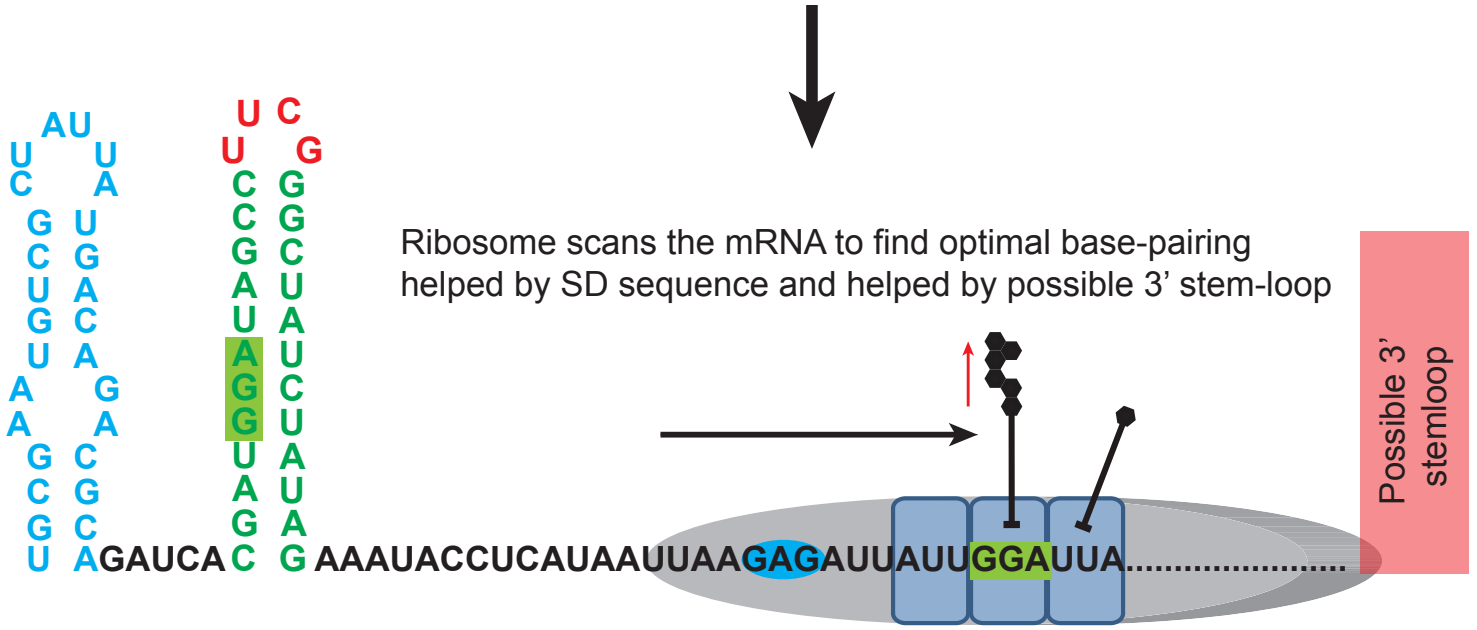


Coupled mRNA rearrangements lead to bypassing



5’ stem-loop blocks backward motion
Relaxation of the unstable state threads mRNA in the 5’ direction.
Refolding of bypass stemloop launches ribosome forward

Coupled mRNA rearrangements lead to bypassing



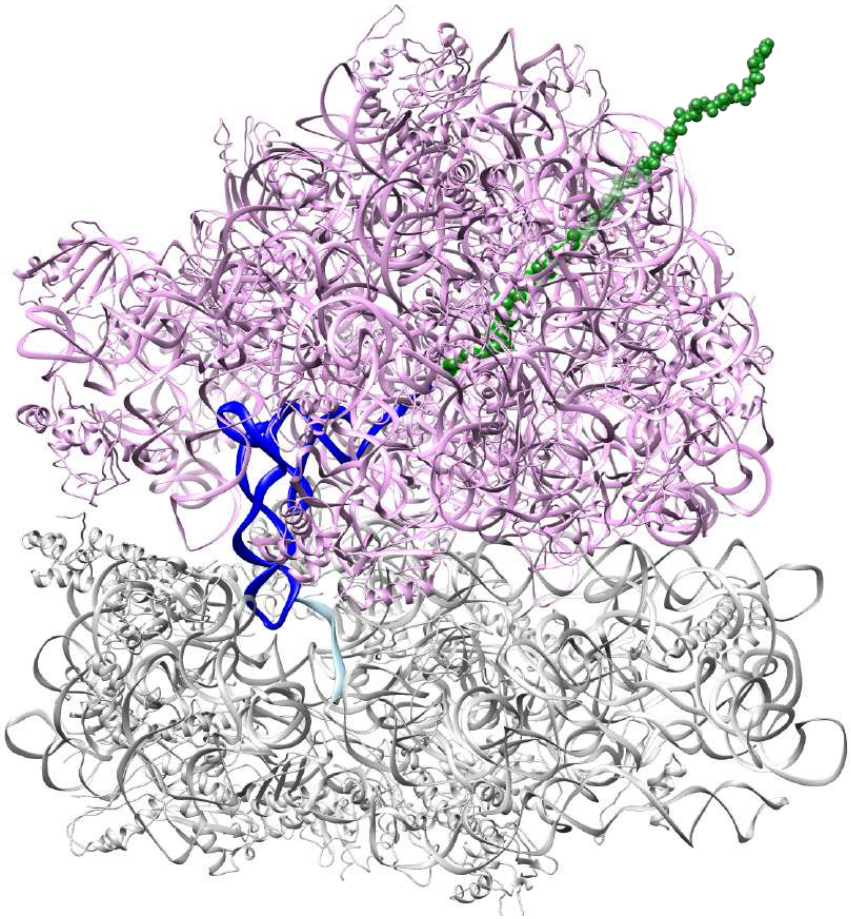
Factors affecting local translational dynamics

1. mRNA sequence

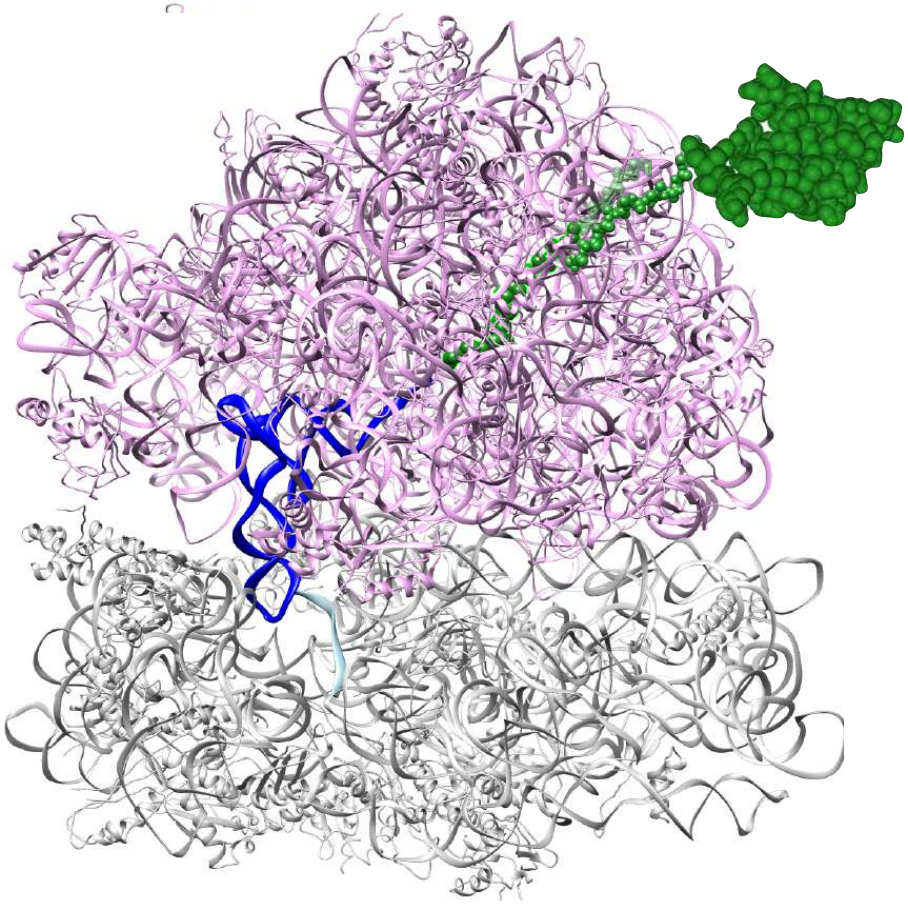
2. mRNA structure

3. nascent peptide chain sequence

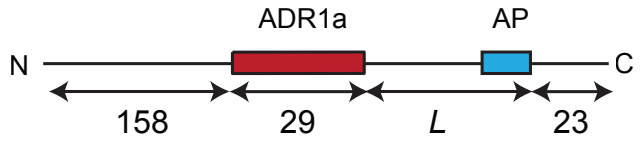
Can folding of nascent protein change dynamics?

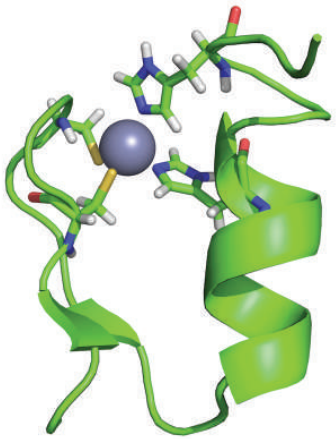


Can folding of nascent protein change dynamics?

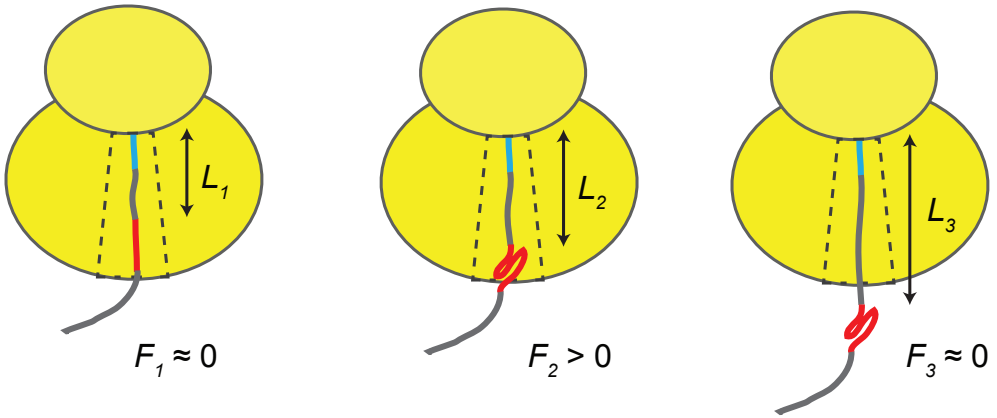


Can folding of nascent protein change dynamics?

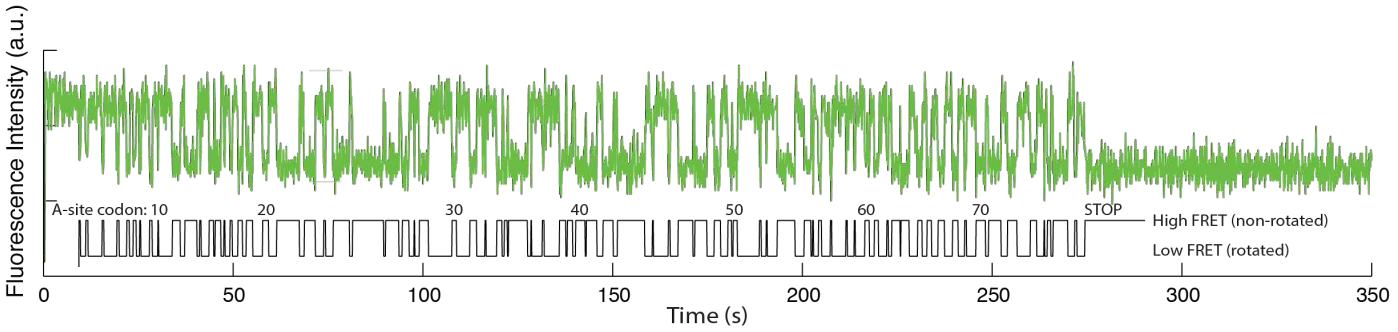


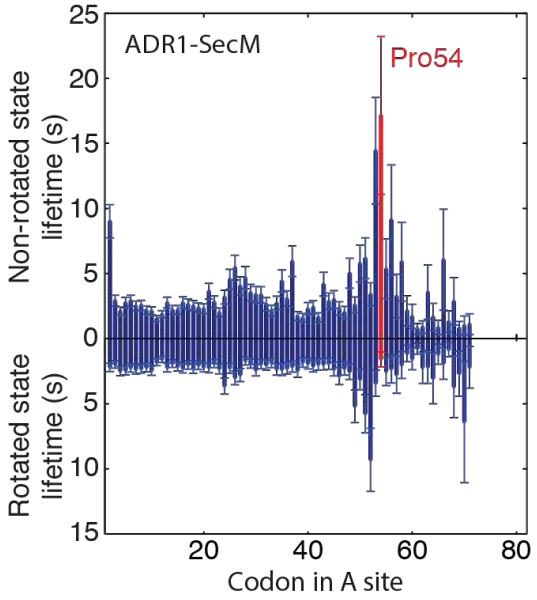


ADR1 folds with Zn2+

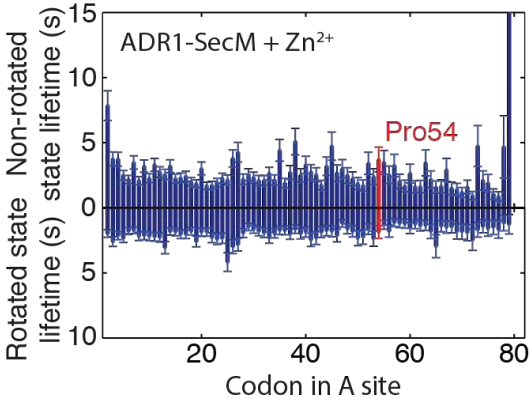


Stalling by SecM sequence relieved by folding at the tunnel exit



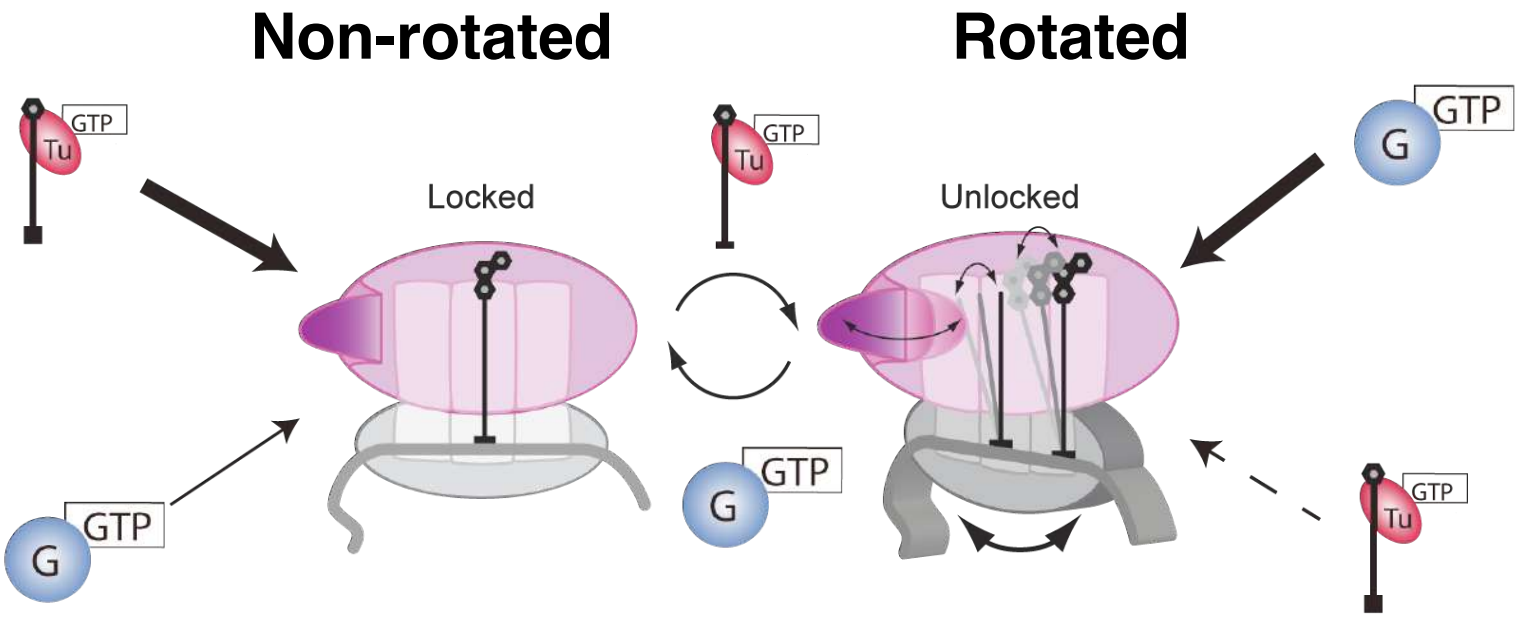


Pausing induced by SecM



Pausing eliminated by folding of ADR1

Pausing in distinct ribosomal states leads to different translational outcomes



- rare codons
- modified codons
- nascent chain interactions

- secondary structures
- ribosomal interactions
- nascent chain interactions
  barriers to movement

Puglisi Group

Albert Tsai
Alexey Petrov
Jin Chen
Junhong Choi
Guy Kornberg
Arjun Prabhakar
Alex Johnson
Magnus Johansson
Sean O'Leary
Hasan Demirci (SLAC)

NIH GM099687, GM51266

Stanford
Peter Sarnow
Gaby Fuchs

Japan
Sotaro Uemura

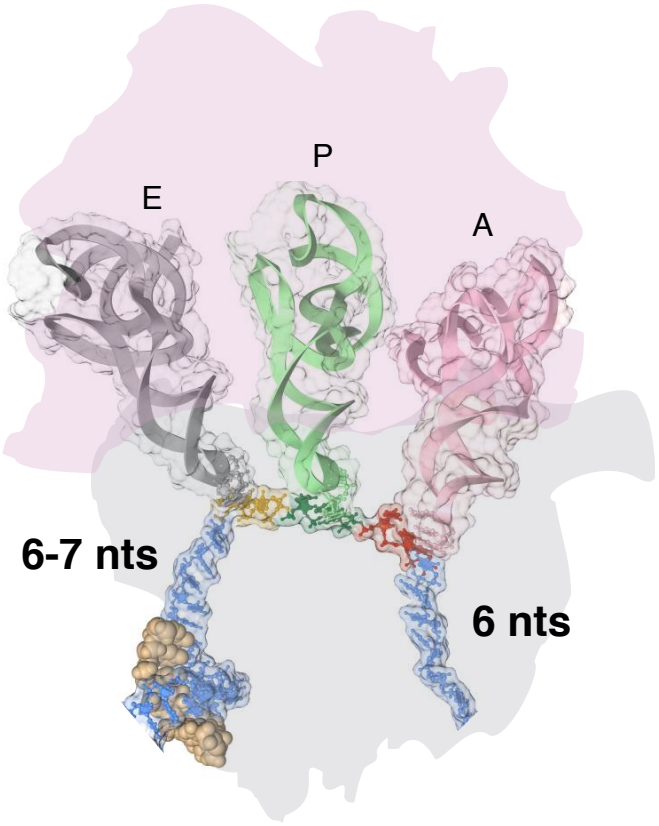
Sweden
Mans Ehrenberg
Kaweng Leong
Gunnar von Heinje
Magnus Johansson

Ireland
John Atkins
Arthur Coakley
Michelle O'Connor

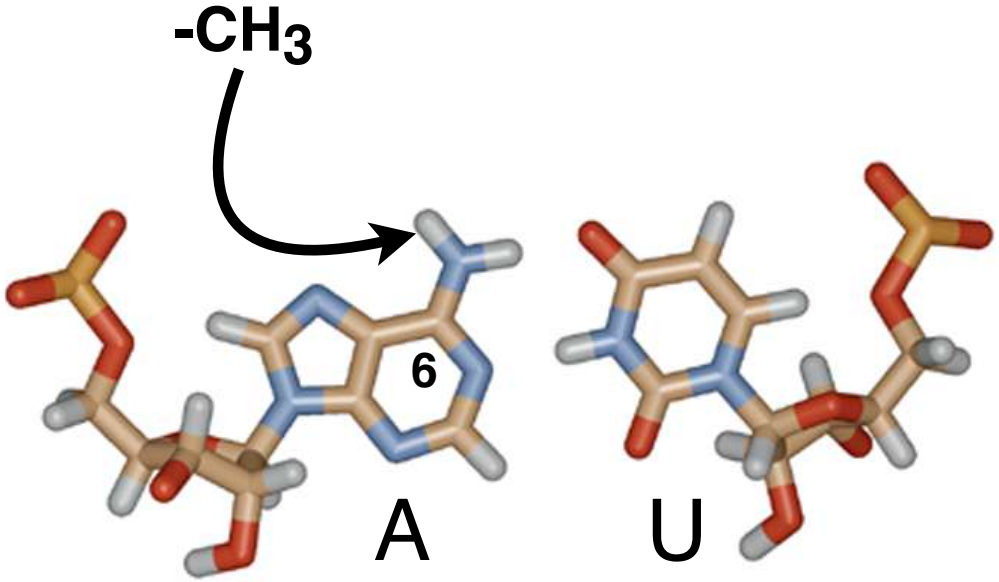
Factors affecting local translational dynamics

1. mRNA sequence

mRNA sequences (Shine-Dalgarno) slow translocation

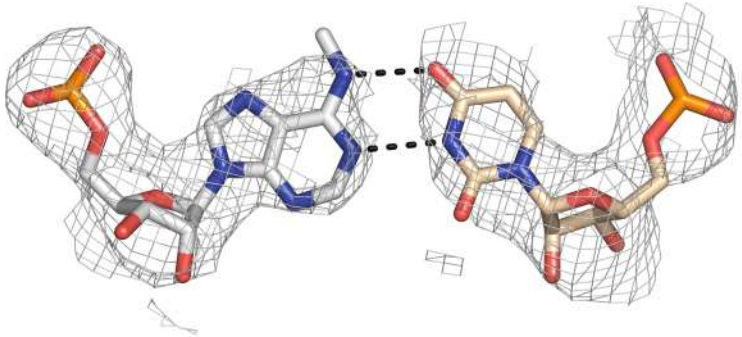


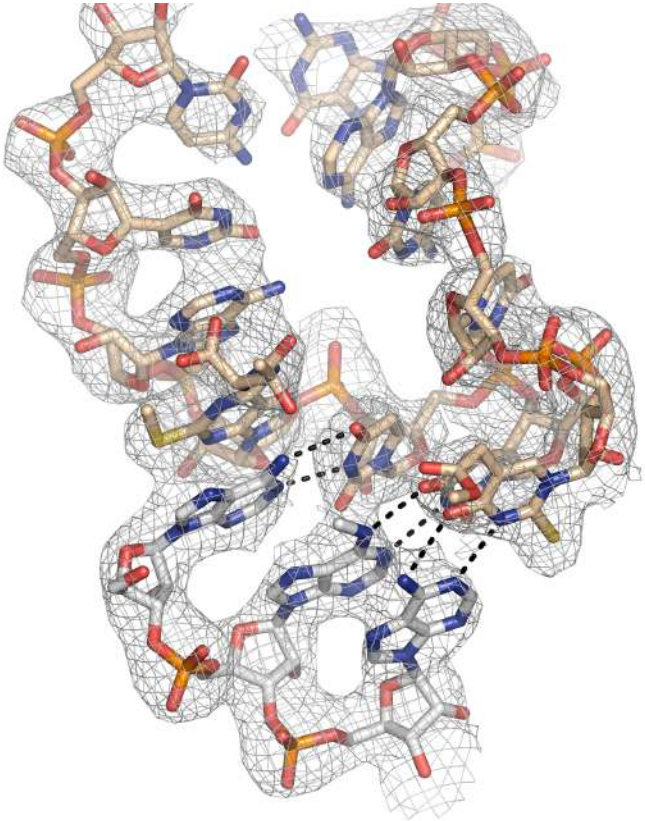
Chemical modification of mRNA: m6A



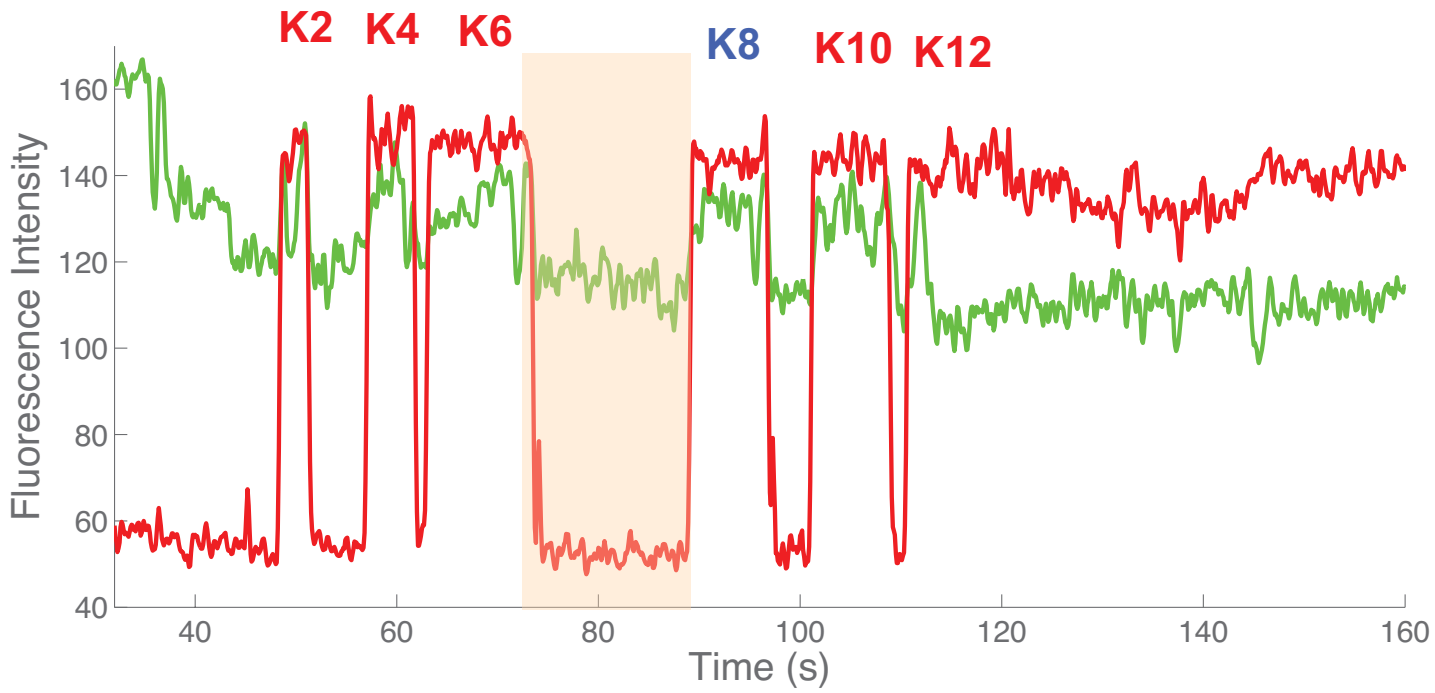
- modified nucleotides found in coding regions of mRNAs
- pseudouridine, 6-methyl-adenosine

How do modifications affect translation?





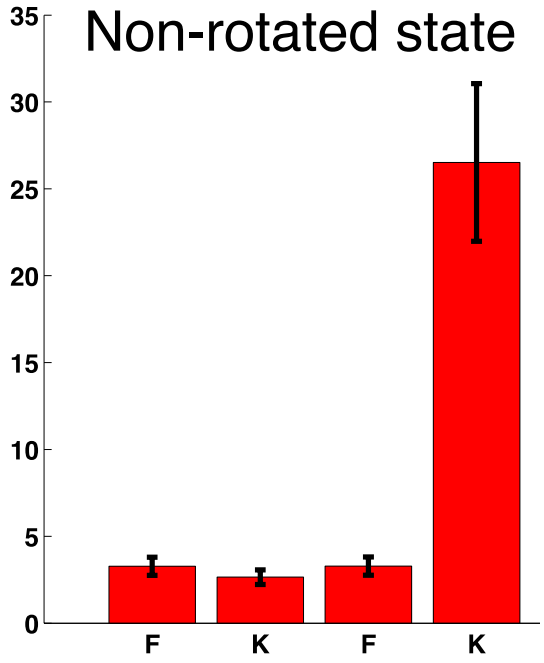
Hasan Demirci, 3.4Å

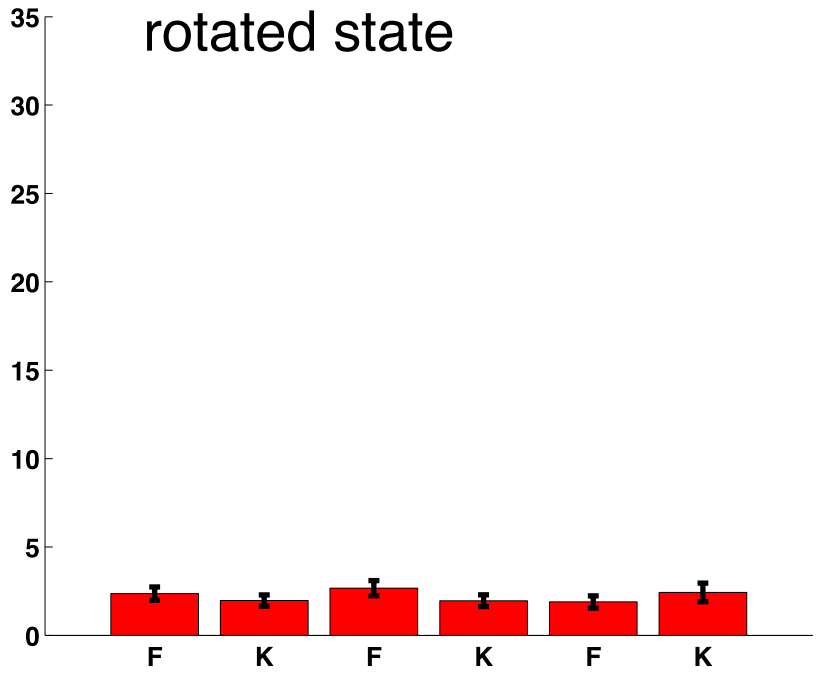


5’…..AUG UUC AAA UUC AAA UUC AAA UUC AA(m6A) UUC AAA UUC AAA UAA……3’

Met0 Phe1 Lys2 Phe3 Lys4 Phe5 Lys6 Phe7 Lys8 Phe9 Lys10 Phe11 Lys12 STOP

m6A modification lengthens the waiting time of ribosomes in the non-rotated state



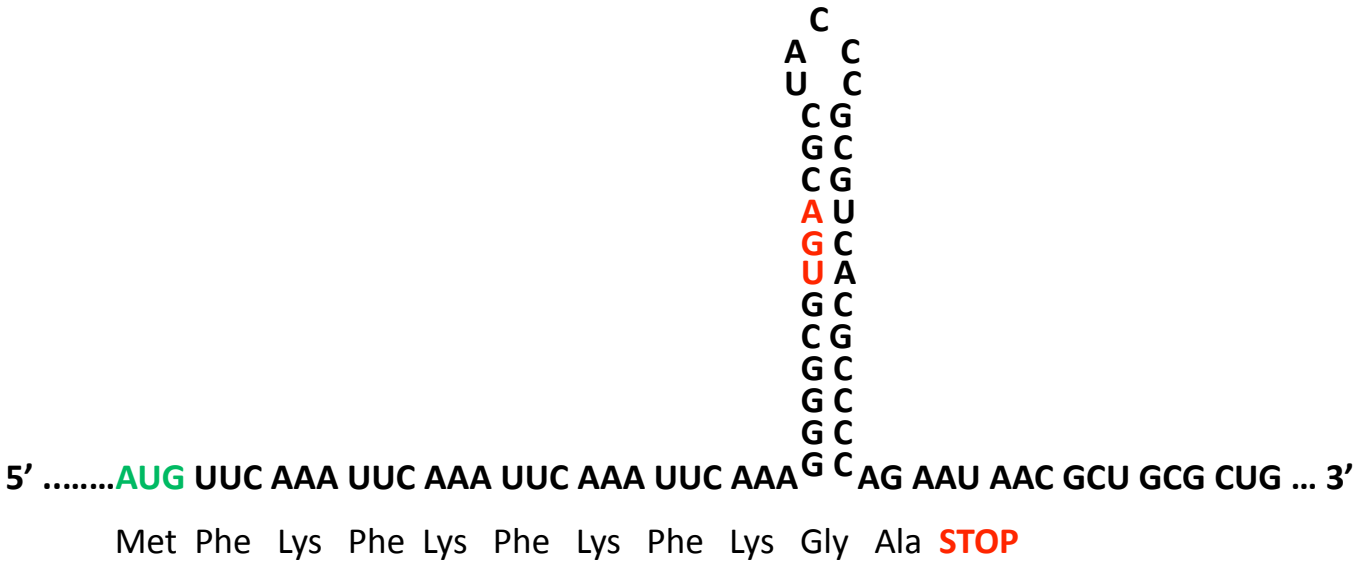


Factors affecting local translational dynamics

2. mRNA structure

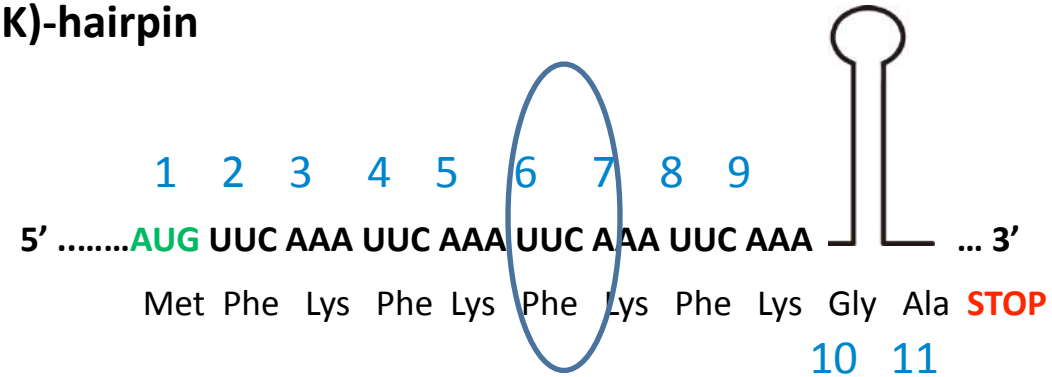
Modulation of translation by mRNA secondary structure

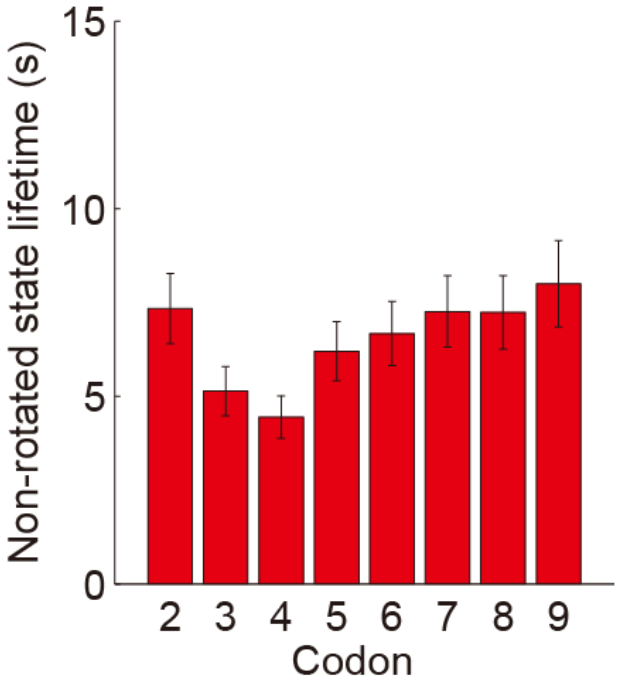
FK sequence with hairpin

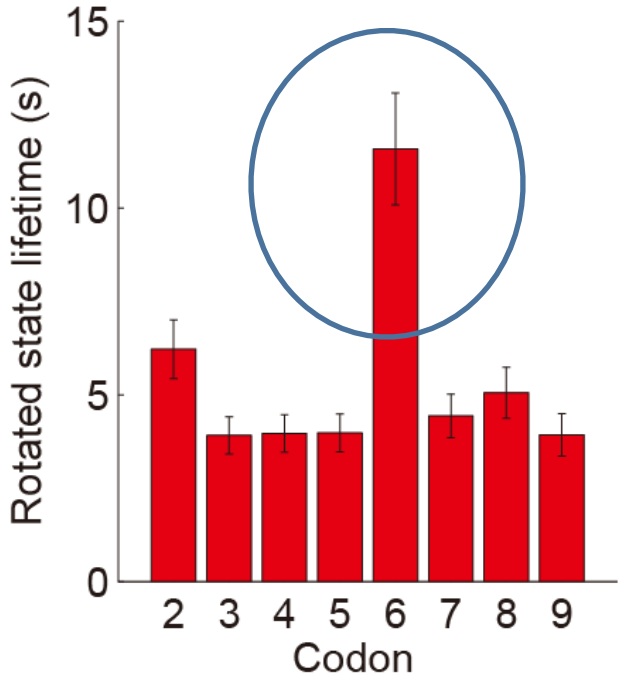


Hairpins pause ribosomes in the rotated pre-translocation state at a single codon

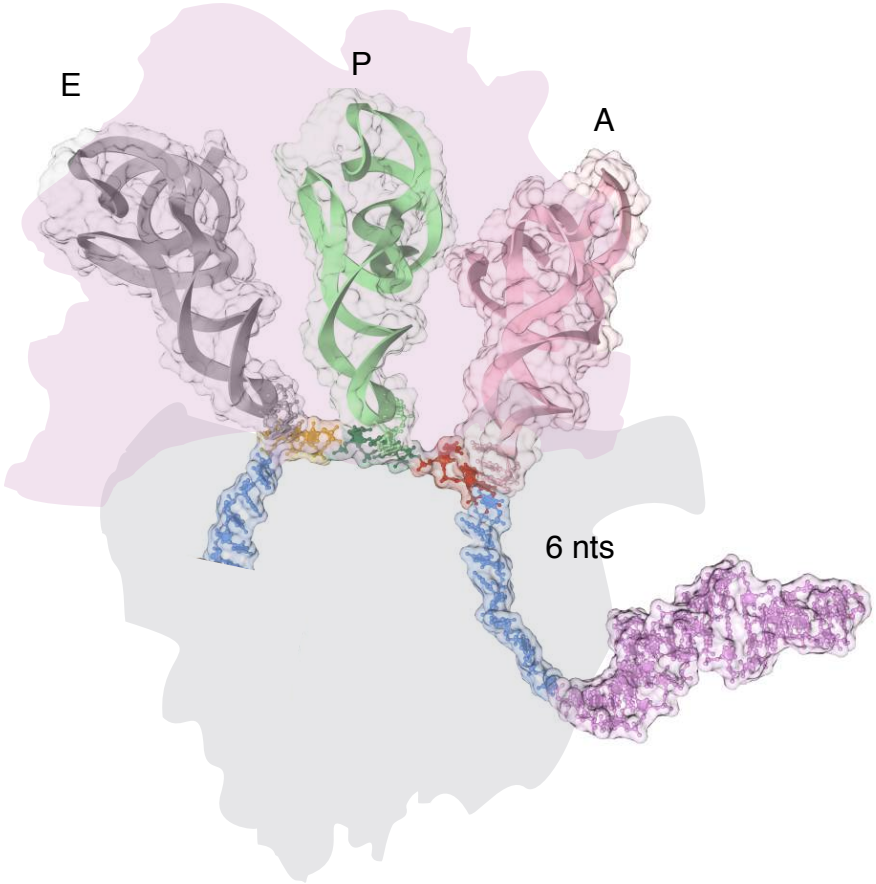
4(FK)-hairpin



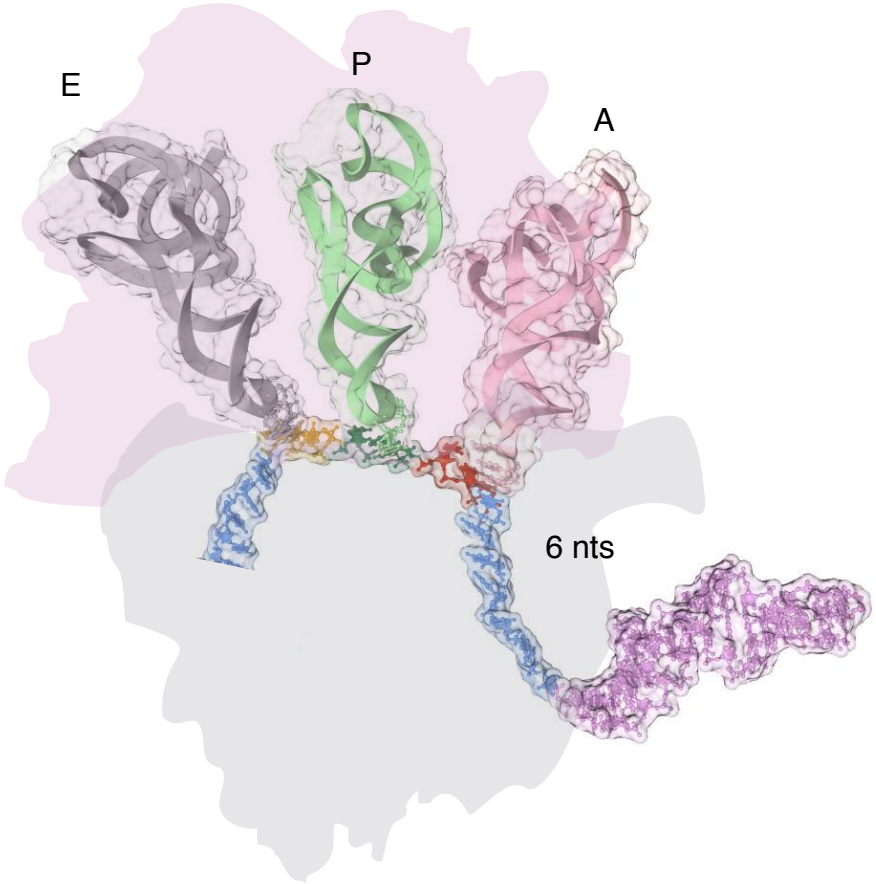




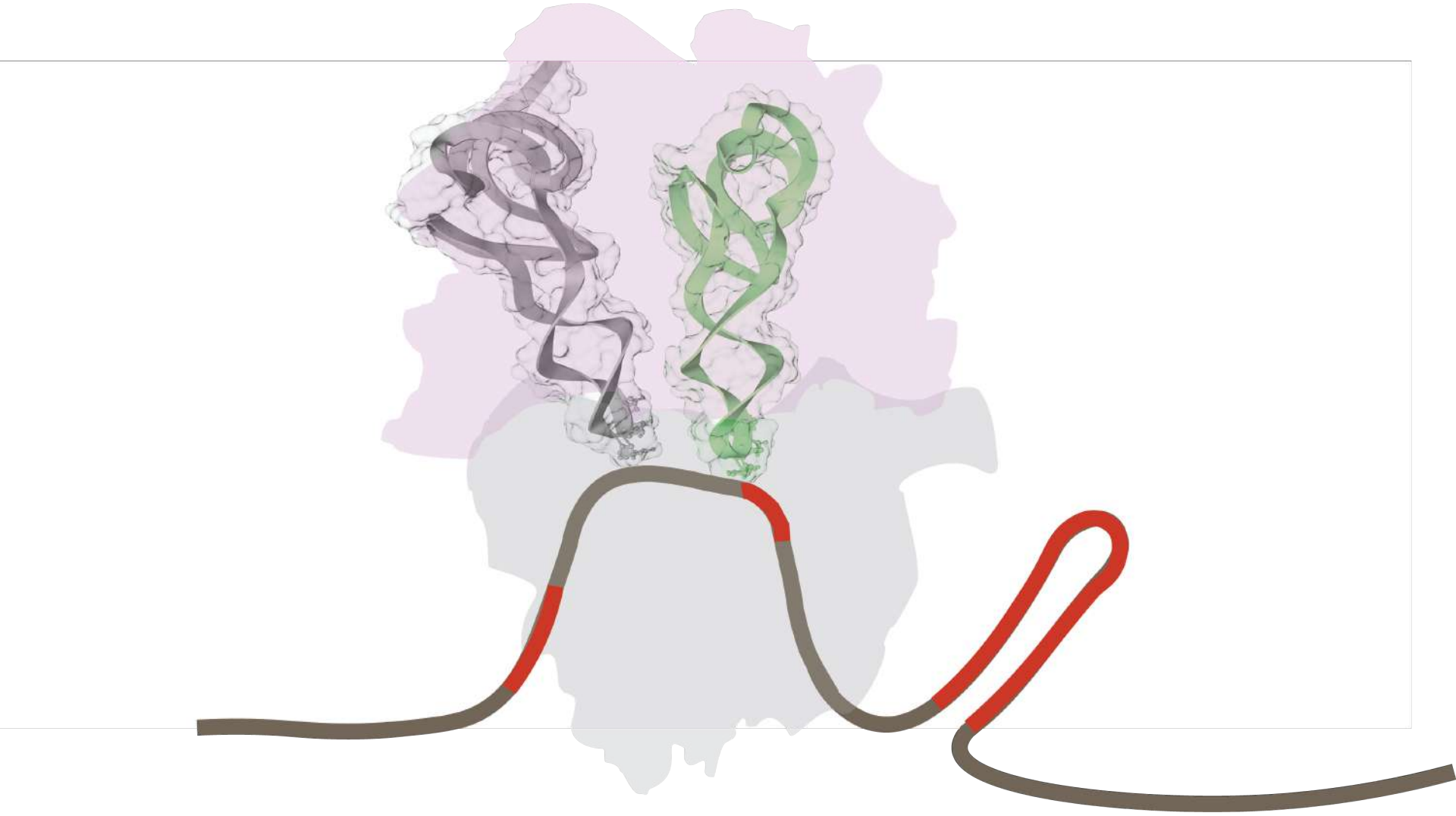
Hairpins are barriers to translocation…..



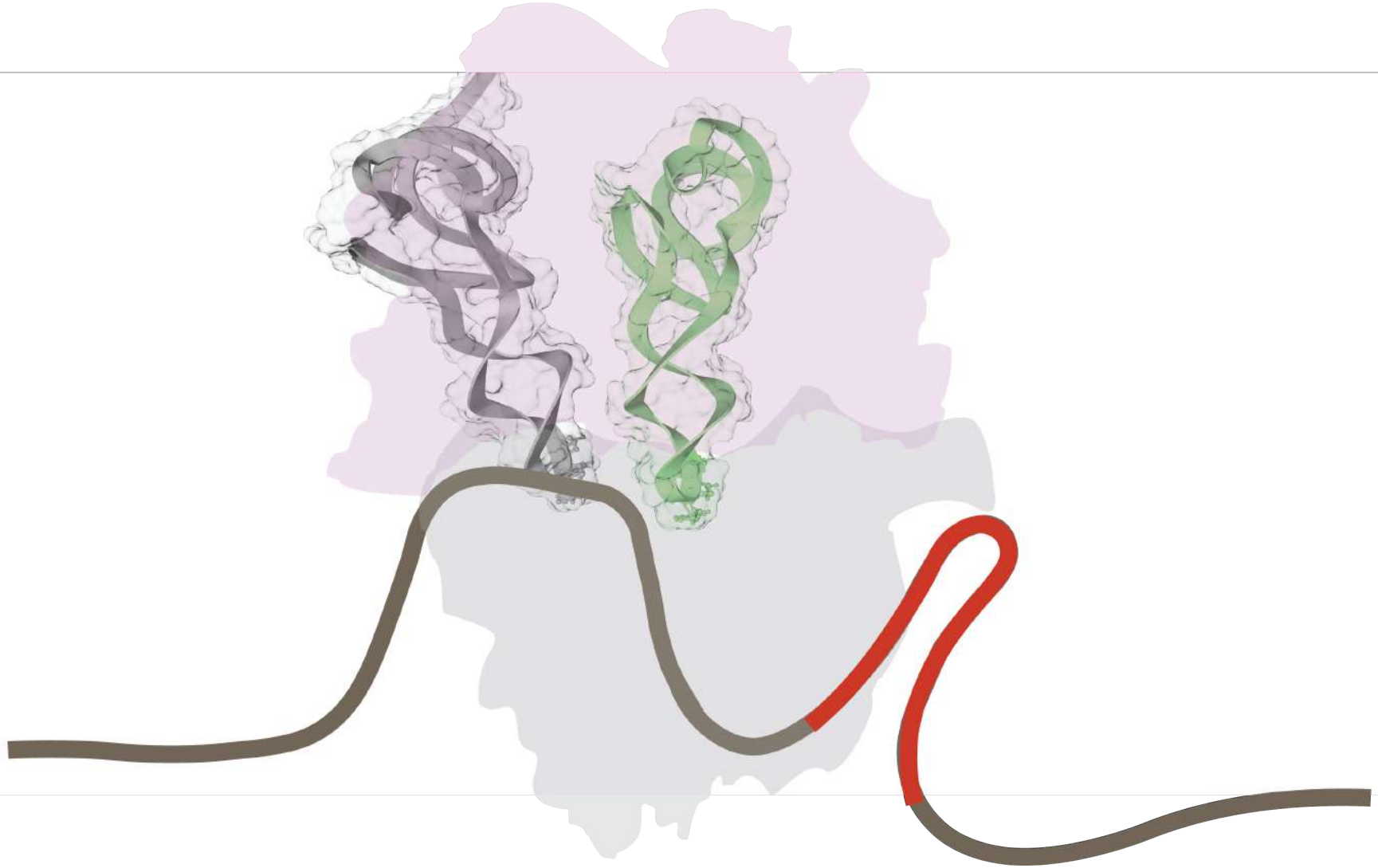
Hairpins are barriers to translocation…..



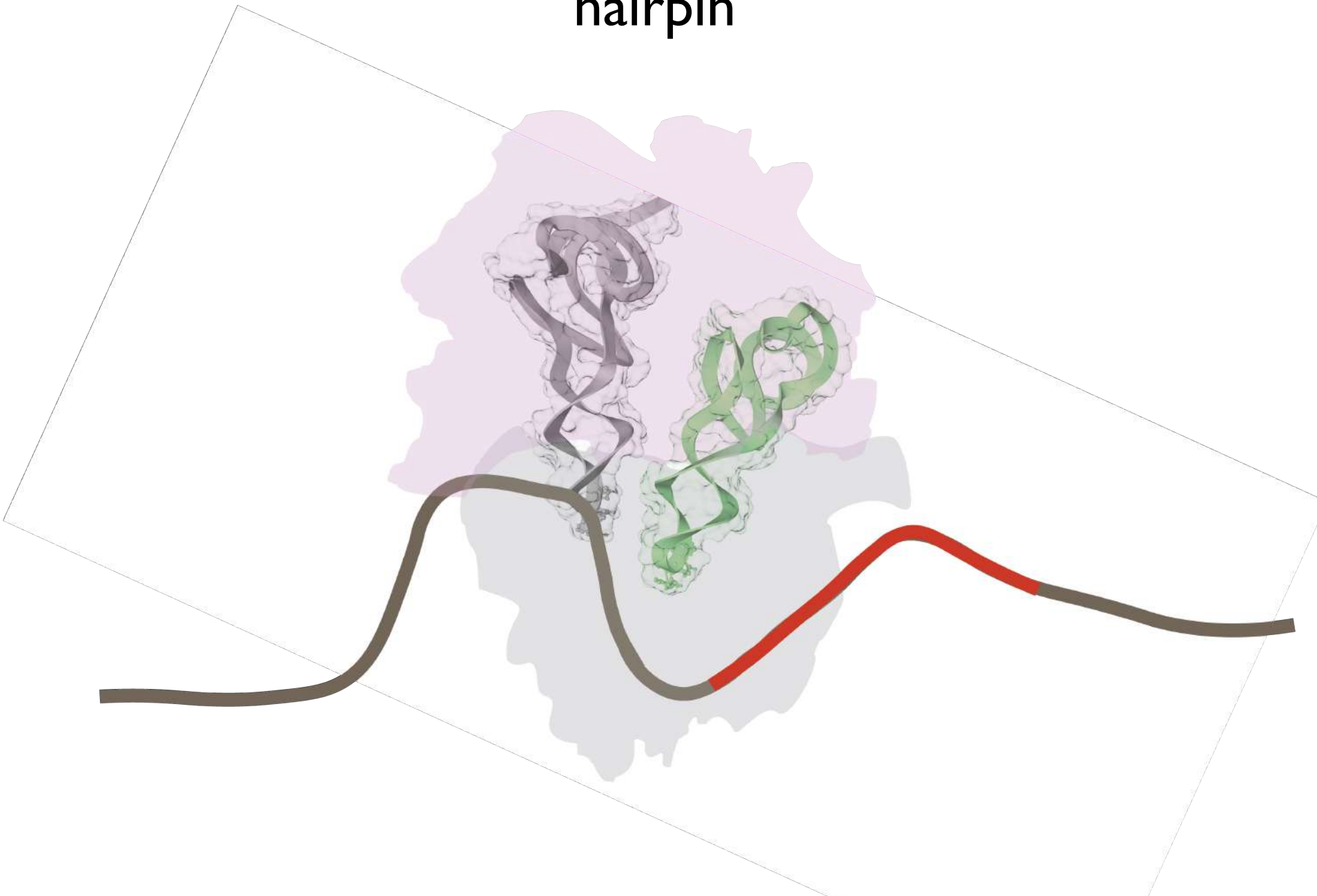
Ribosomes pause only at the base of the hairpin



Ribosomes pause only at the base of the hairpin



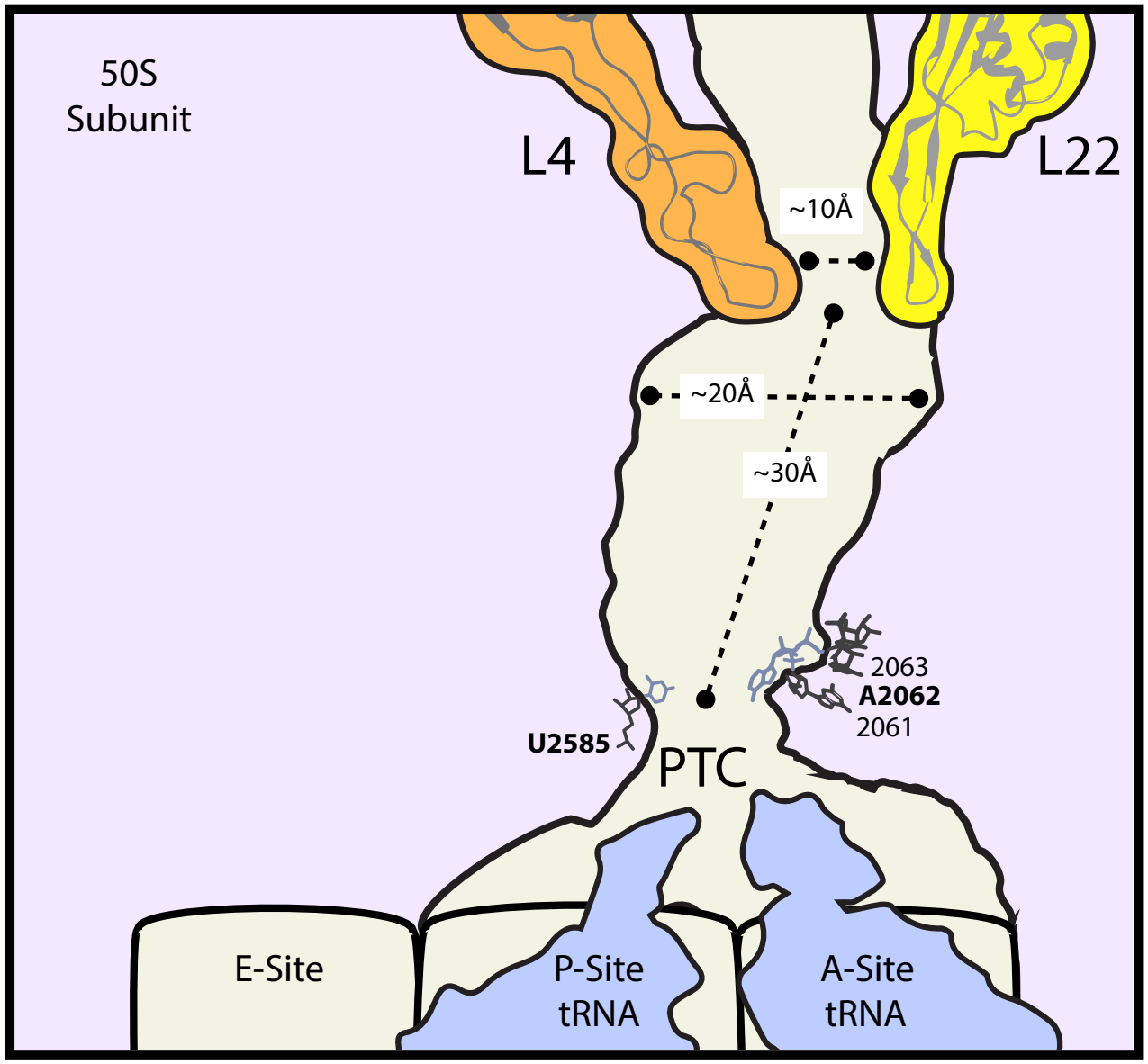
Ribosomes pause only at the base of the hairpin



Factors affecting local translational dynamics

3. Nascent peptide sequence

Is the exit-tunnel a passive conduit?



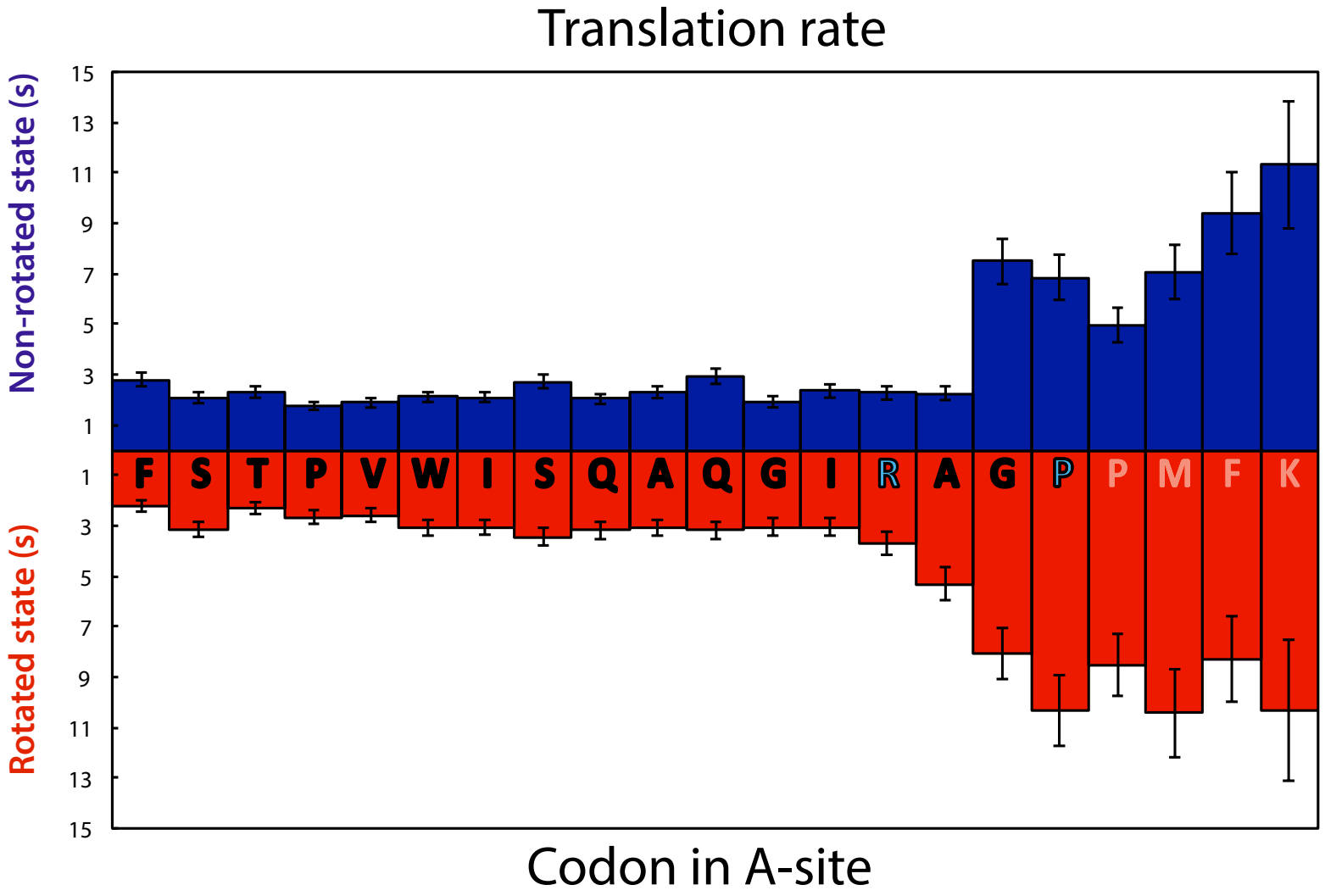
Nascent Chain Stalling Sequences

K. Ito et al. / Biochemical and Biophysical Research Communications 393 (2010) 1–5

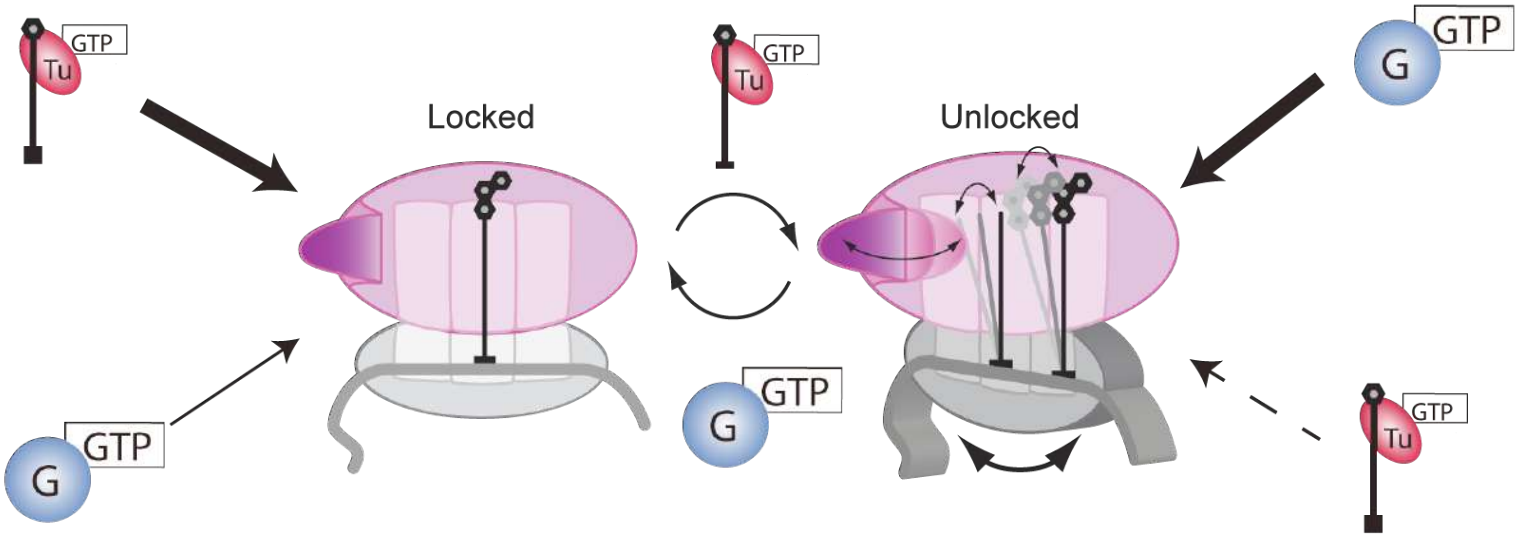
---- *L22/L4 constriction* -------- ---------- *PTC* -----

	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	P	A	
Inducible class																						
1								Erythromycin +				**M**	**x**	**x**	**x**	**x**	**I**	**F**	**V**	**I**	*s*	ErmCL
2					Tryptophan +			**W**	**x**	**x**	**x**	**D**	**x**	**x**	**I**	**x**	**x**	**x**	**x**	**P**	*	TnaC *Ec*
3					Tryptophan +			**W**	**x**	**x**	**x**	**D**	**x**	**x**	**L**	**x**	**x**	**x**	**x**	**P**	*k*	TnaC *Pv*
Intrinsic class																						
4					**F**	**x**	**x**	**x**	**x**	**W**	**I**	**x**	**x**	**x**	**x**	**G**	**I**	**R**	**x**	**G**	**P**	SecM *Ec*
5					**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**P**	***P***	**I**	**R**	**x**	**G**	**P**	SecM mut
6				**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**H**	**A**	***P***	**I**	**R**	**G**	**S**	**P**	SecM *Ms*
7												**F**	**x**	**x**	**Y**	**x**	**I**	**W**	**P**	**P**	**P**	Evolved
8	**R**	**I**	**x**	**x**	**W**	**I**	**x**	**x**	**x**	**x**	**x**	**M**	**N**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	MifM *Bs*

SecM nascent chain interactions cause complex translational pausing

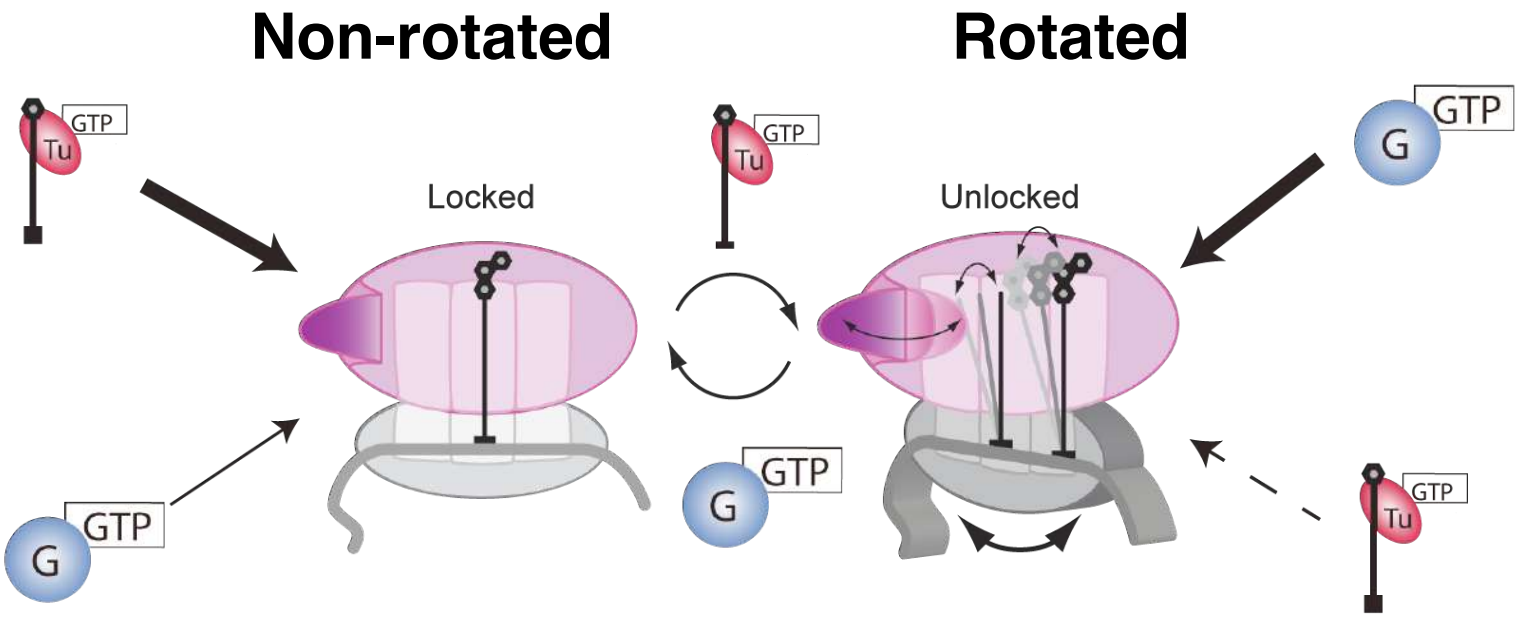


Are all pauses the same?



can different ribosomal conformations shunt to different translational pathways?

Two states, different properties

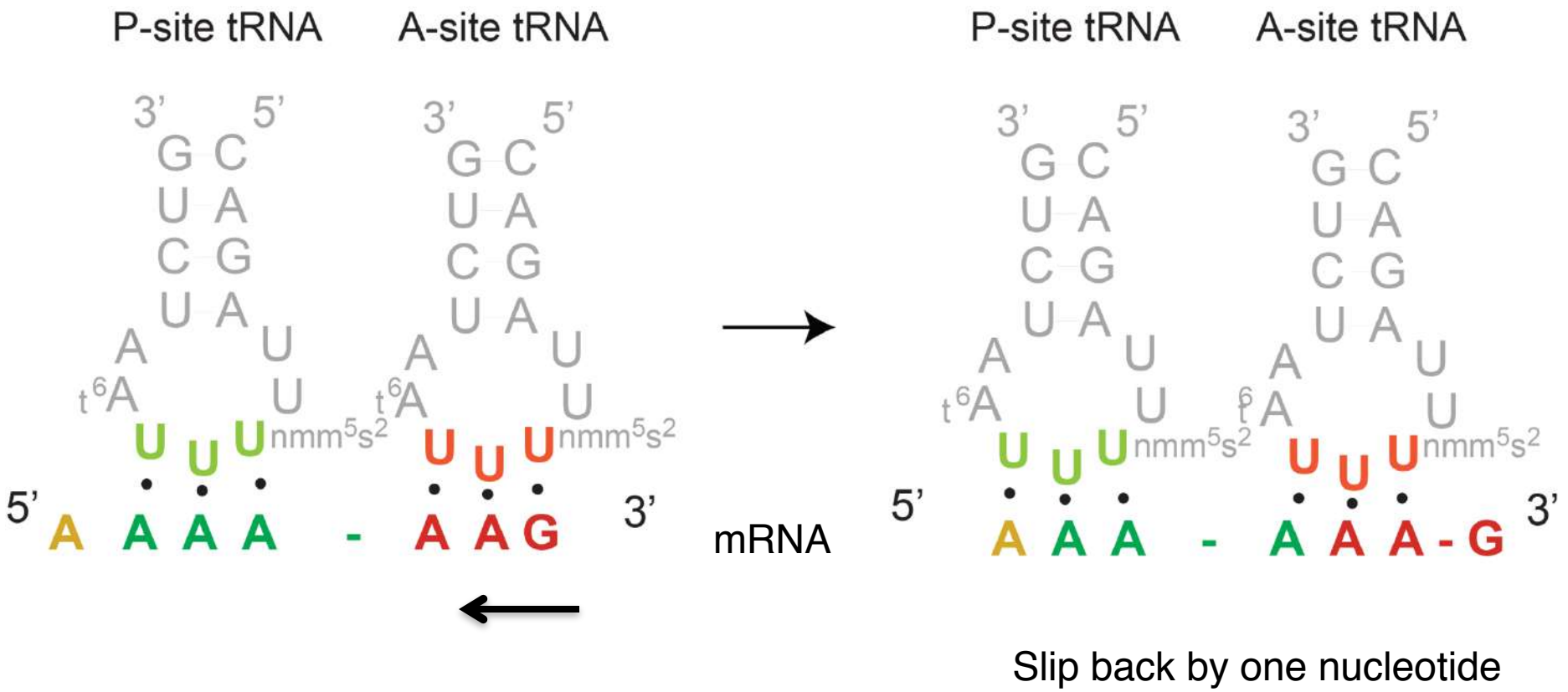


High affinity for tRNAs
High affinity for mRNA

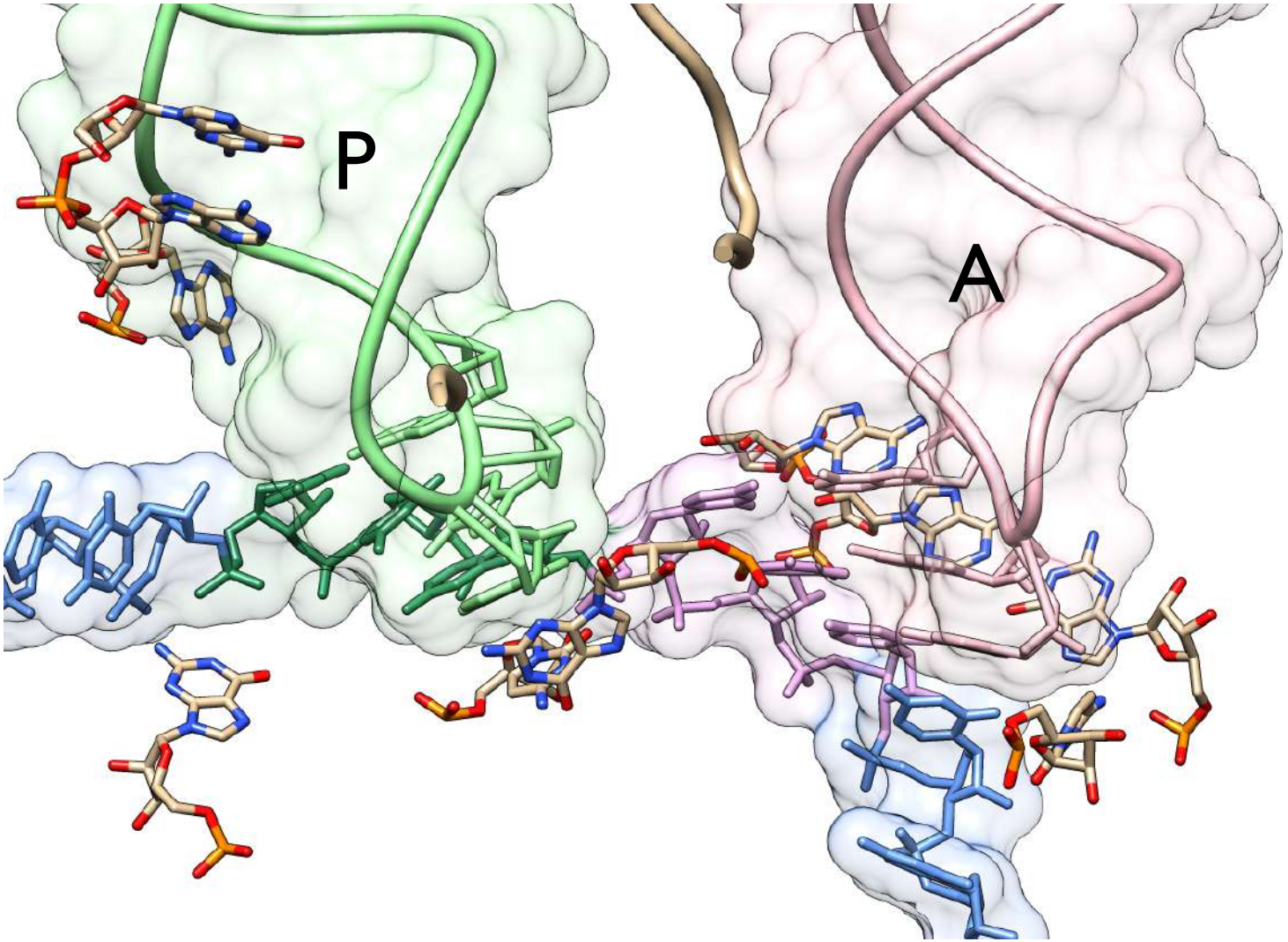
Lower affinity for tRNAs
Lower affinity for mRNA

Recoding—frameshifting and bypassing

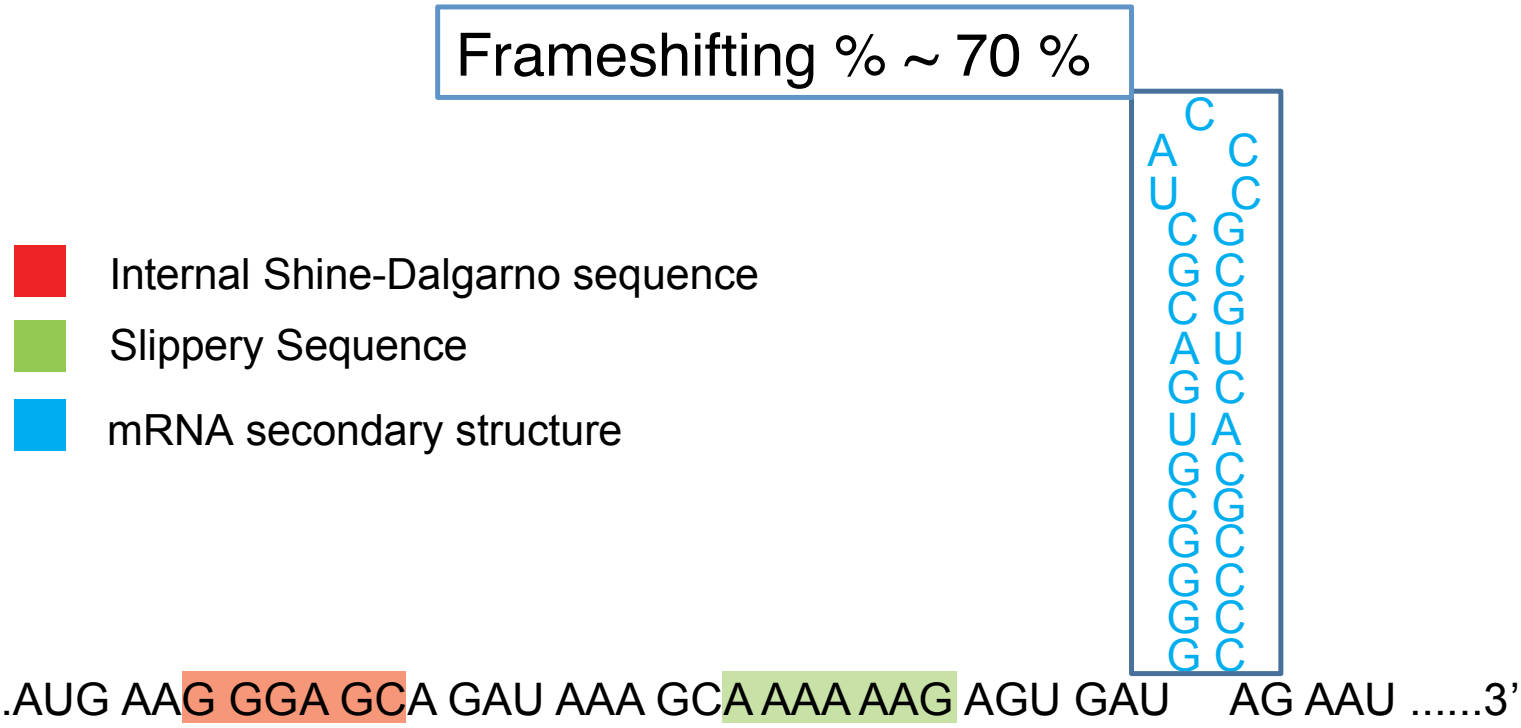
Frameshifting requires breaking of tRNA-mRNA interactions and slippage



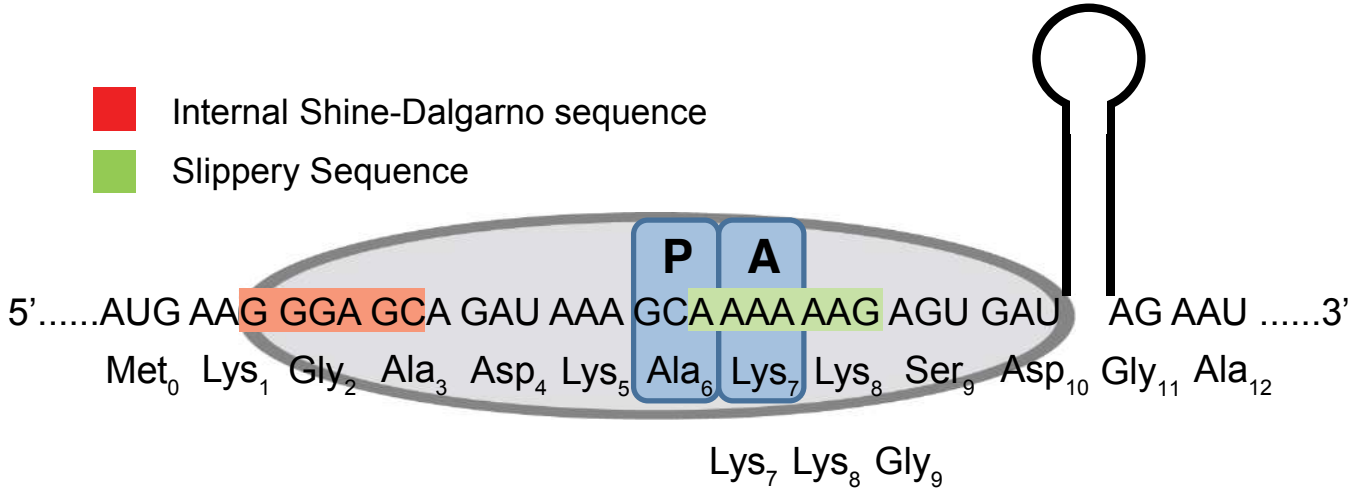
codon-anticodon interactions and reading frame

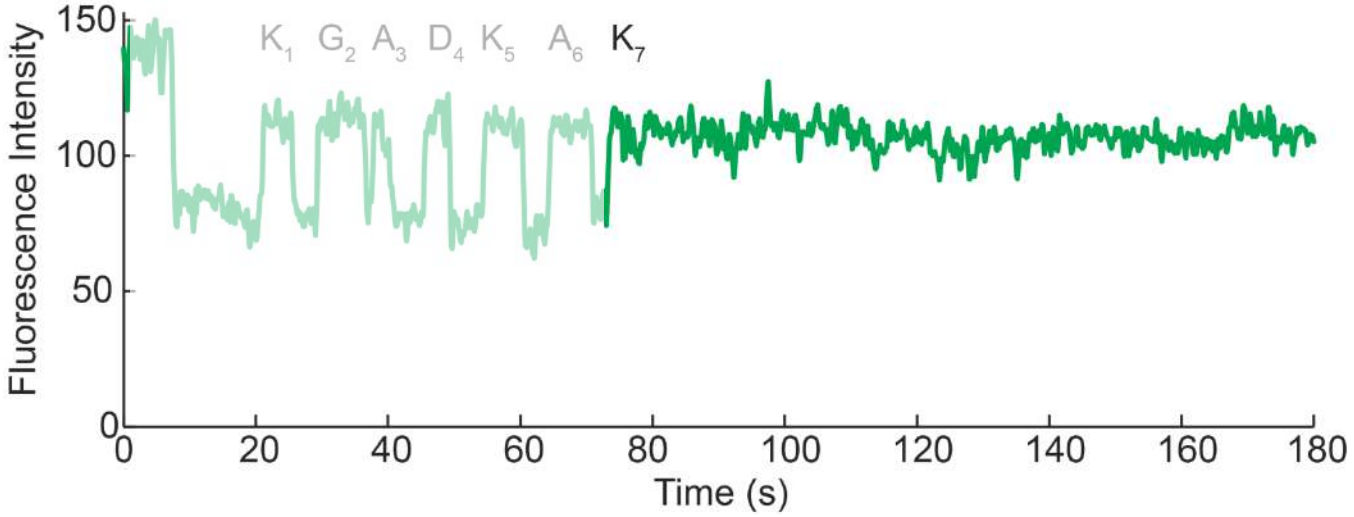


mRNA features stimulate frameshifting

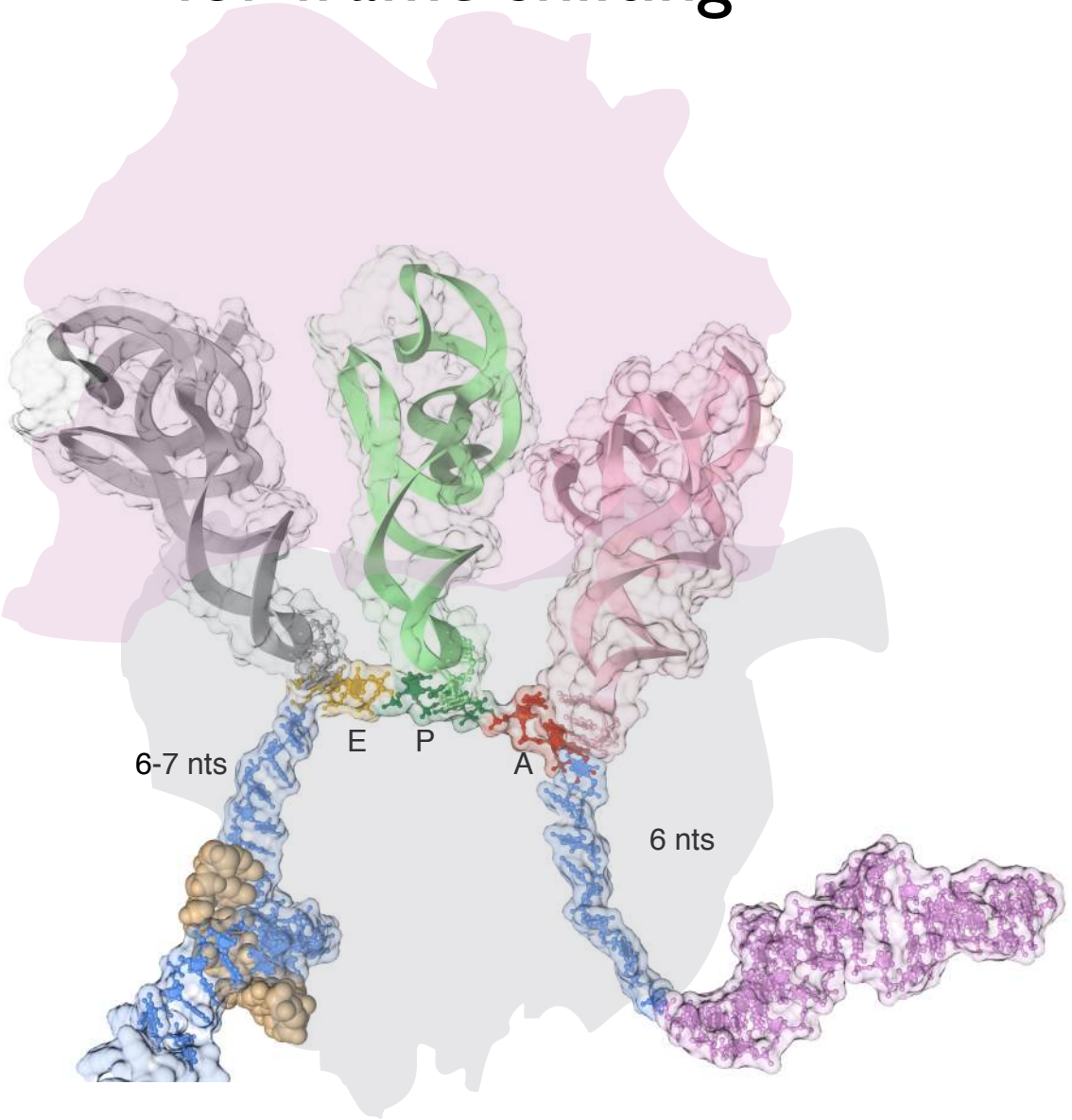


The P site is positioned 3 nucleotides upstream of the slippery sequence

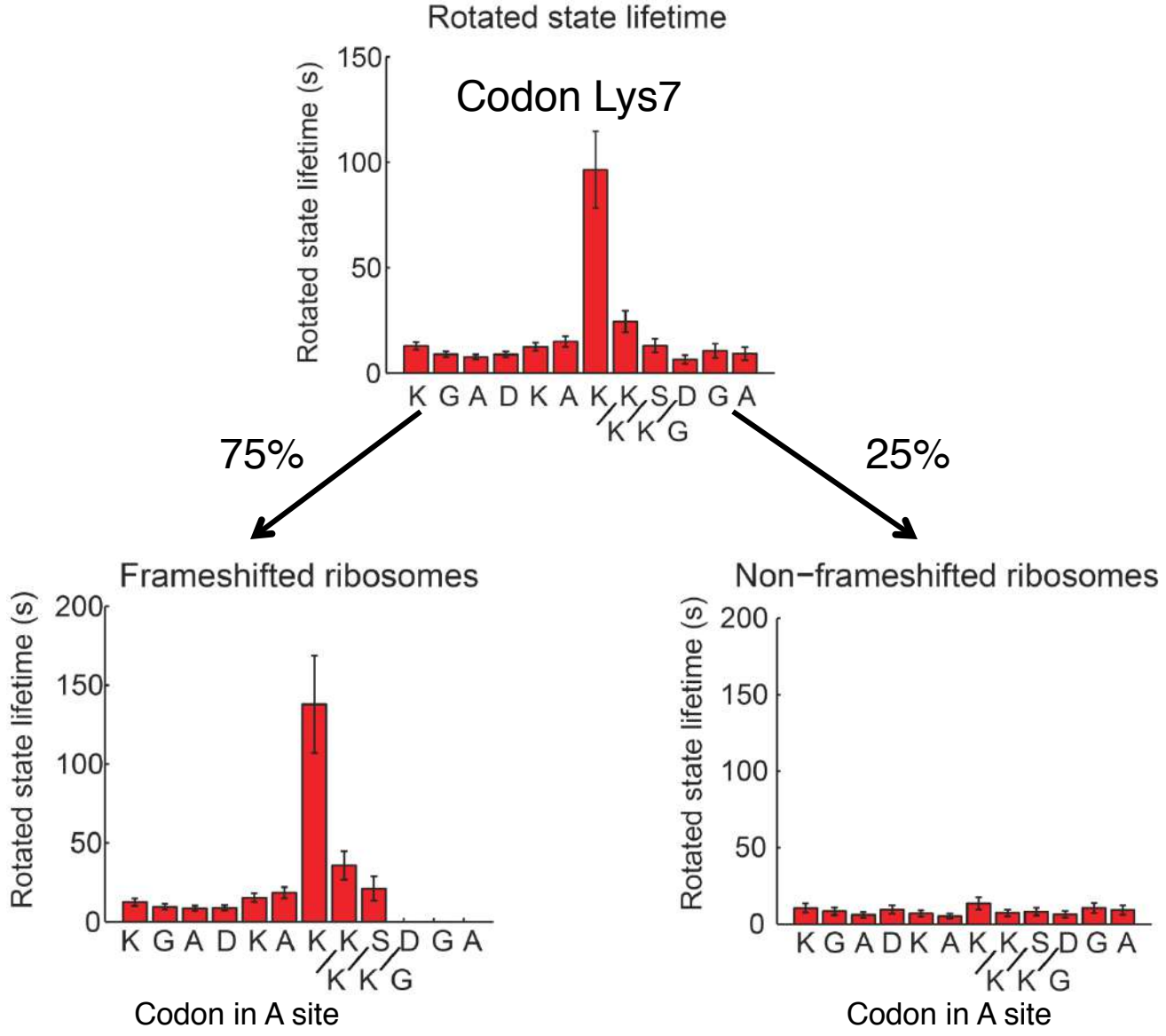




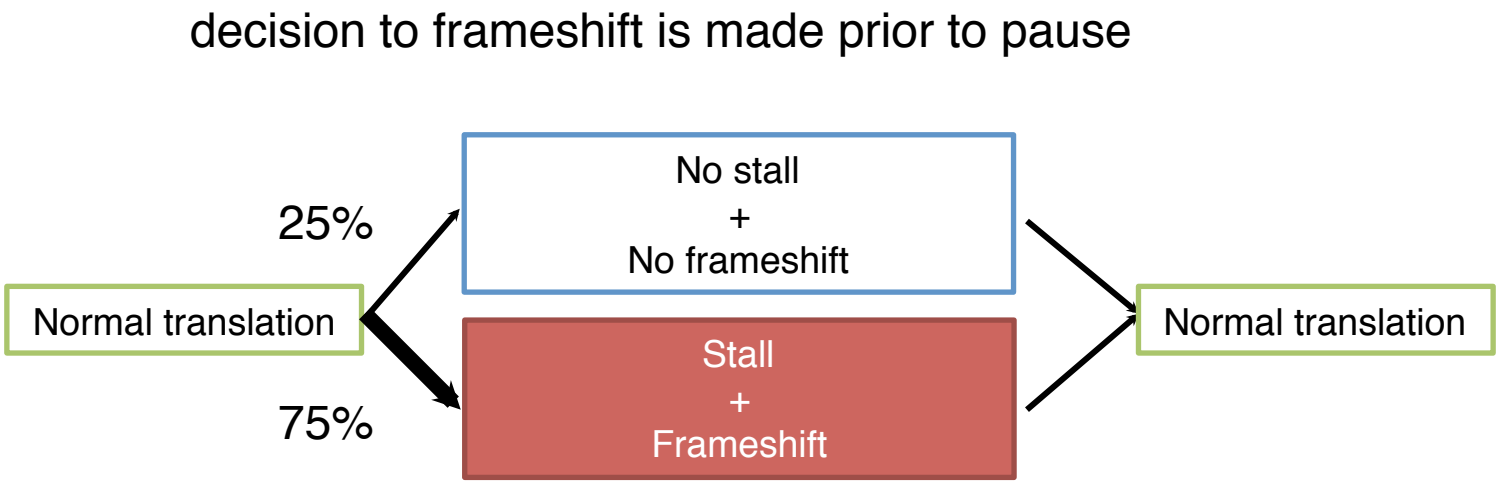
Barriers created by SD and hairpin set the stage for frame shifting



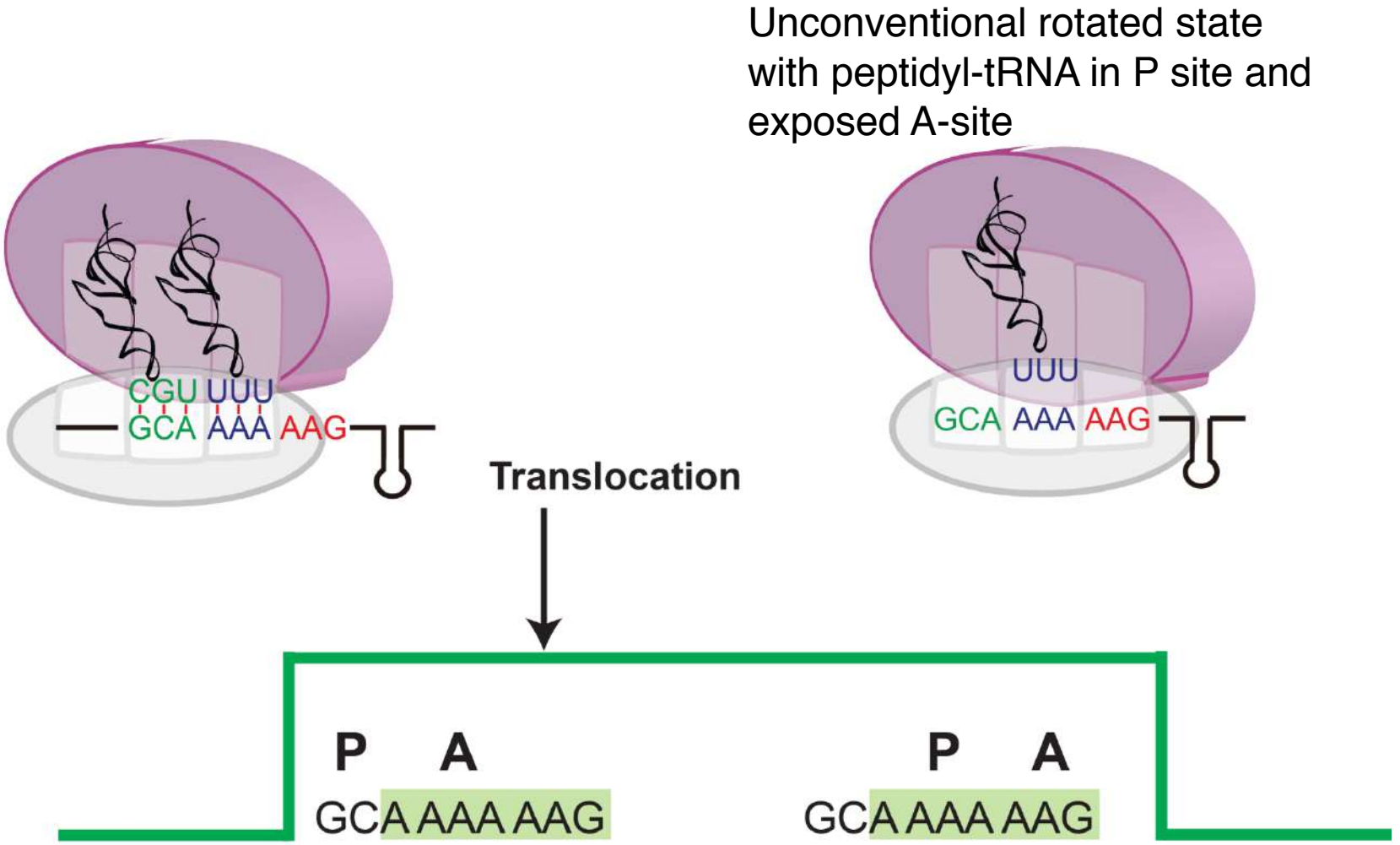
The long rotated state pause is characteristic of frameshifted ribosomes



Branchpoint of pathways in -1 frameshifting



Uncoupled translocation leaves the ribosome in an unconventional rotated state

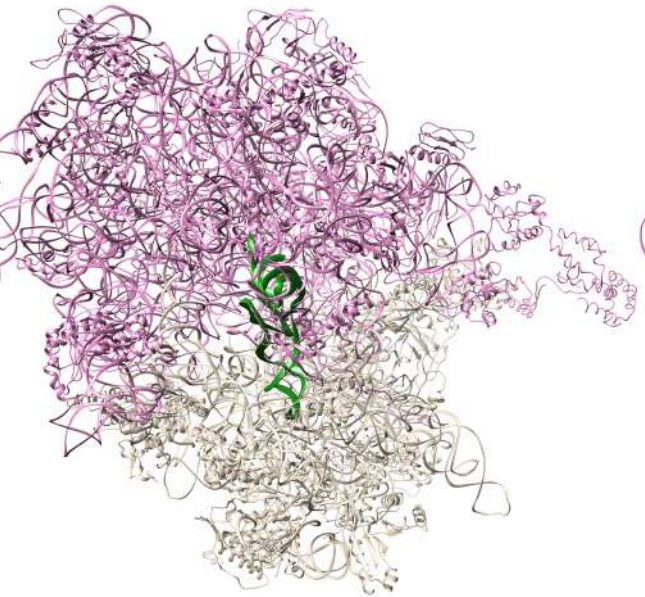


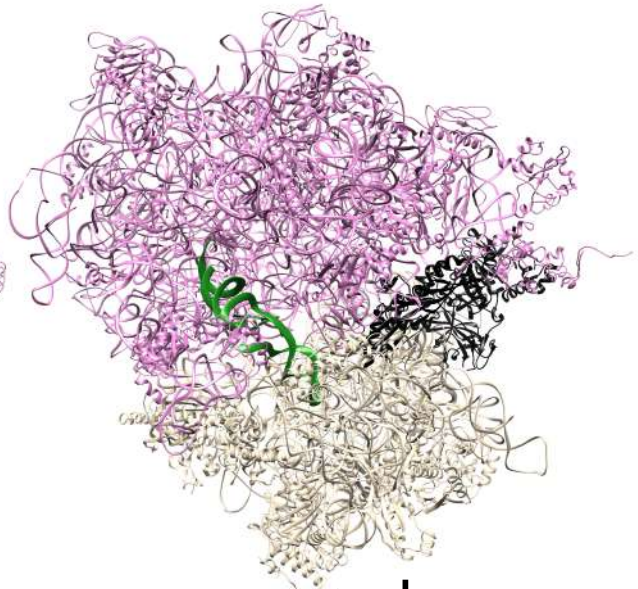
Uncoupled translocation leaves the ribosome in an unconventional rotated state

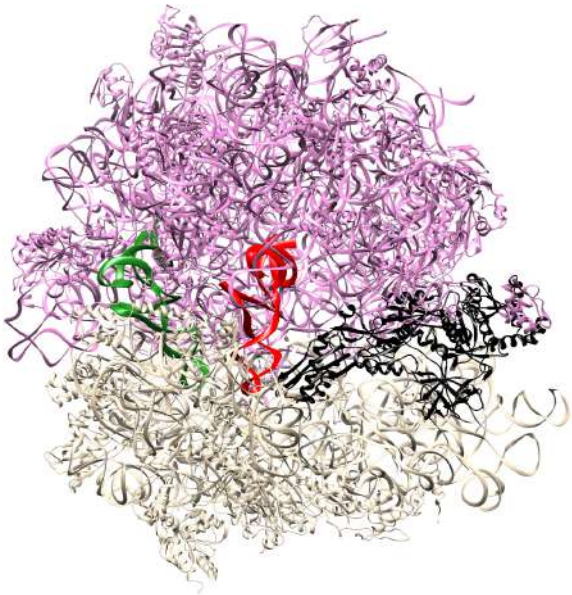
P classical

P/E hybrid

A/P, P/E hybrid







non-rotated

rotated

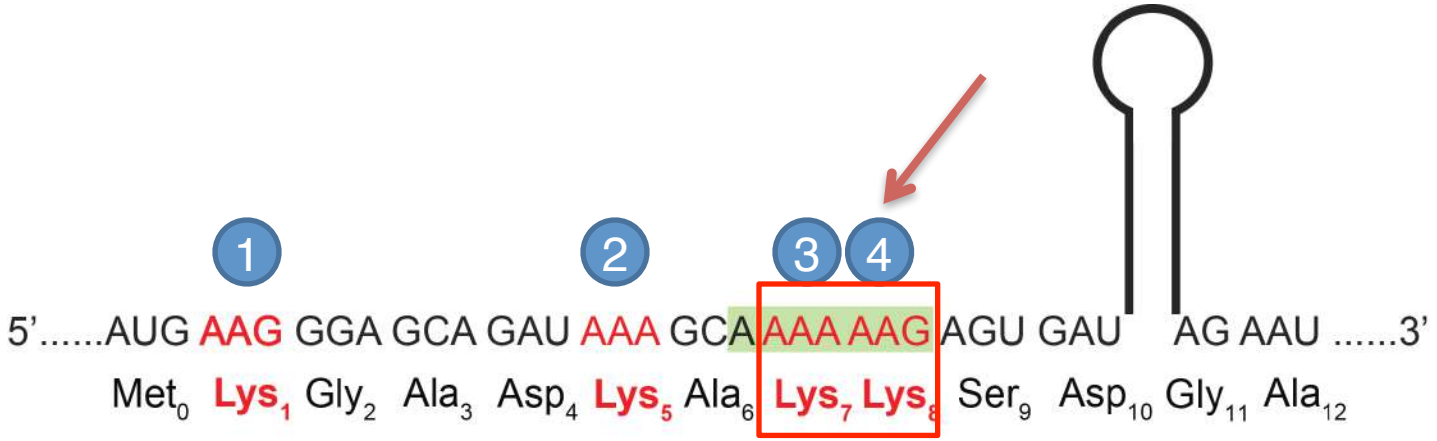
rotated

EF-Tu-tRNA substrate

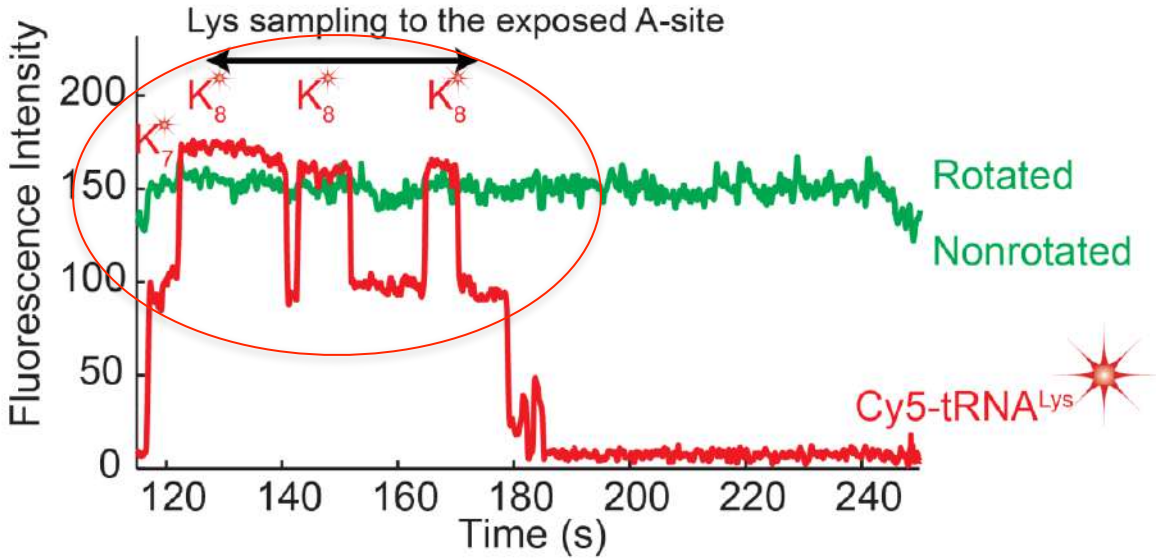
frustrated, non-canonical state

EF-G substrate

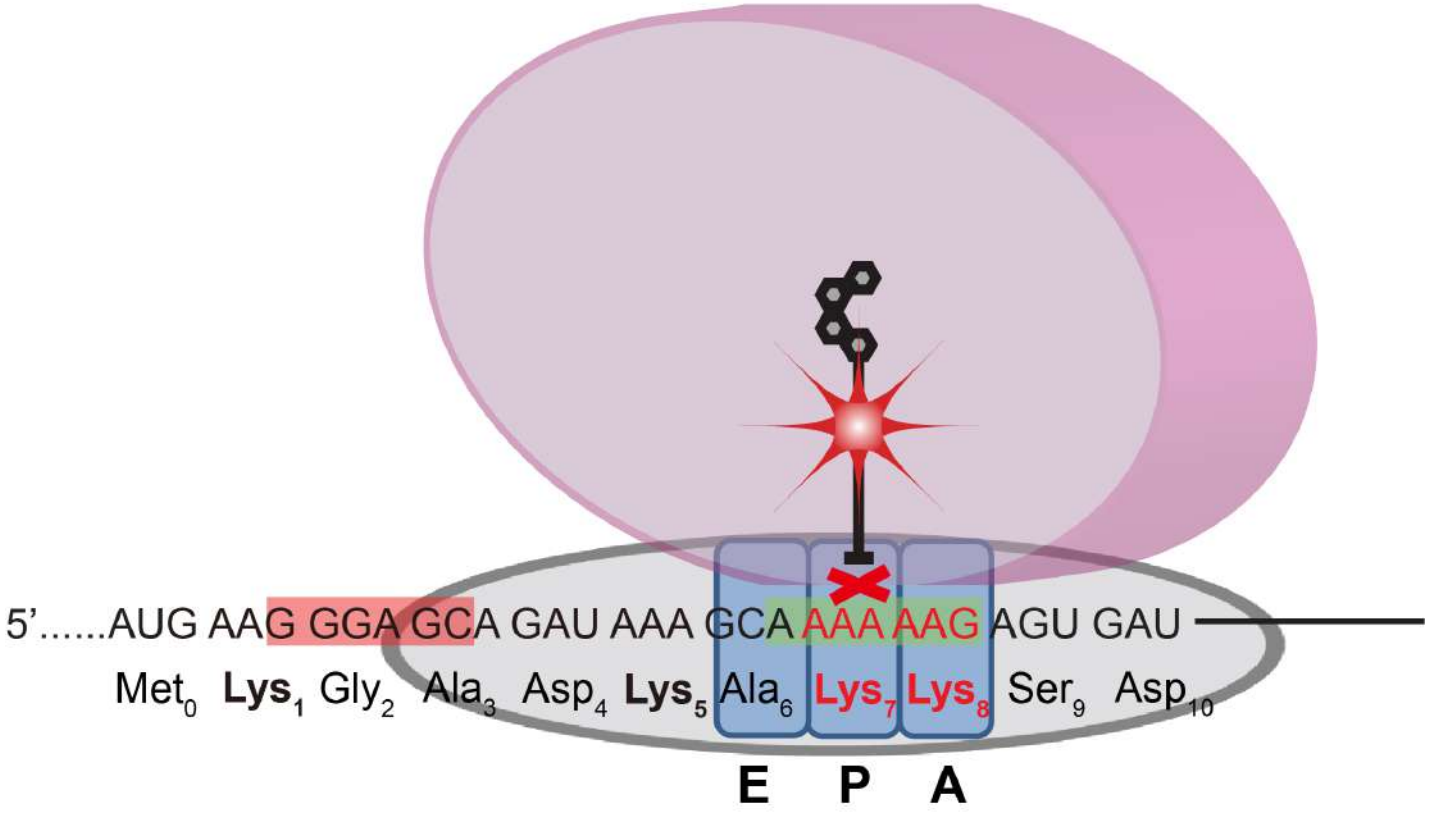
tRNA binds the ribosome multiple times during the rotated state pause



long rotated state pause at codon Lys7

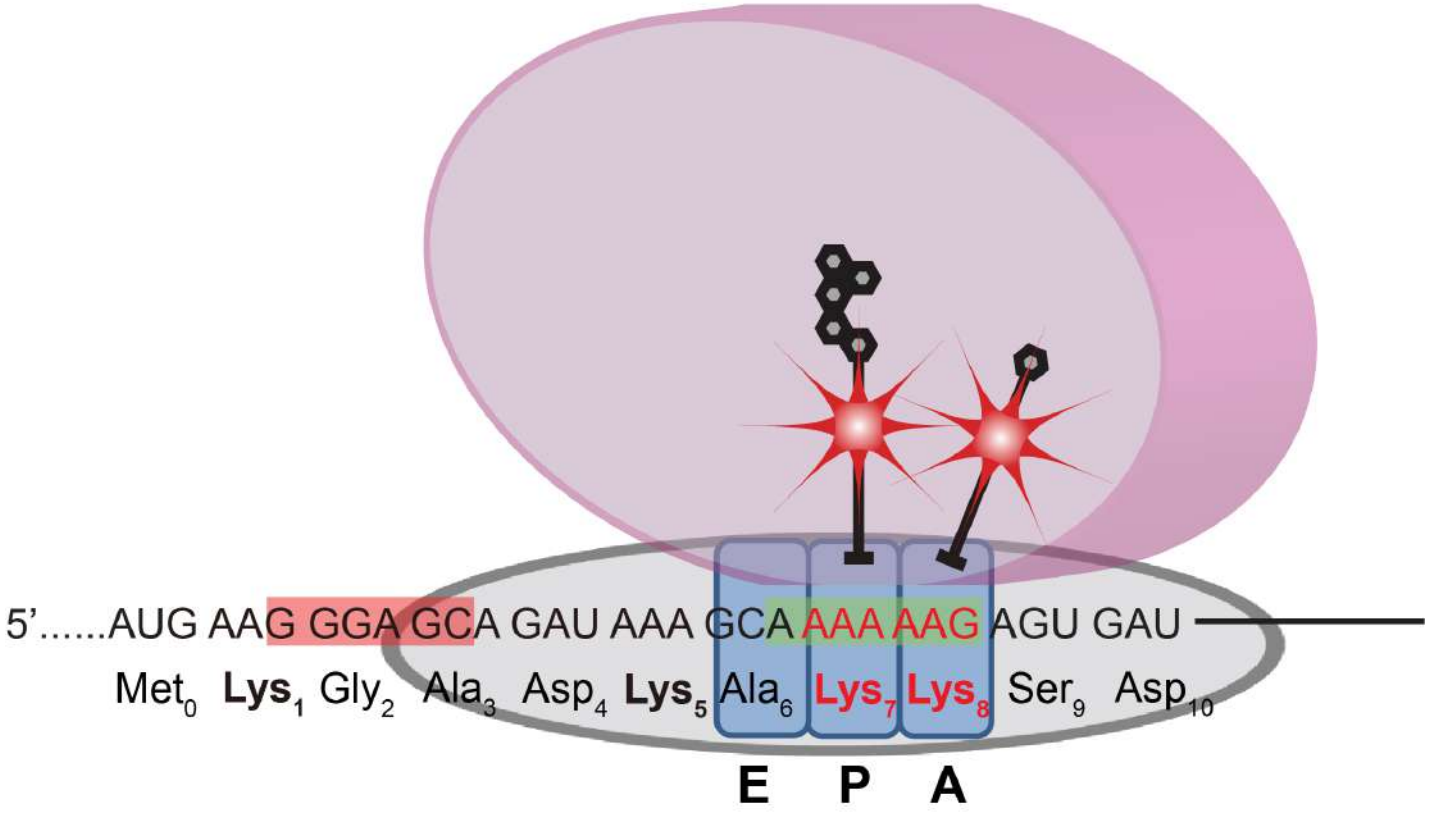


Uncoupled translocation leaves the ribosome in a rotated state with exposed A site

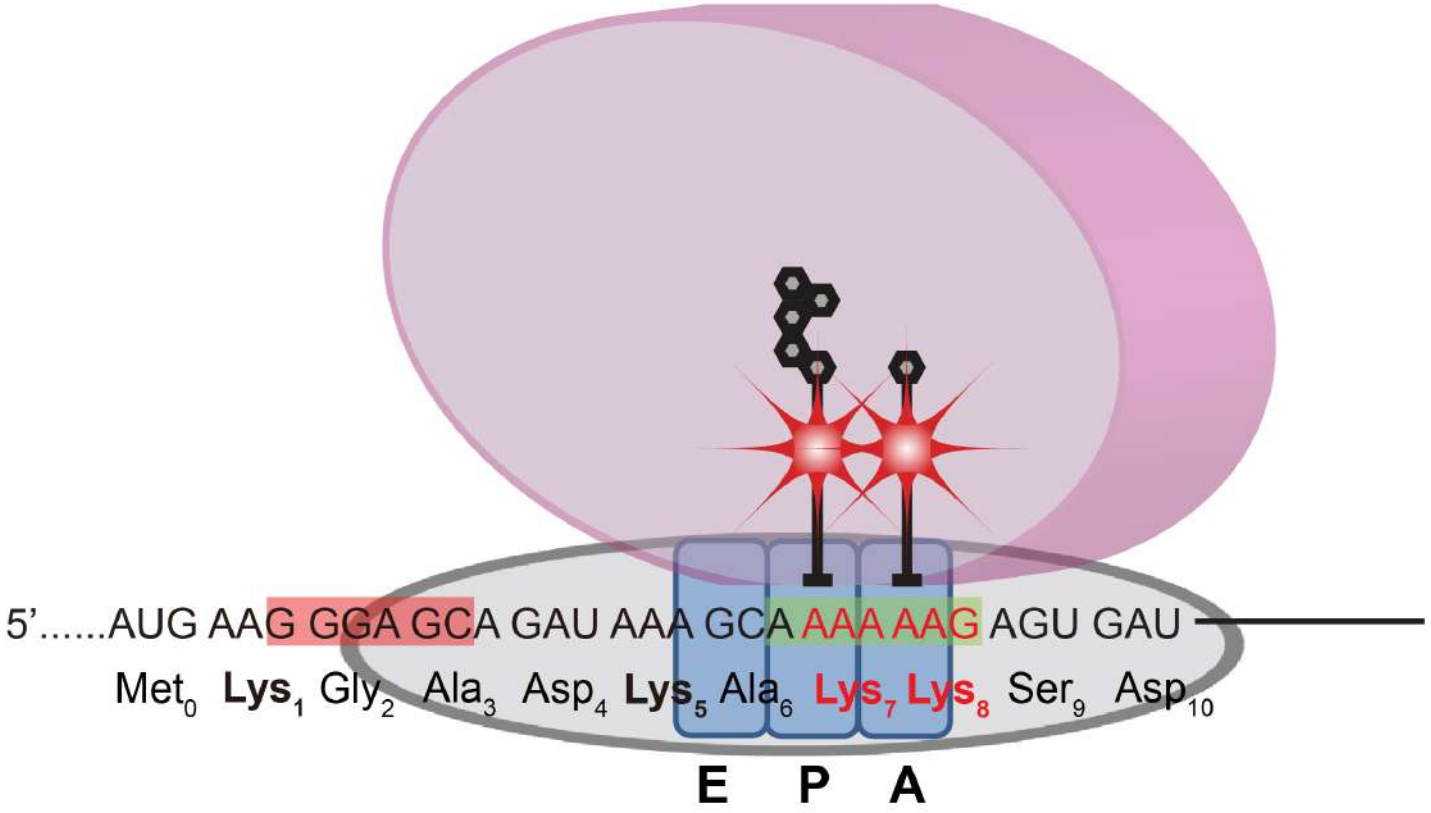


Chen, Jin, et al. ***Nature*** 512.7514 (2014)

Binding of tRNA to the A site actively drives the shift to the -1 frame



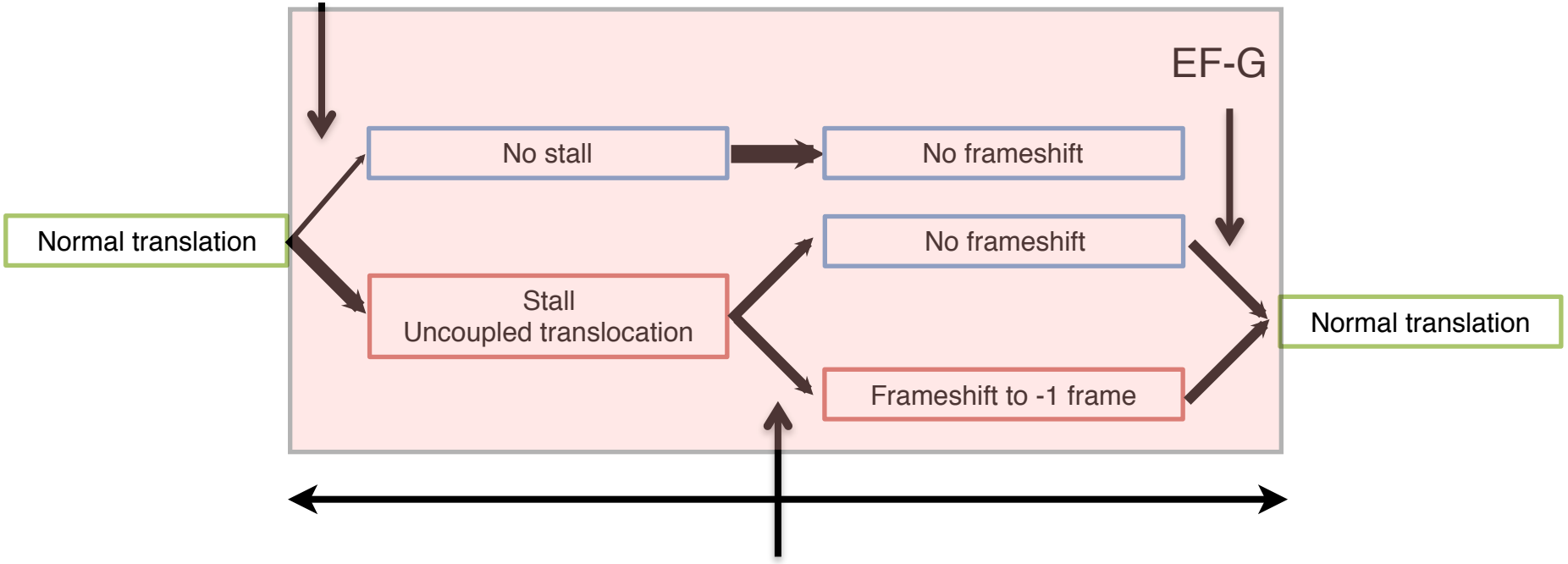
Binding of tRNA to the A site actively drives the shift to the -1 frame



Reading frame changes during long "equilibration" period

Hairpin open/close
rRNA-SD engagement

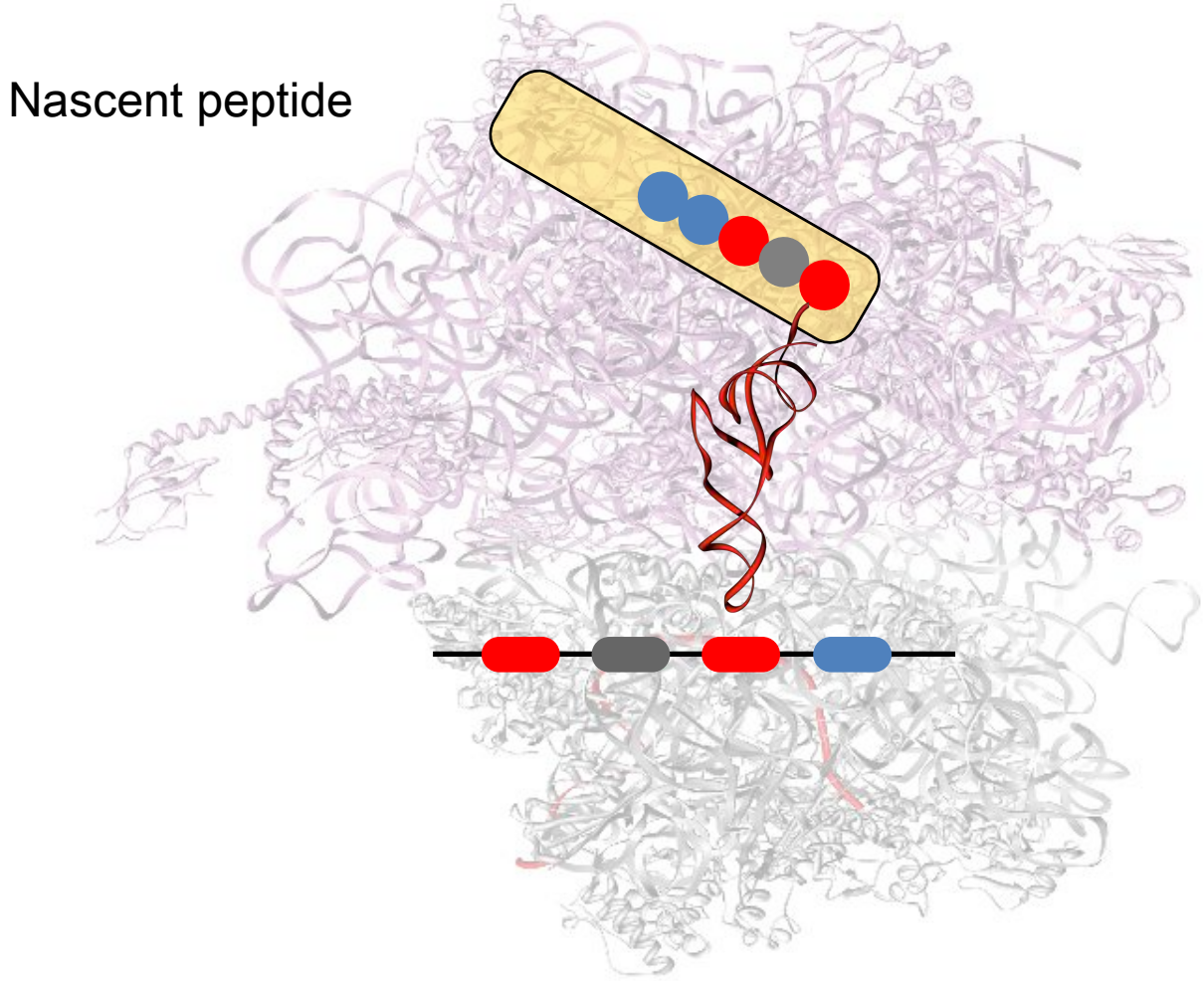
100-150 s



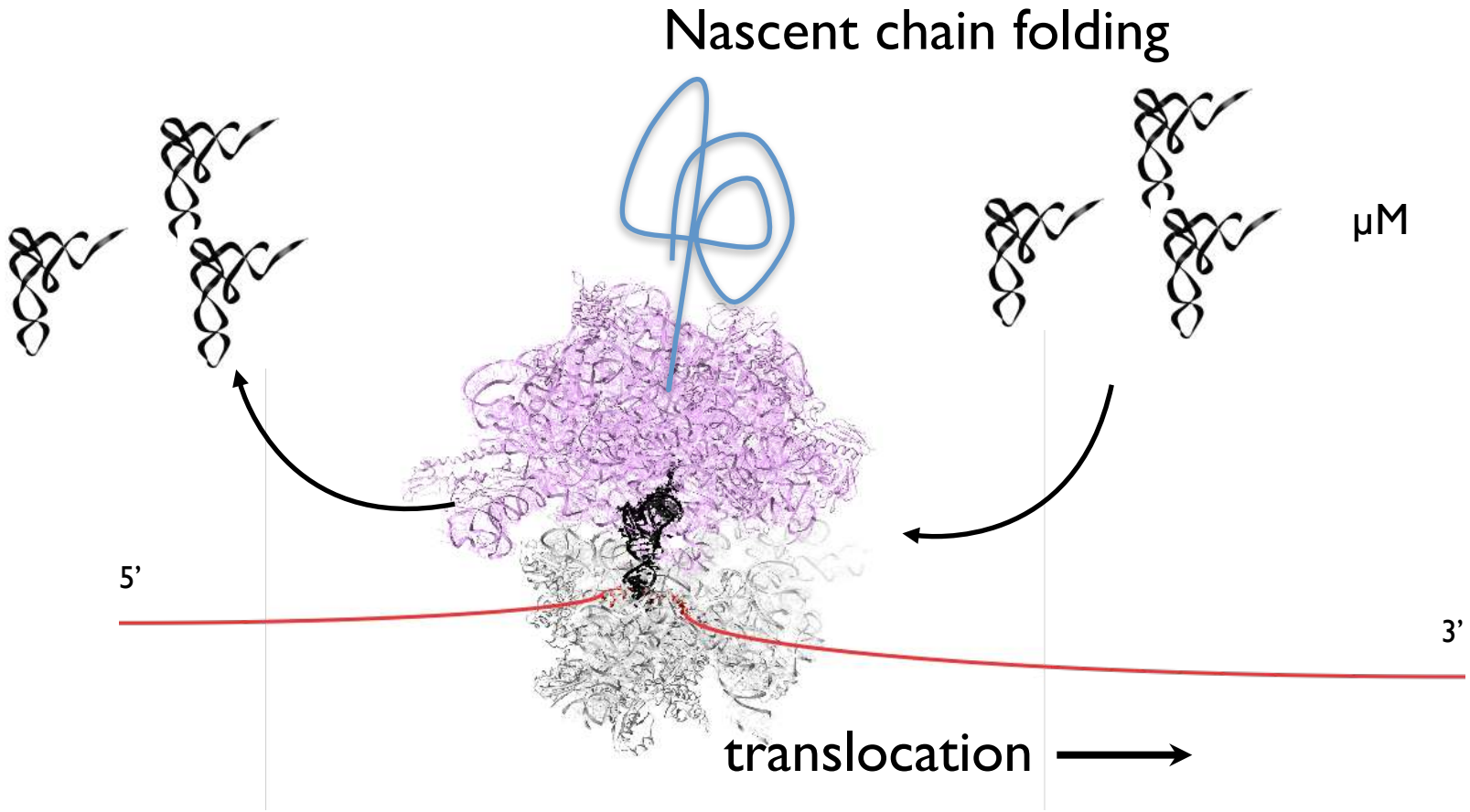
tRNA slippage into -1 frame
Or redefining into 0 frame

Changes in frame/decoding may occur generally during long pauses observed here

Nascent peptide exits through a tunnel in the 50S subunit



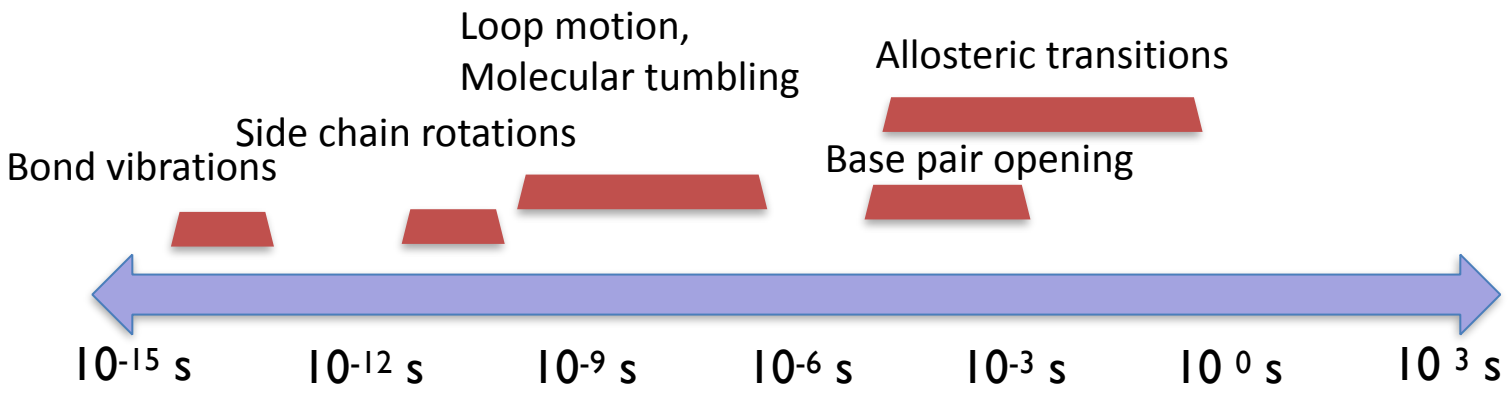
Time evolution of biological systems



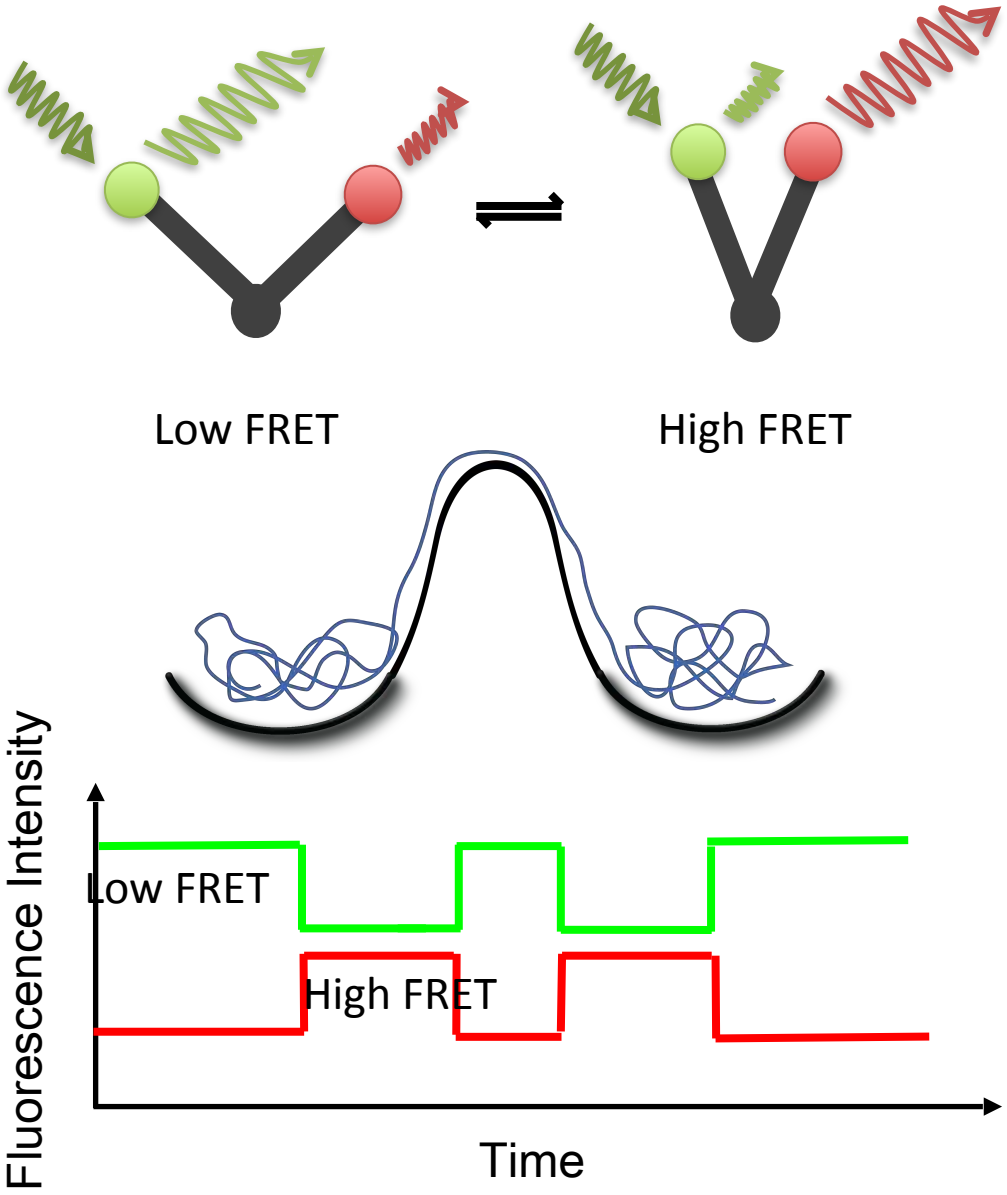
milliseconds - seconds

1-20 aa/s in vivo, 1-3 aa/s in vitro

Timescales for biomolecular dynamics

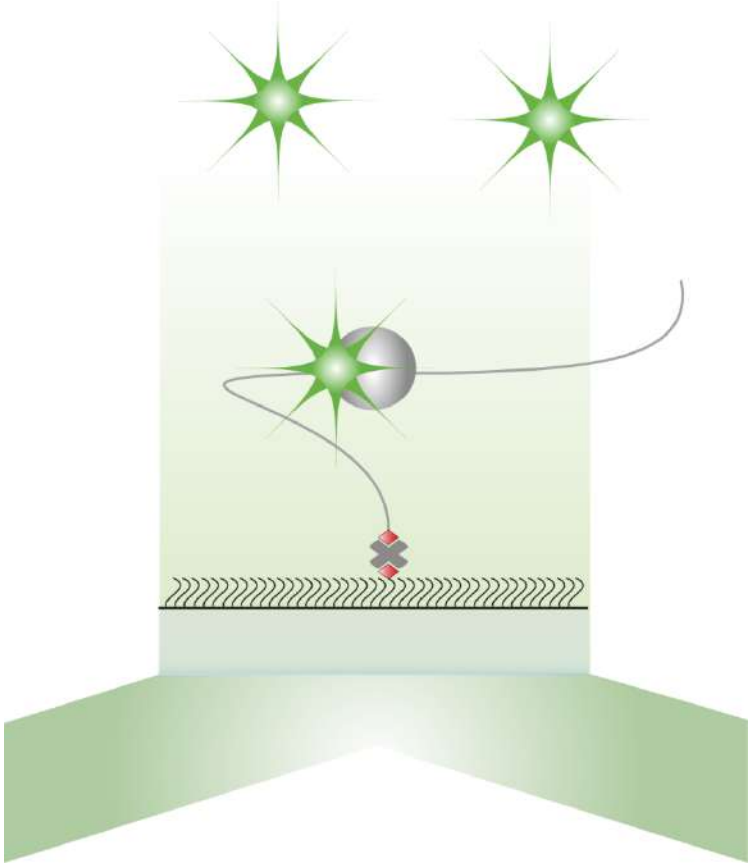


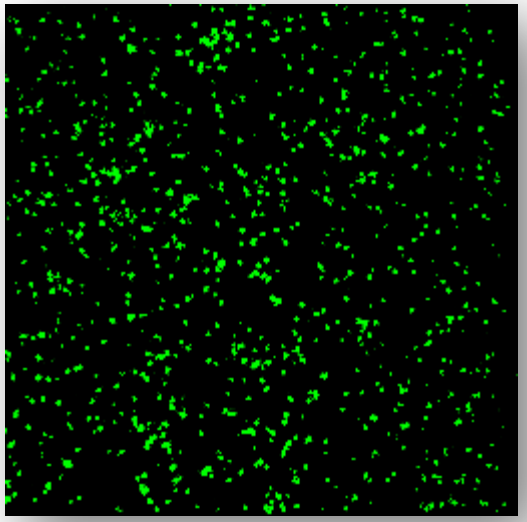
Single-Molecule FRET



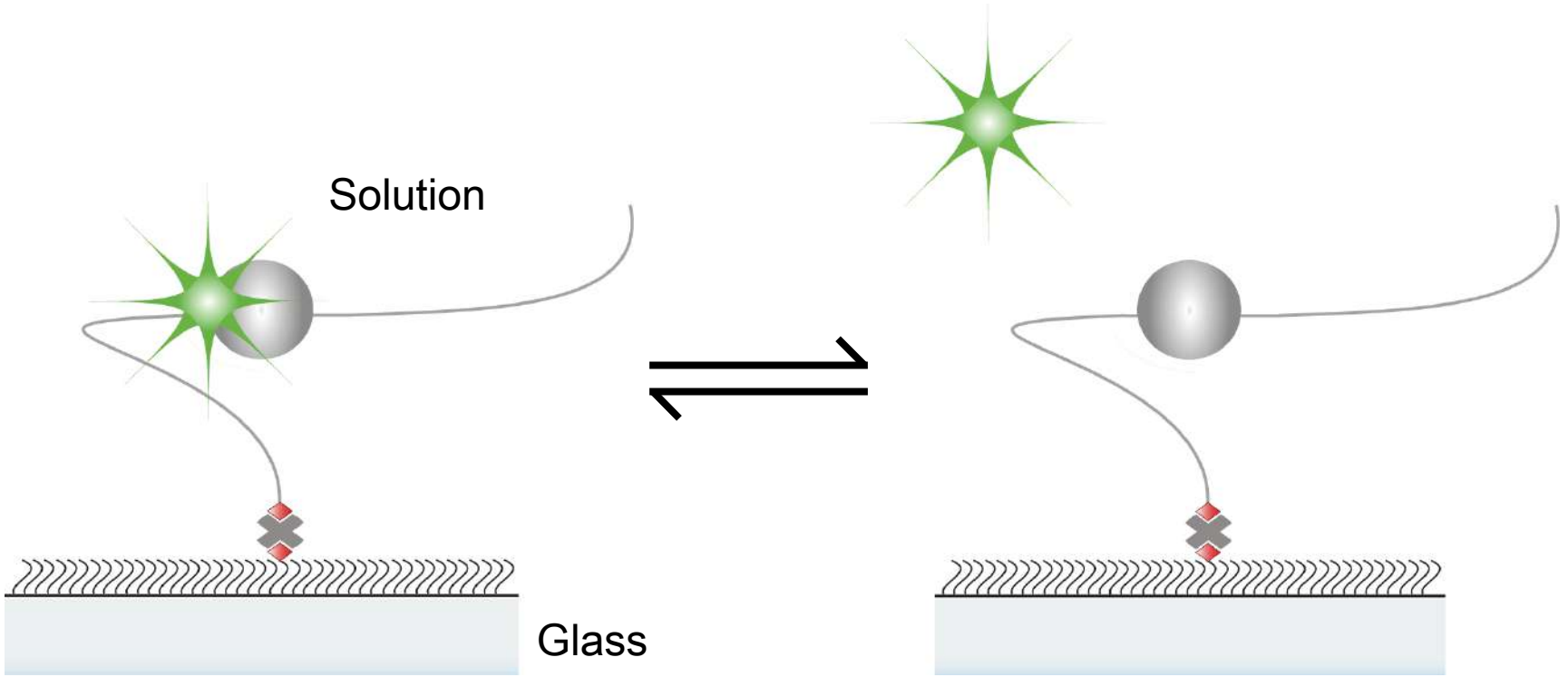
Observing signal molecule binding kinetics using TIRF

Total **I**nternal **R**eflection **F**luorescent microscope

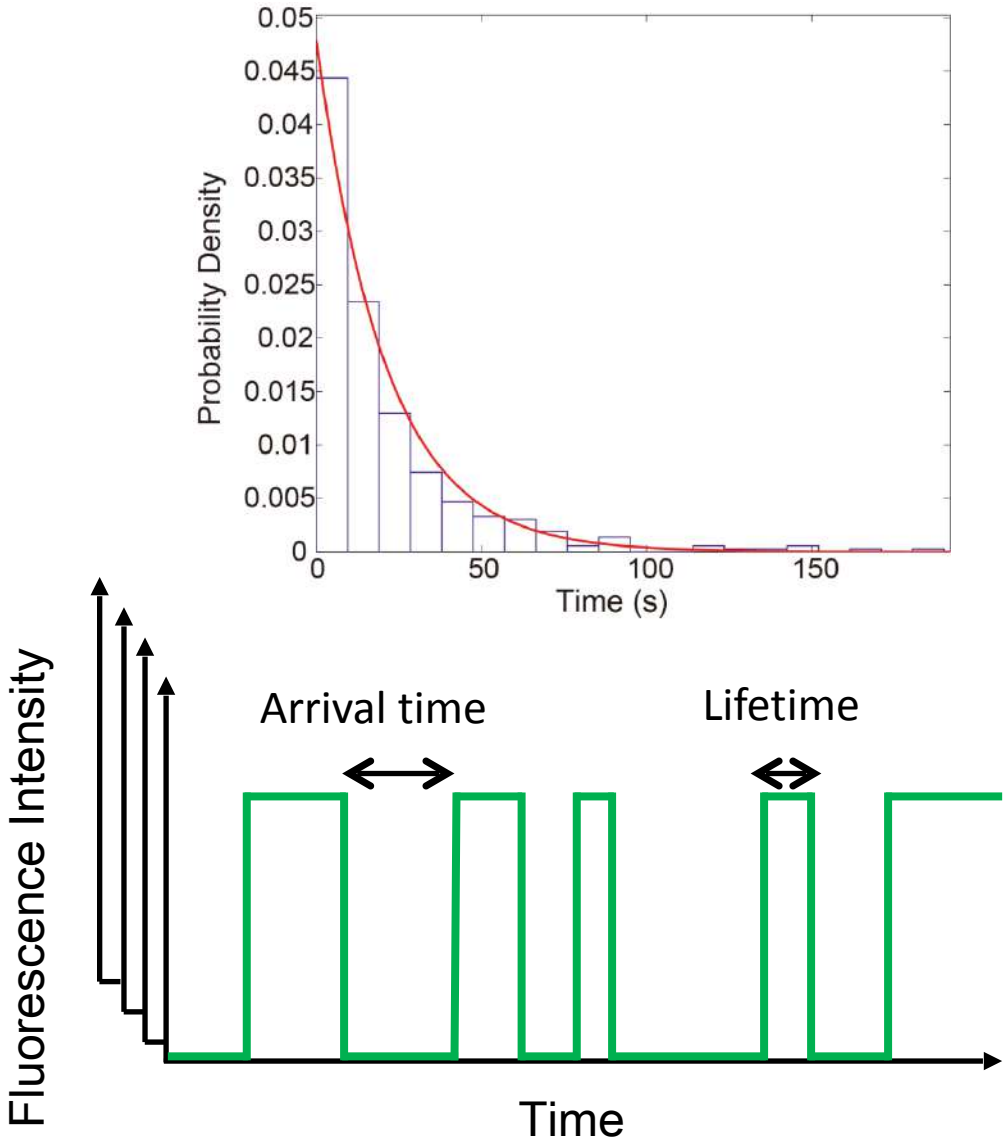




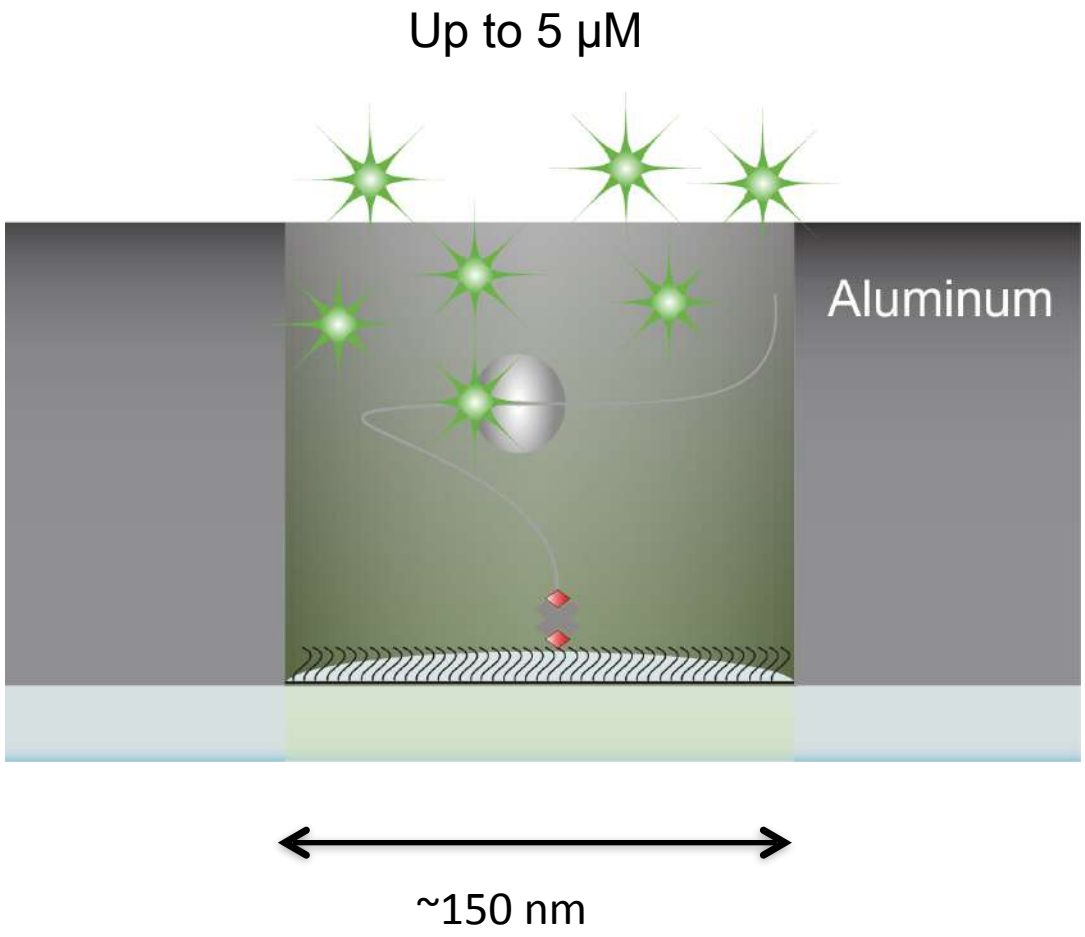
Observing signal-molecule binding kinetics

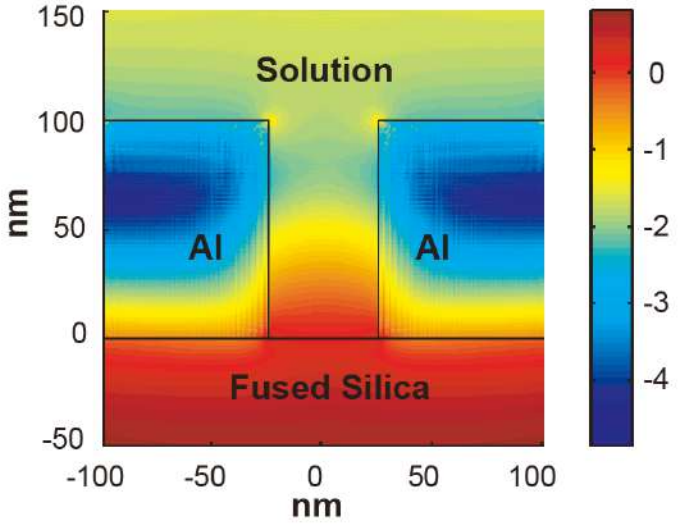


Statistically analyze the distributions to obtain kinetics



Overcoming the concentration barrier with the zero-mode waveguide (ZMW)

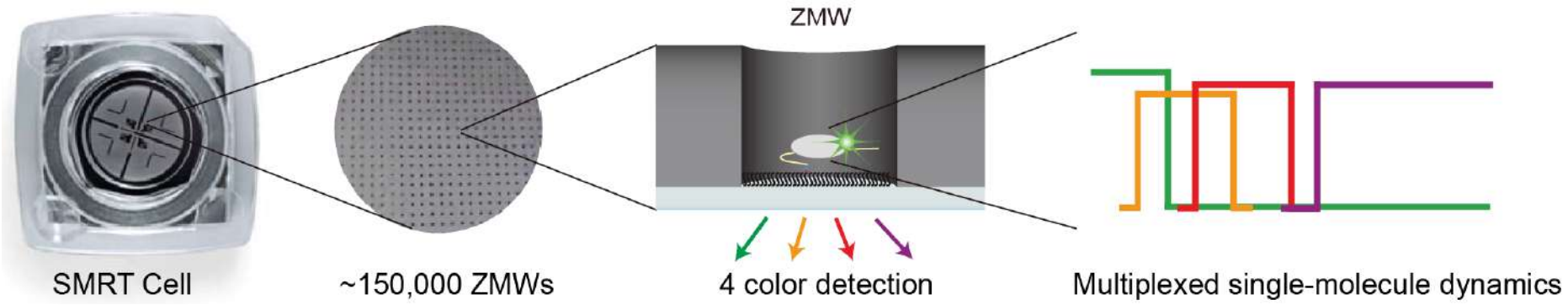




Chen, Jin, Dalal, Ravi, et al. ***PNAS*** 111.2 (2014)

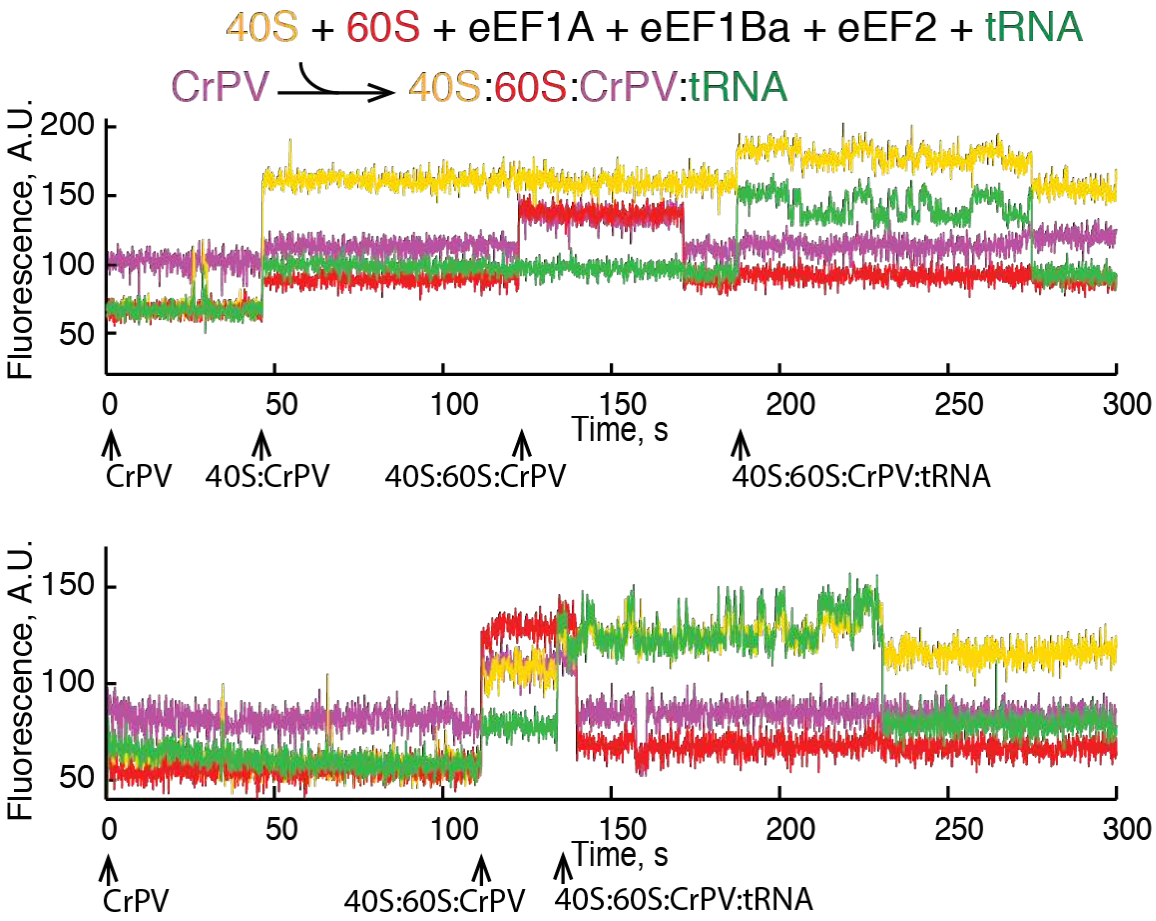
High-throughput multicolor single-molecule dynamics on the PacBio RS

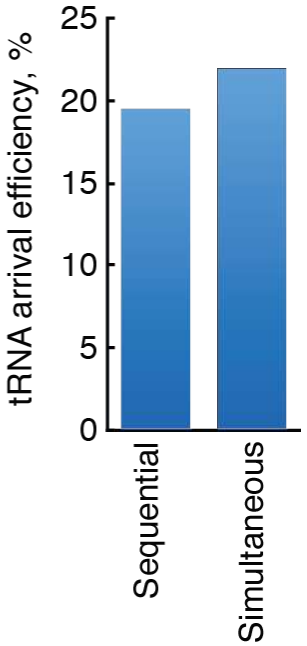
PACIFIC BIOSCIENCES™



Chen, Jin, Dalal, Ravi, et al. ***PNAS*** 111.2 (2014)

Real-time observation of translation initiation in yeast





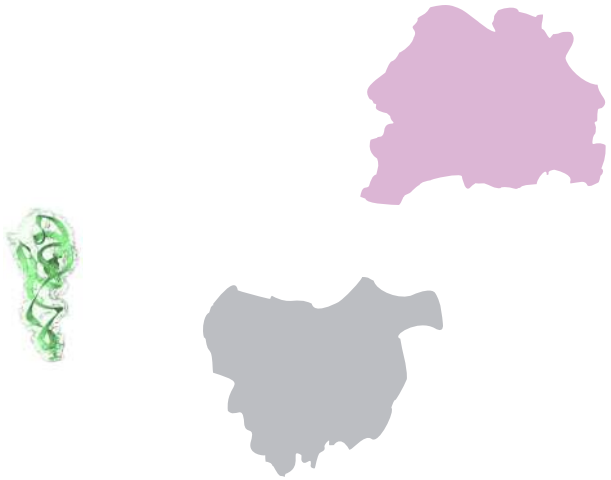
Beware the elegant meal.....

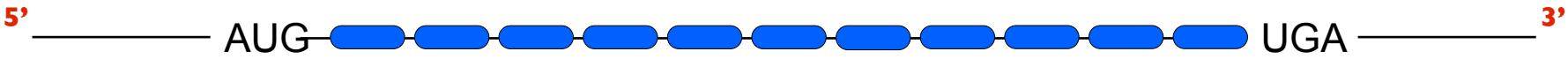
Beware the elegant meal.....

Beware the elegant meal.....

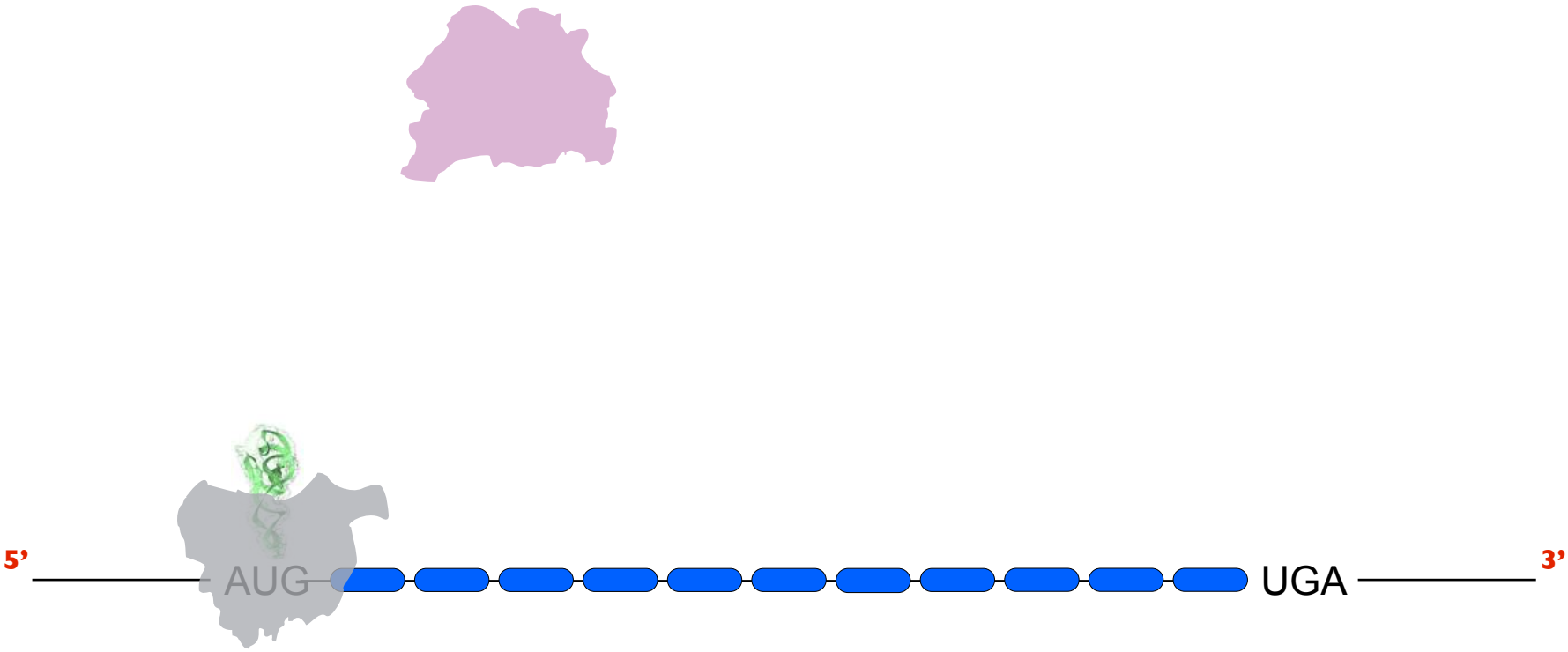
- labeling
- surface
- photophysics
- concentrations

Dynamics of Translation

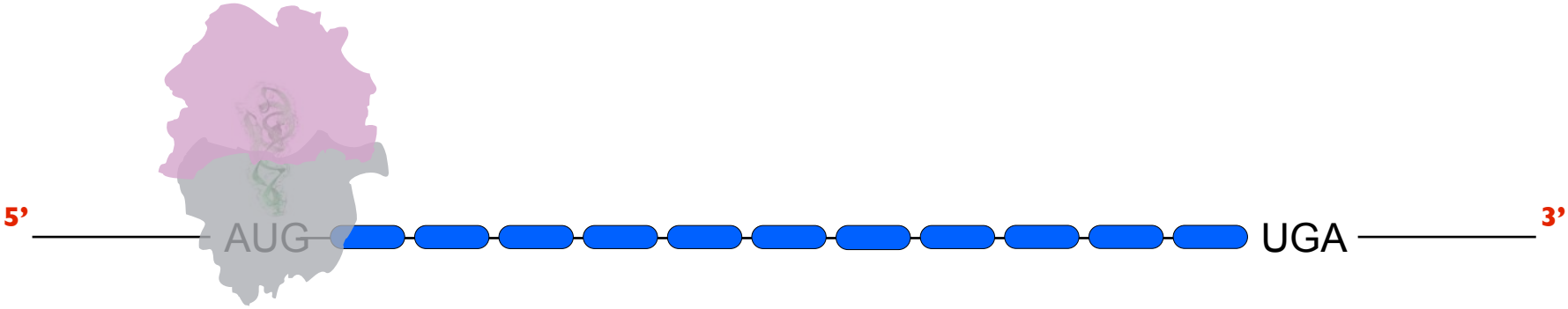




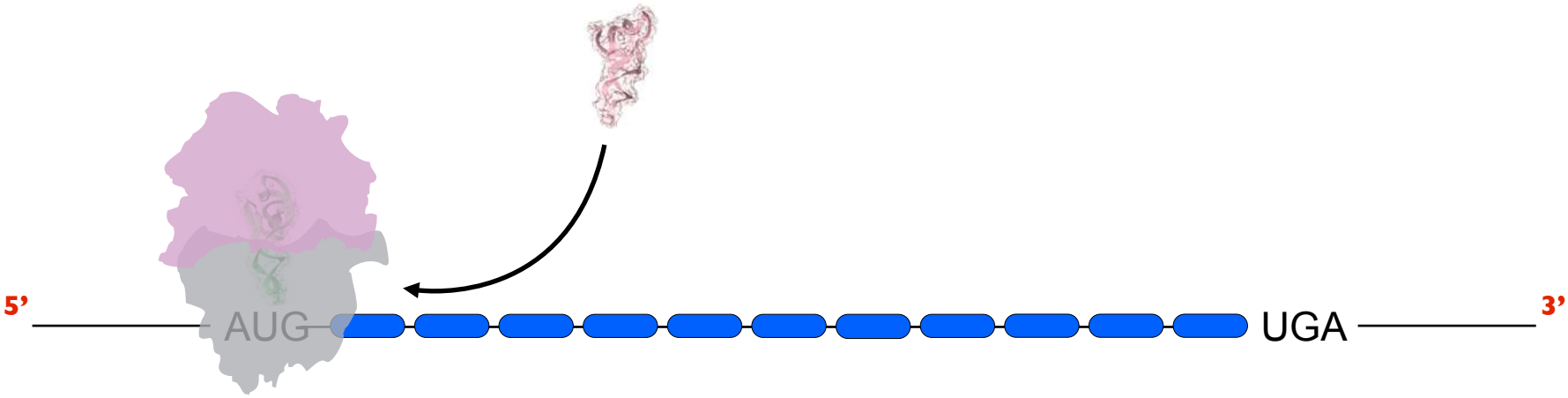
Dynamics of Translation



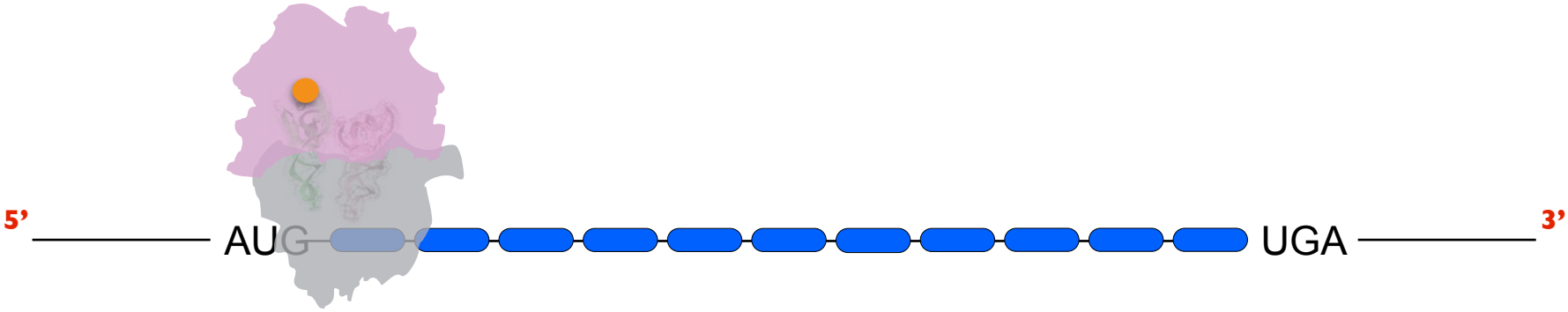
Dynamics of Translation



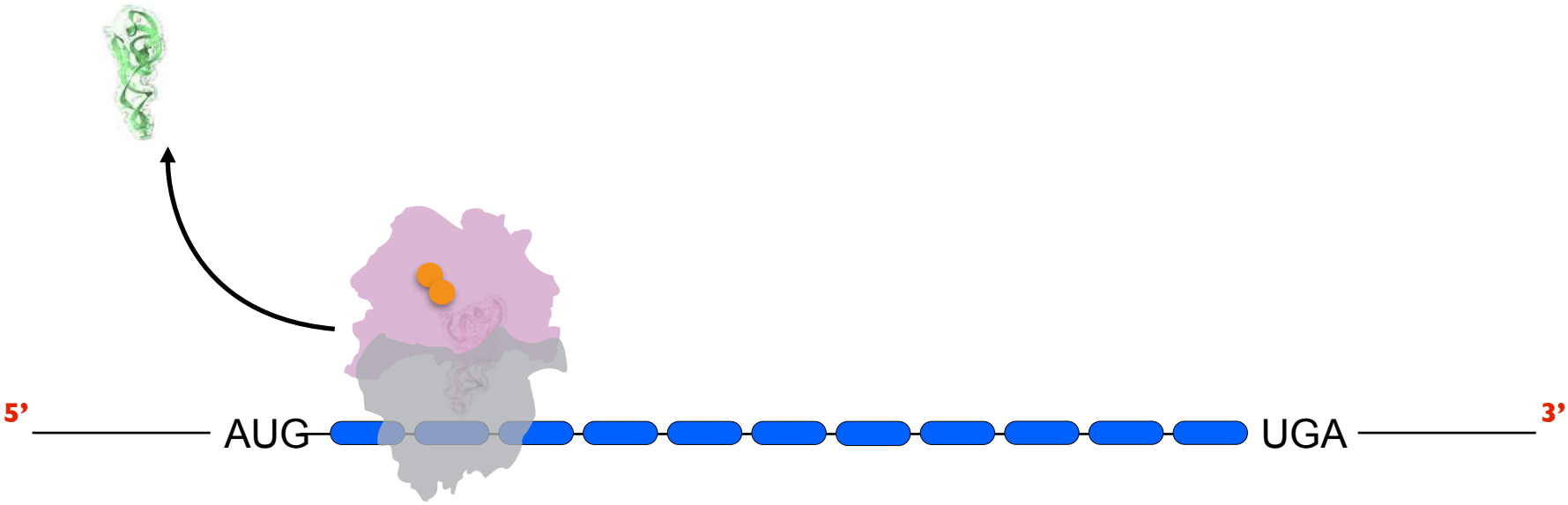
Dynamics of Translation



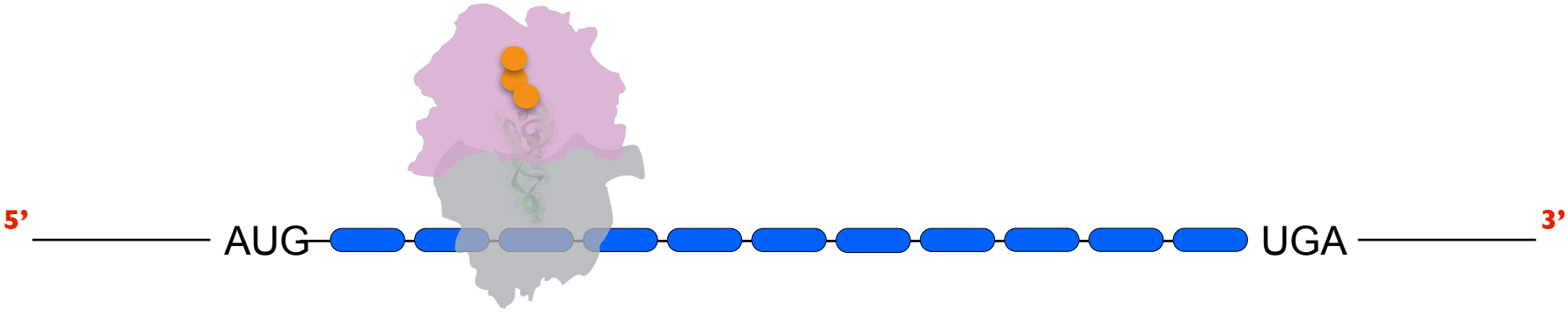
Dynamics of Translation



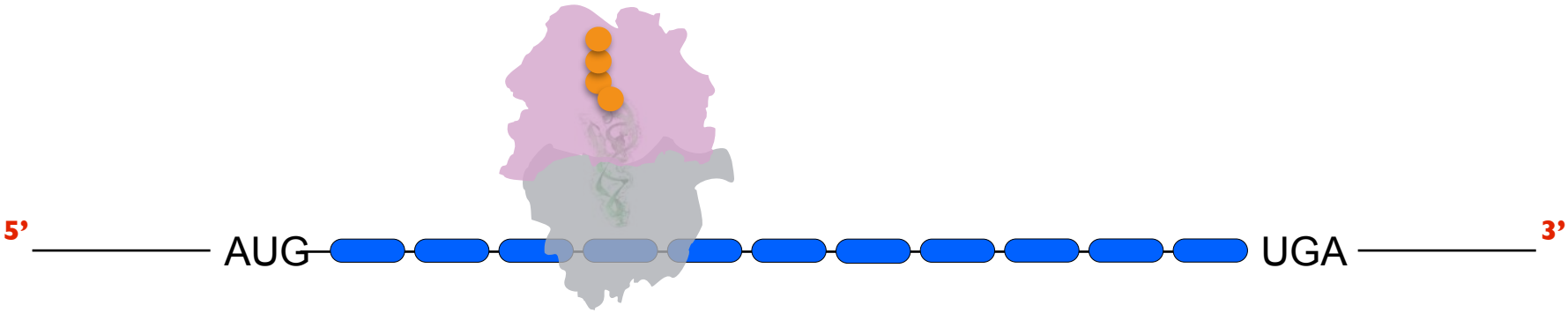
Dynamics of Translation



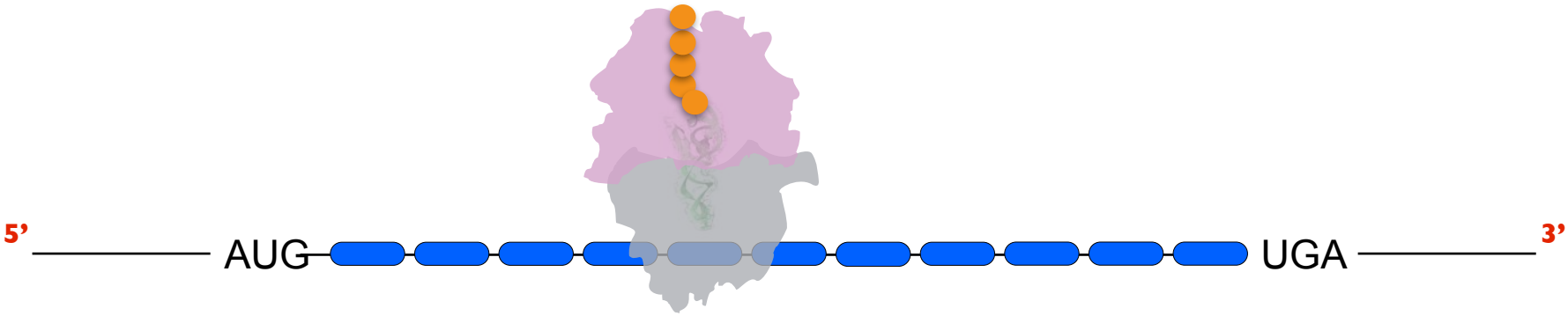
Dynamics of Translation



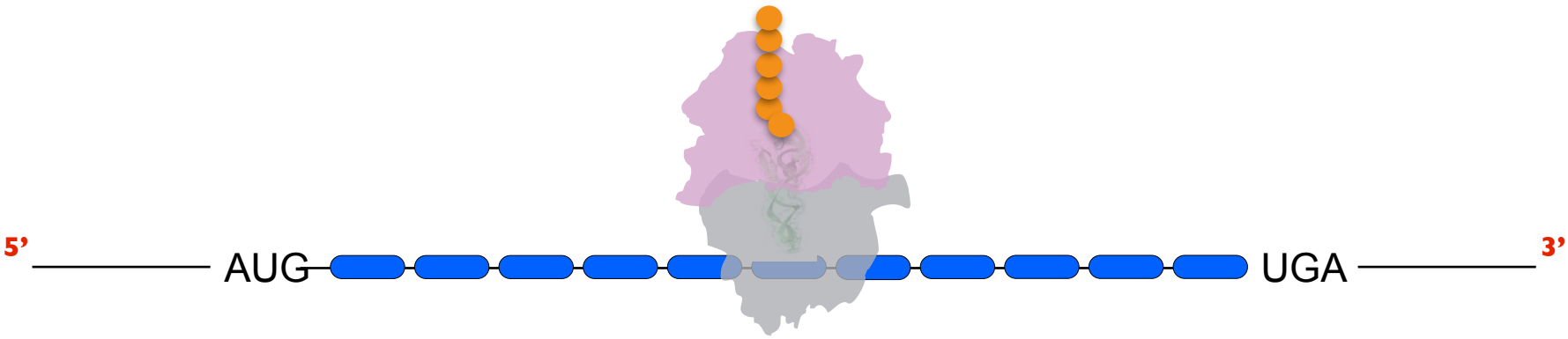
Dynamics of Translation



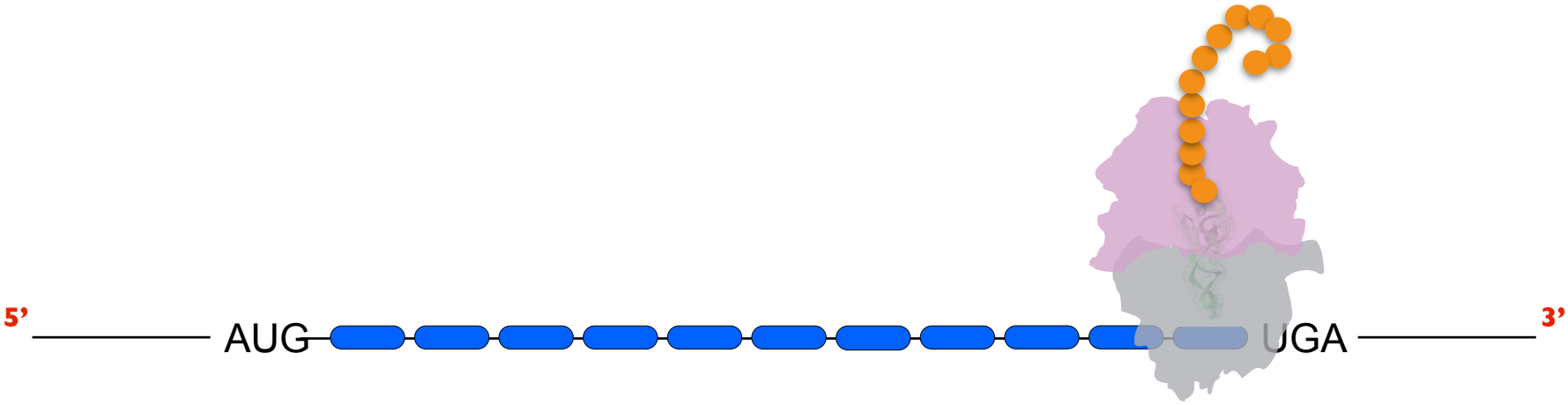
Dynamics of Translation



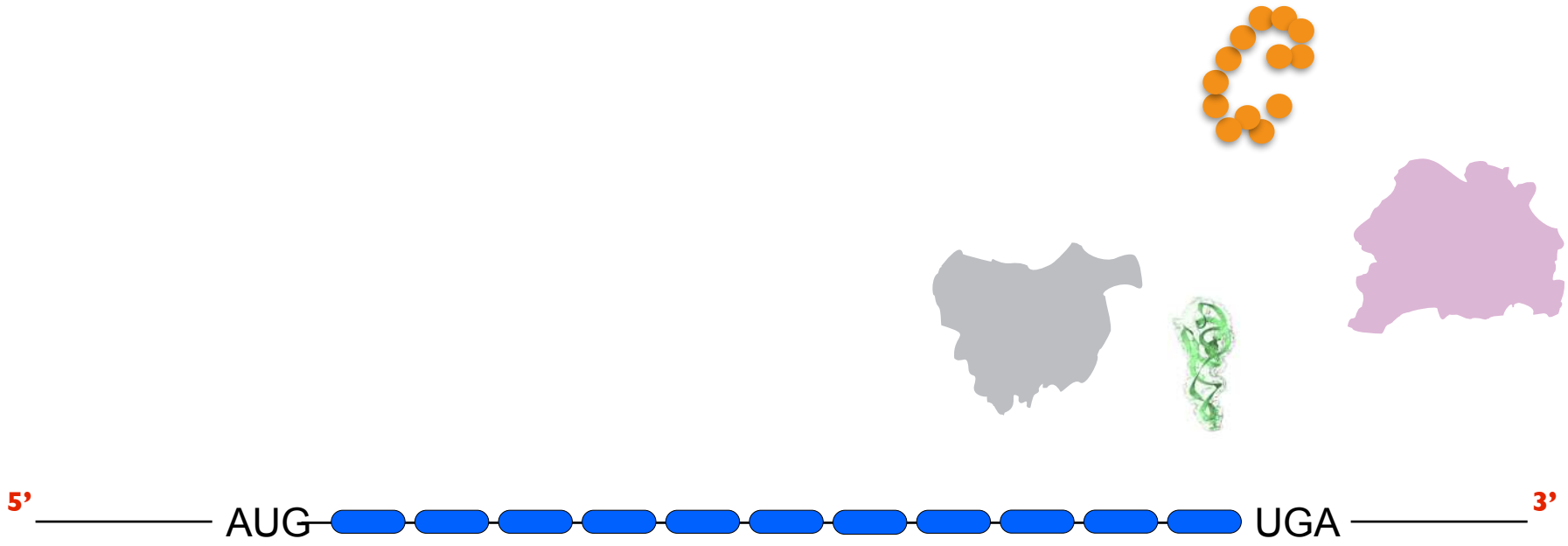
Dynamics of Translation



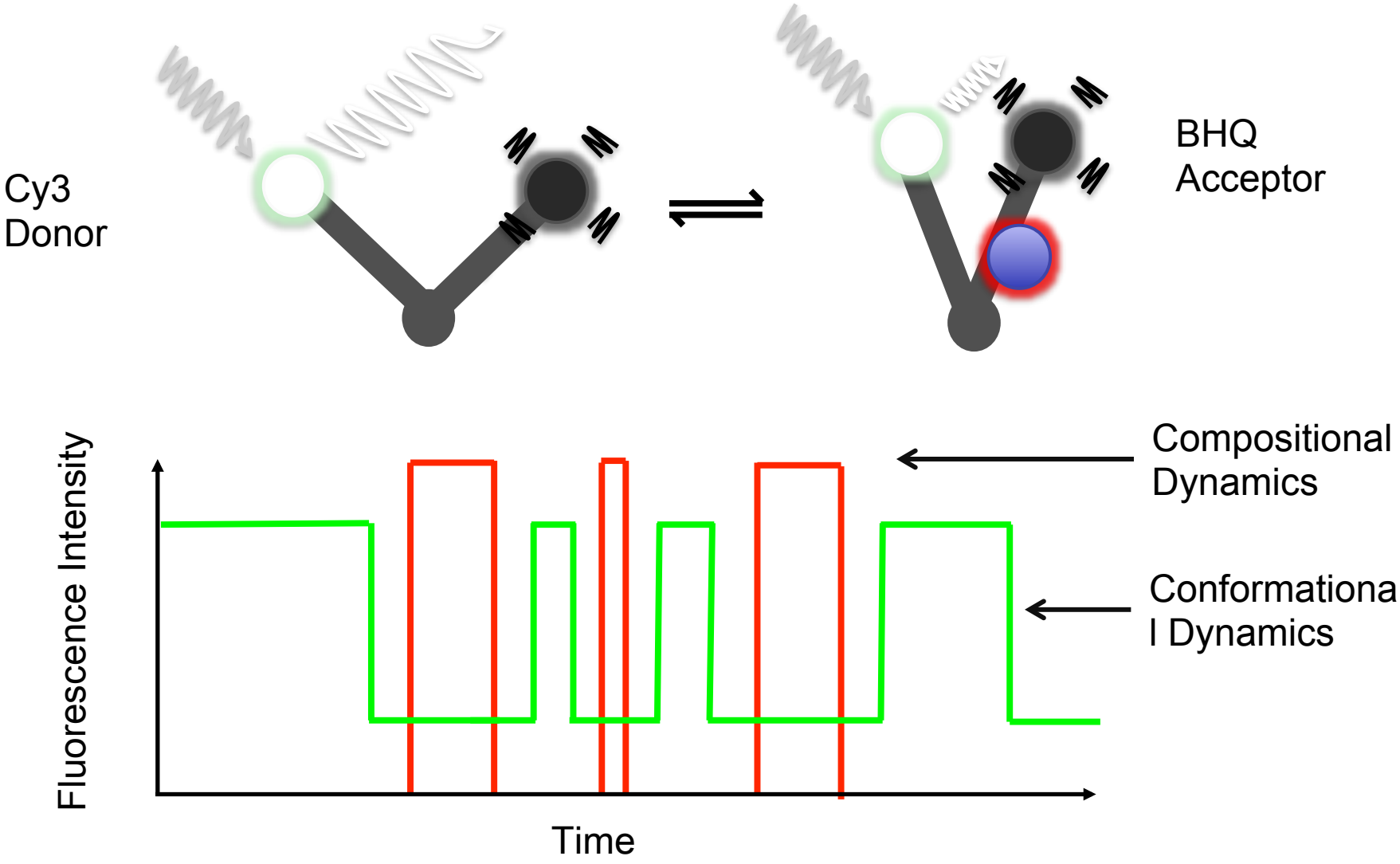
Dynamics of Translation



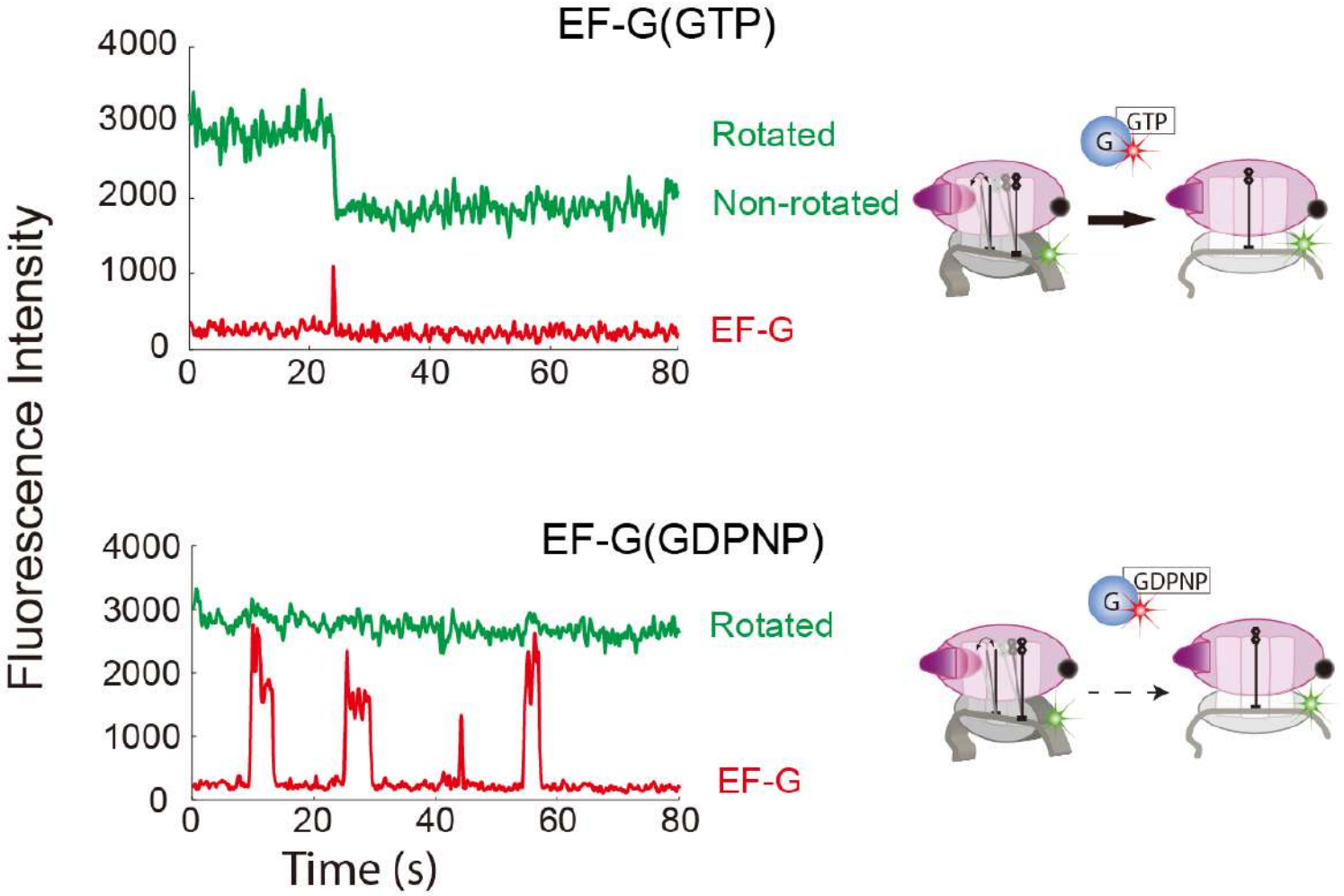
Dynamics of Translation



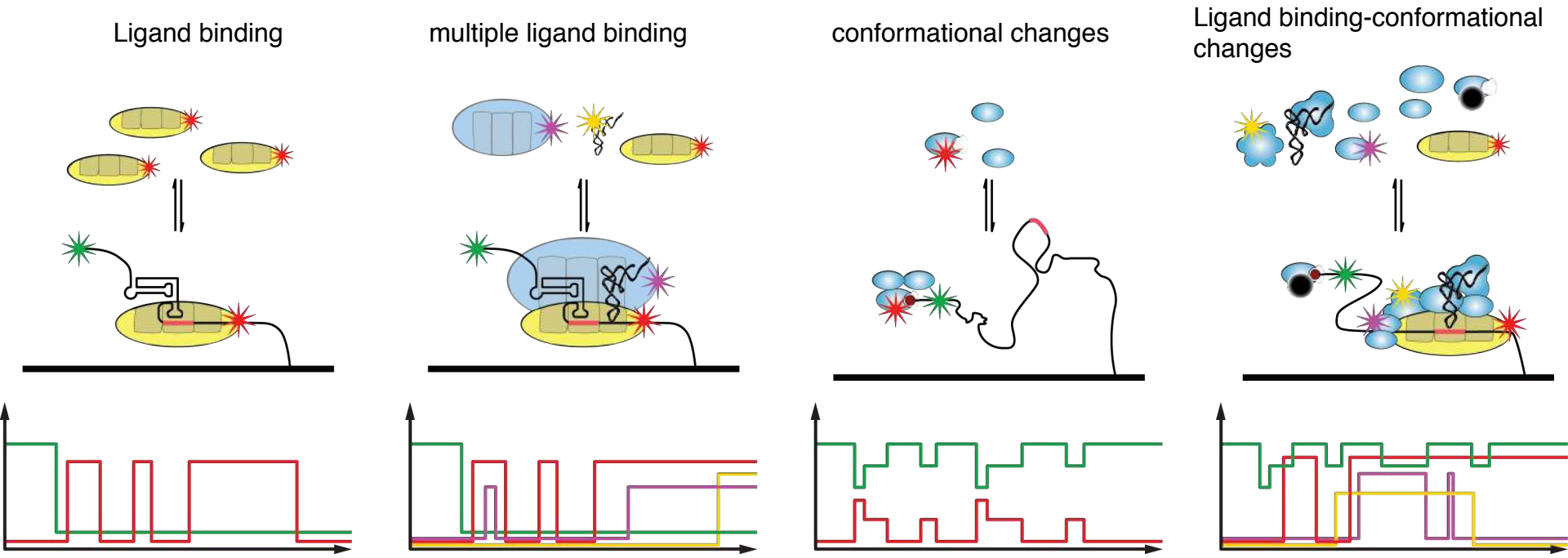
BHQ allows for correlation studies of conformation and composition



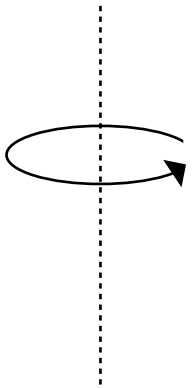
EF-G binding and GTP hydrolysis reset ribosome conformation

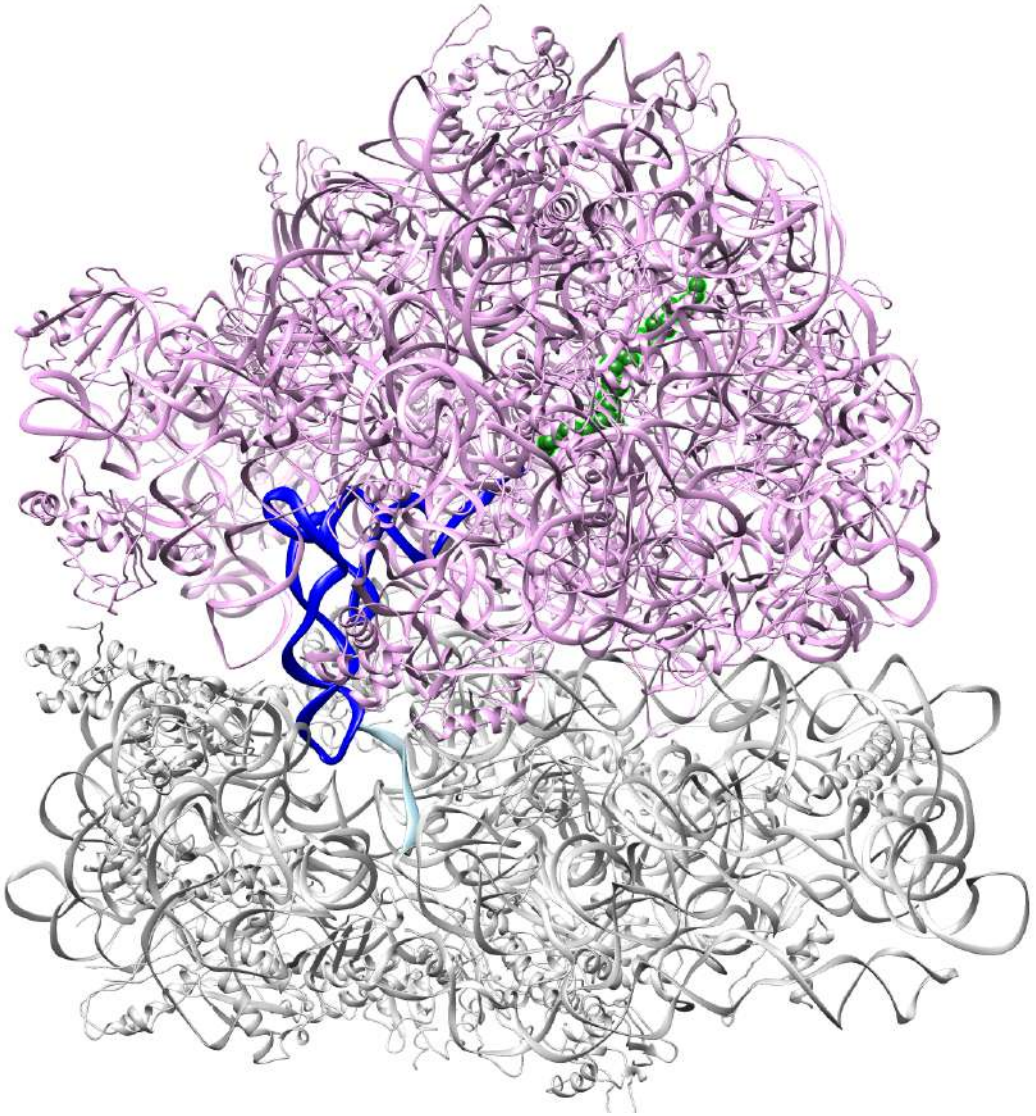


Experiments to map composition and conformation of biological systems

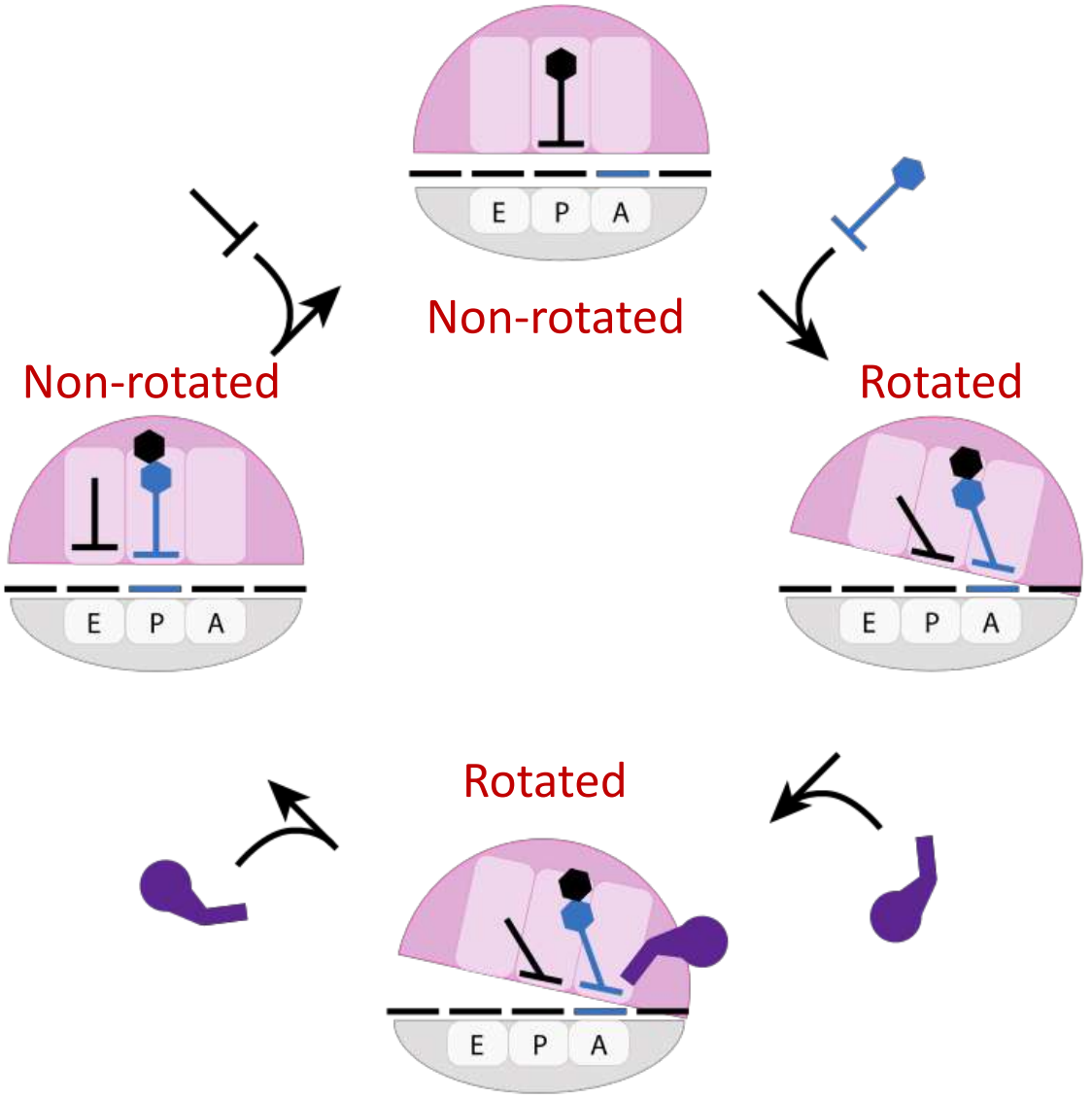


The nascent chain and folding might change dynamics?

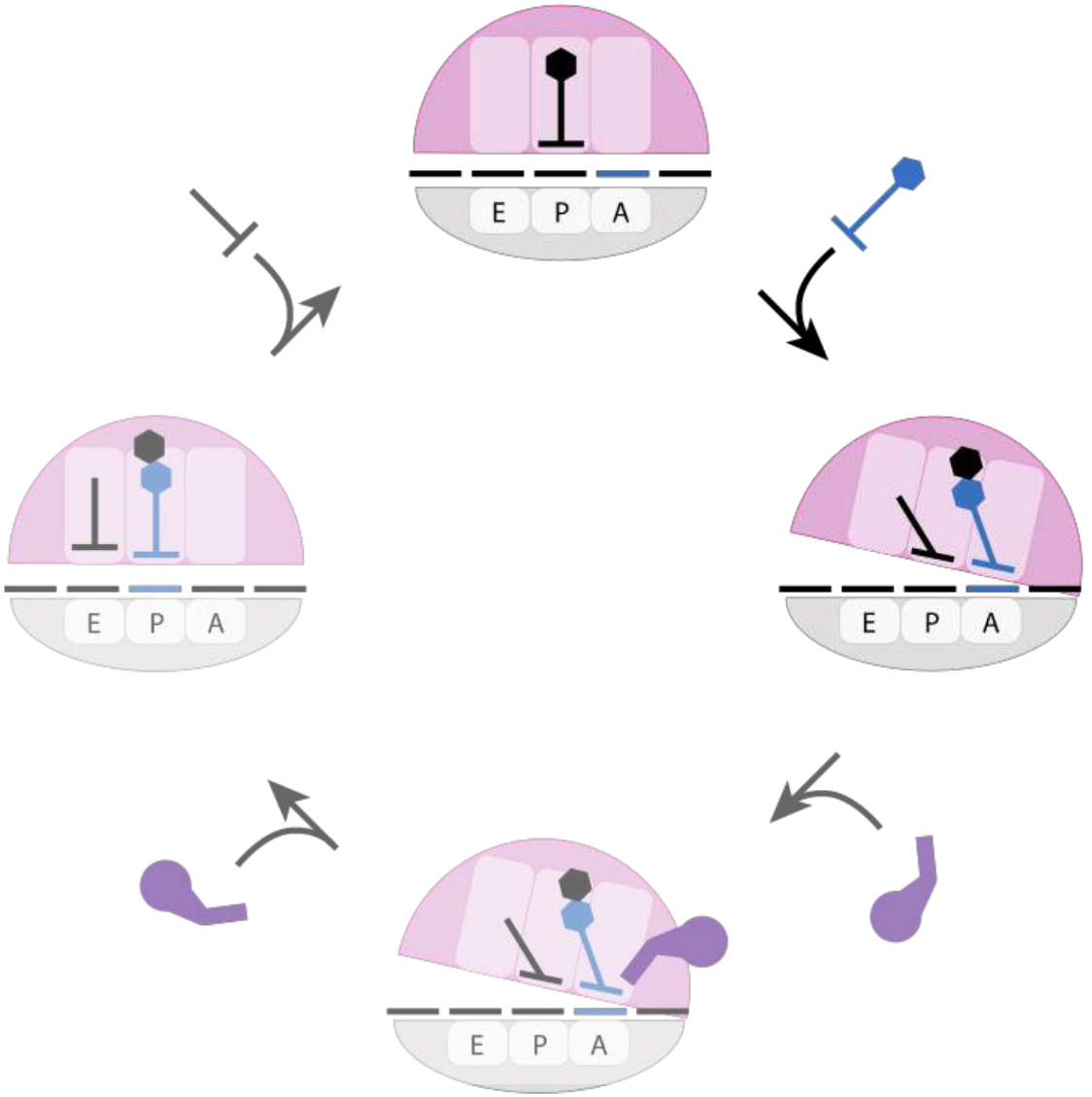


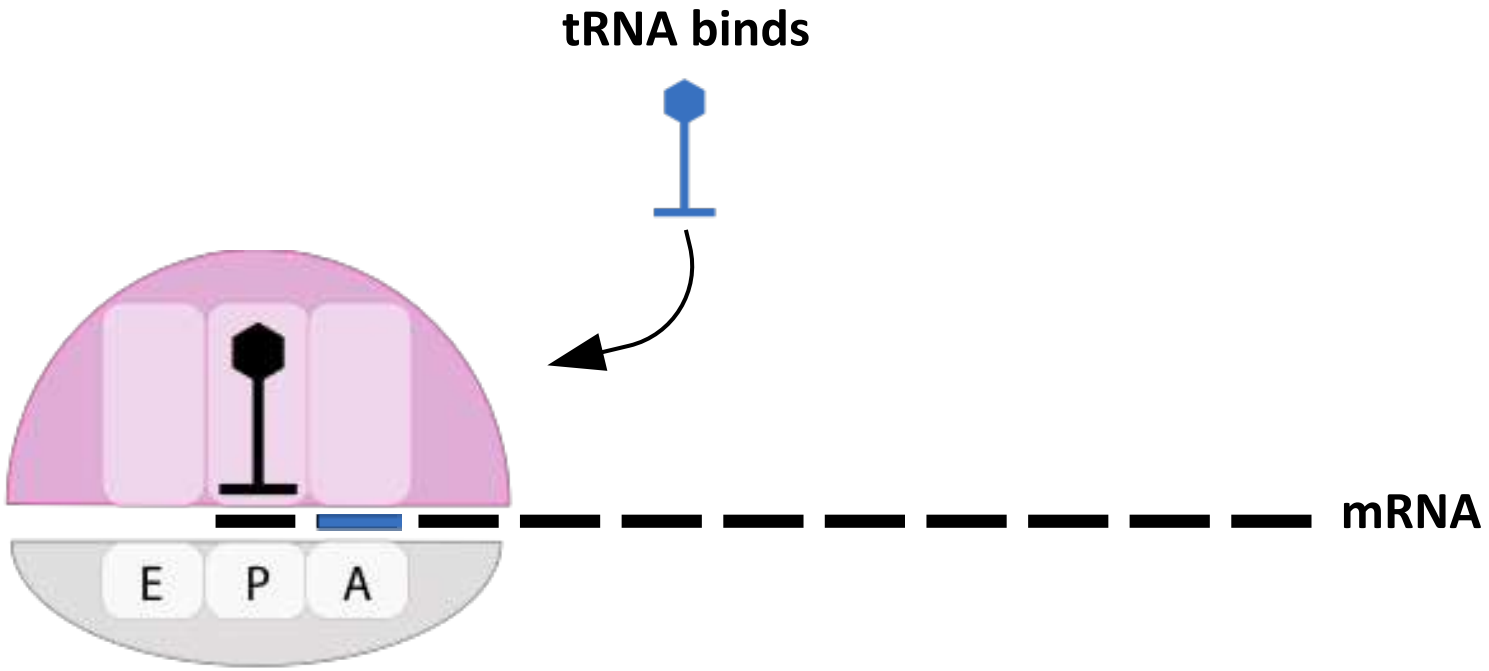


How the message affects translational dynamics is missing

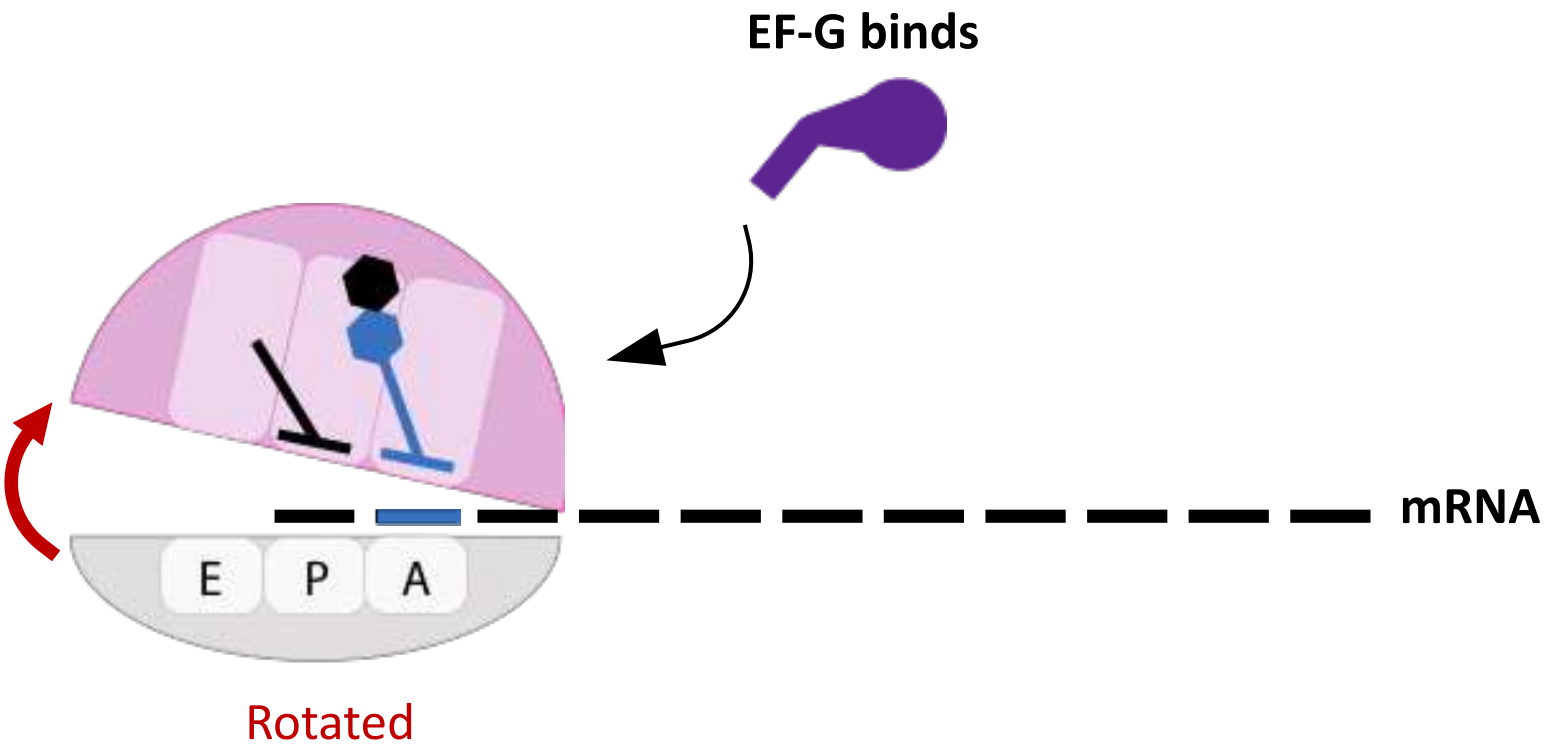


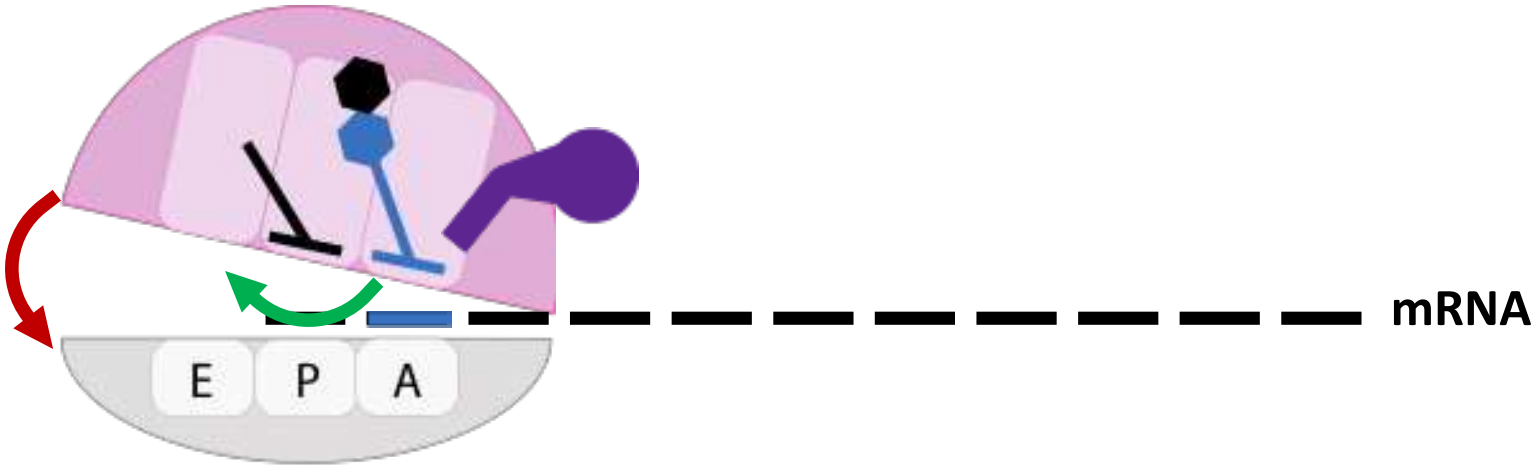
Current model: tRNA binding controls elongation dynamics



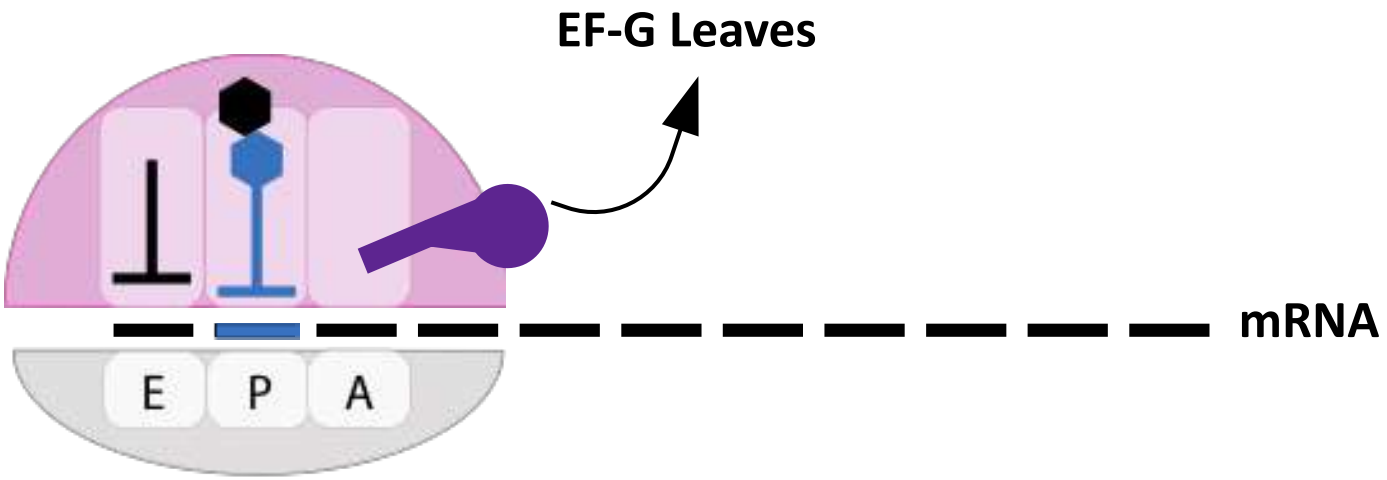


Non-rotated


EF-G binds
mRNA
E
P
A
Rotated

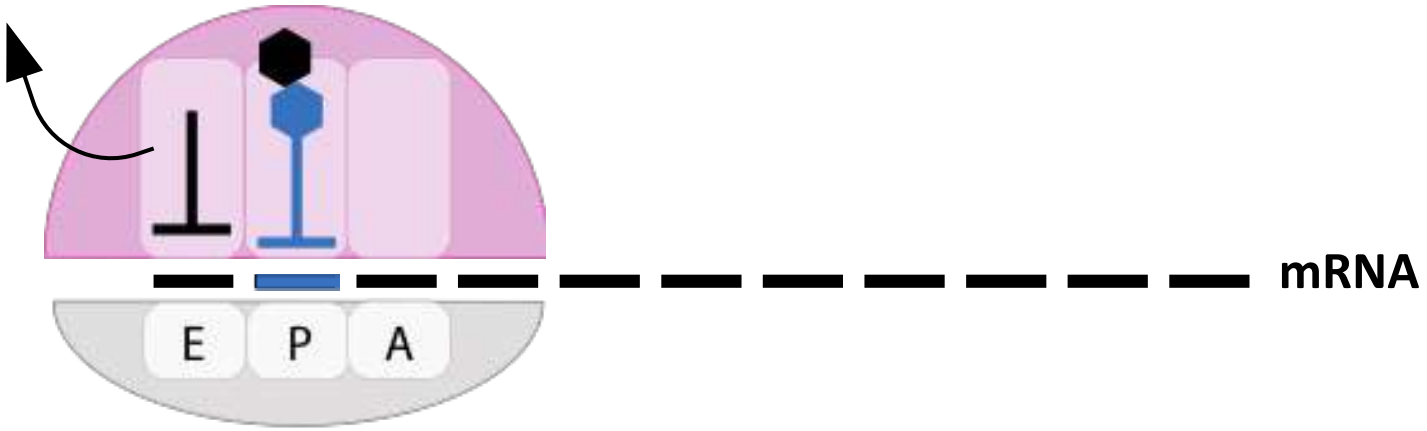


Rotated



Non-rotated

tRNA leaves



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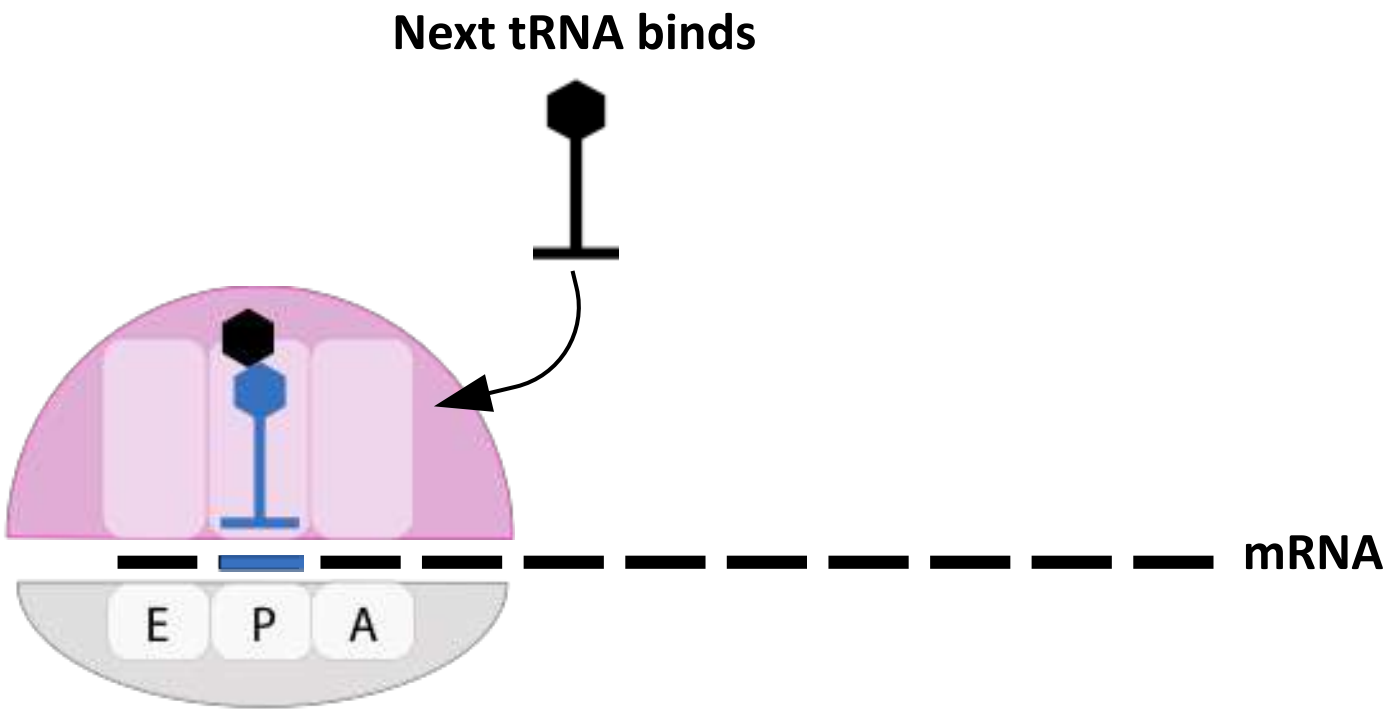

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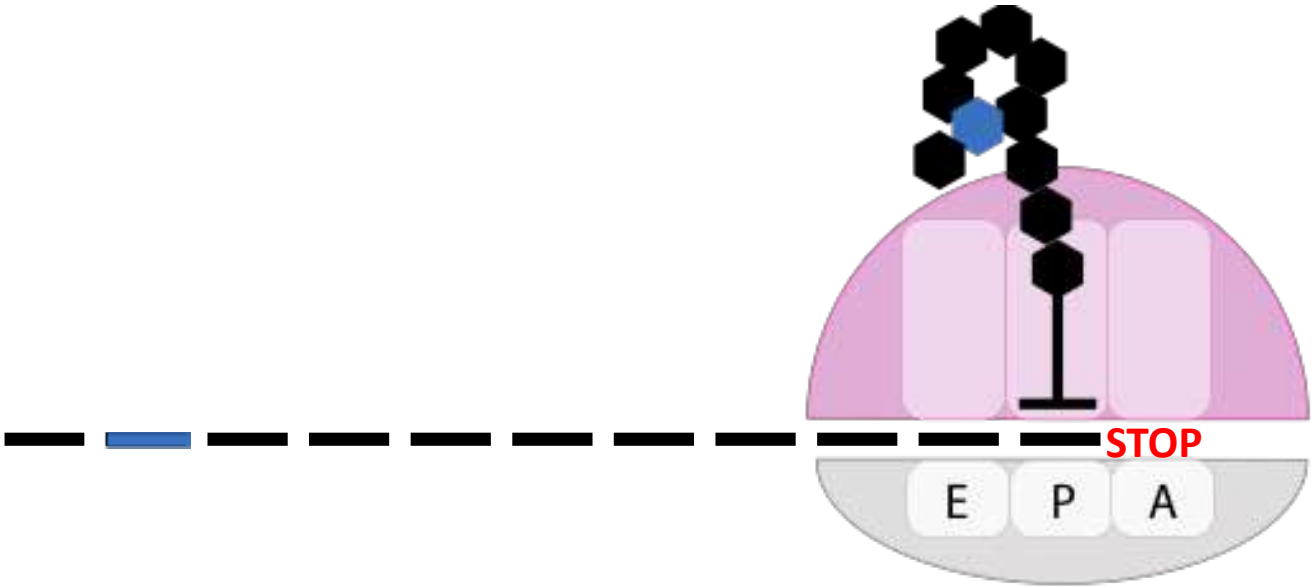
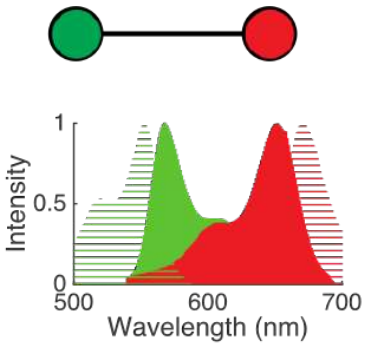
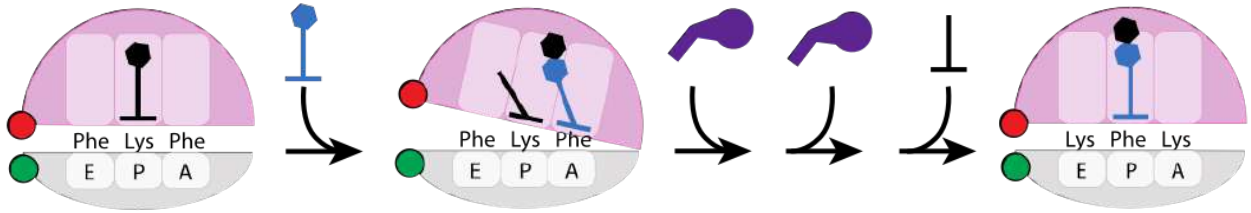

STOP
E
P
A

Following the states of elongation using intersubunit FRET

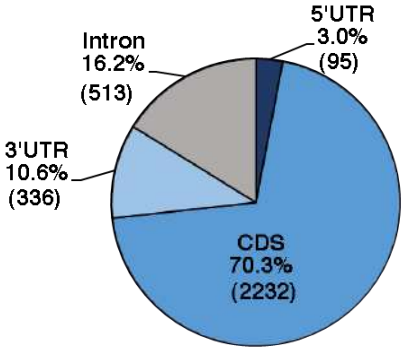
Cy3 Cy5 Ribosome

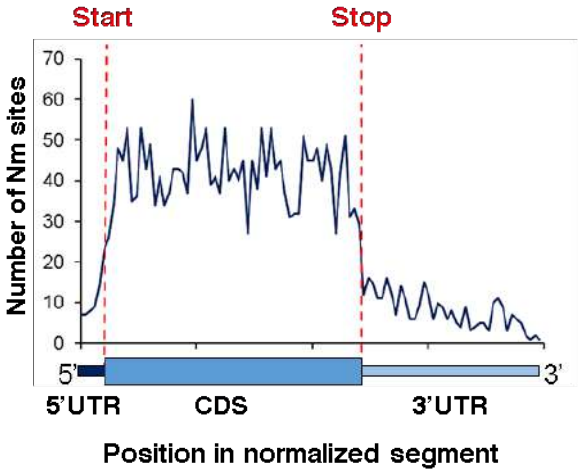




Aitken & Puglisi, *NSMB* 2010

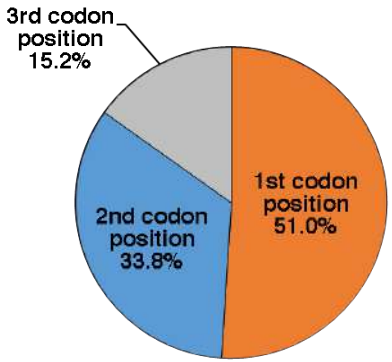
2’-Omethylation (Nm) within the coding region

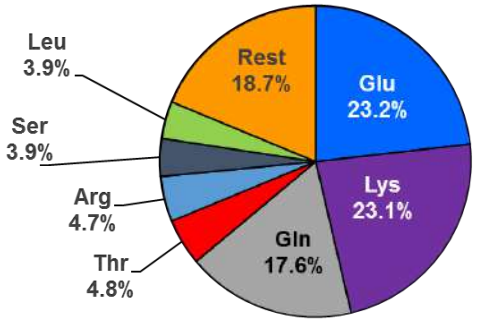


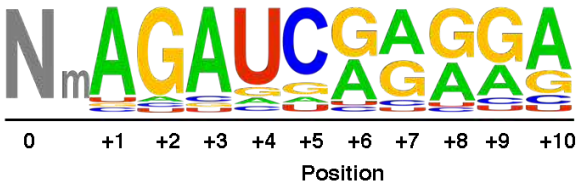


- Chuan He / Gidi Rechavi groups (in press) developed a new method to detect 2’-O-methylated RNA bases with a high sensitivity (their results were provided via personal communication).
- The majority of the modification occurs in the coding region of mRNAs.

Modification occurs more frequently in certain codons

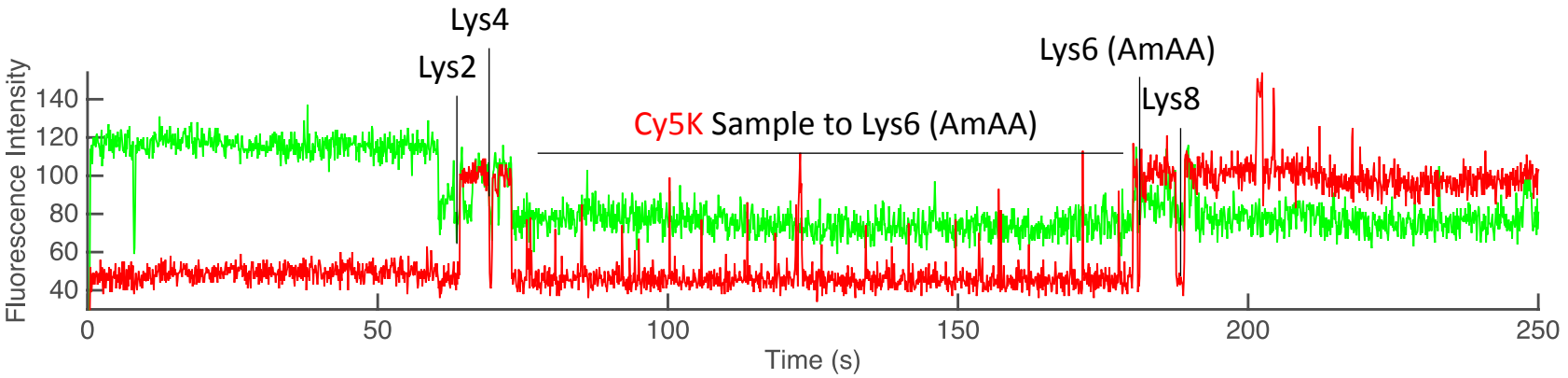






We applied our single-molecule methods to lysine codons……

2’-O-methylation of mRNA delays tRNA decoding

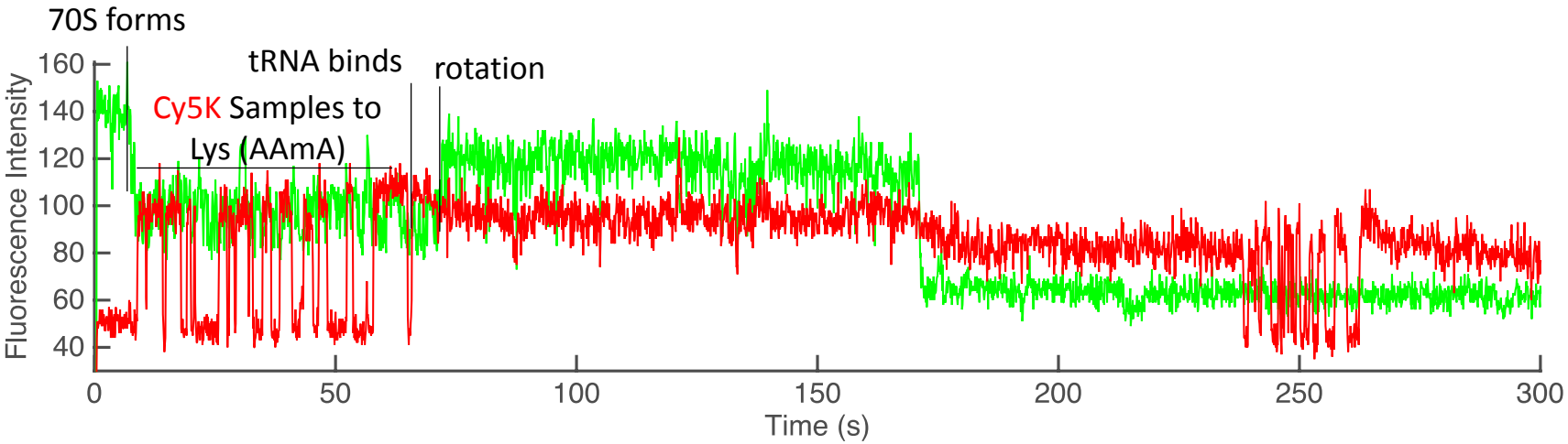


Representative experimental trace (AmAA at 5mM Mg^{2+}):

- Start-Phe^{1}-Lys^{2}-Phe^{3}-Lys^{4}-Phe^{5}-Lys^{6}(AmAA)-Phe^{7}-Lys^{8}-STOP

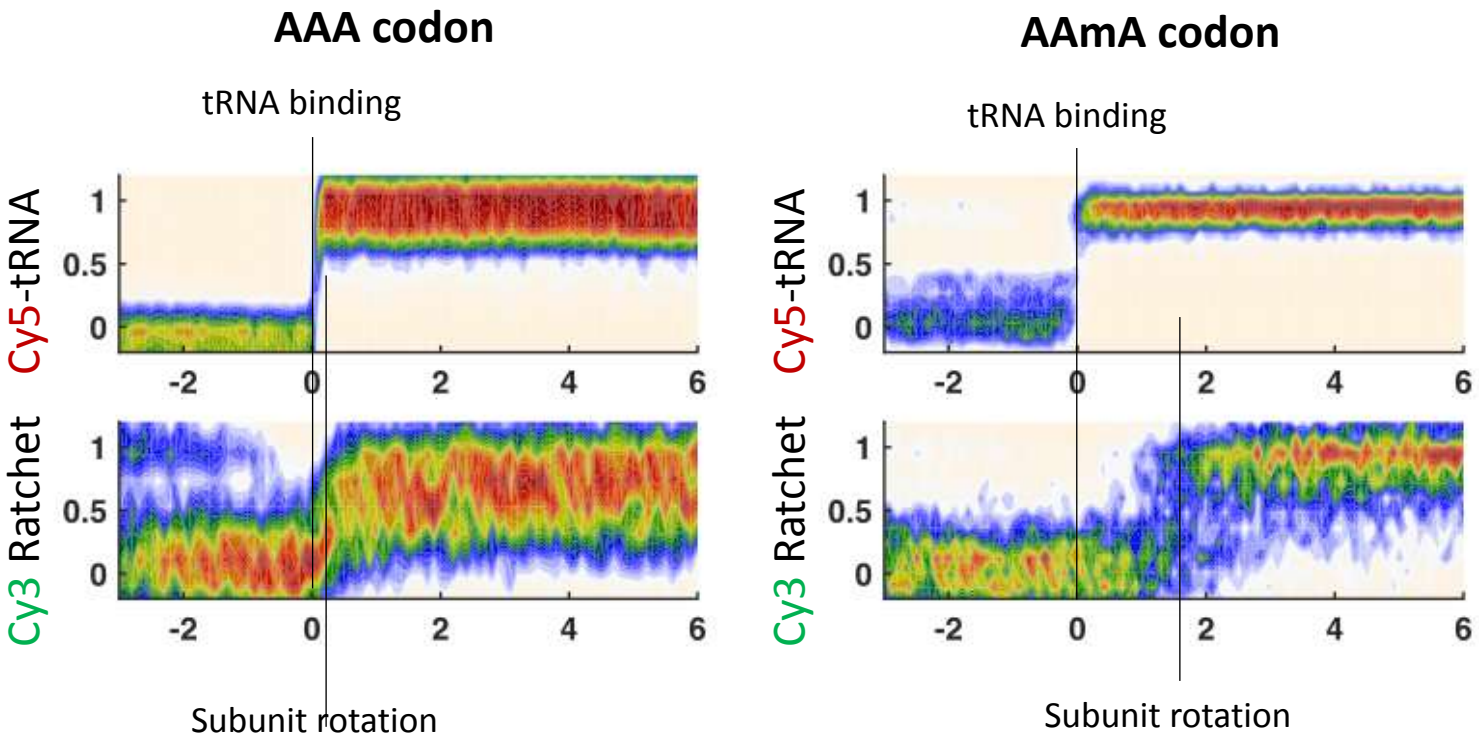
2'-O-methylation of mRNA delays tRNA decoding

(A**Am**A at 15 mM Mg^{2+}):

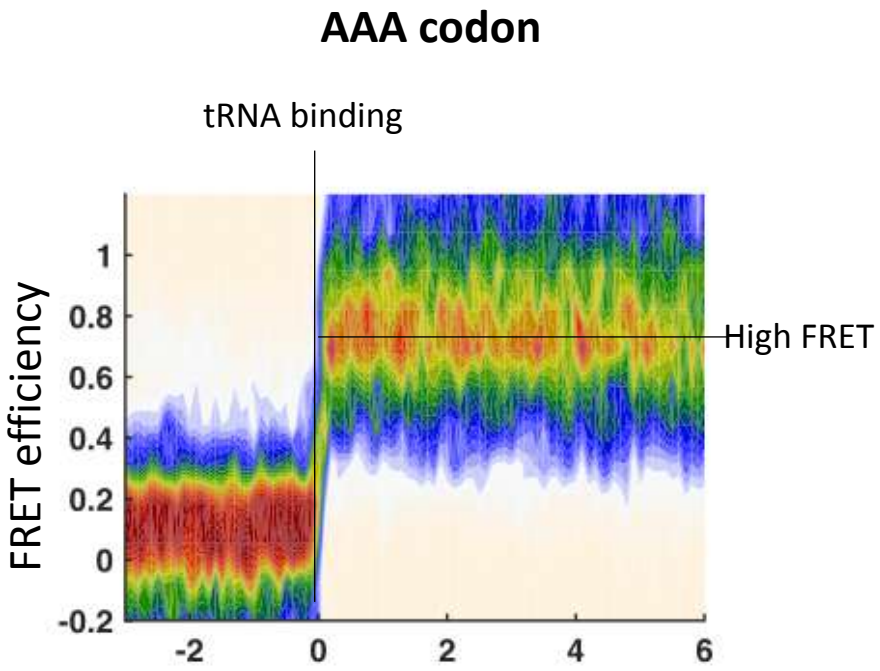


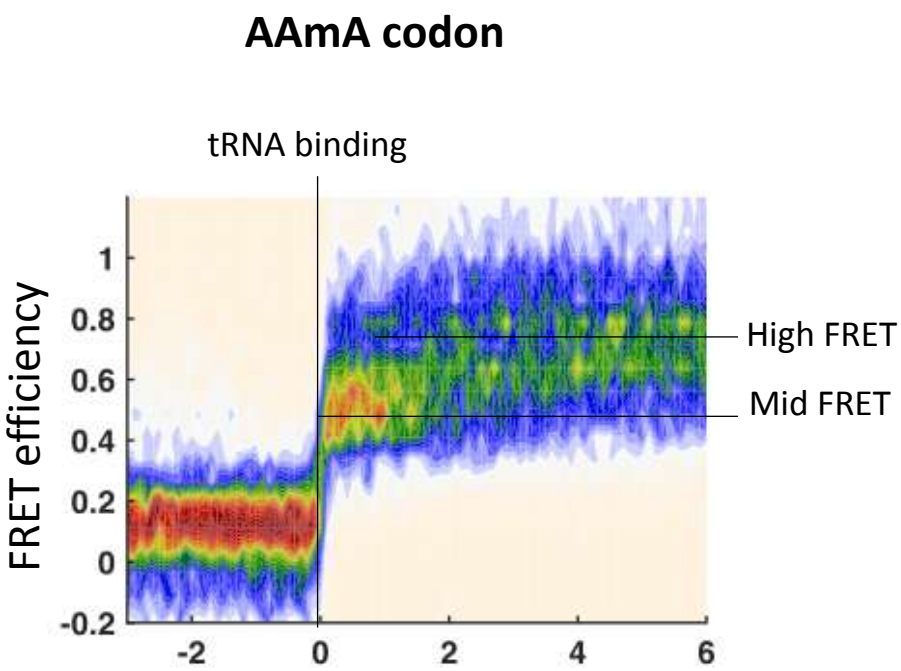
Similar effects at positions 1 and 2

Time lag for tRNA accommodation at 2'OMethyl codons

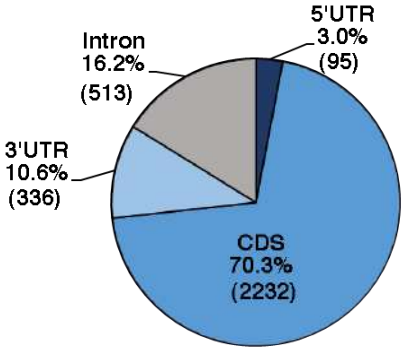


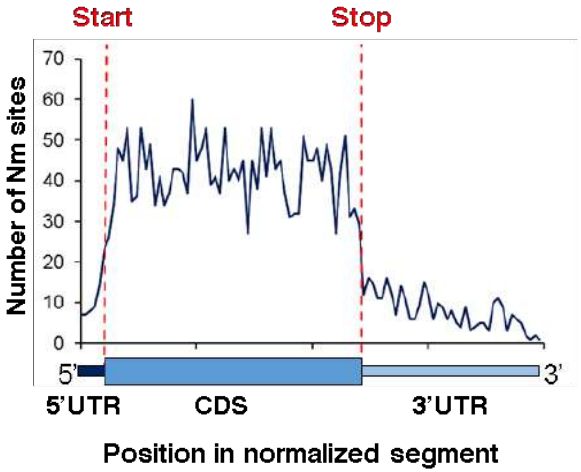
tRNA-tRNA FRET shows a disruption after GTP hydrolysis by EF-Tu





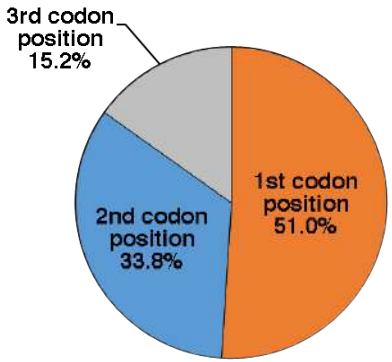
2’-Omethylation (Nm) within the coding region

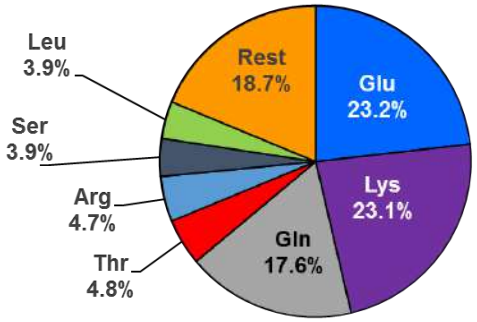


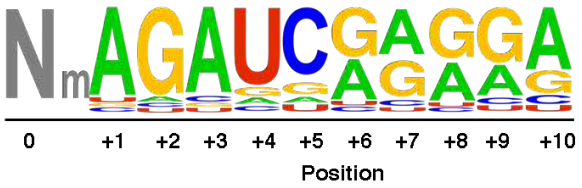


- Chuan He / Gidi Rechavi groups (in press) developed a new method to detect 2’-O-methylated RNA bases with a high sensitivity (their results were provided via personal communication).
- The majority of the modification occurs in the coding region of mRNAs.

Modification occurs more frequently in certain codons

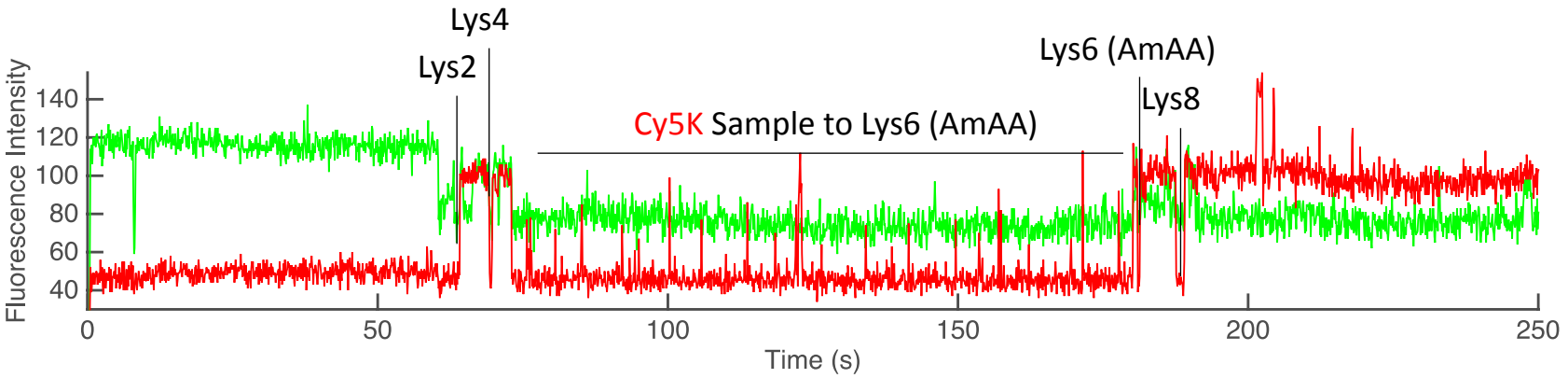






We applied our single-molecule methods to lysine codons……

2’-O-methylation of mRNA delays tRNA decoding

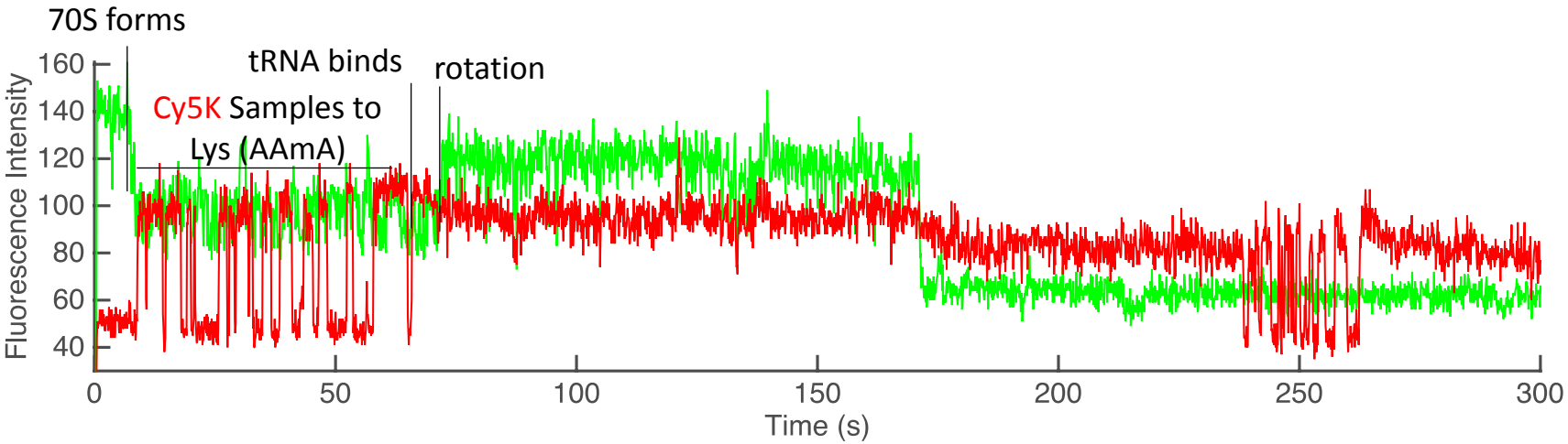


Representative experimental trace (AmAA at 5mM Mg^{2+}):

- Start-Phe^{1}-Lys^{2}-Phe^{3}-Lys^{4}-Phe^{5}-Lys^{6}(AmAA)-Phe^{7}-Lys^{8}-STOP

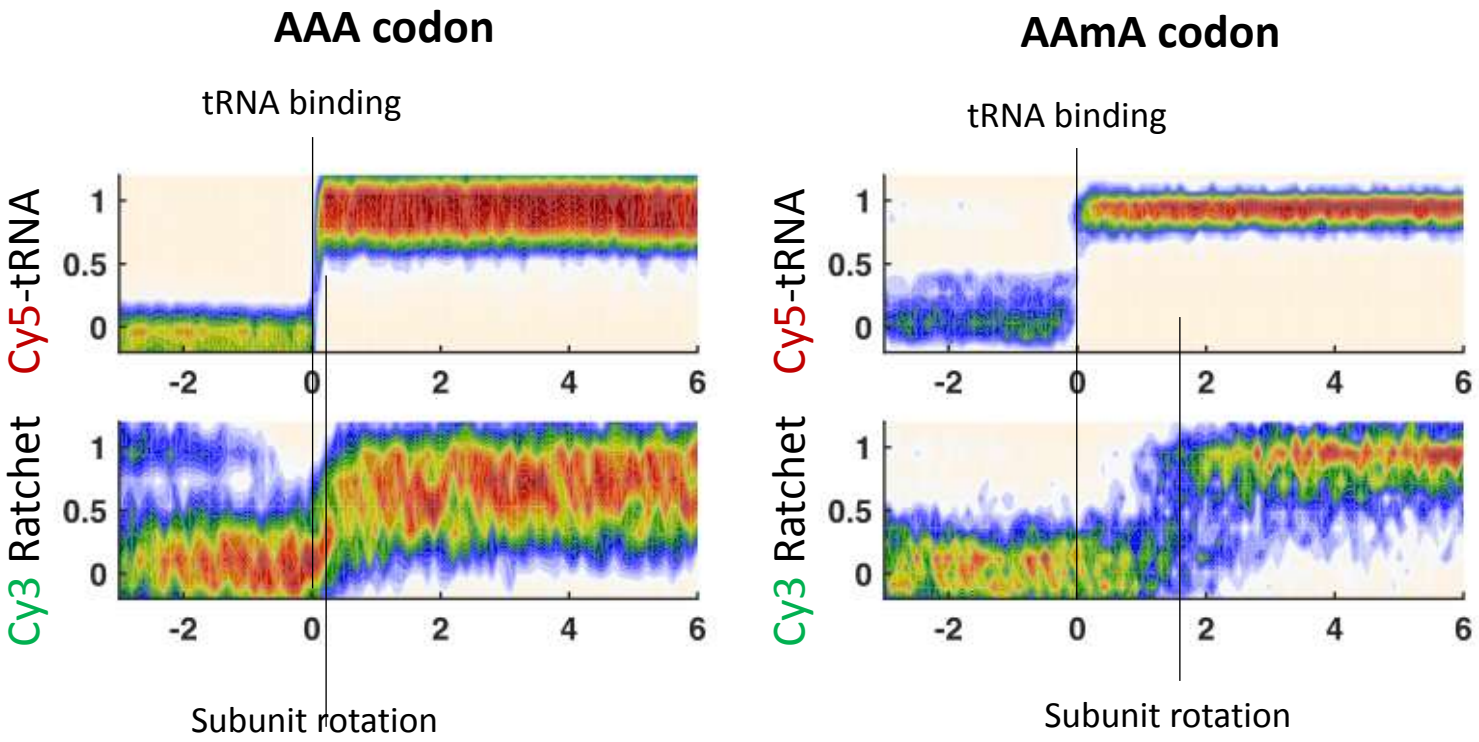
2'-O-methylation of mRNA delays tRNA decoding

(A**Am**A at 15 mM Mg^{2+}):

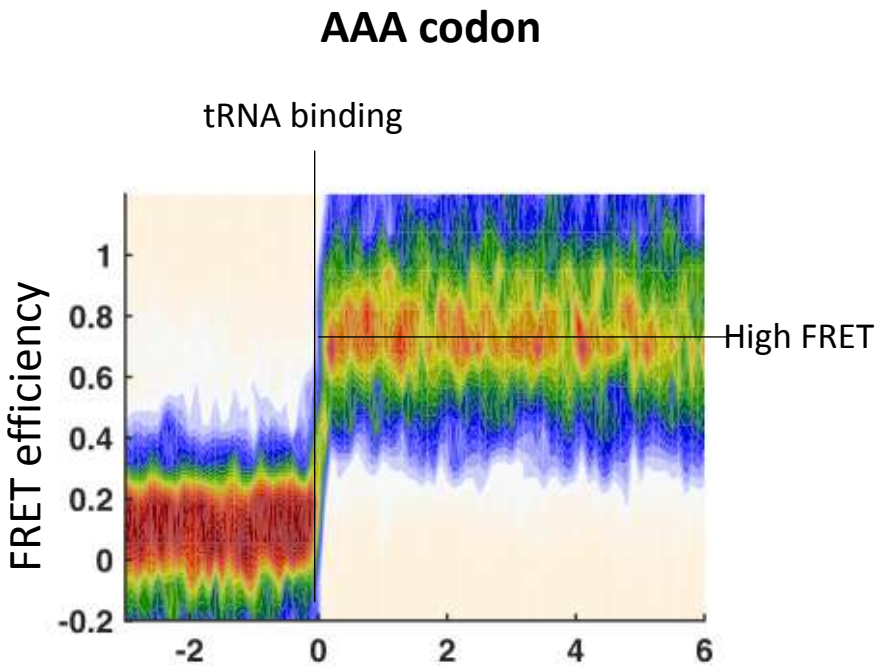


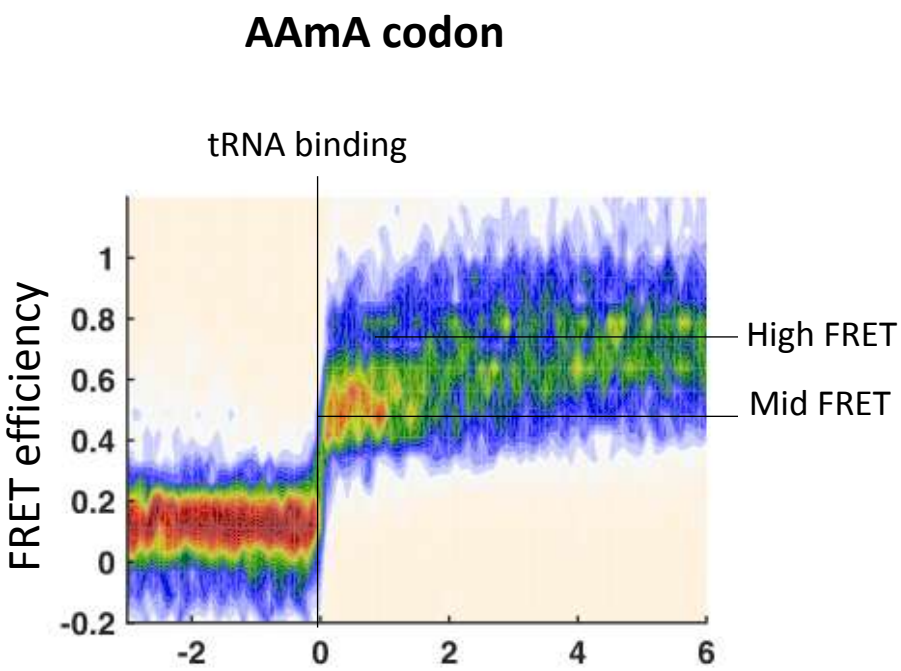
Similar effects at positions 1 and 2

Time lag for tRNA accommodation at 2'OMethyl codons

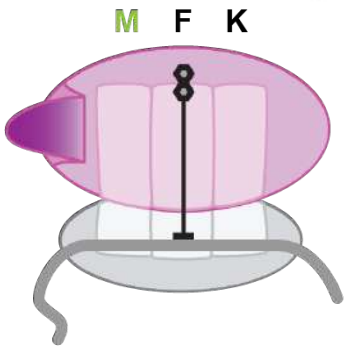


tRNA-tRNA FRET shows a disruption after GTP hydrolysis by EF-Tu

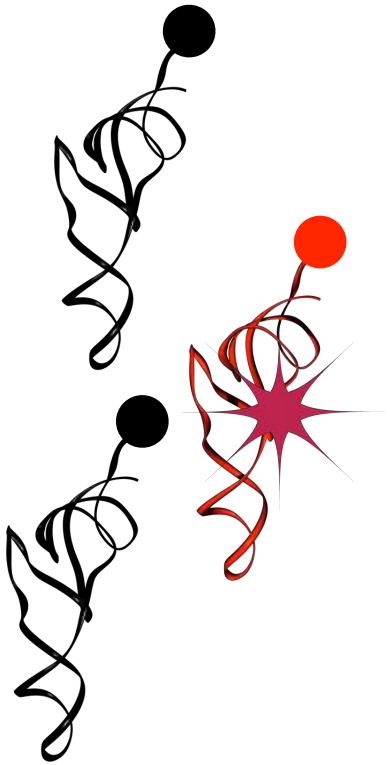




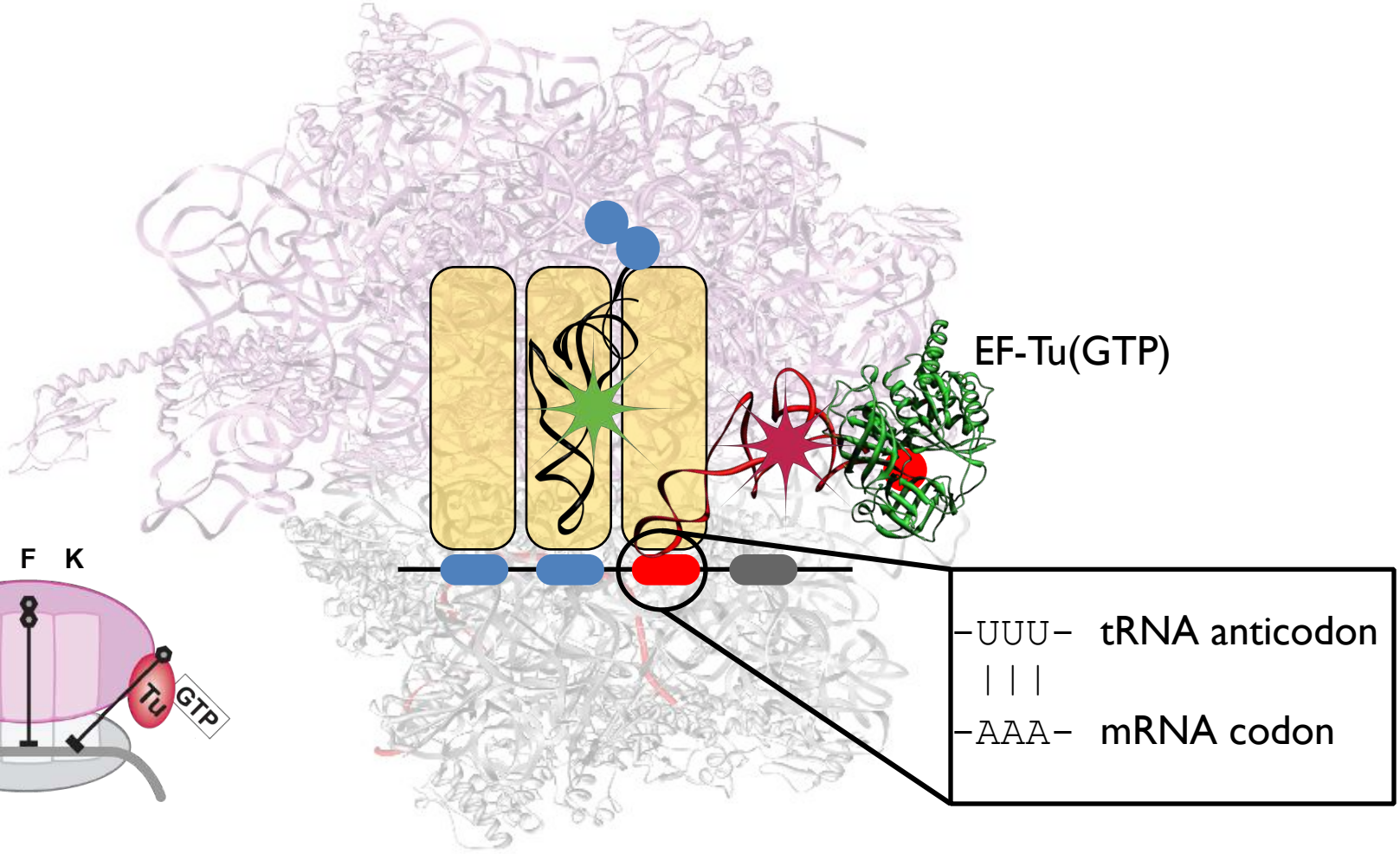
Translation elongation



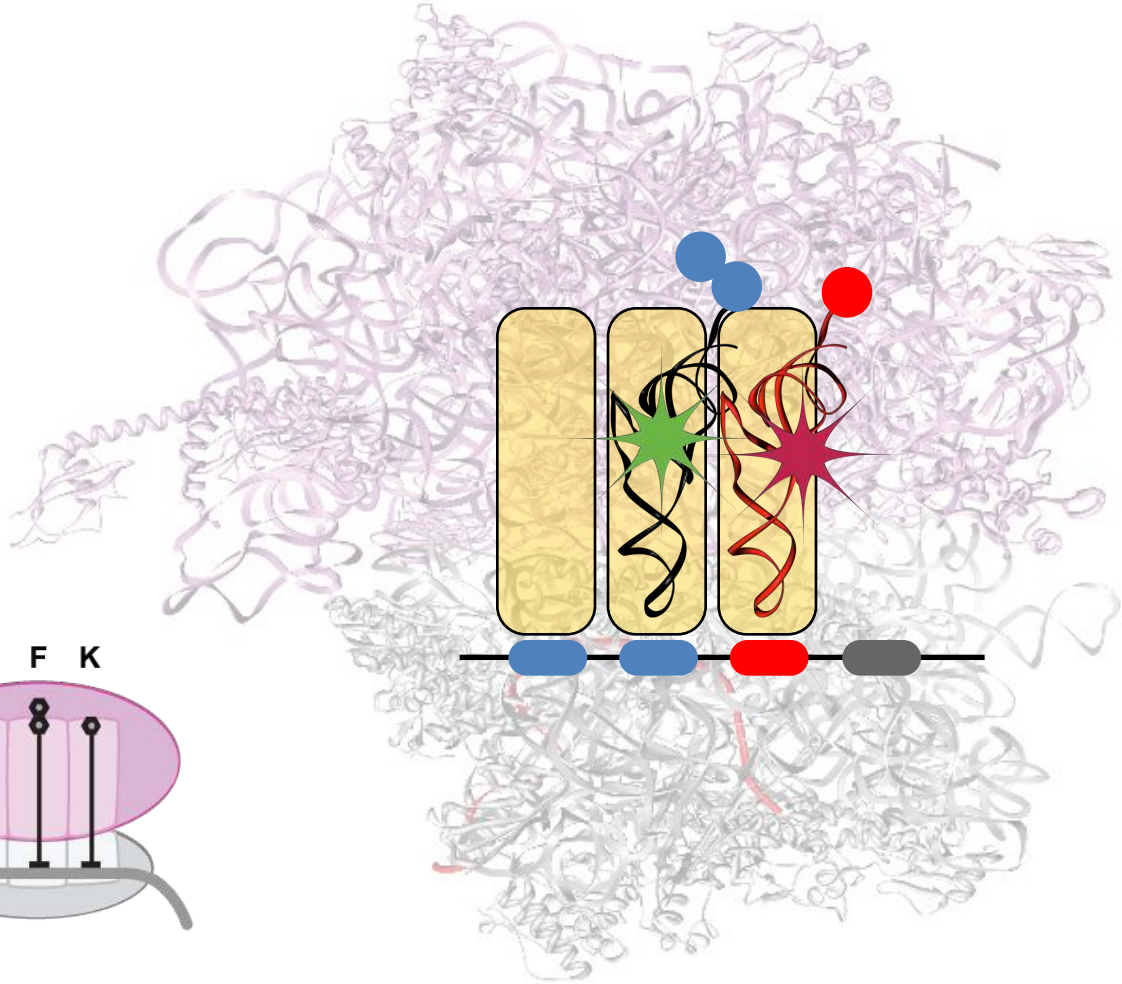
Non-rotated



tRNA selection

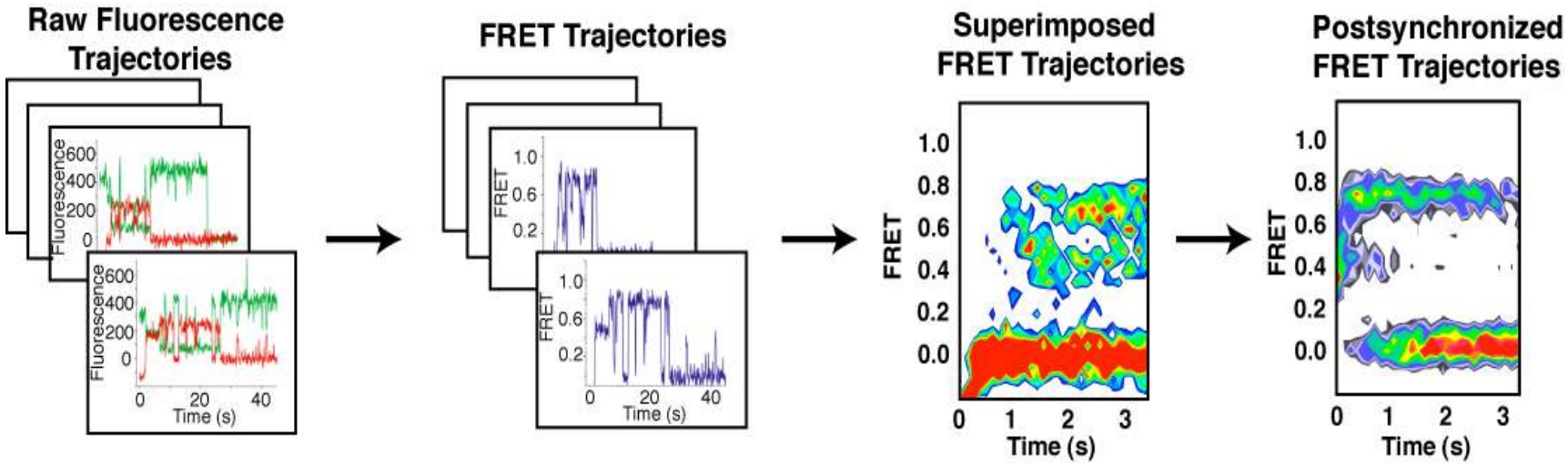


tRNA accommodation



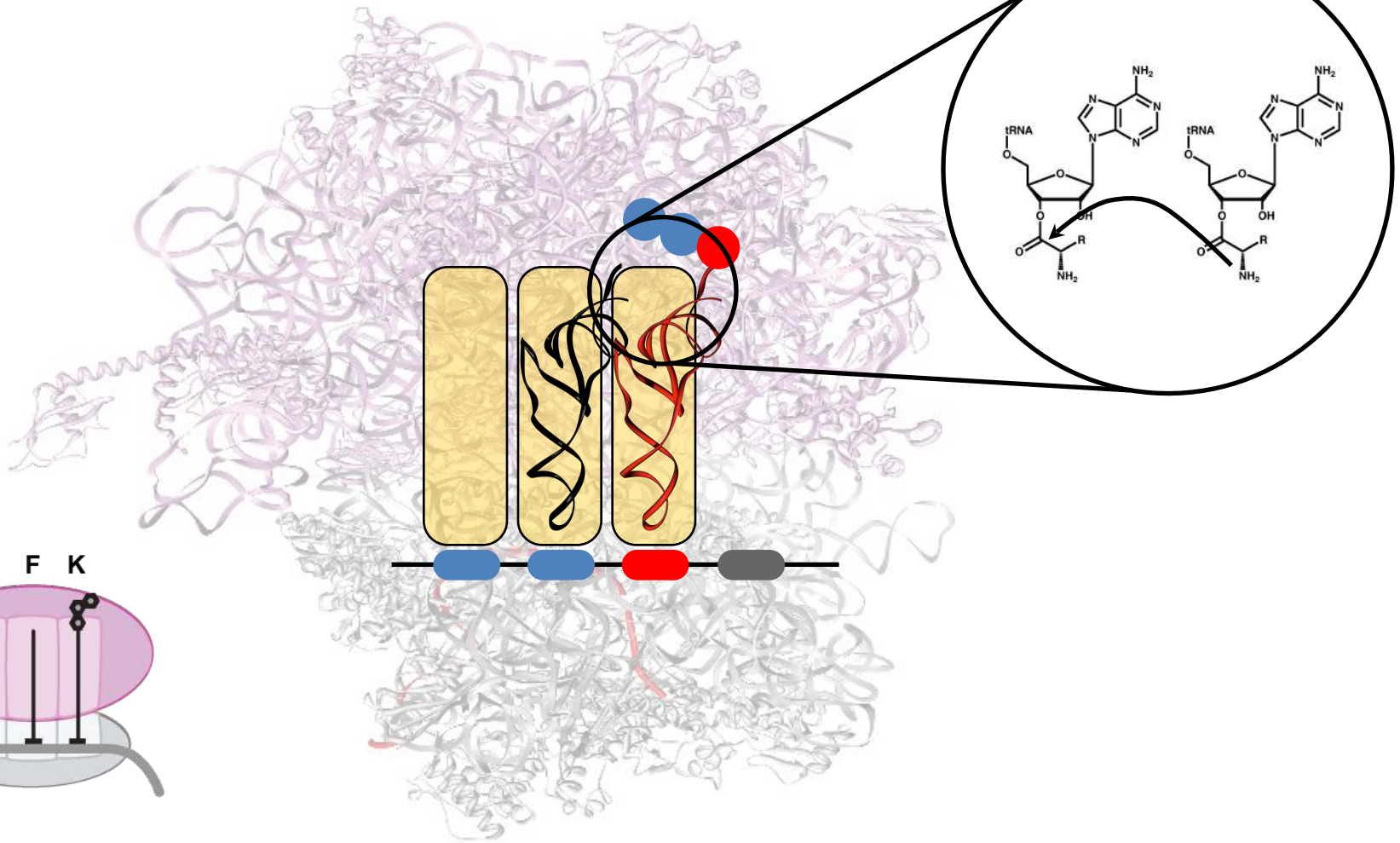
Non-rotated

Pathway of tRNA selection and accommodation



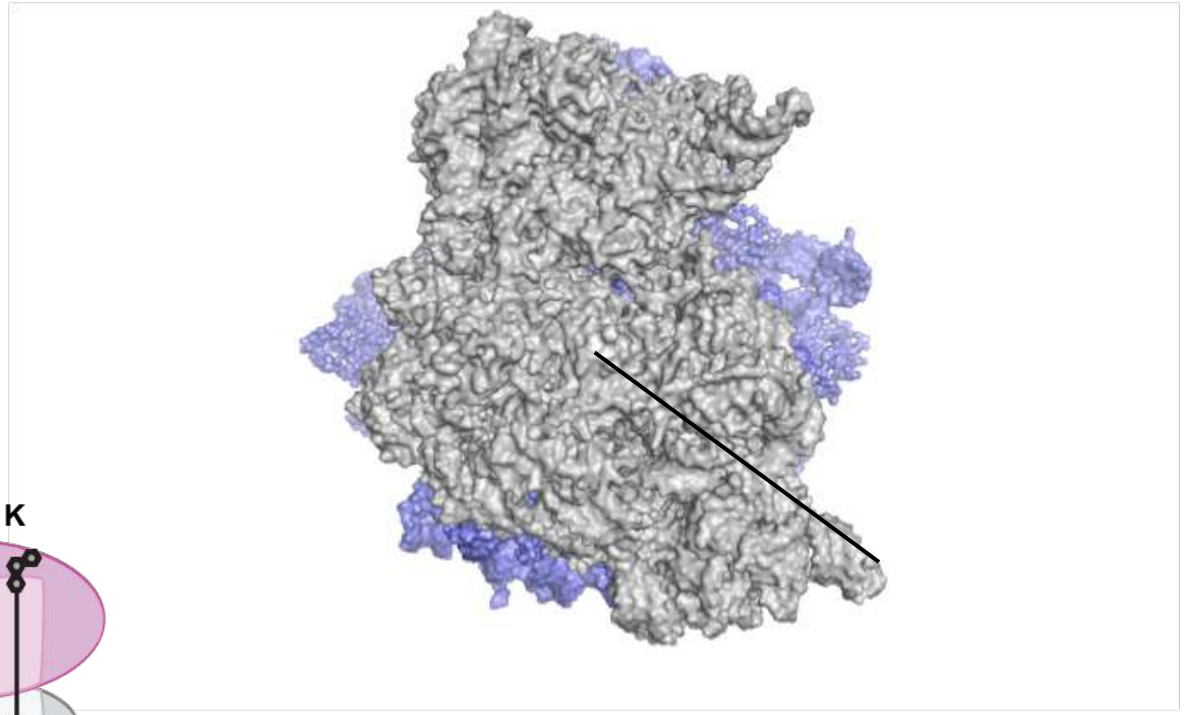
Blanchard et al. NSMB, 2004

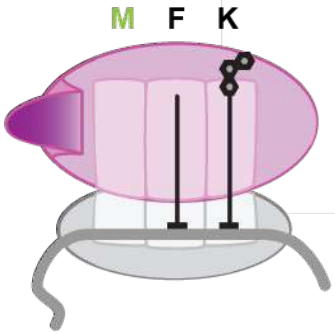
Peptide bond formation



Non-rotated

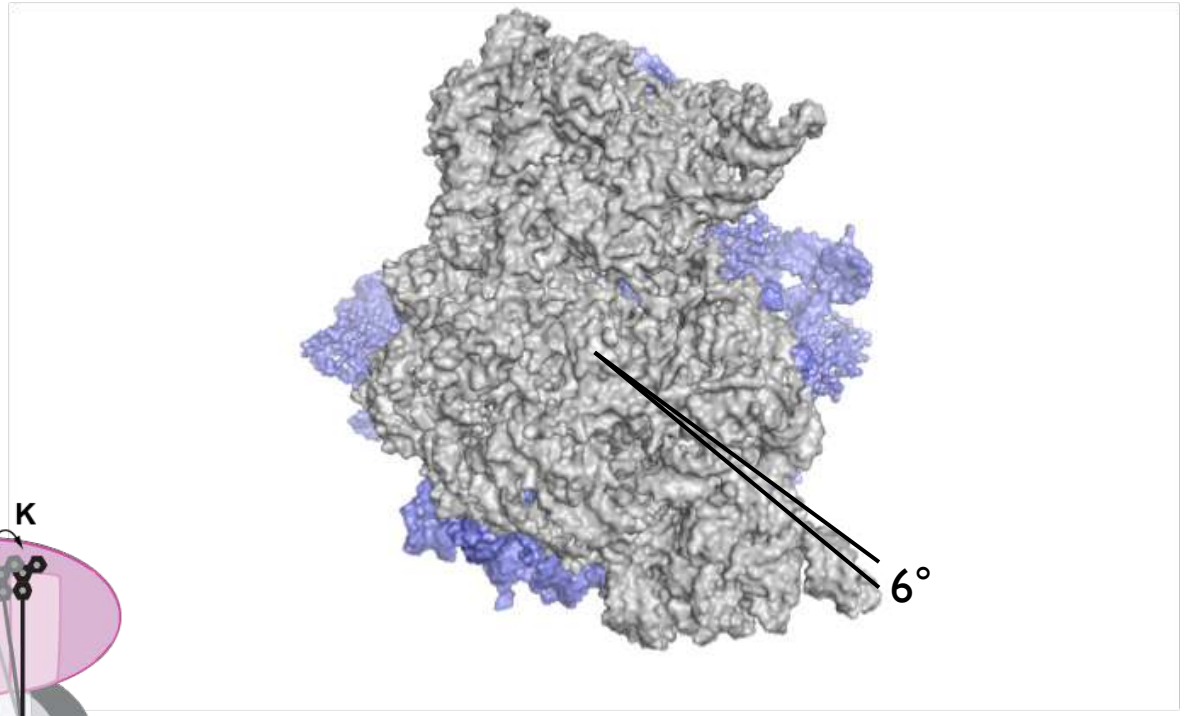
Subunit rotation

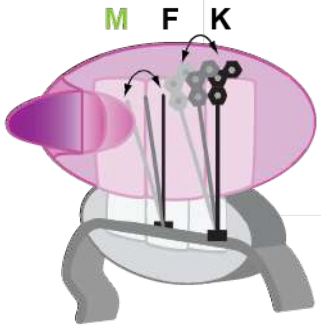




Non-rotated

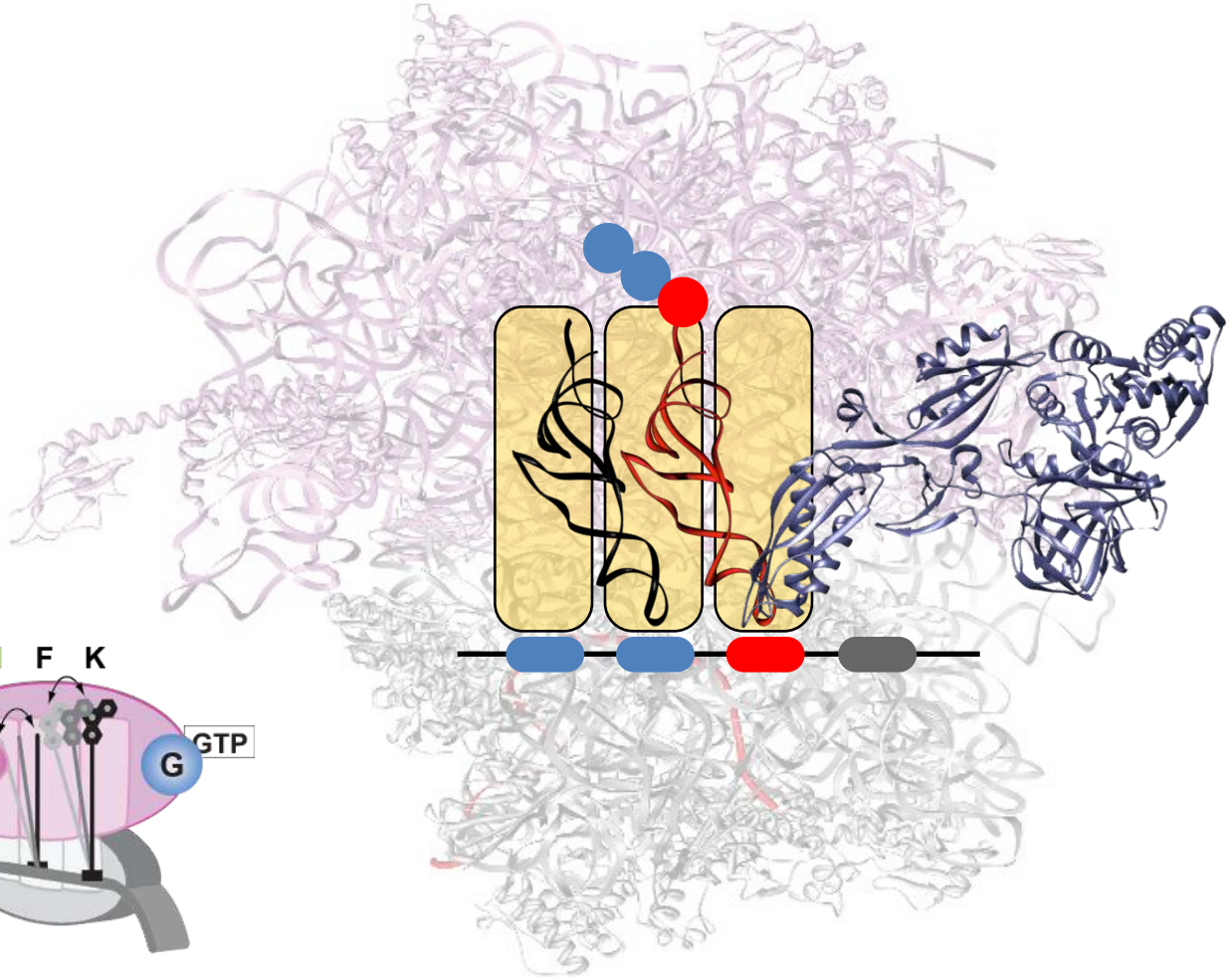
Subunit rotation





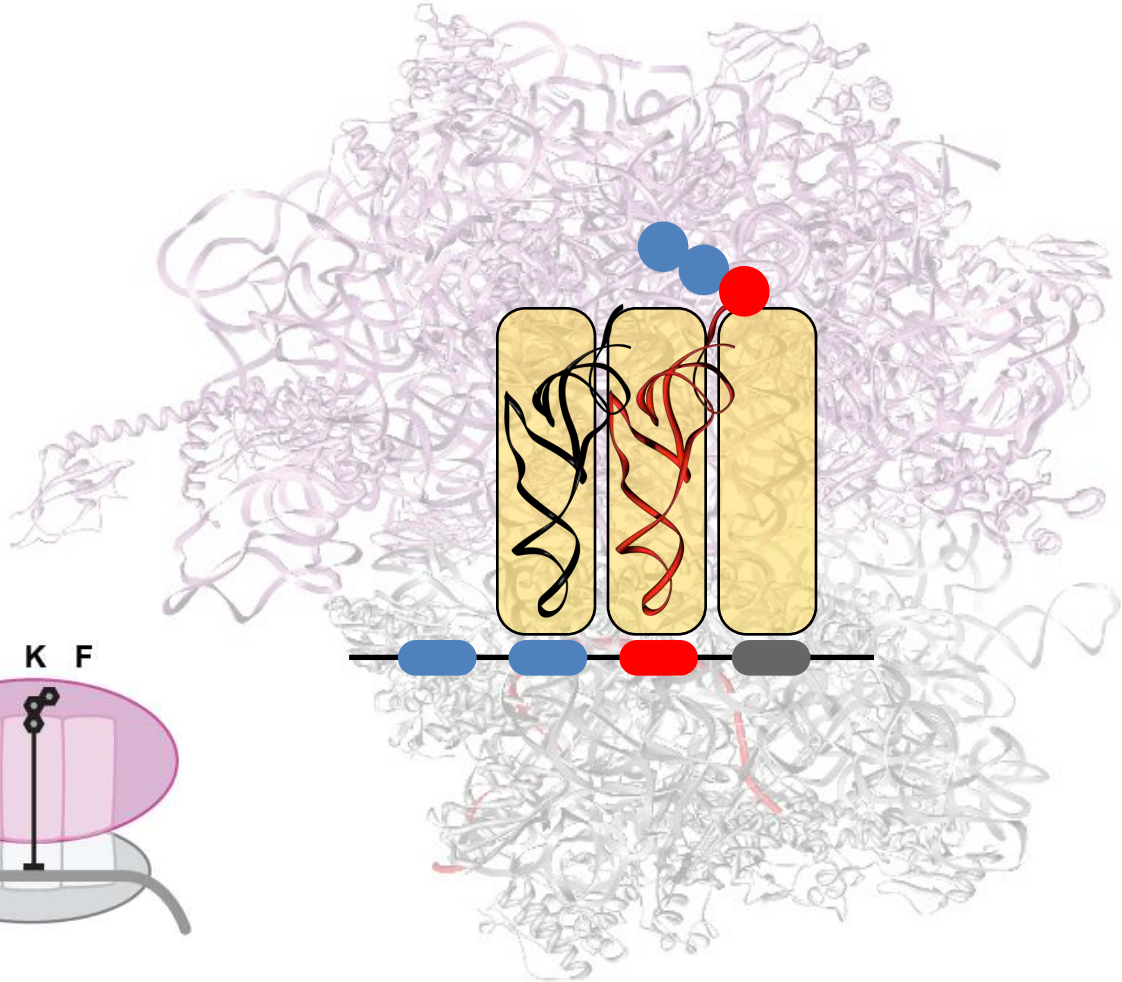
Rotated

Preparation for translocation



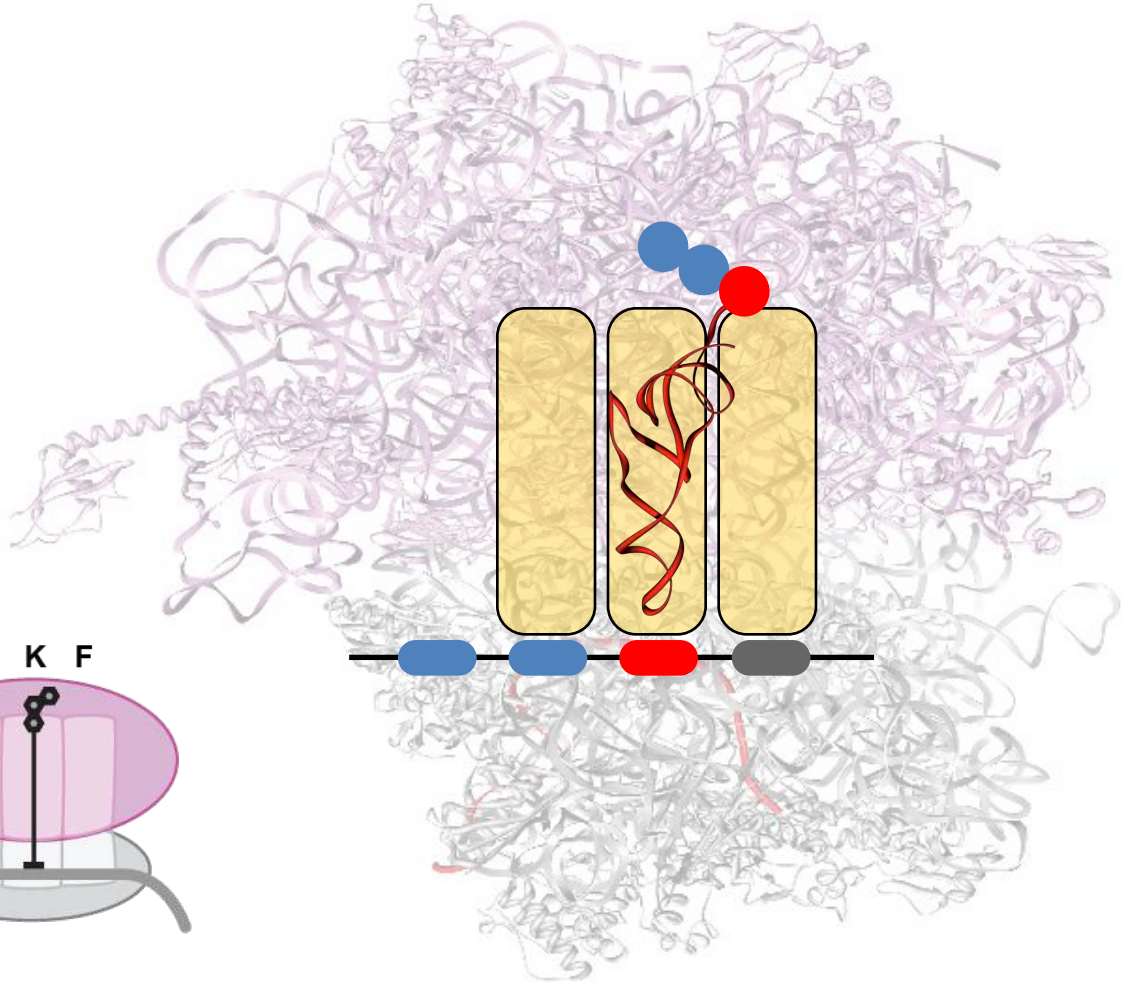
Rotated

Translocation



Non-rotated

E-site tRNA exits



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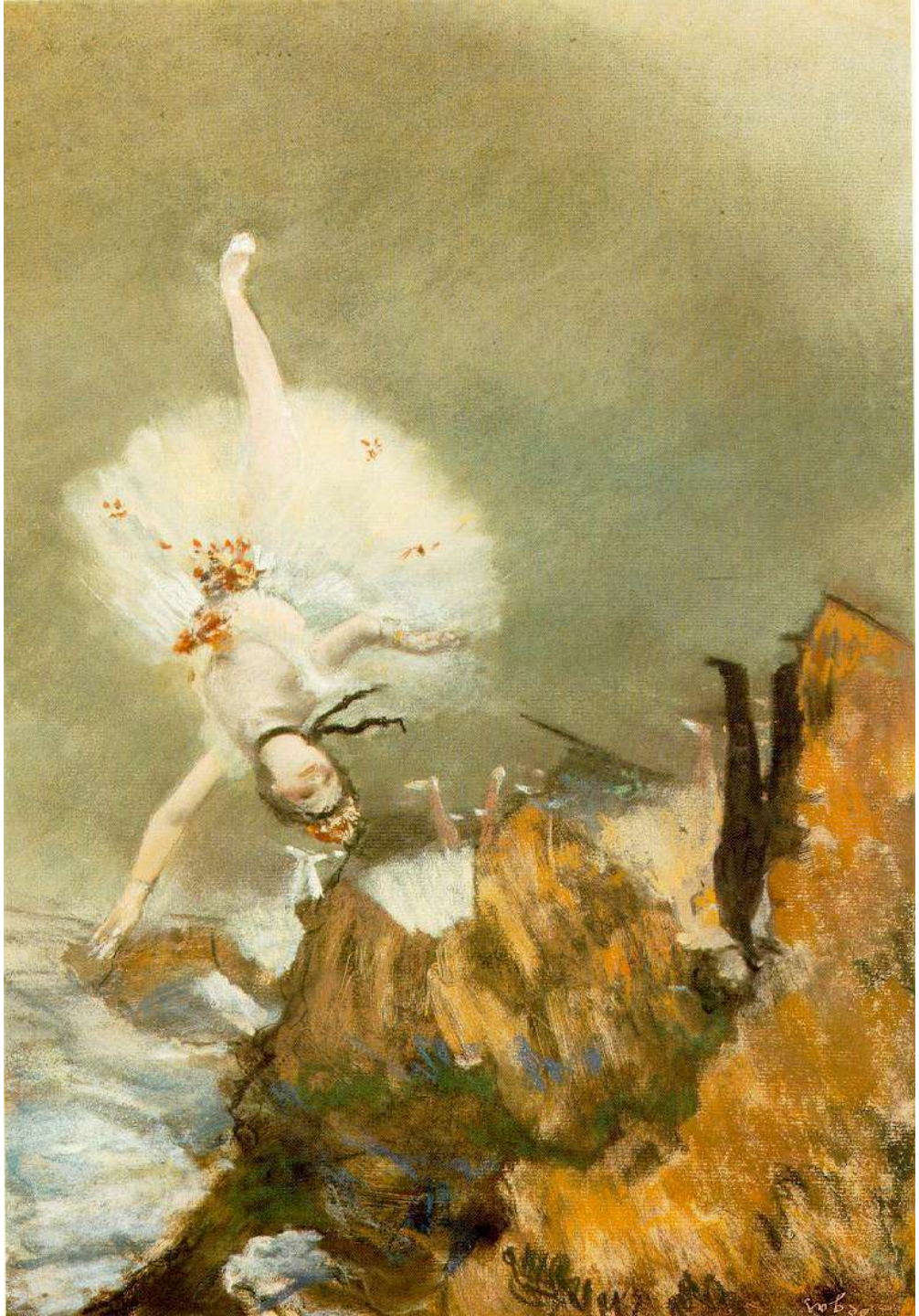
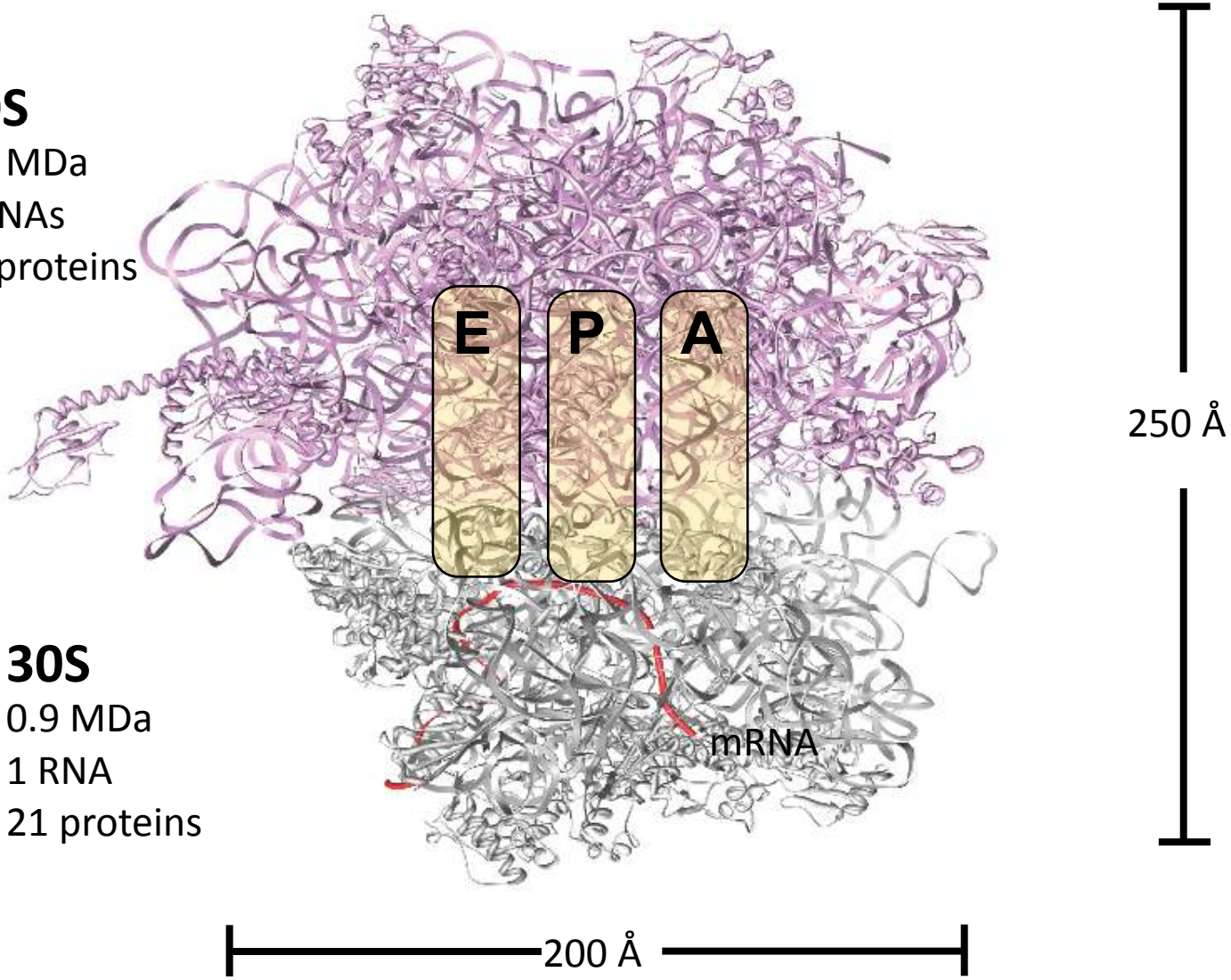
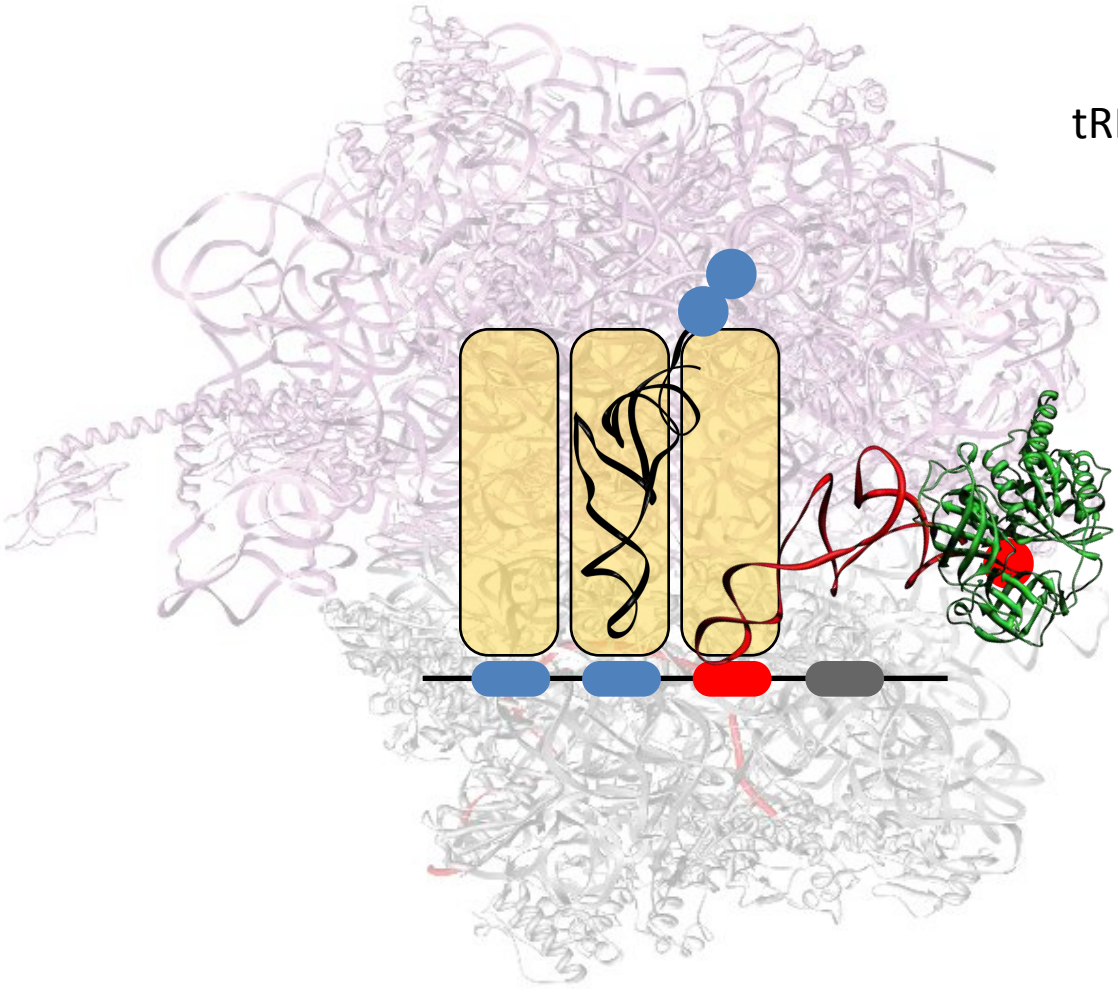


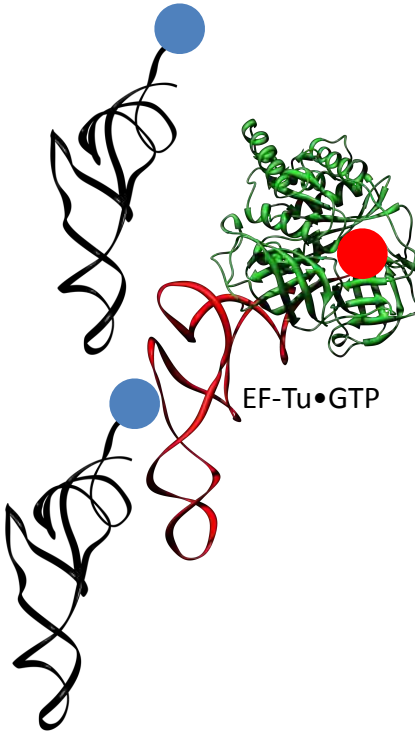
The ribosome



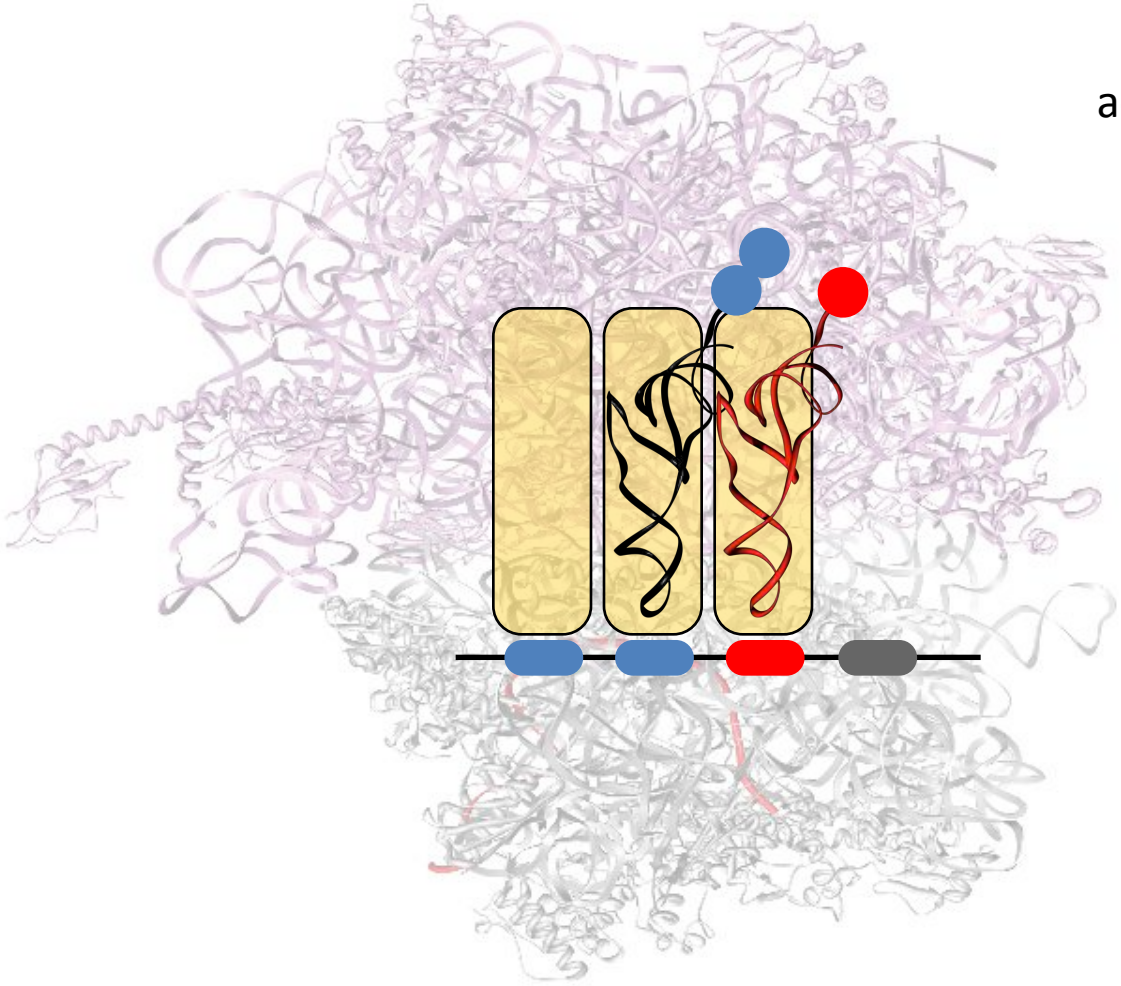
The Ribosome Selects tRNA for Catalysis



tRNA selection

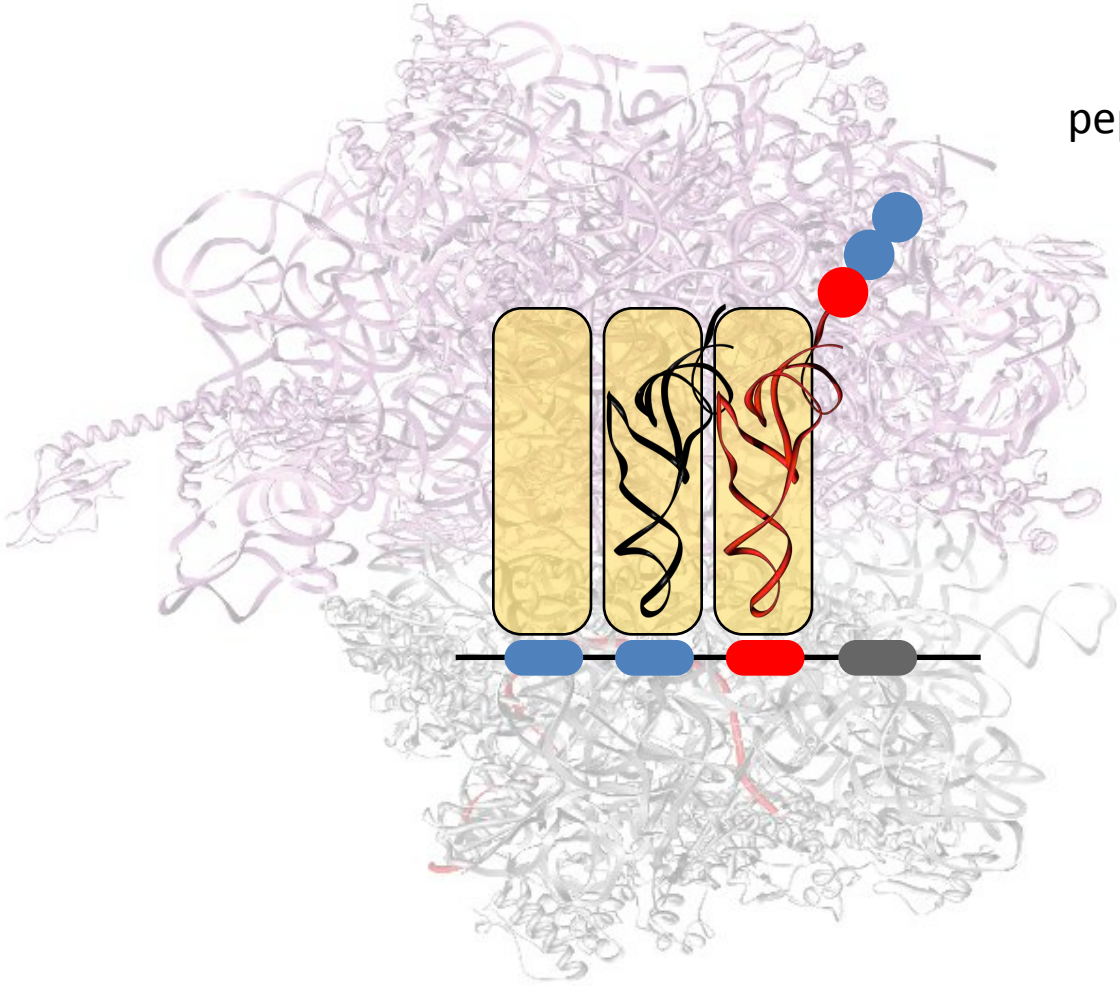


The Ribosome Selects tRNA for Catalysis



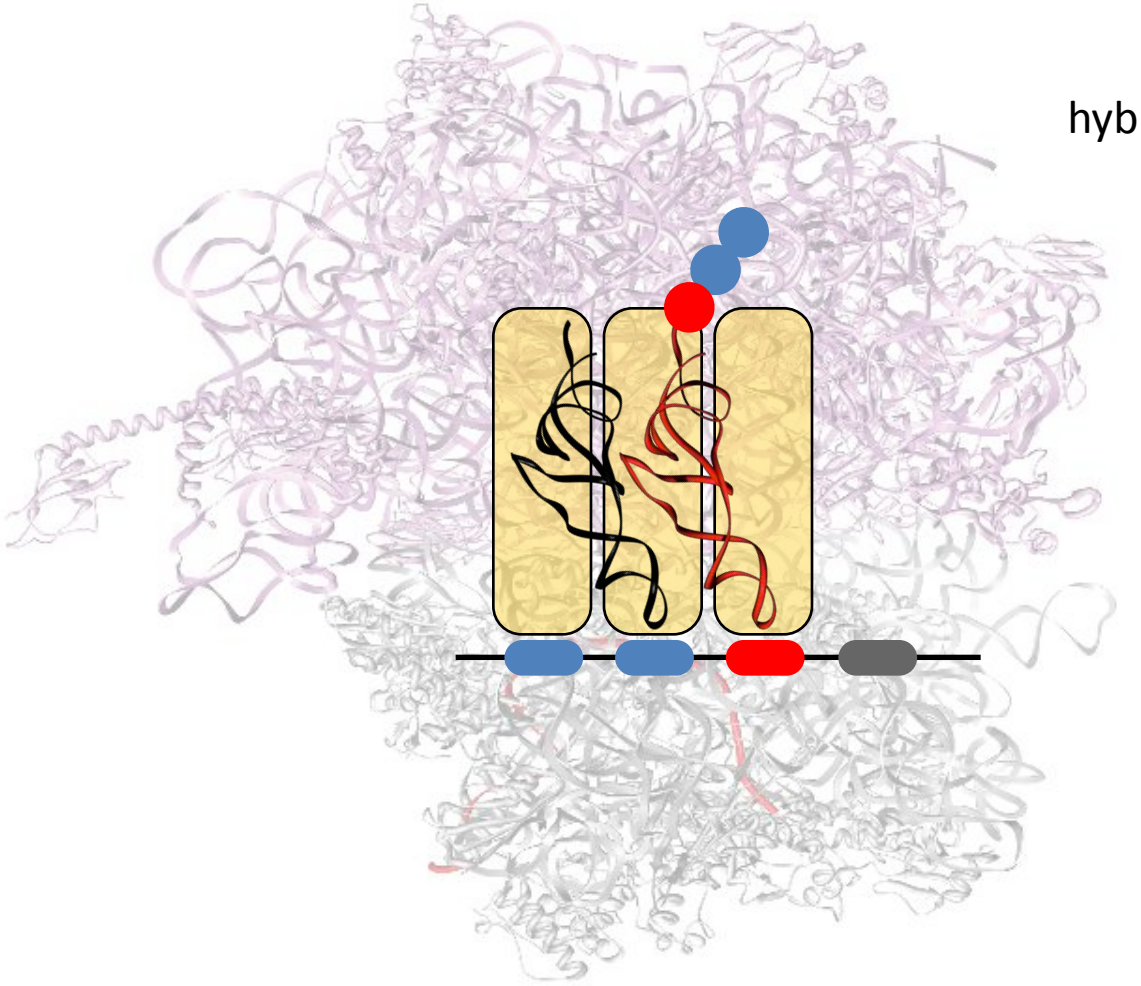
accommodation

The Ribosome Selects tRNA for Catalysis



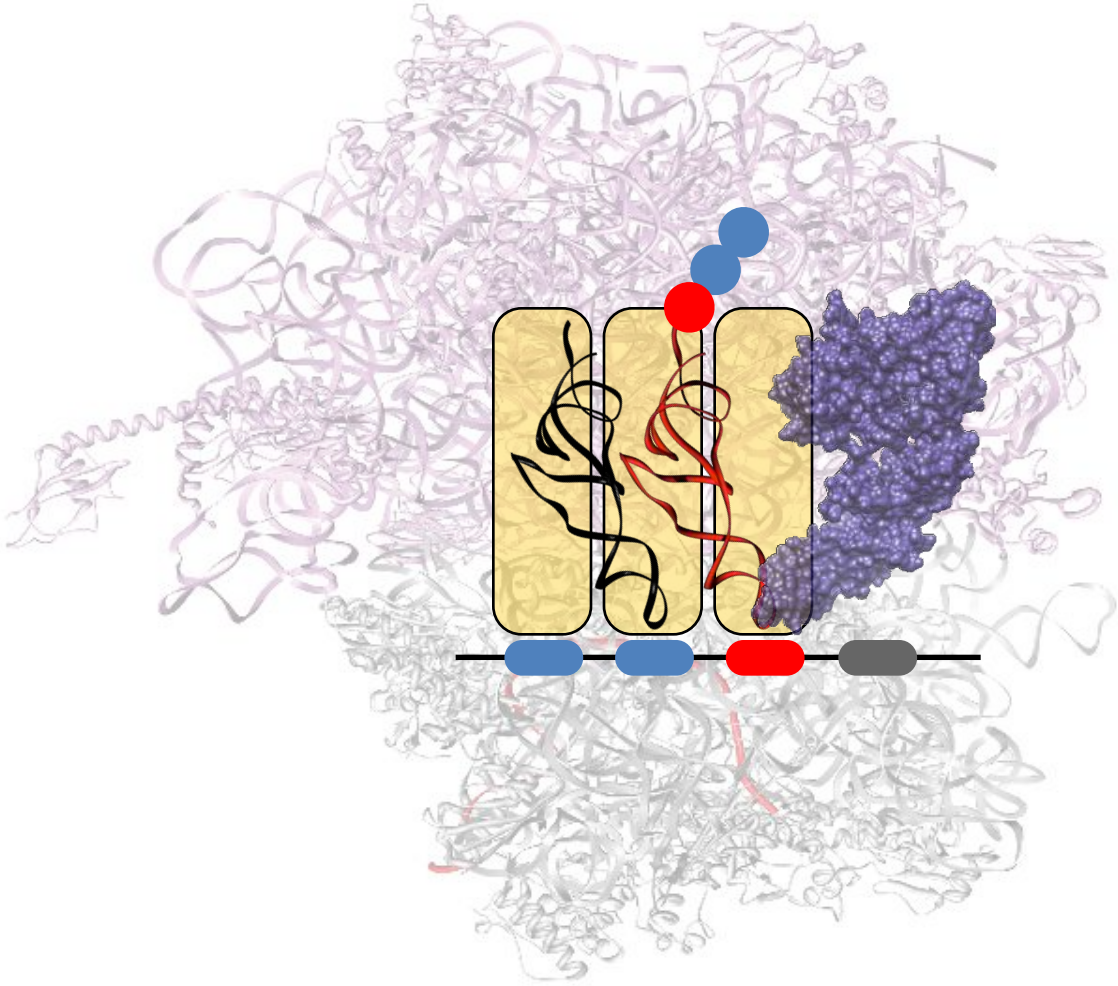
peptide bond formation

Translocation Moves the Ribosome



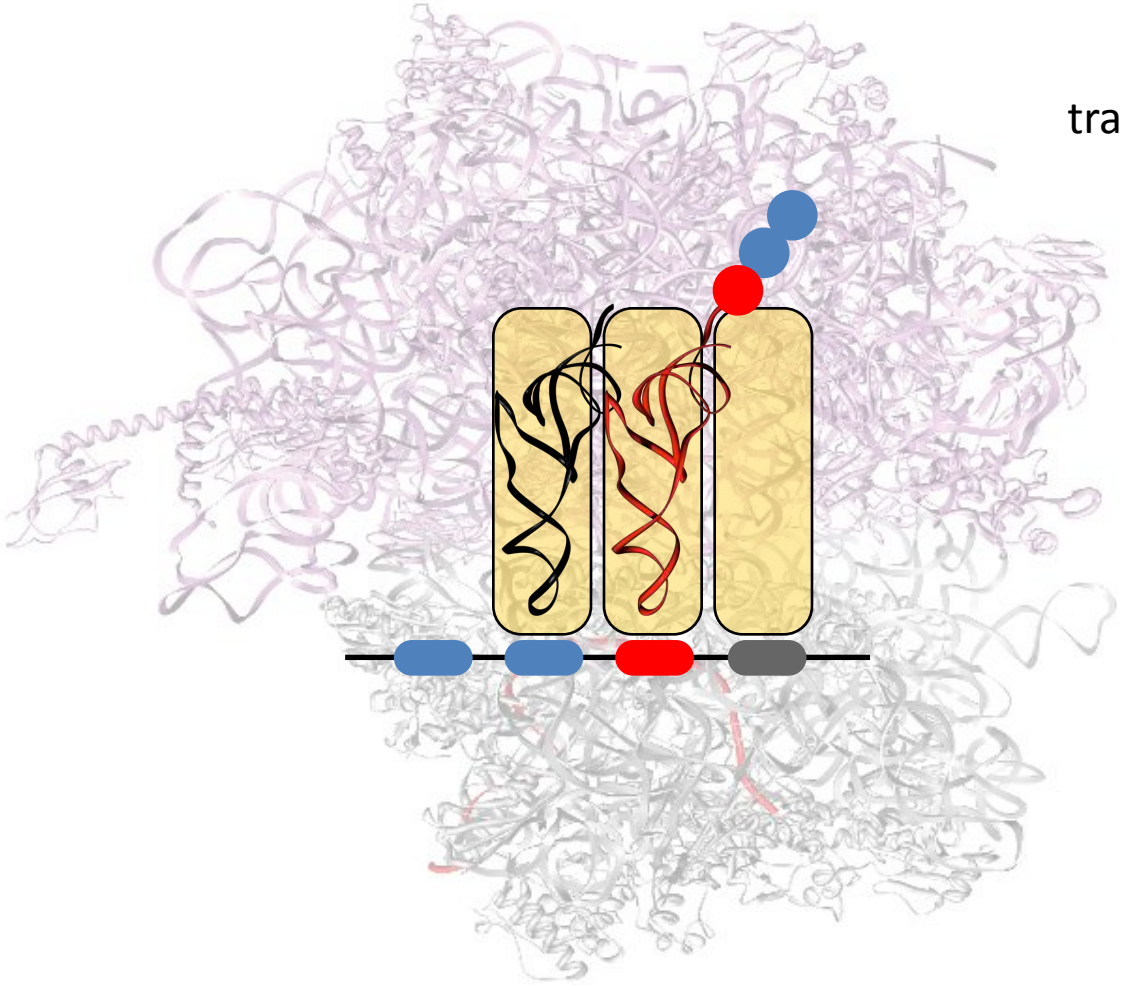
hybrid state formation

Translocation Moves the Ribosome



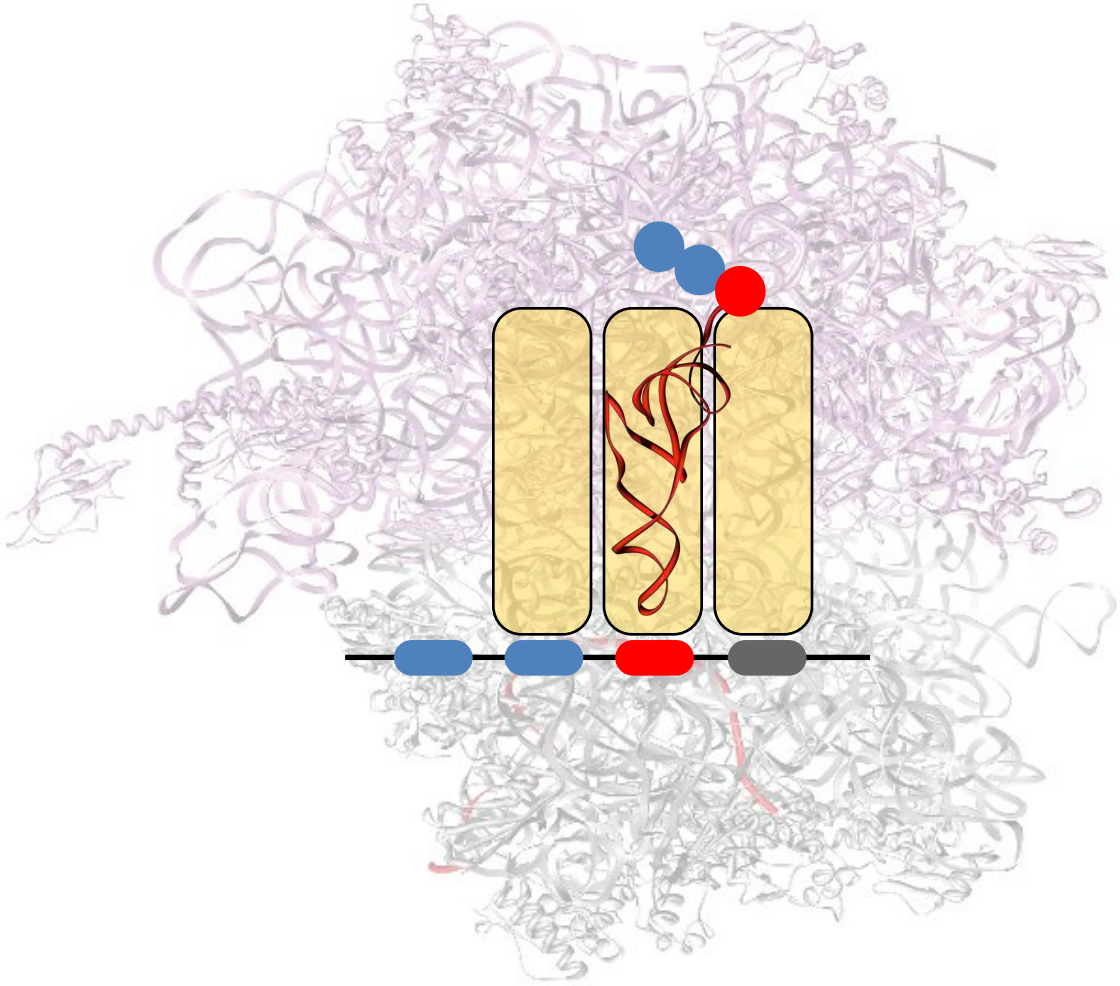
EF-G binds in the A site and catalyzes movement of tRNA-mRNA complexes on the ribosome

Translocation Moves the Ribosome

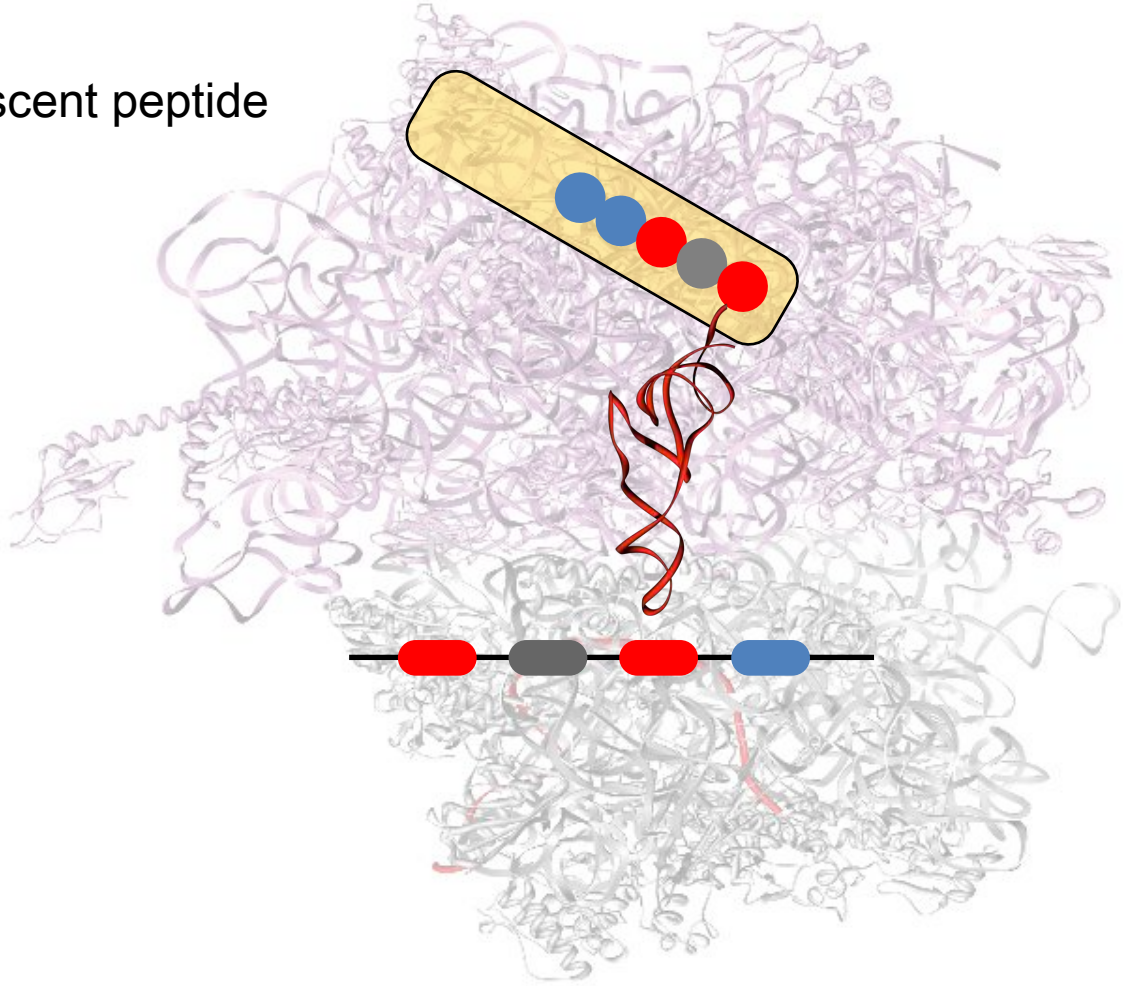


translocation

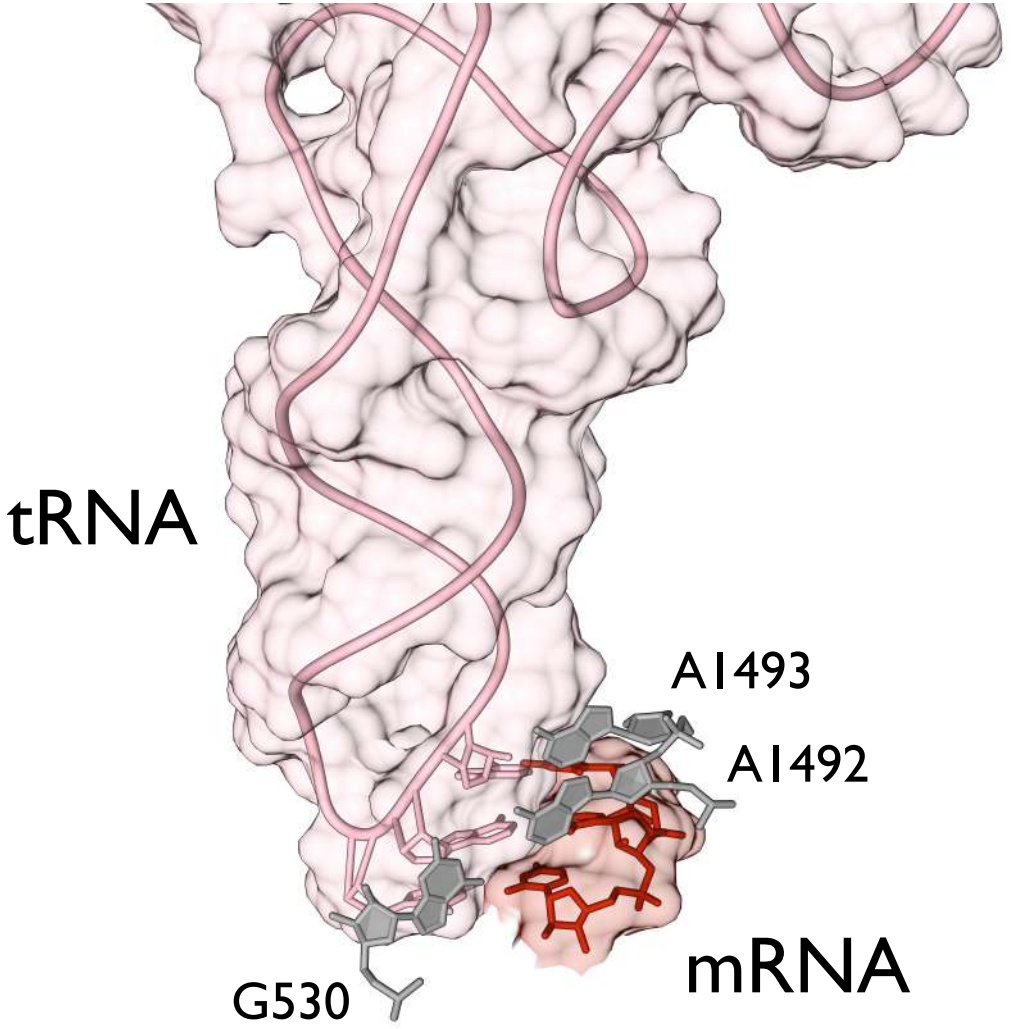
E-site tRNA departs……and process can repeat



Nascent peptide exits through a tunnel in the 50S subunit



Ribosomal RNA recognizes the shape of the codon-anticodon helix in the A site



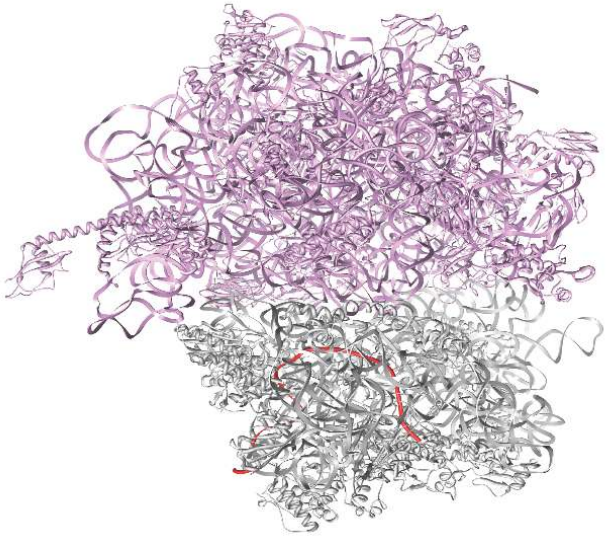
Beware the elegant meal.....

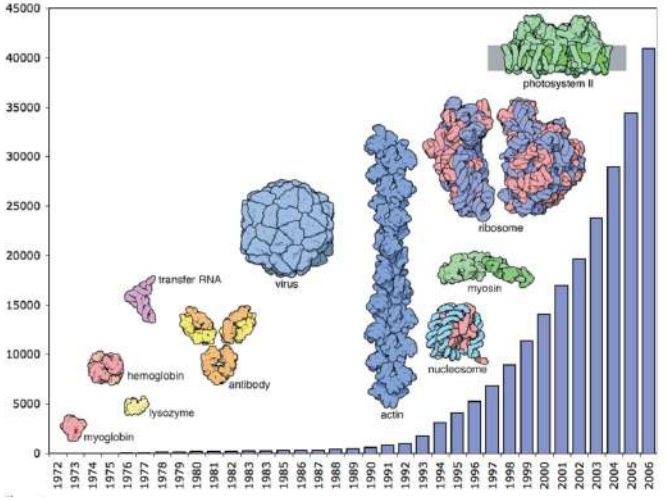
Beware the elegant meal.....

Beware the elegant meal.....

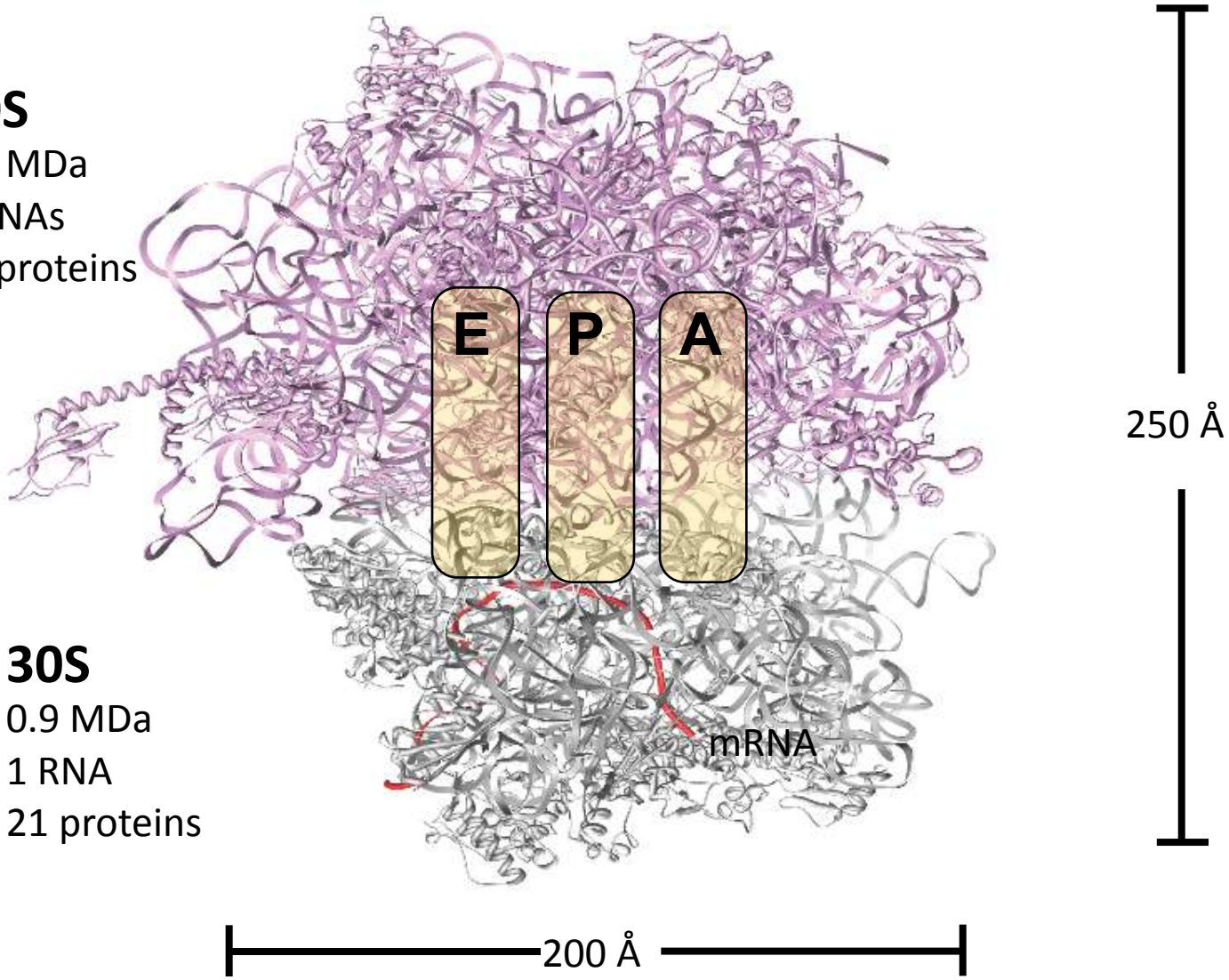
- labeling
- surface
- photophysics
- concentrations

The choreography of biological processes

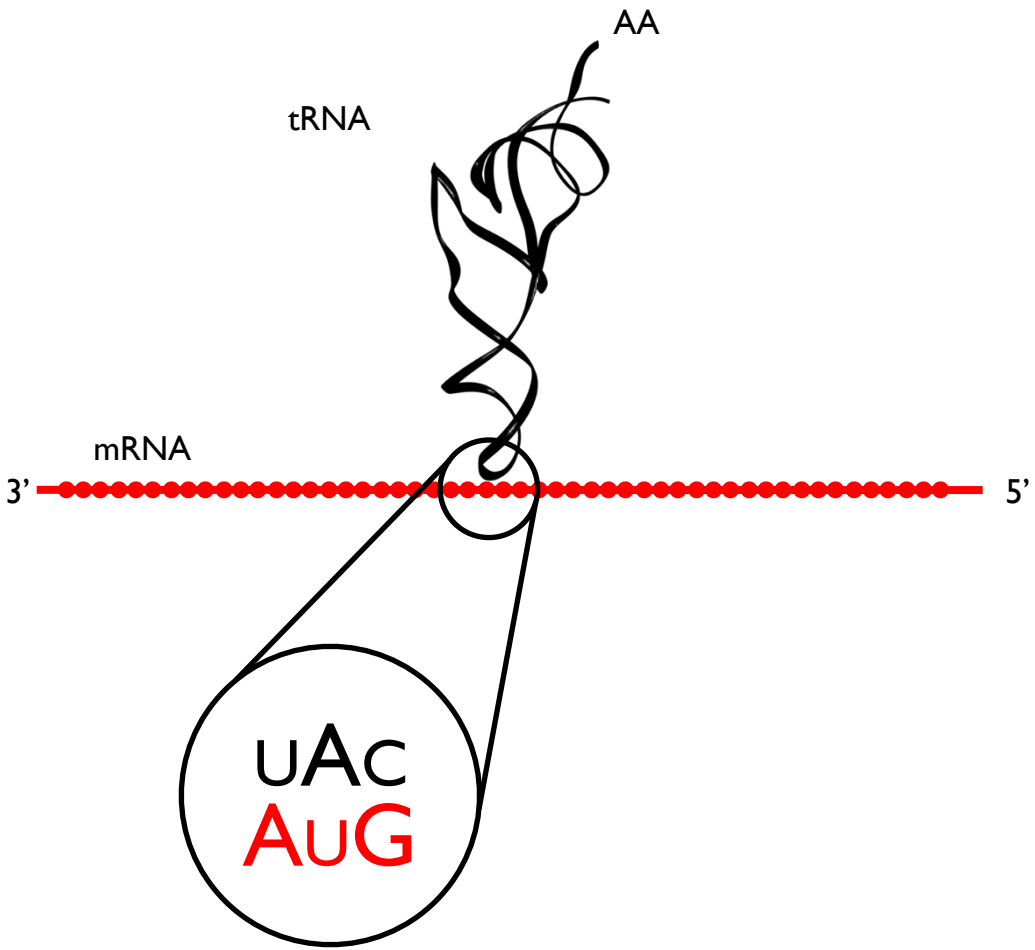




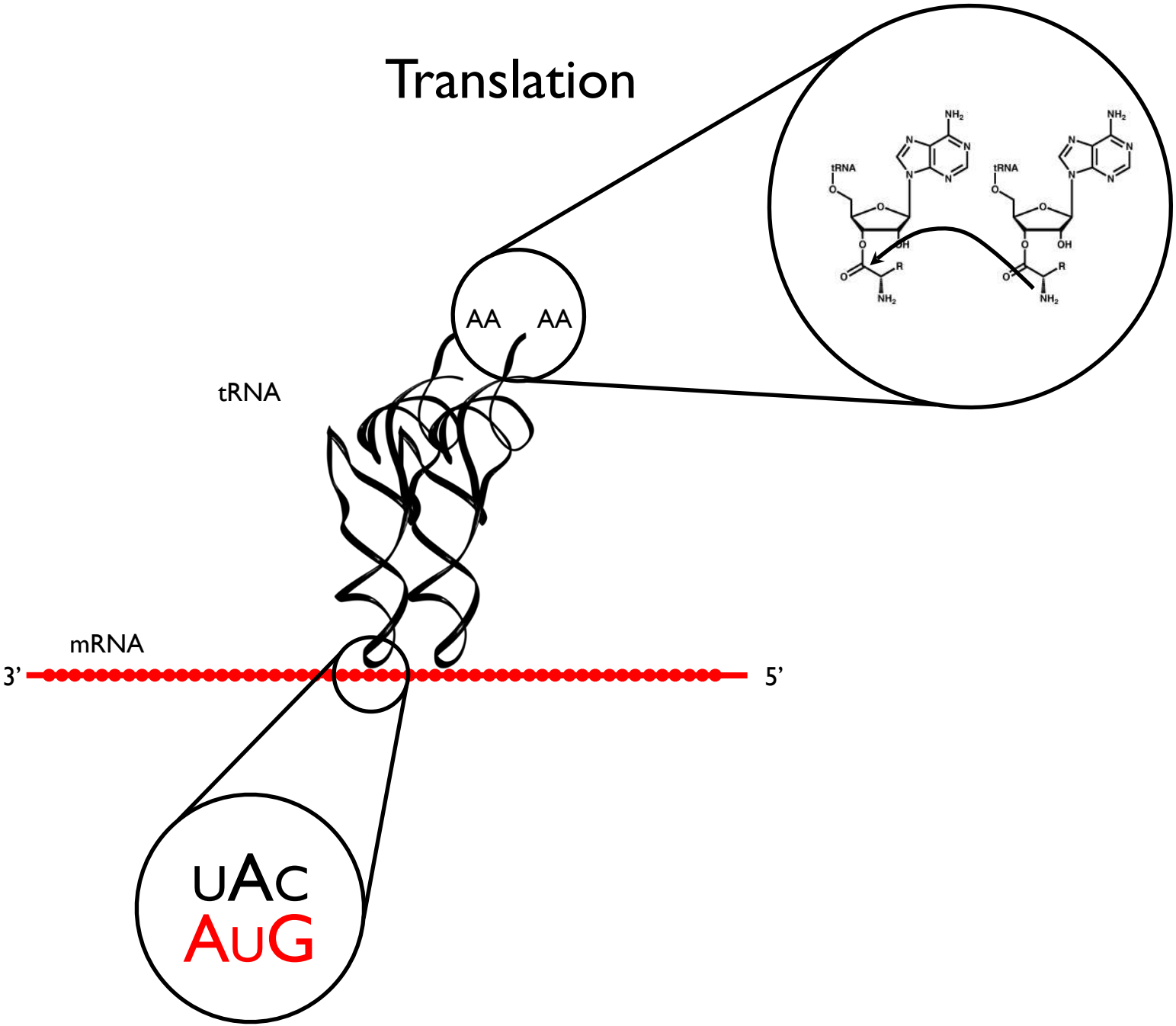
The ribosome



tRNA Links the Nucleotide and Amino Acid Codes



Translation



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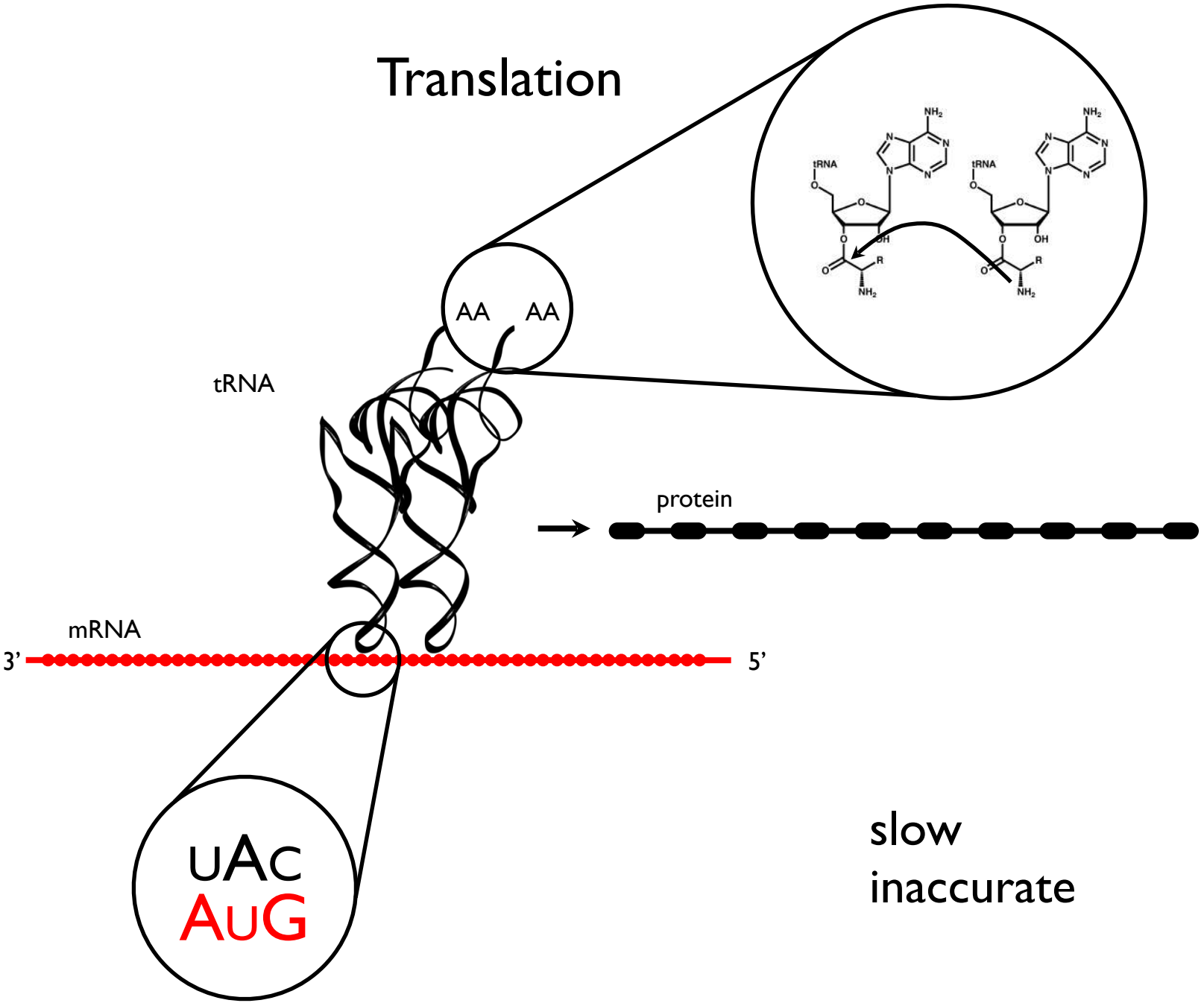

slow
inaccurate

Translation