Ribosomes: Machines that Synthesize Proteins

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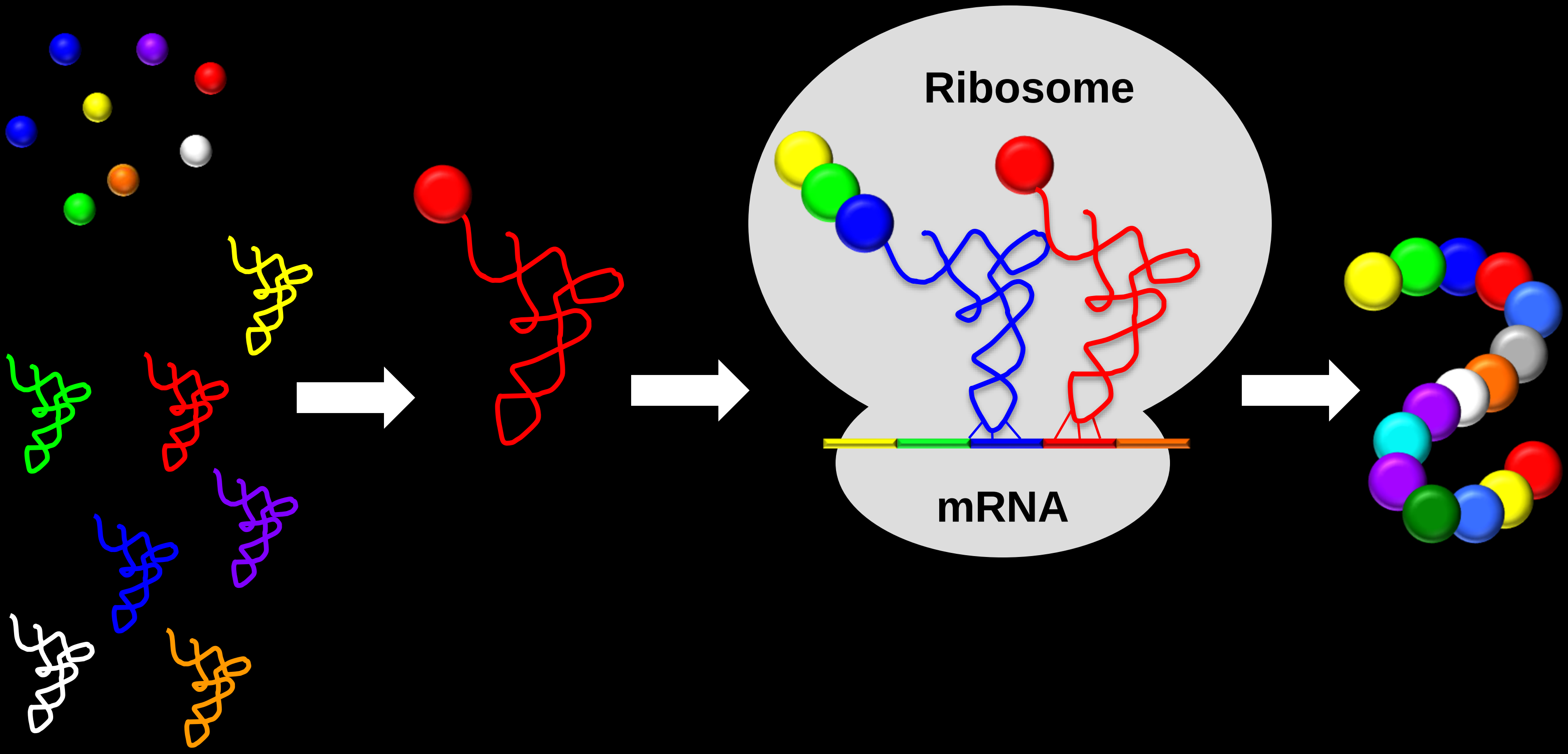
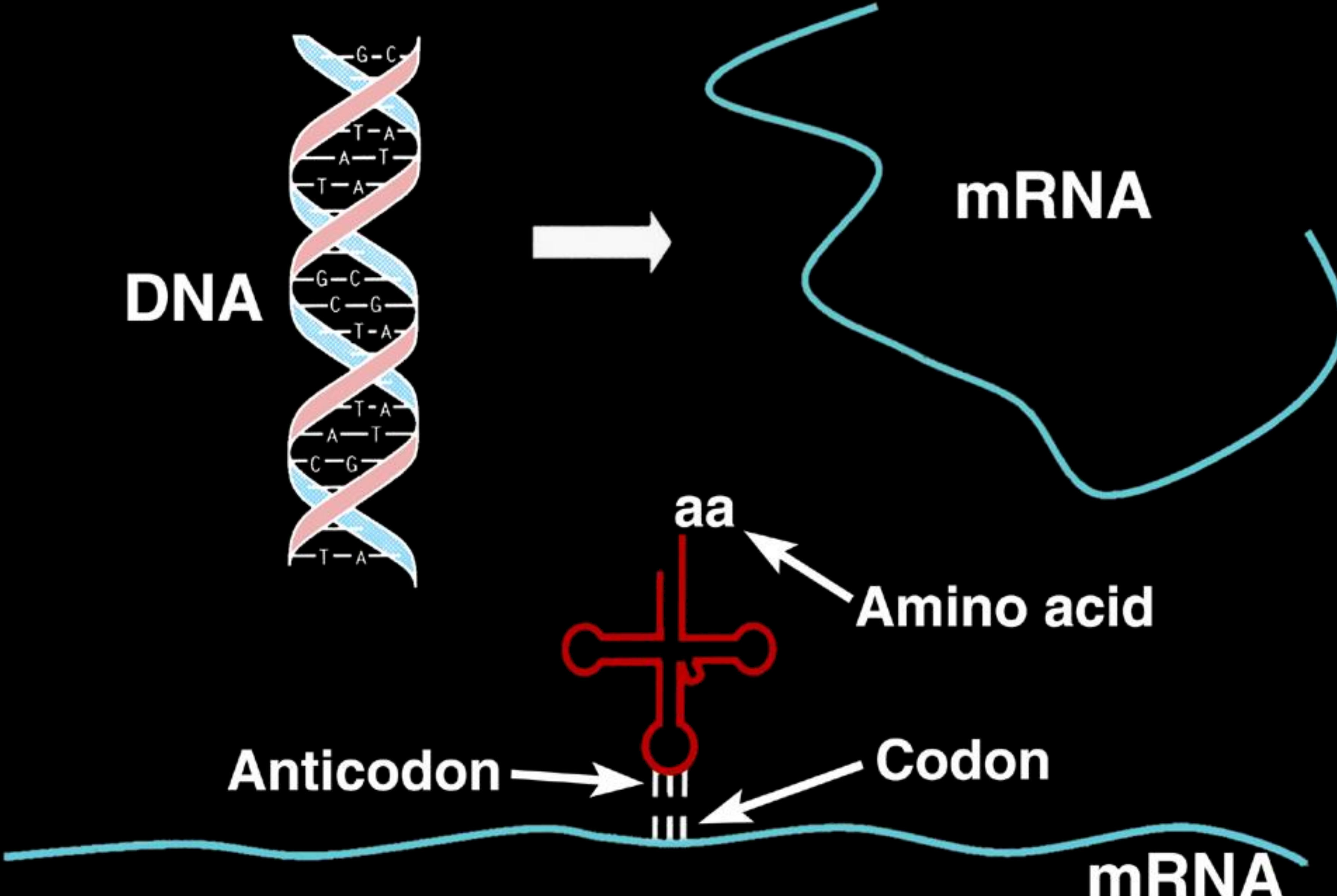
Protein Translation



Flow of Genetic Information



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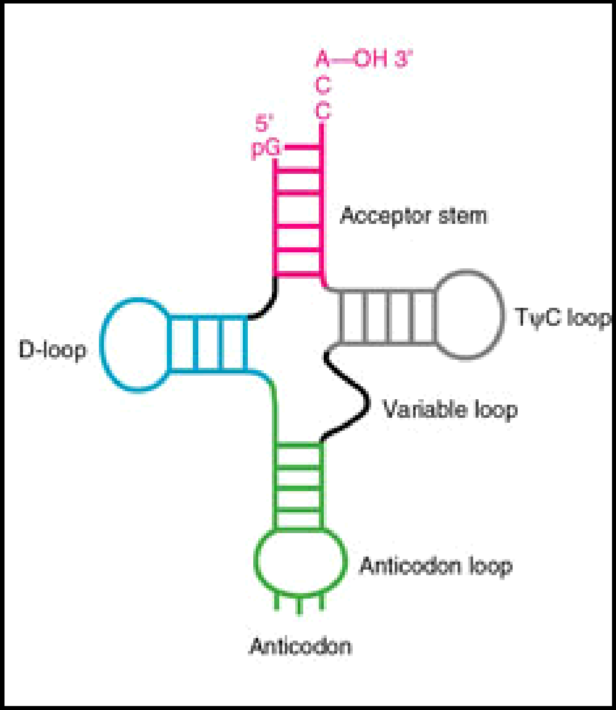
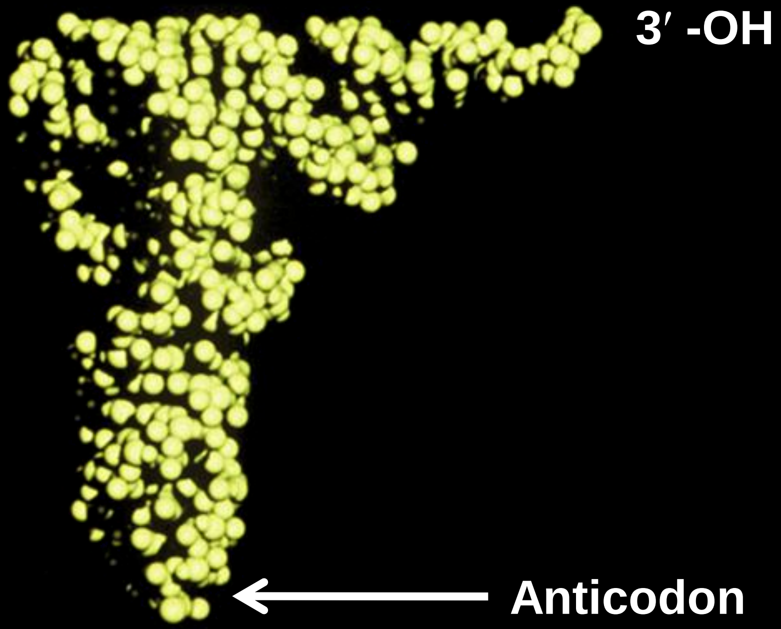
The Genetic Code

First Position (5')	Second Position: U	C	A	G	Third Position (3')
U	PHE	SER	TYR	CYS	U
	PHE	SER	TYR	CYS	C
	LEU	SER	Stop	Stop	A
	LEU	SER	Stop	TRP	G
C	LEU	PRO	HIS	ARG	U
	LEU	PRO	HIS	ARG	C
	LEU	PRO	GLN	ARG	A
	LEU	PRO	GLN	ARG	G
A	ILE	THR	ASN	SER	U
	ILE	THR	ASN	SER	C
	ILE	THR	LYS	ARG	A
	MET	THR	LYS	ARG	G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	GLY	A
	VAL	ALA	GLU	GLY	G

Singer & Berg

Transfer RNA





Secondary structure

Tertiary structure

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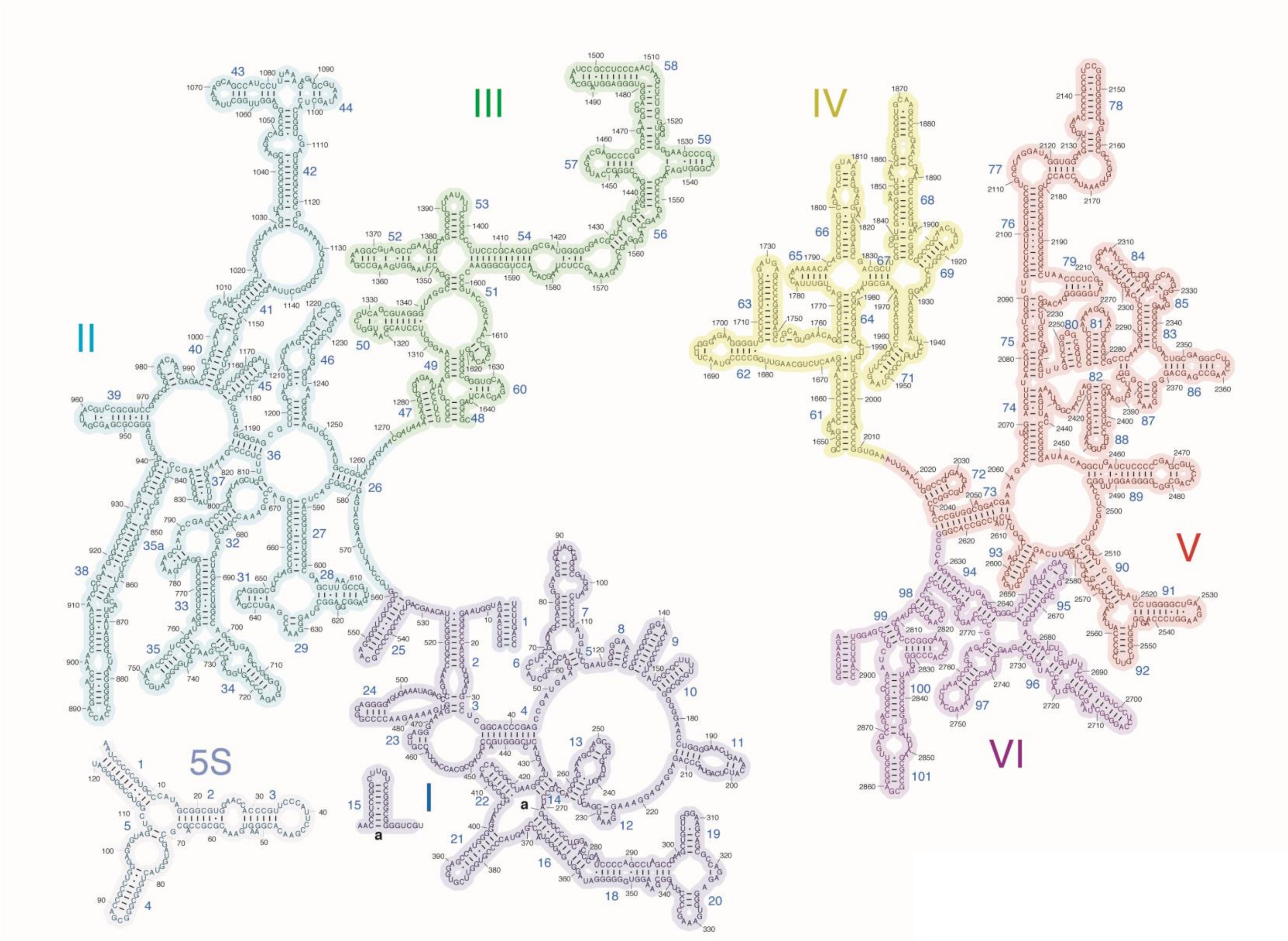
The ribosome is an extremely large molecular machine

Bacterial ribosomes are comprised of:

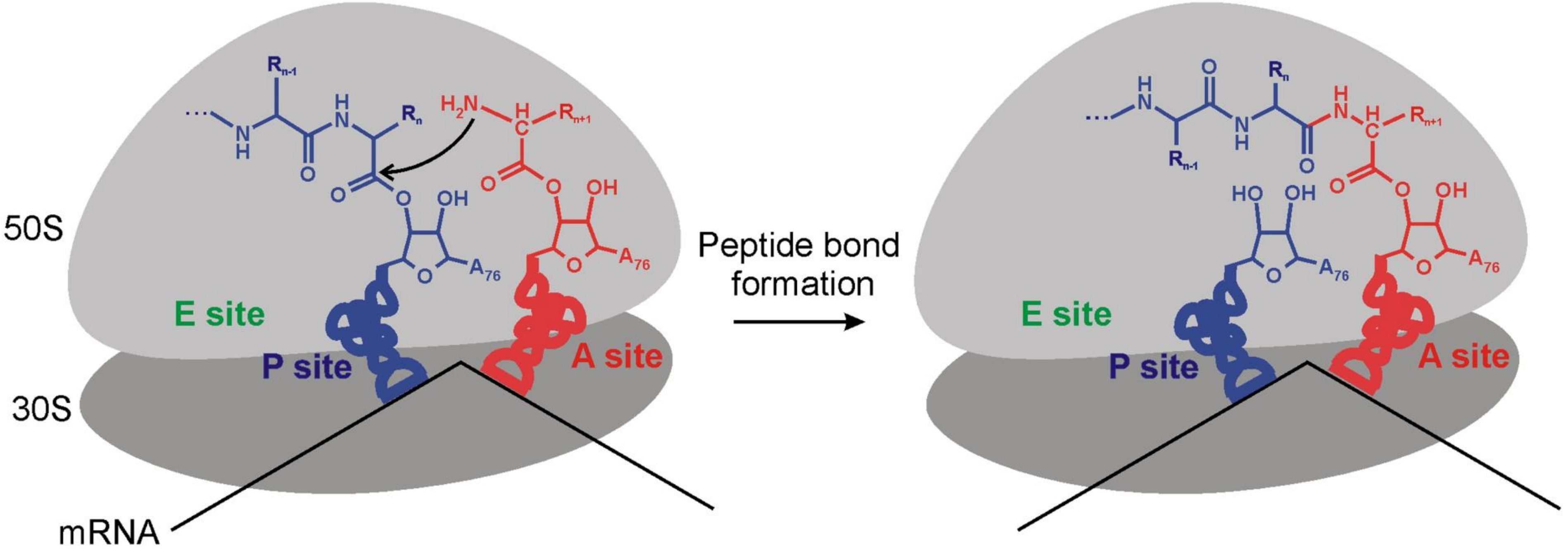
- 2 subunits: 50S large subunit & 30S small subunit
- 3 RNA molecules >4500 nucleotides in length
- >50 different proteins
- Molecular weight of 2.5 million daltons

Secondary structure of large subunit ribosomal RNA from *Thermus thermophilus*



Noller, H. http://rna.ucsc.edu/rnacenter

Peptidyl transferase reaction catalyzed by the ribosome



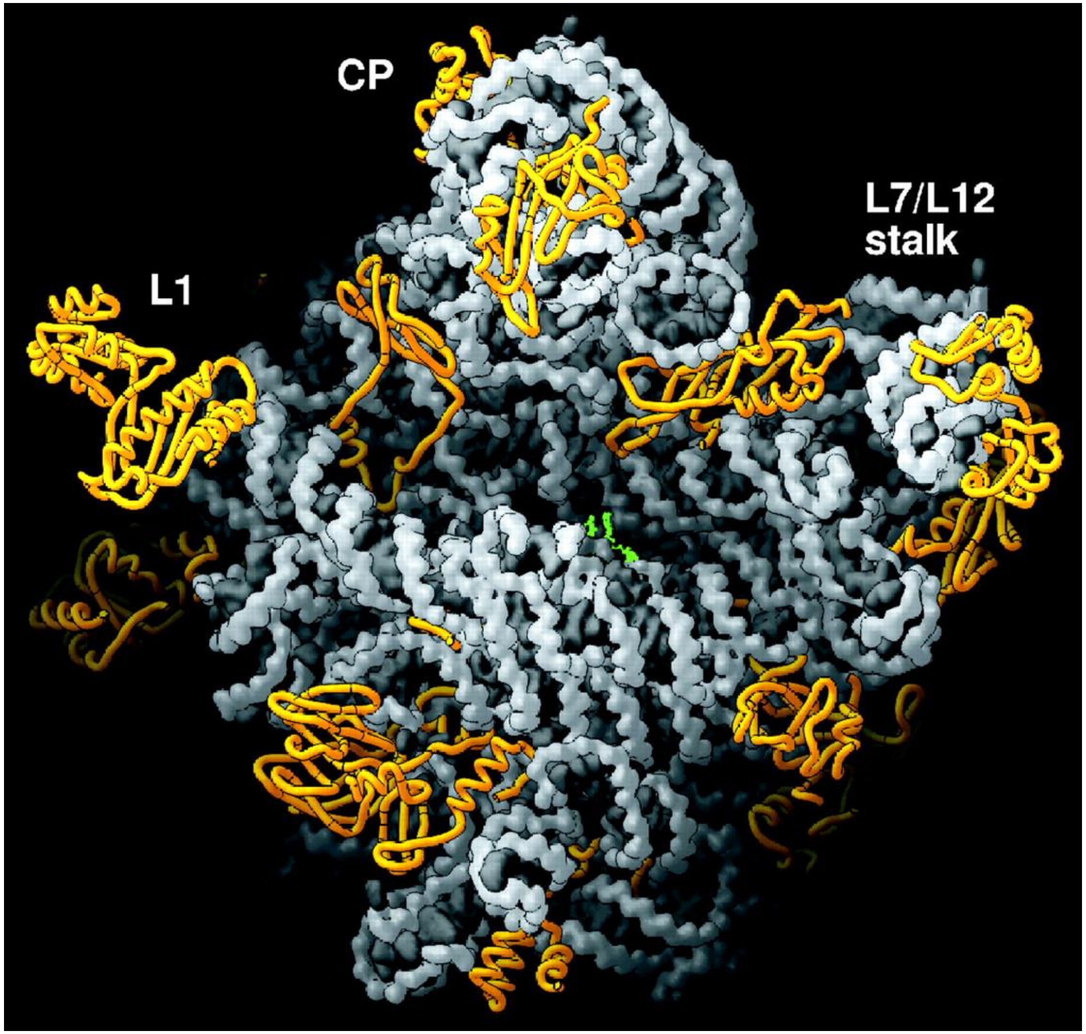
Beringer & Rodnina (2007). *Mol. Cell.* 26: 311-321

Biophysical techniques used to study the ribosome

- X-ray crystallography
- Cryo-electron microscopy
- smFRET

If the ribosome requires proteins to function, where did the proteins come from to make the first ribosome?

The ribosome is a ribozyme

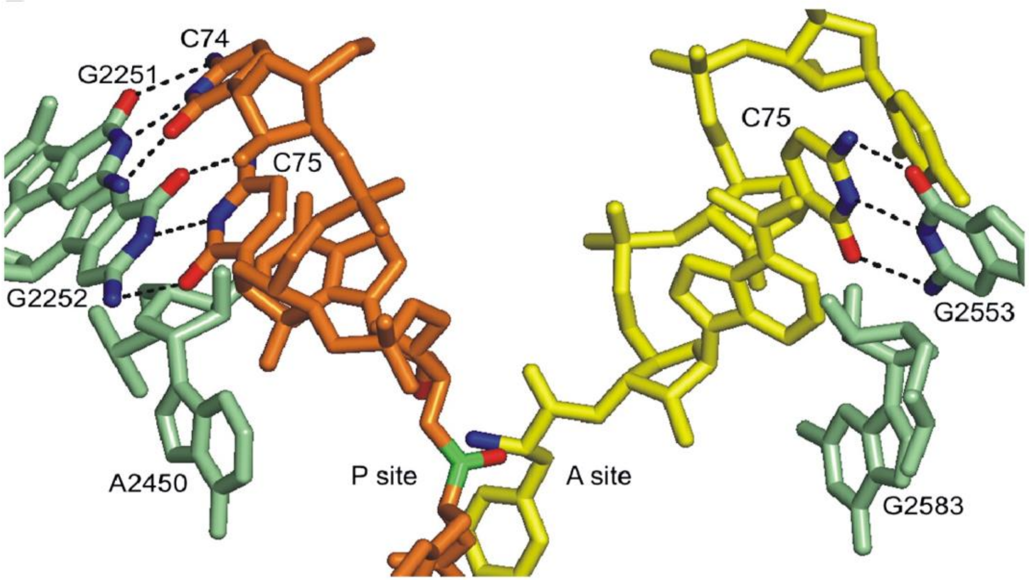


The *H. marismortui* large ribosomal subunit

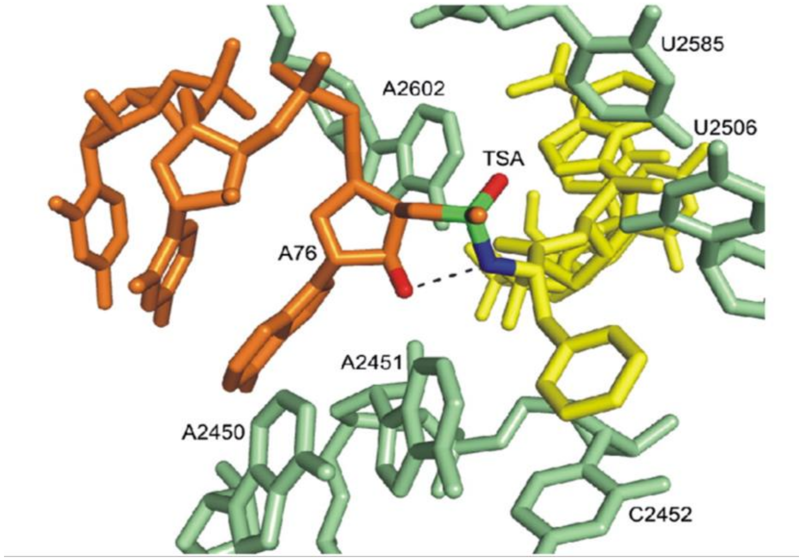
N Ban et al. Science 2000;289:905-920

Structure of the peptidyl transferase center in the 50S subunit of the ribosome

Peptidyl- & aminoacyl-tRNA substrate analogs

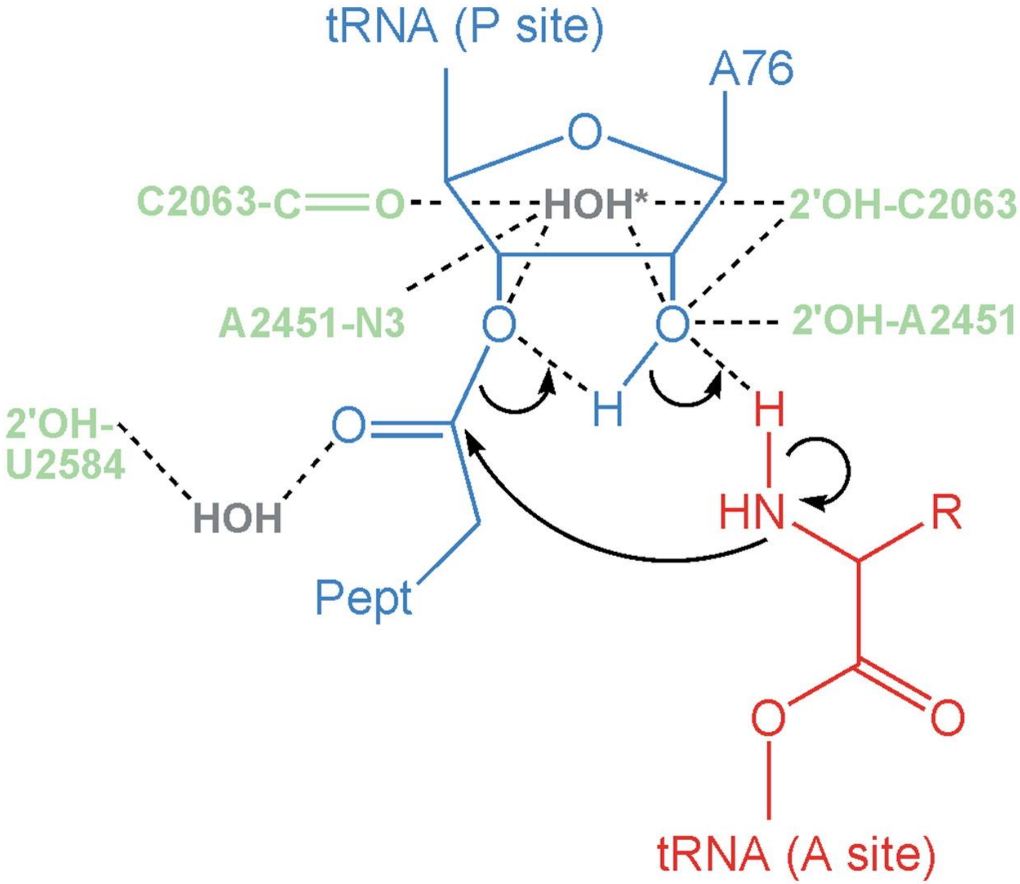


Transition state analog



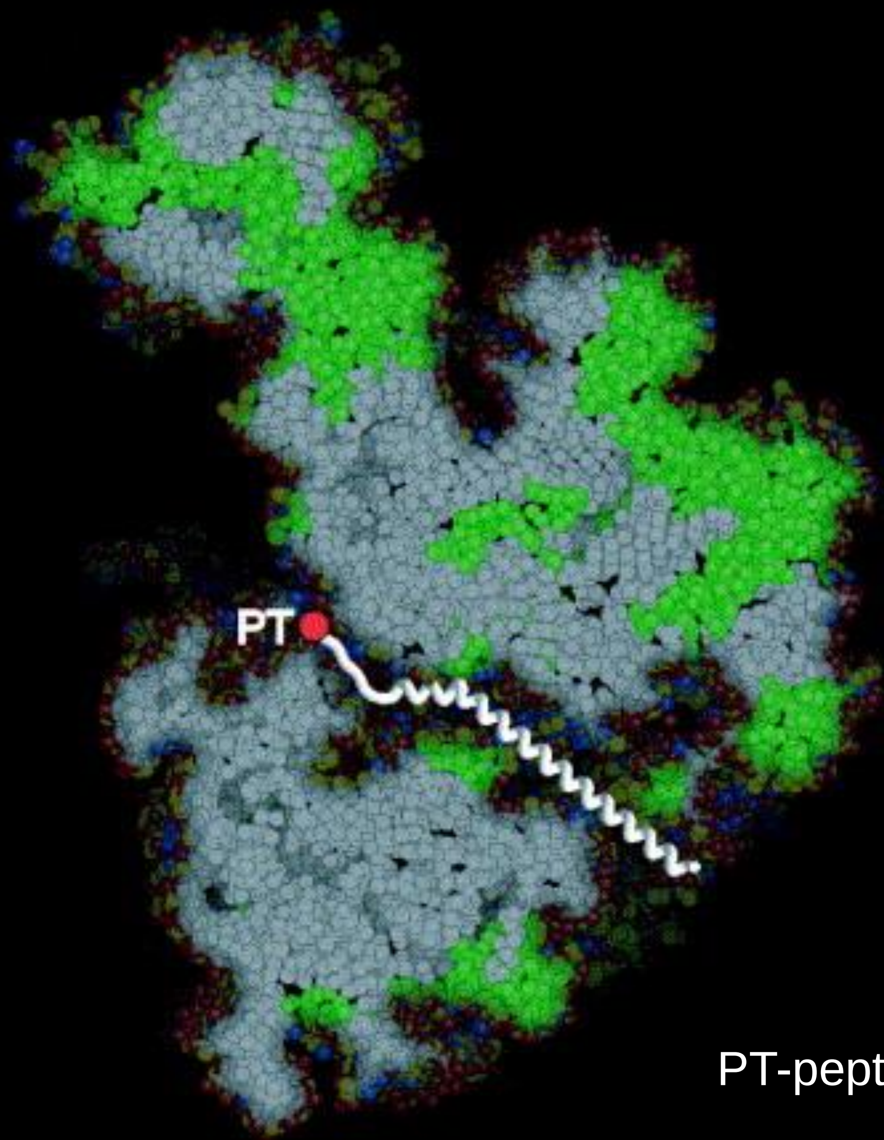
Beringer & Rodnina (2007). *Mol. Cell.* 26: 311-321

Proposed proton shuttling mechanism for the peptidyl transferase reaction



Beringer & Rodnina (2007). *Mol. Cell.* 26: 311-321

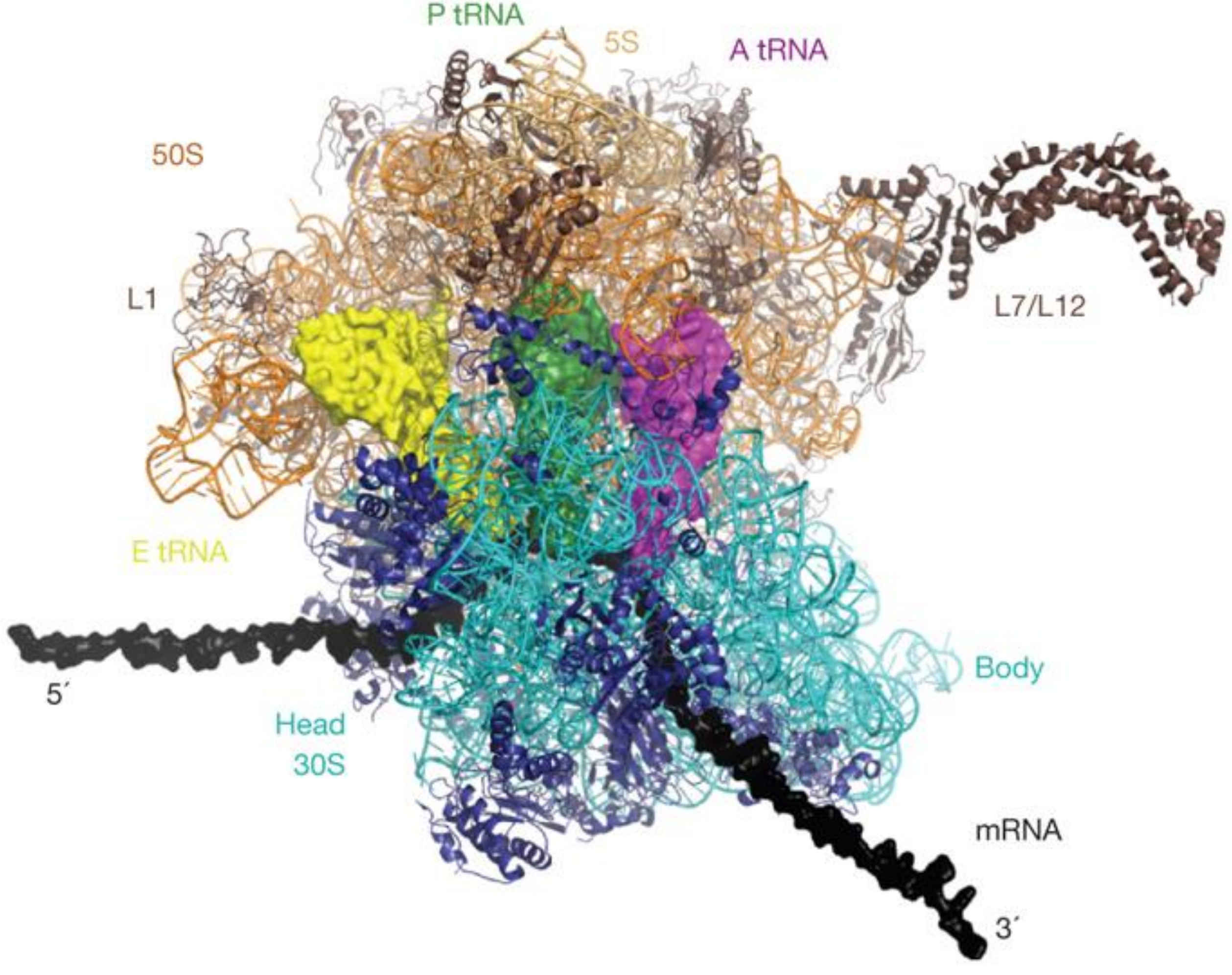
Polypeptide tunnel



PT-peptidyl transferase center

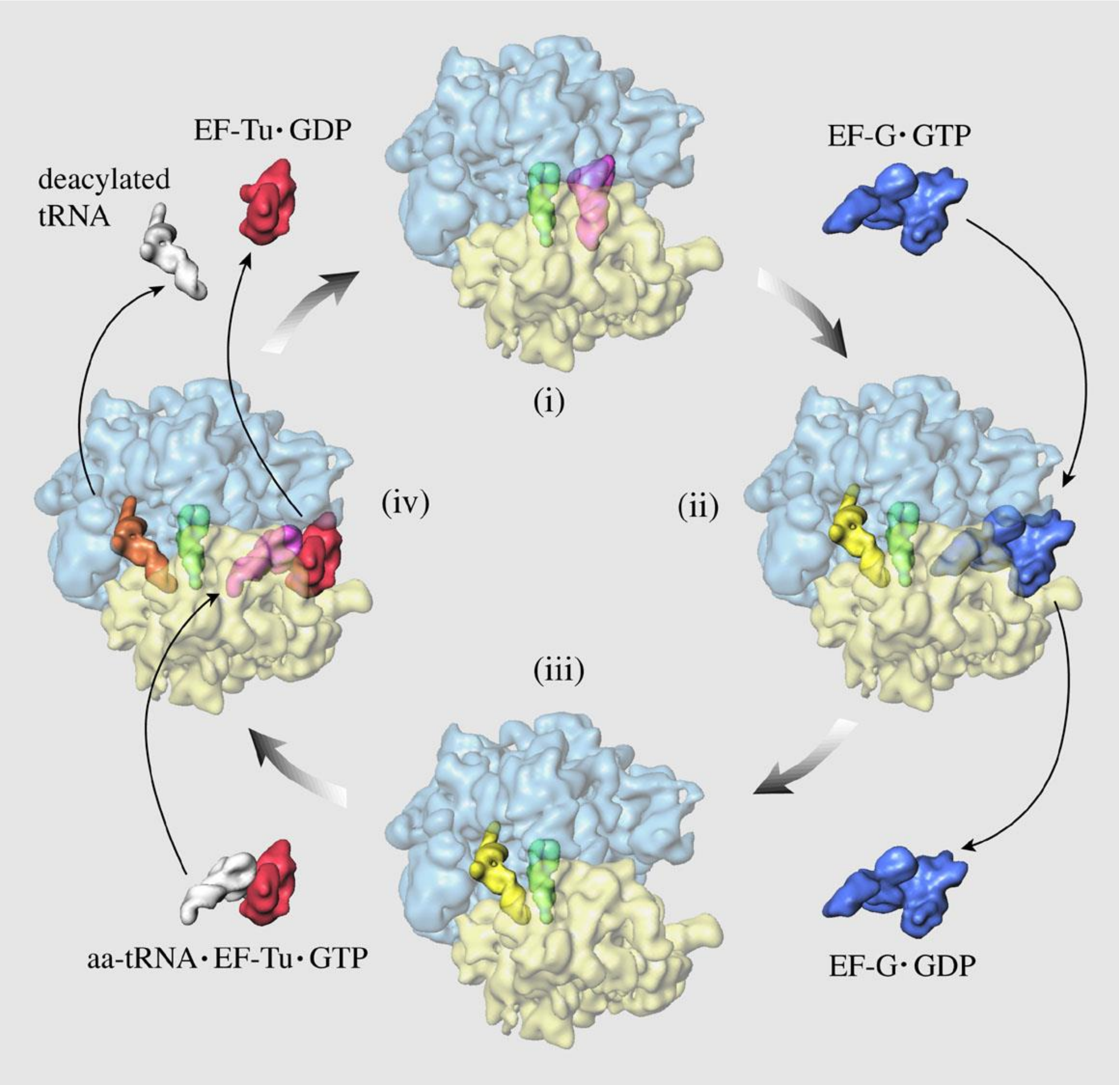
Mankin, AS (2006) TIBS Vol 31(1):11-13

Complete structure of the ribosome



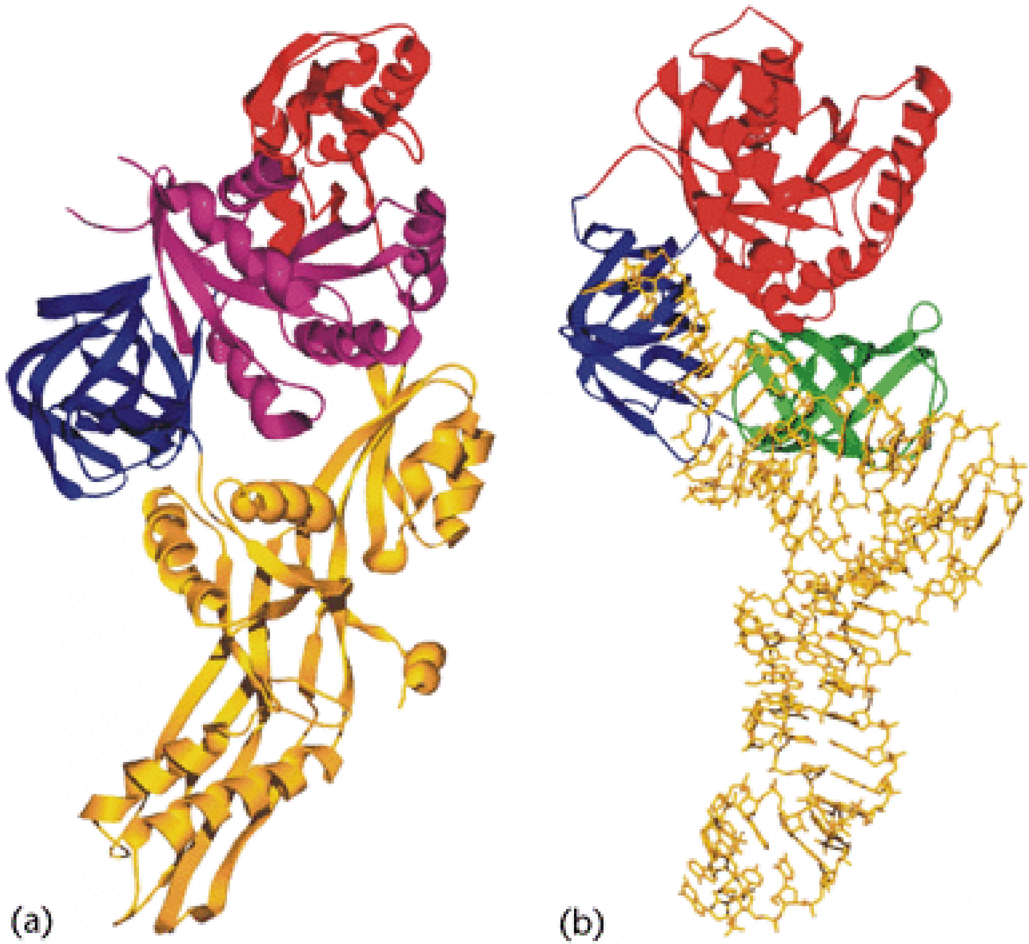
TM Schmeing & V Ramakrishnan (2009) *Nature* **461**, 1234-42

Translation elongation cycle



Frank, J. http://www.columbia.edu/cu/franklab/index.html

Molecular mimicry by Elongation factors

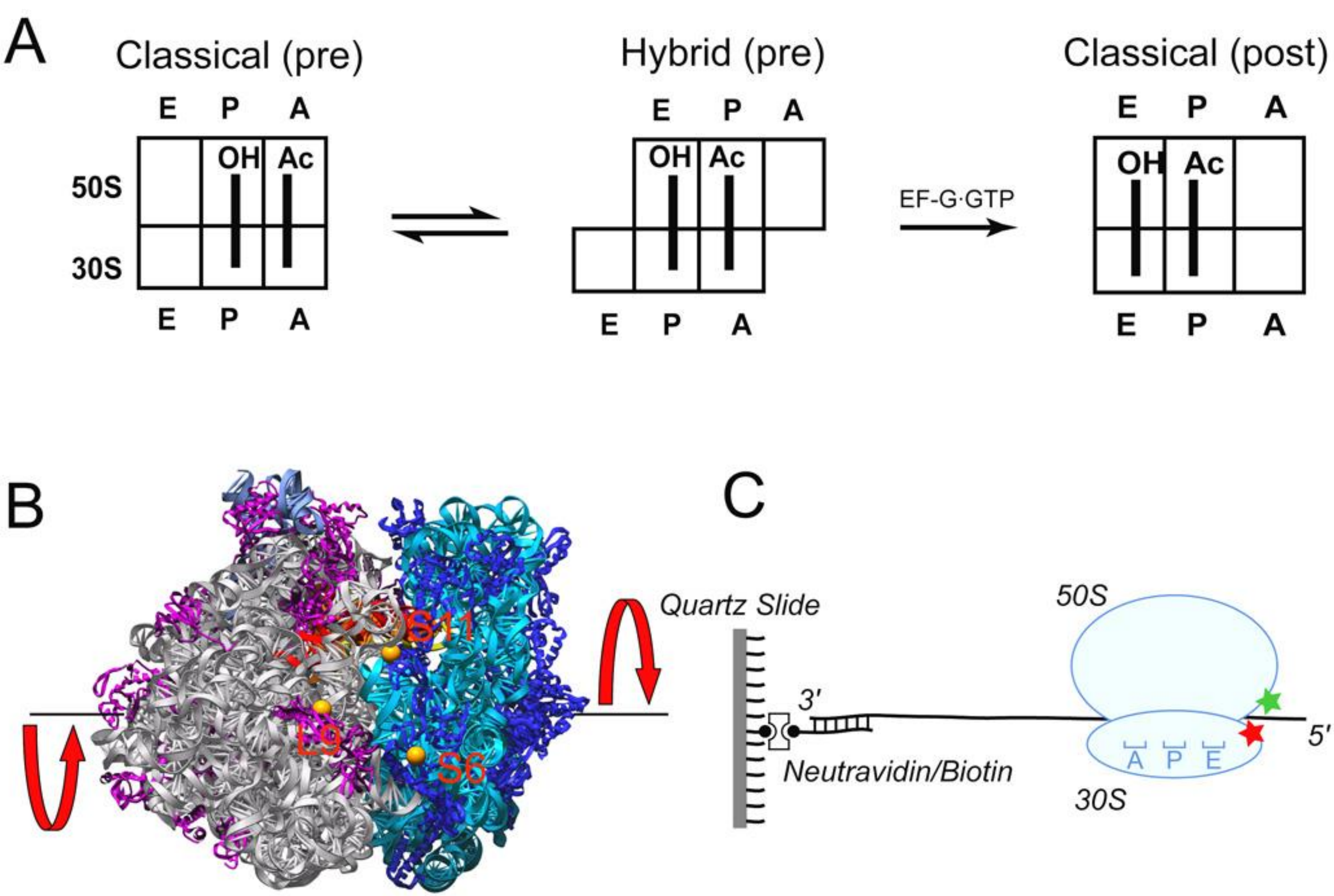


EF-G

EF-TU•GTP*
+ aminoacyl-tRNA

Neirhaus, K. & Wilson D (2005) Enc. of Life Sciences:1-10

Ribosome Translocation- a ratchet mechanism involving EF-G



Cornish, P et al., (2008) *Mol. Cell.* 30(5): 578-588

The Nobel Prize in Chemistry 2009

“*for studies of the structure and function of the ribosome*”.

Venki Ramakrishnan

Tom Steitz

Ada Yonath

Nobelprize.org. 2 Jun 2011 http://nobelprize.org/nobel_prizes/chemistry/laureates/2009/

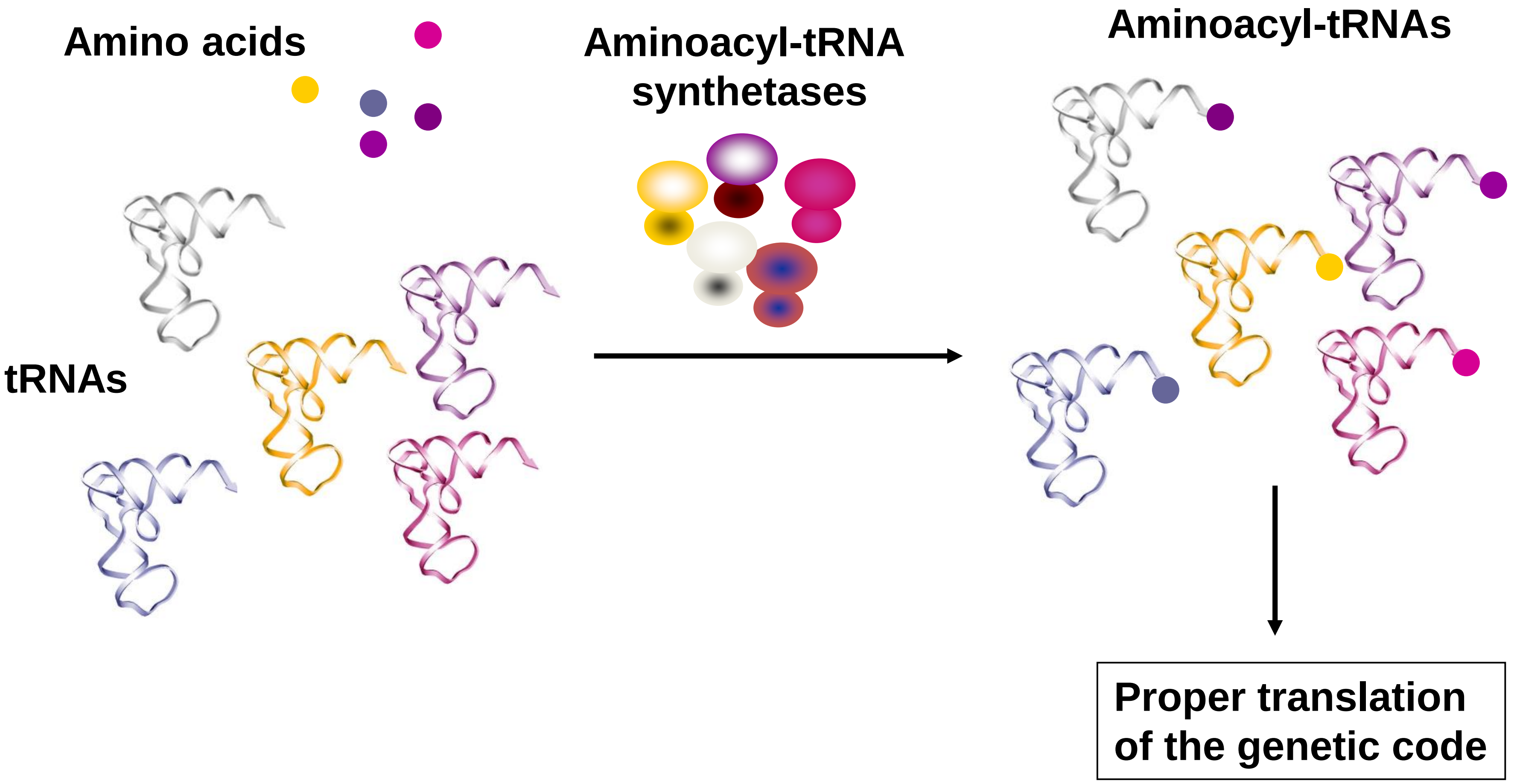
Big questions in protein translation

What is the origin of the ribosome ?

How did the genetic code evolve ?

Which amino acids came first in proteins ?

Role of Aminoacyl-tRNA Synthetases in Protein Biosynthesis



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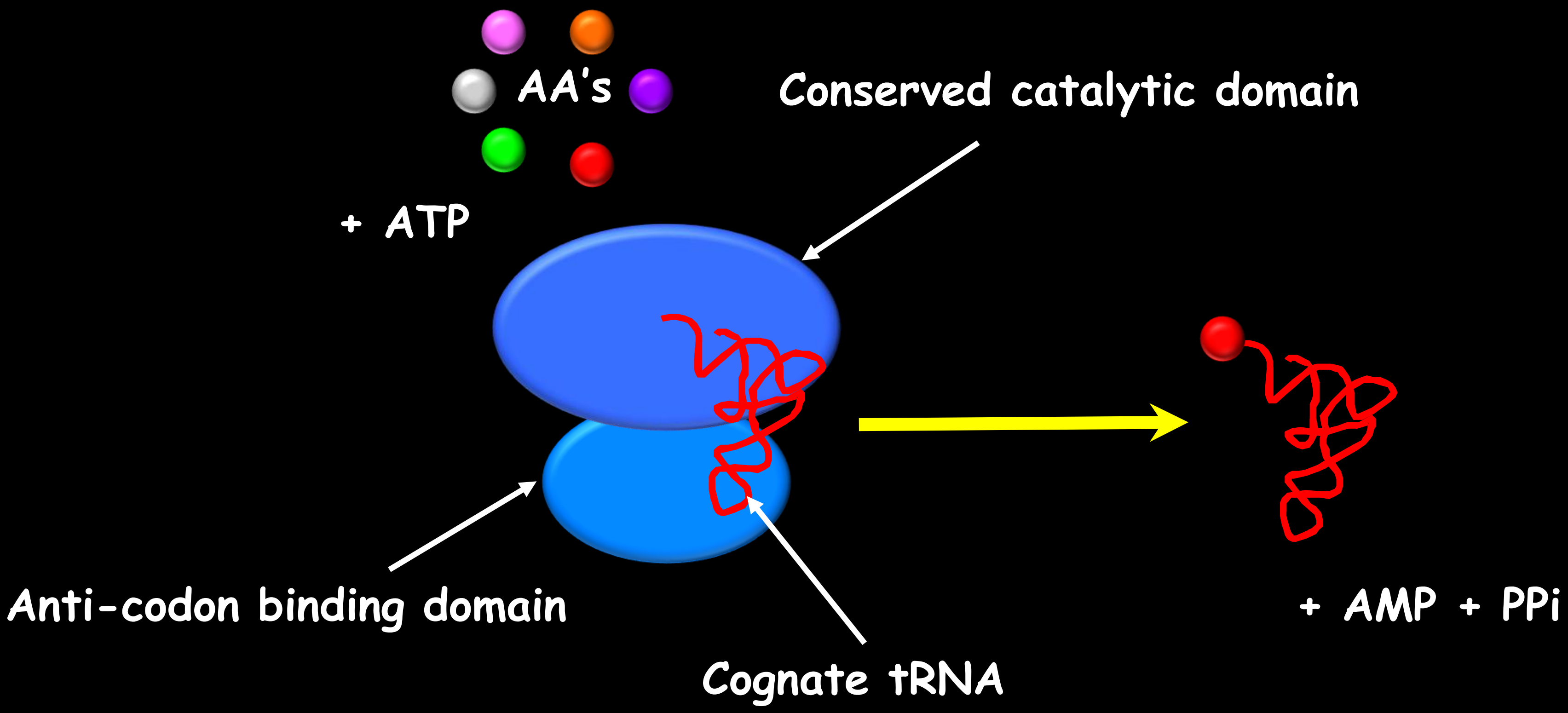
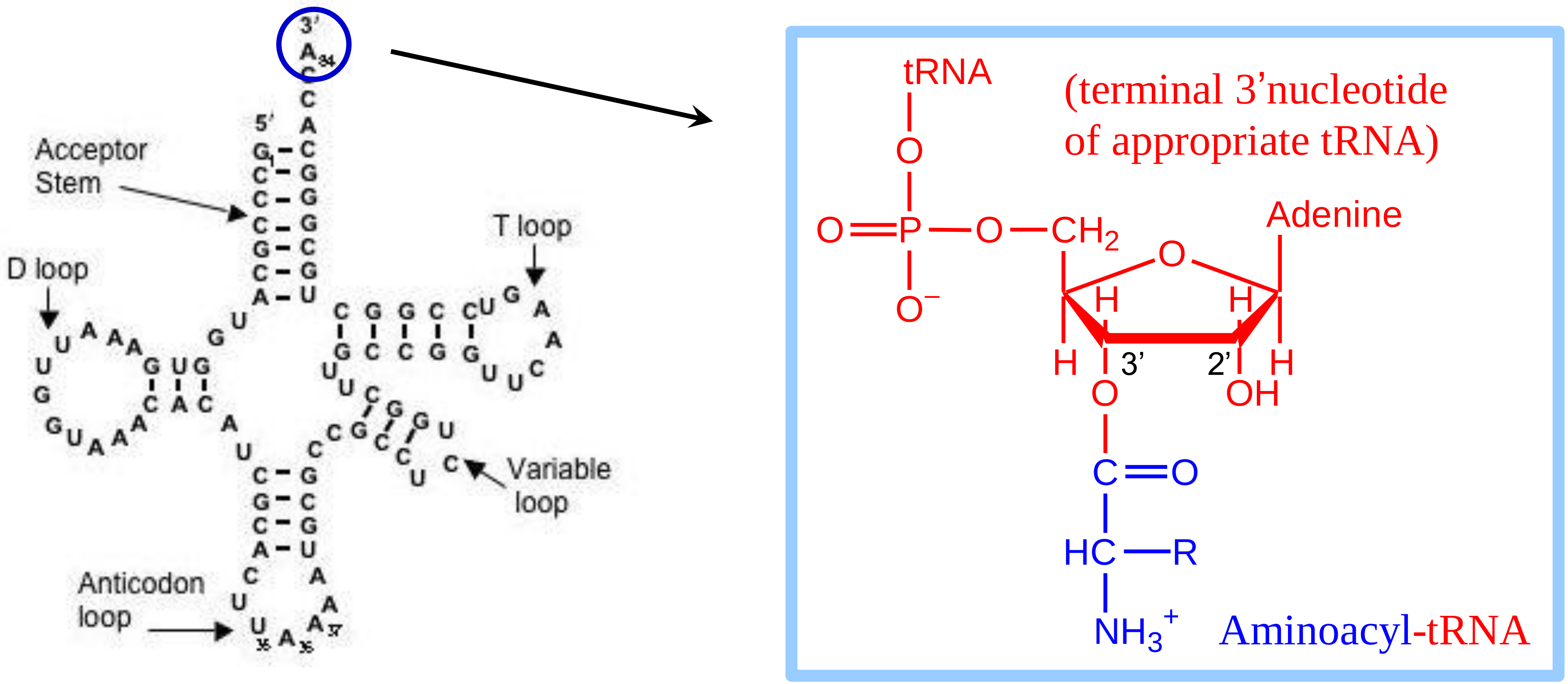
The 20 aminoacyl-tRNA synthetases all share a common mechanism



(1) E + AA + ATP $\leftrightarrow$ E(AA-AMP) + PPi

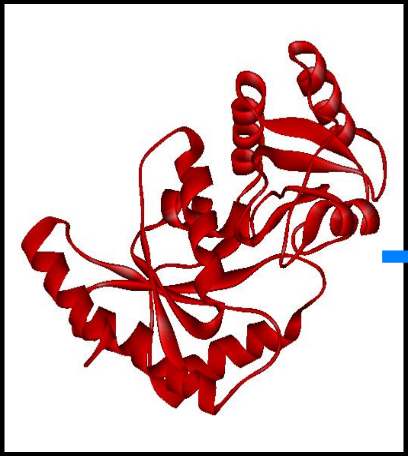
(2) E(AA-AMP) + tRNA $\leftrightarrow$ E + AA-tRNA + AMP

Reaction Catalyzed by AARSs

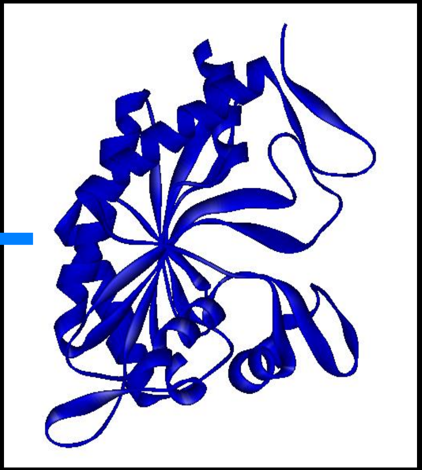


Aminoacyl-tRNA synthetases enzymes can be divided into 2 different classes

Class I	Class II
MetRS	SerRS
ValRS	ThrRS
LeuRS	AlaRS
IleRS	GlyRS
CysRS	ProRS
ArgRS	HisRS
GluRS	AspRS
GlnRS	AsnRS
TyrRS	LysRS
TrpRS	PheRS

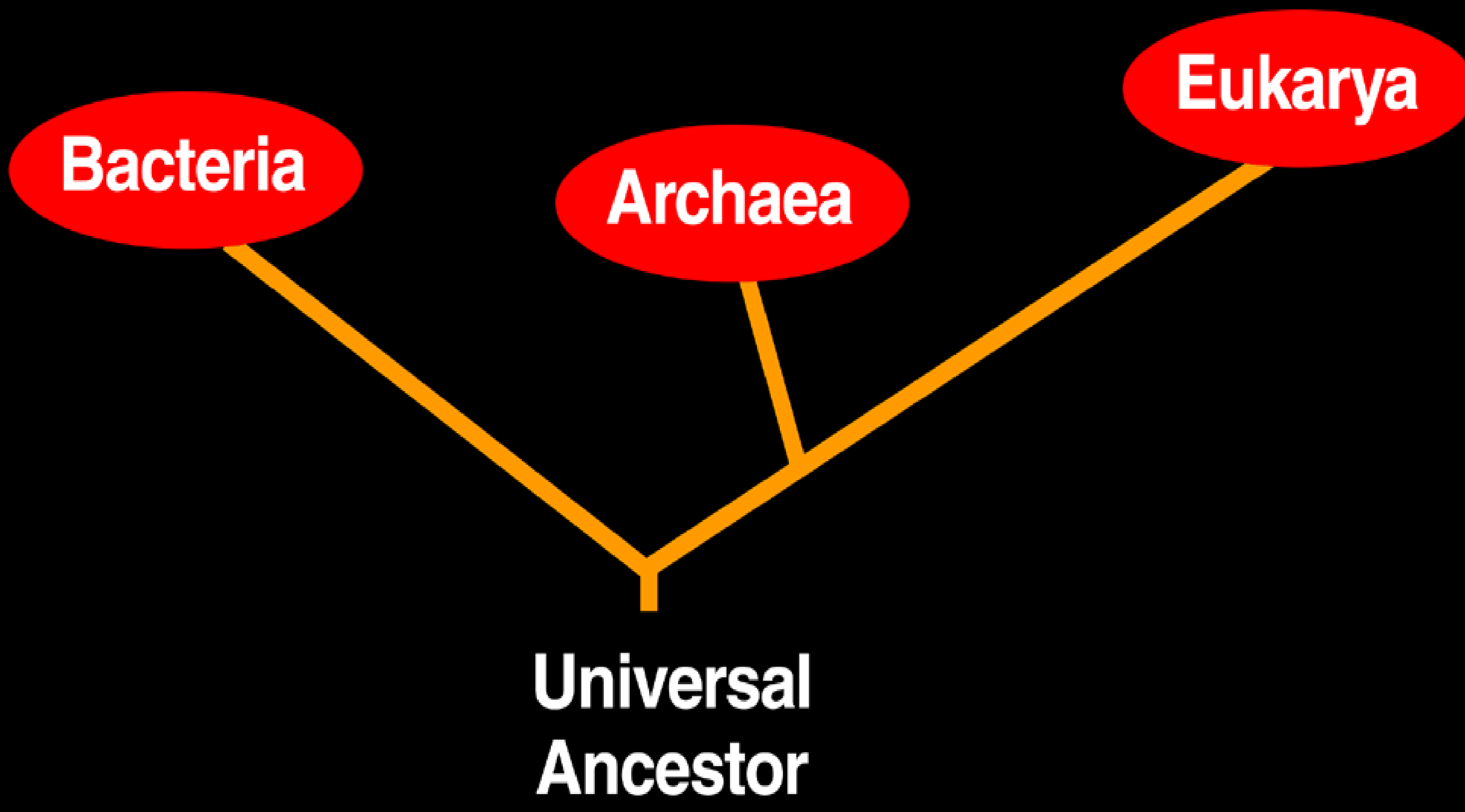


Aminoacylates tRNA 2′ OH

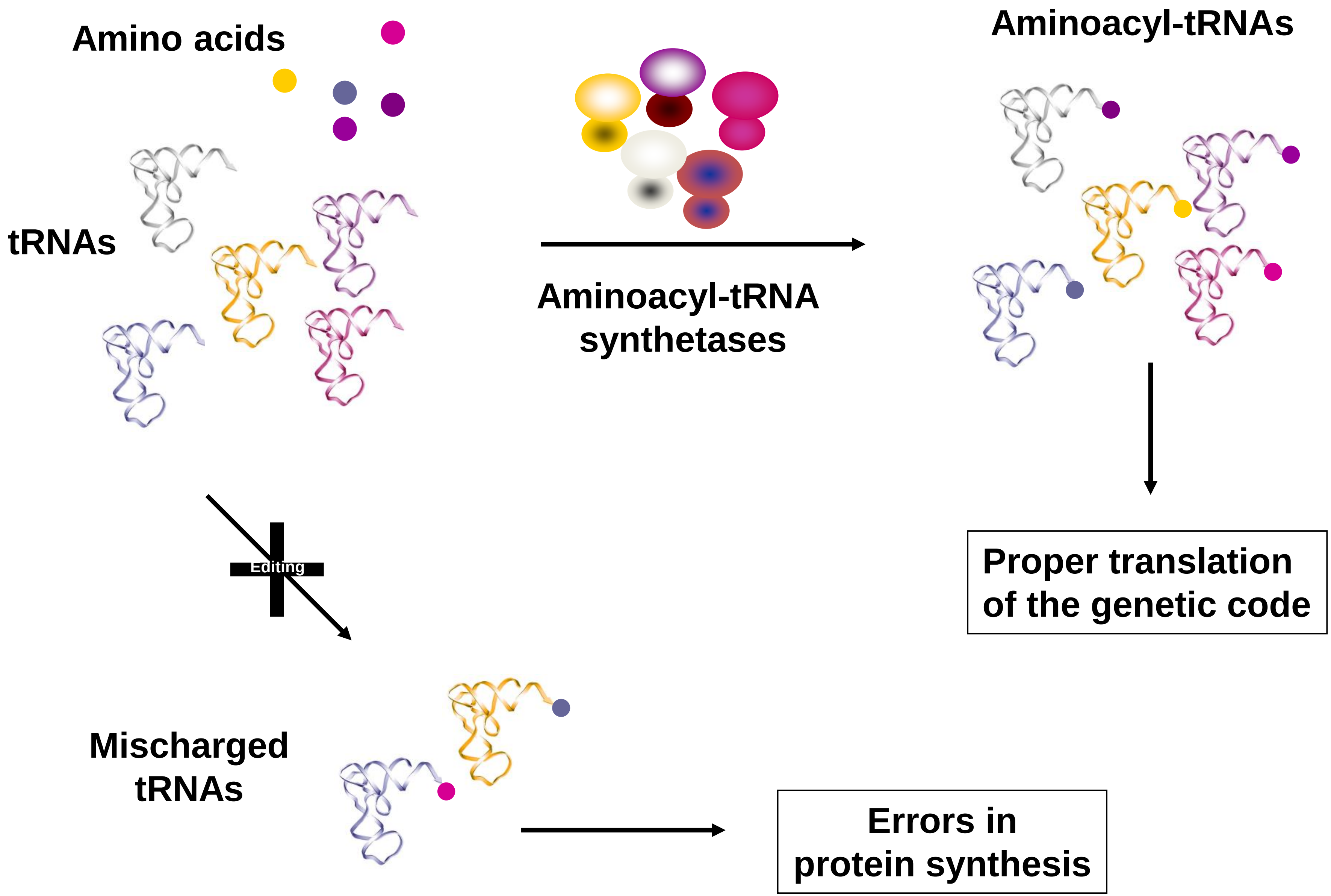


Aminoacylates tRNA 3′ OH

Ribas de Pouplana & Schimmel (2001)


Bacteria
Archaea
Eukarya
Universal
Ancestor

Role of Aminoacyl-tRNA Synthetases in Protein Biosynthesis



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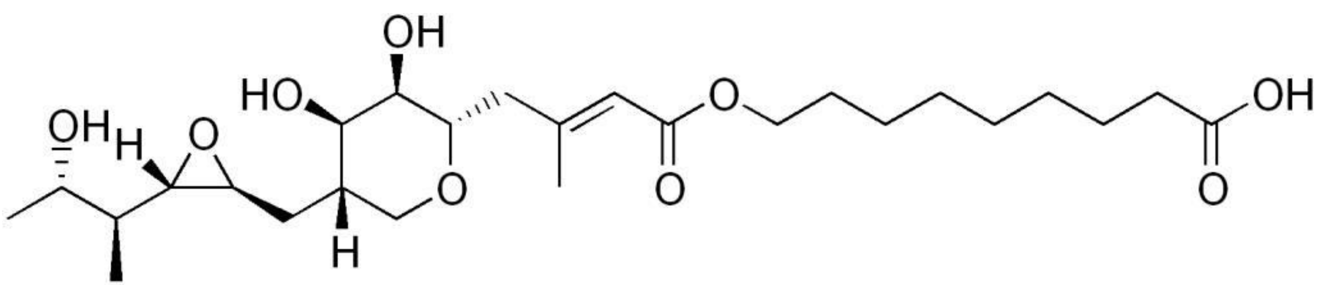
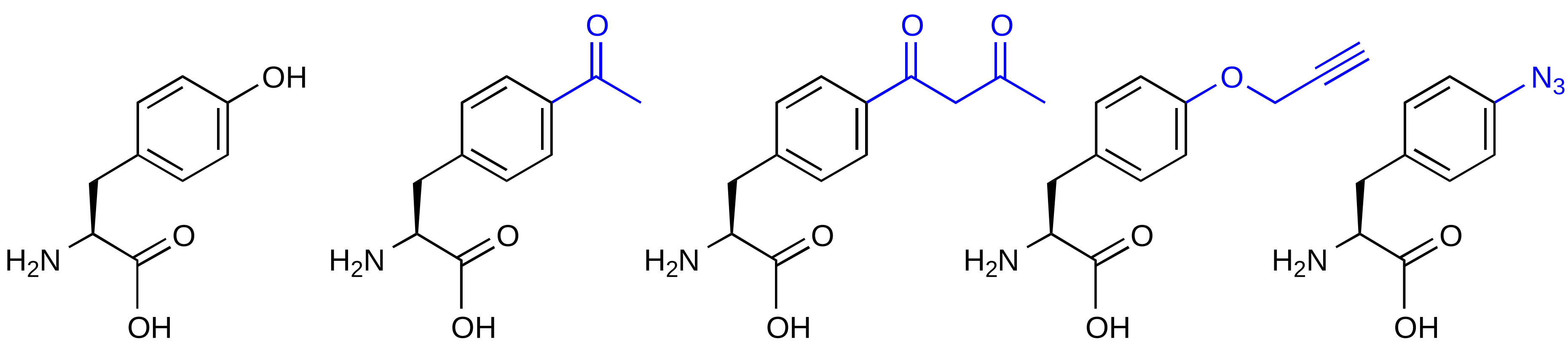
Protein Translation Studies: practical applications

- Development of Antibiotics



- Expanding the genetic code



P.G. Schultz, TSRI

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ARTICLE

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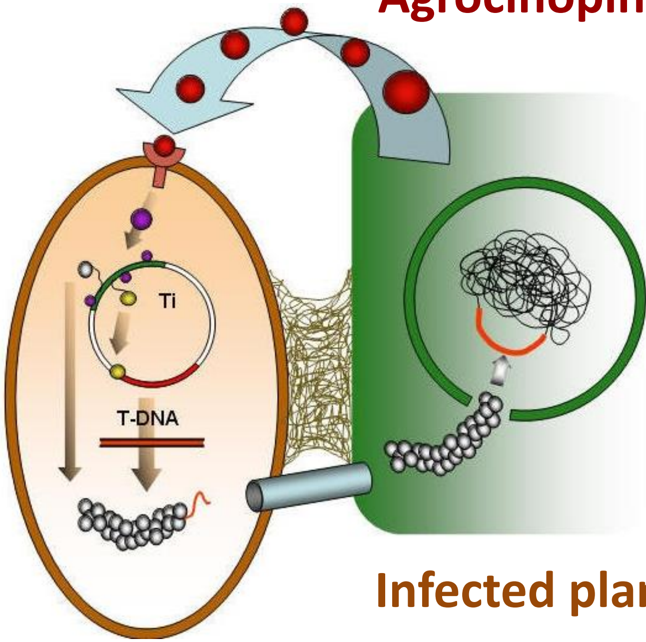
Plant tumour biocontrol agent employs a tRNA-dependent mechanism to inhibit leucyl-tRNA synthetase

Shaileja Chopra[1,*], Andrés Palencia[2,*], Cornelia Virus[1], Ashutosh Tripathy[3], Brenda R. Temple[4], Adrian Velazquez-Campoy[5], Stephen Cusack[2] & John S. Reader[1]

Pathogenic *Agrobacterium tumefaciens* causes crown gall tumors in plants

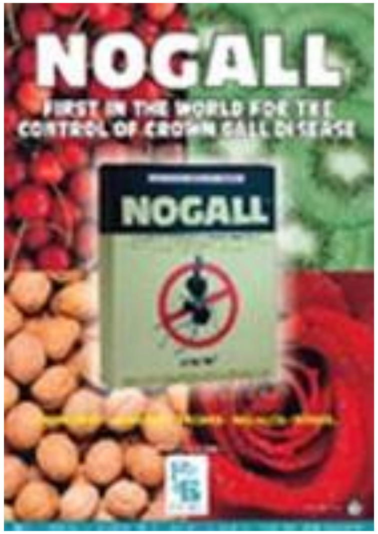
Agrocinopine



Infected plant cell

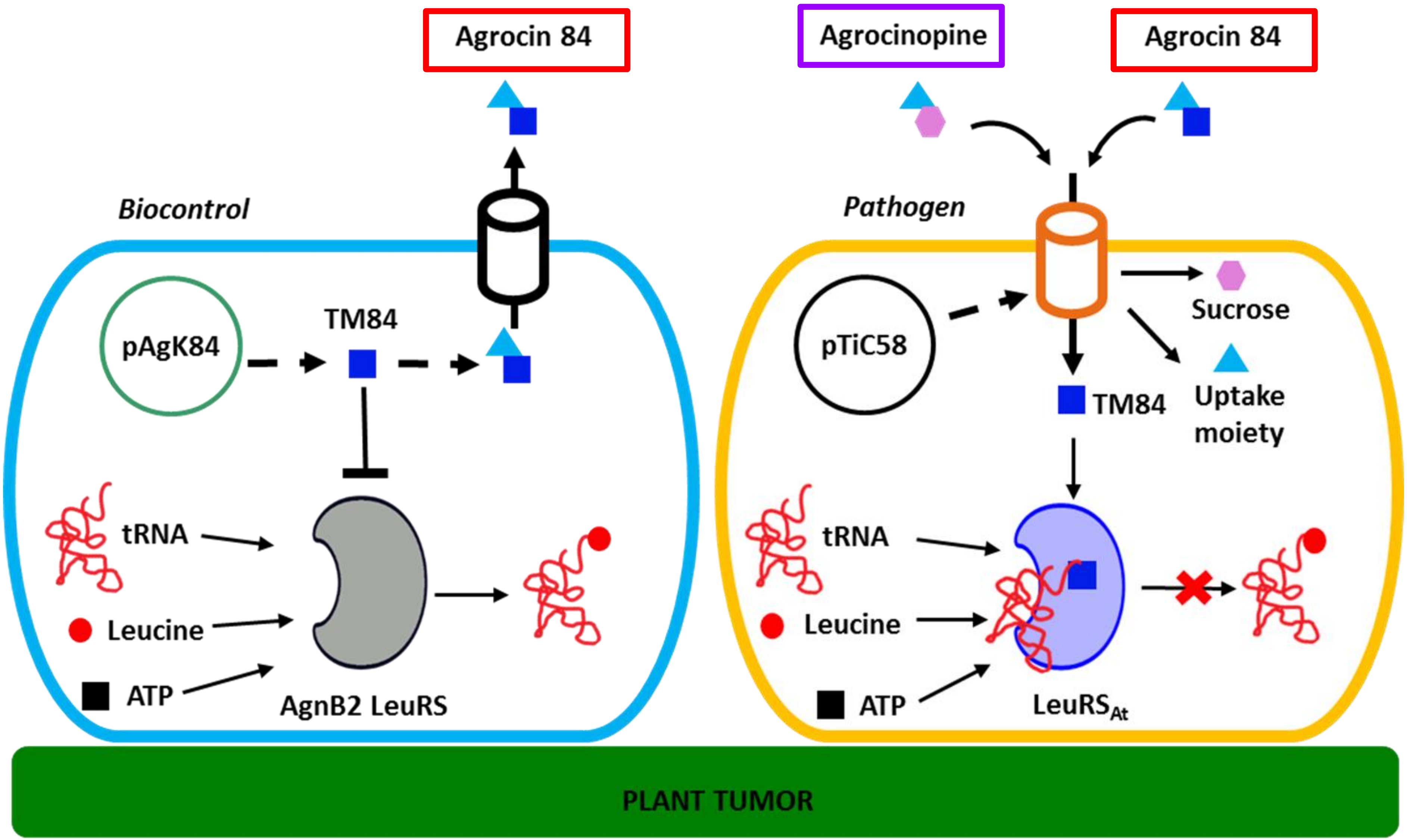
Mullins et al. 2001

Pathogenic
Agrobacterium tumefaciens

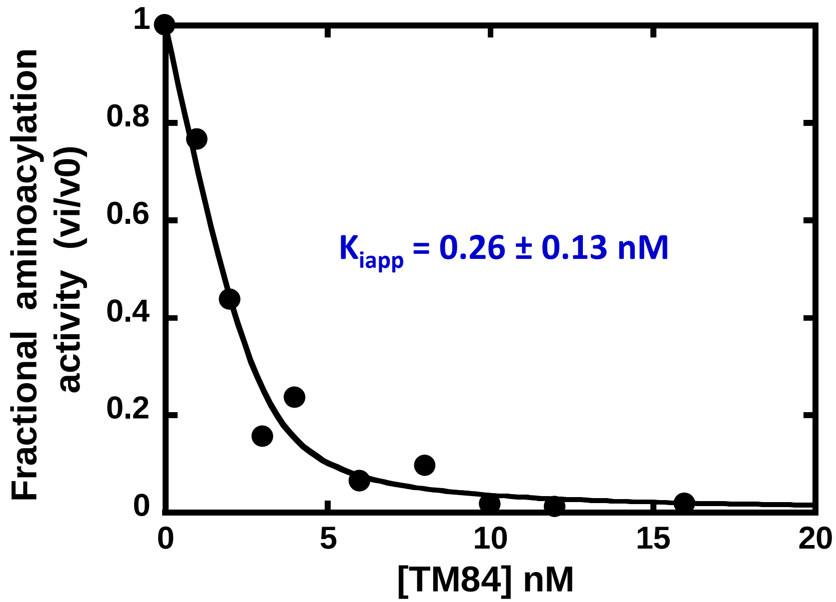


***Agrobacterium radiobacter* (Biocontrol)**

A. radiobacter (plant biocontrol agent) produces Agrocin 84 to compete with *A. tumefaciens* (pathogen)



TM84 is a potent inhibitor of leucyl tRNA synthetases (LeuRSs)



1) Aminoacyl adenylate formation:

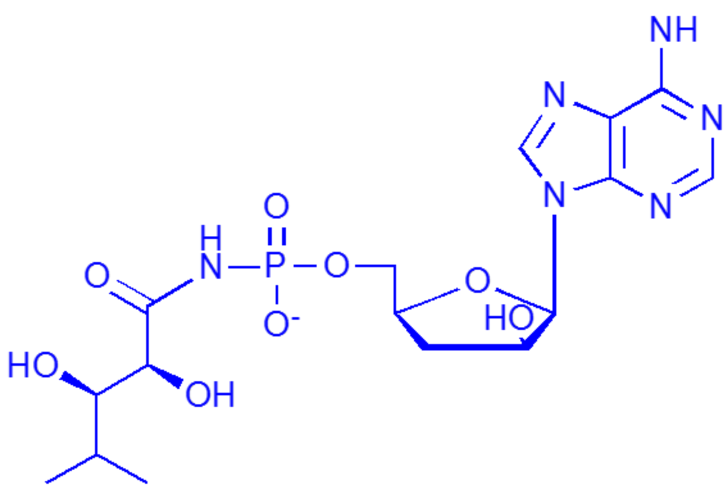
LeuRS + Leu + ATP ⟷ LeuRS (Leu-AMP) + PPi

LeuRS Reaction:

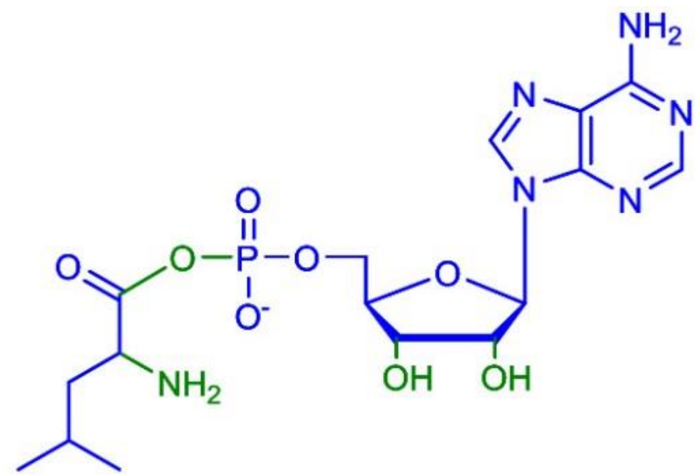
2) Aminoacyl transfer:

LeuRS (Leu-AMP) + $tRNA^{Leu}$ ⟷ LeuRS + Leu-$tRNA^{Leu}$ + AMP

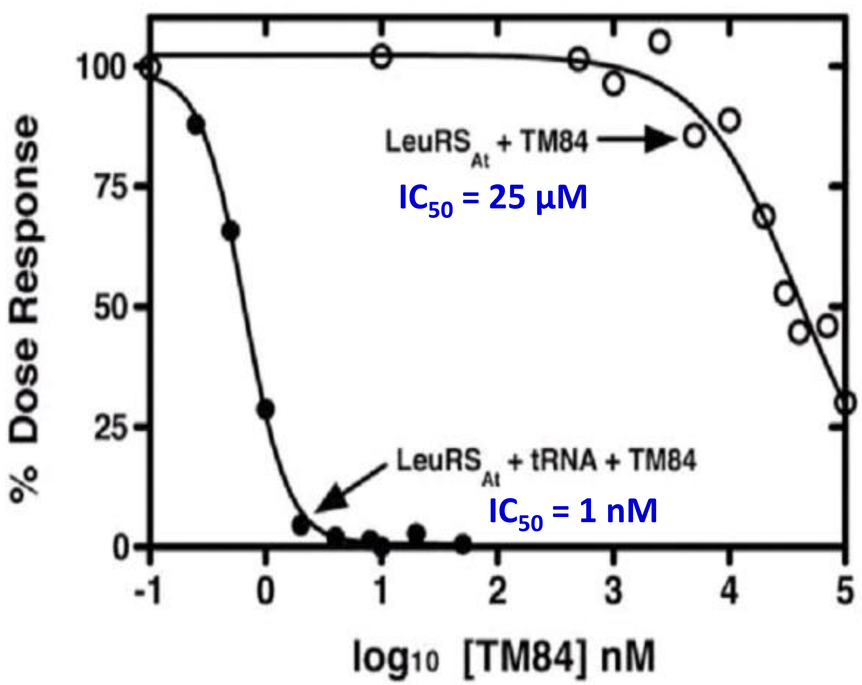
TM84 closely resembles Leu-AMP



TM84 (Toxic Moiety 84)

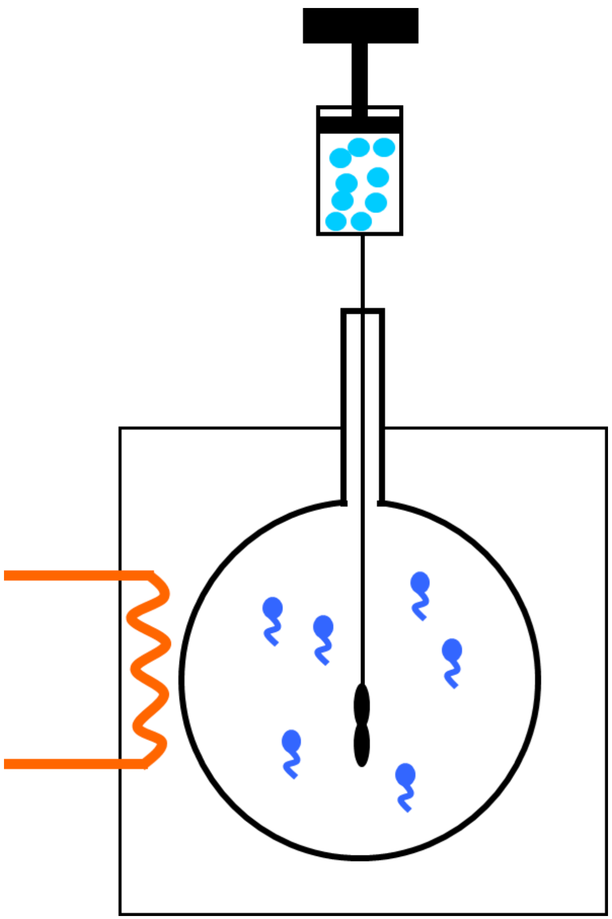


Leu-AMP

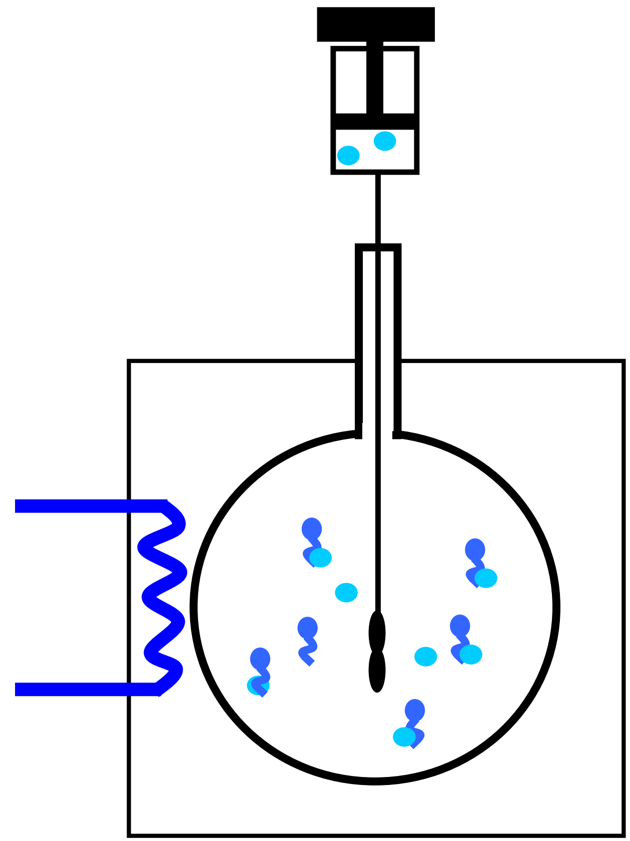


$tRNA^{Leu}$ is essential for the tight-binding of TM84!

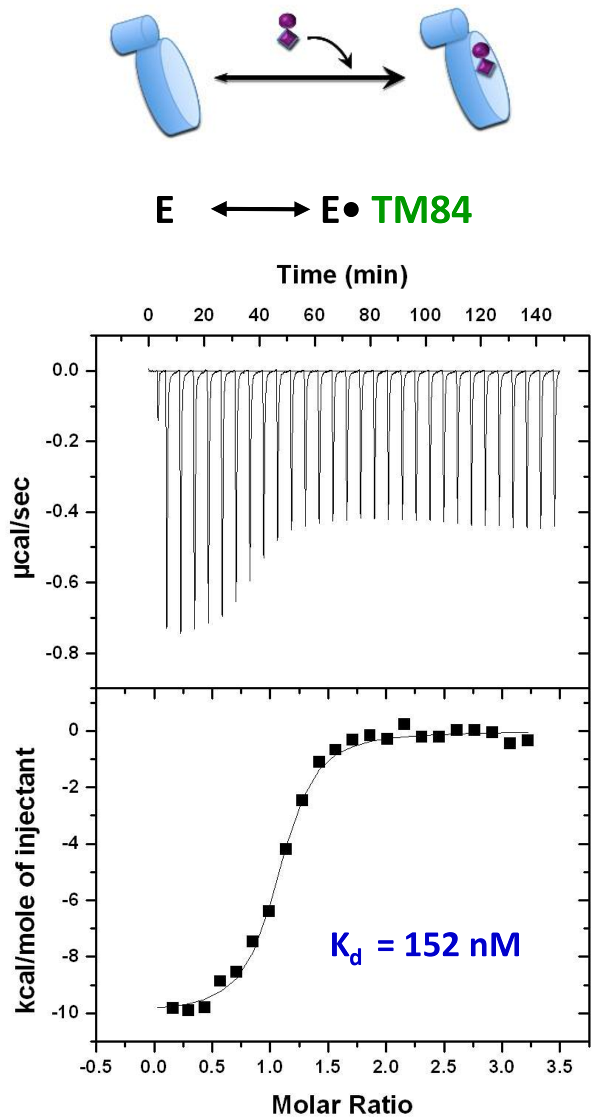
Utilizing Isothermal Titration Calorimetry (ITC) to dissect the mechanism of inhibition by TM84:

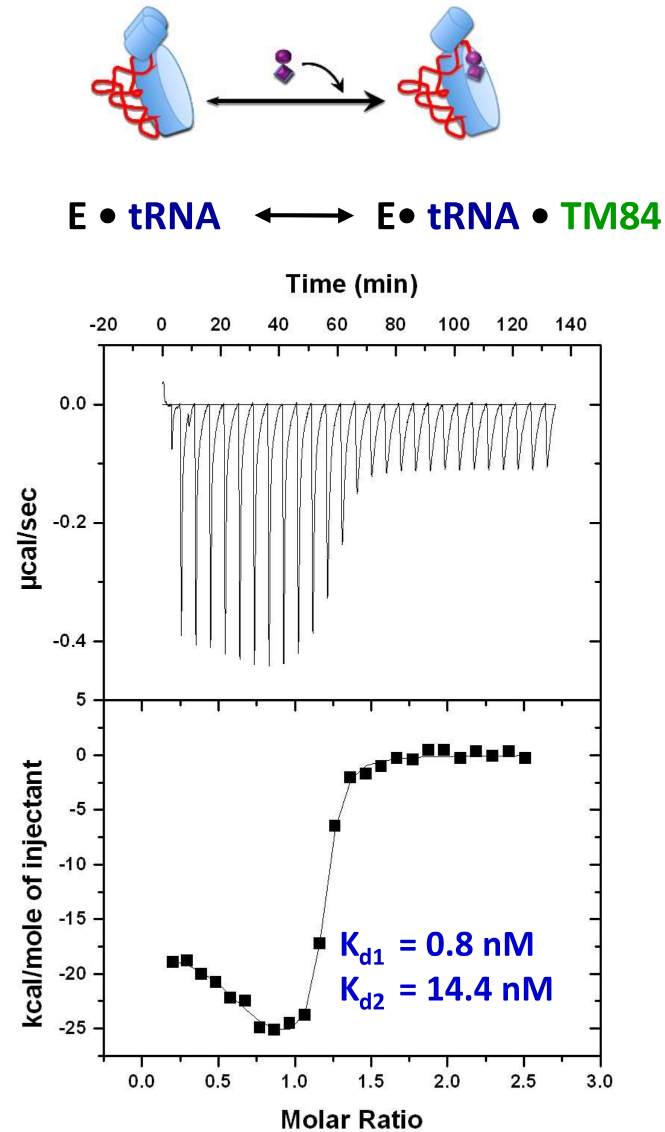


Unbound ligand



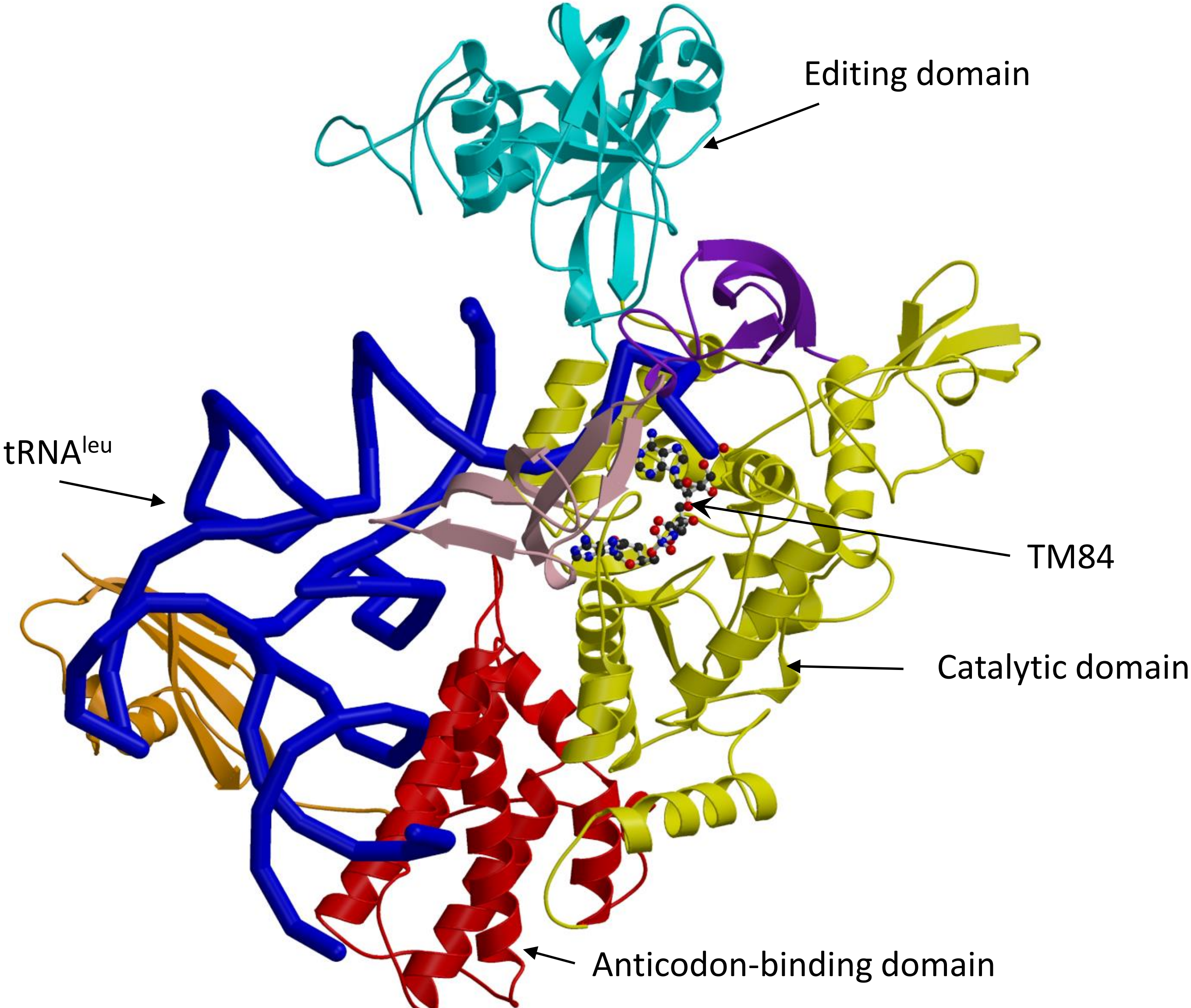
Bound ligand

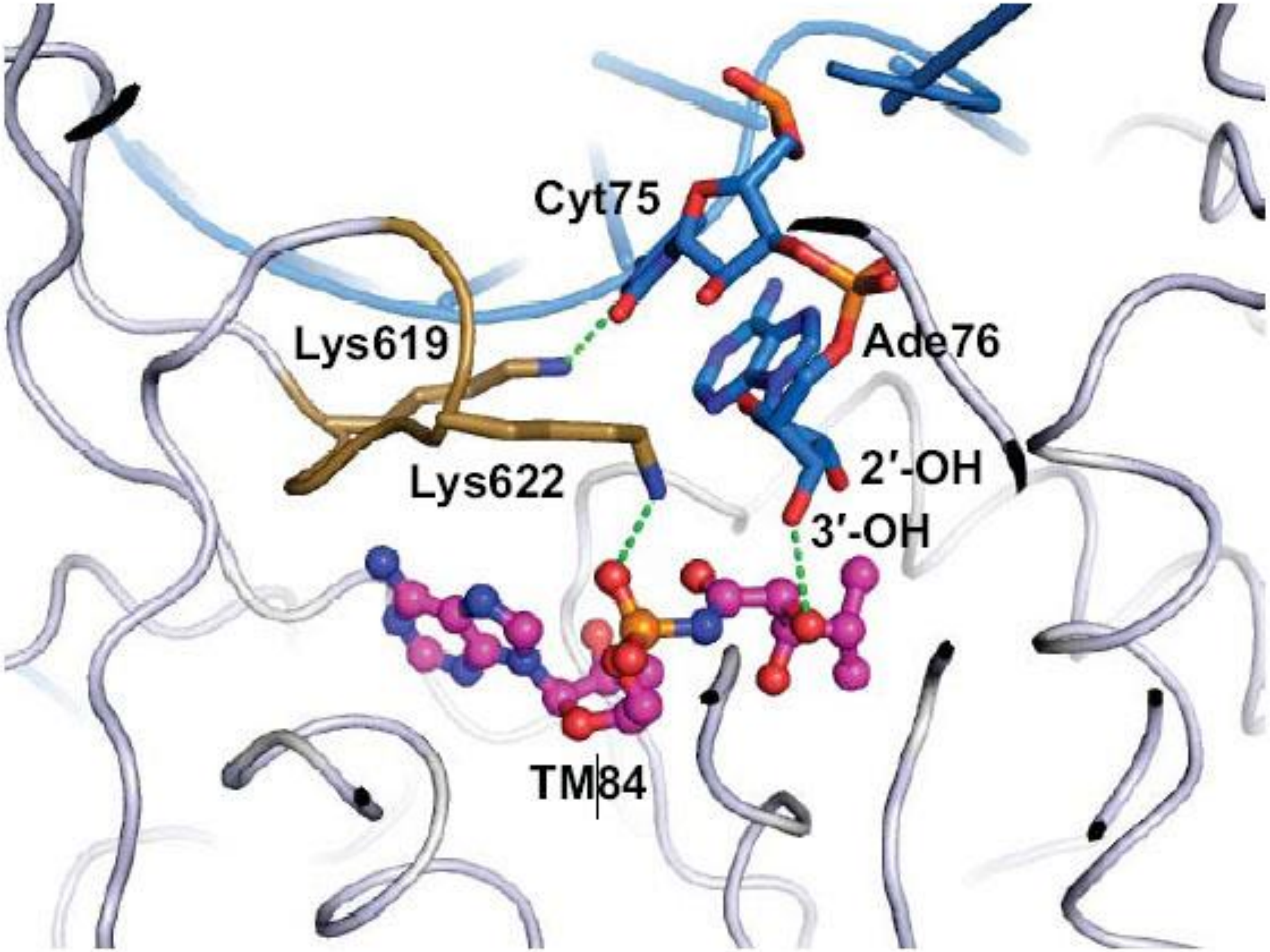




$tRNA^{Leu}$ is essential for tight-binding of TM84

TM84 binds to *E. coli* LeuRS-tRNA^{Leu} in the aminoacylation-like conformation!





Cyt75
Lys619
Ade76
Lys622
2′-OH
3′-OH
TM84