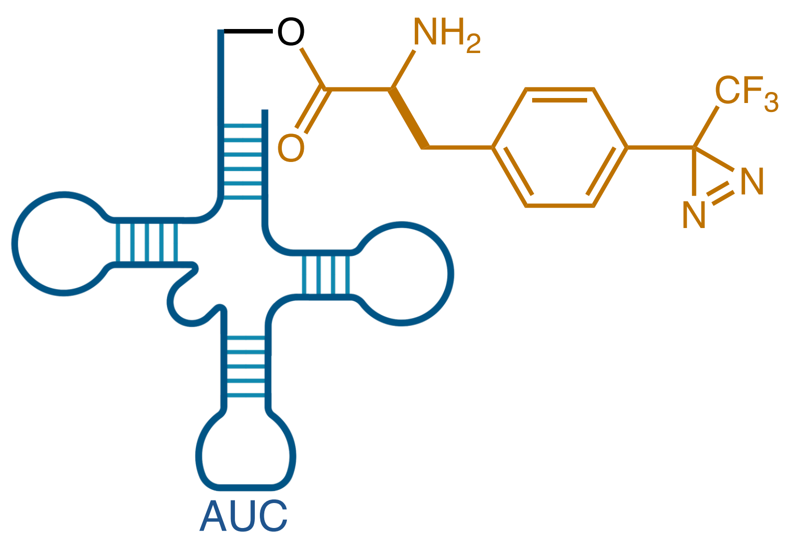
Genetic Code Expansion



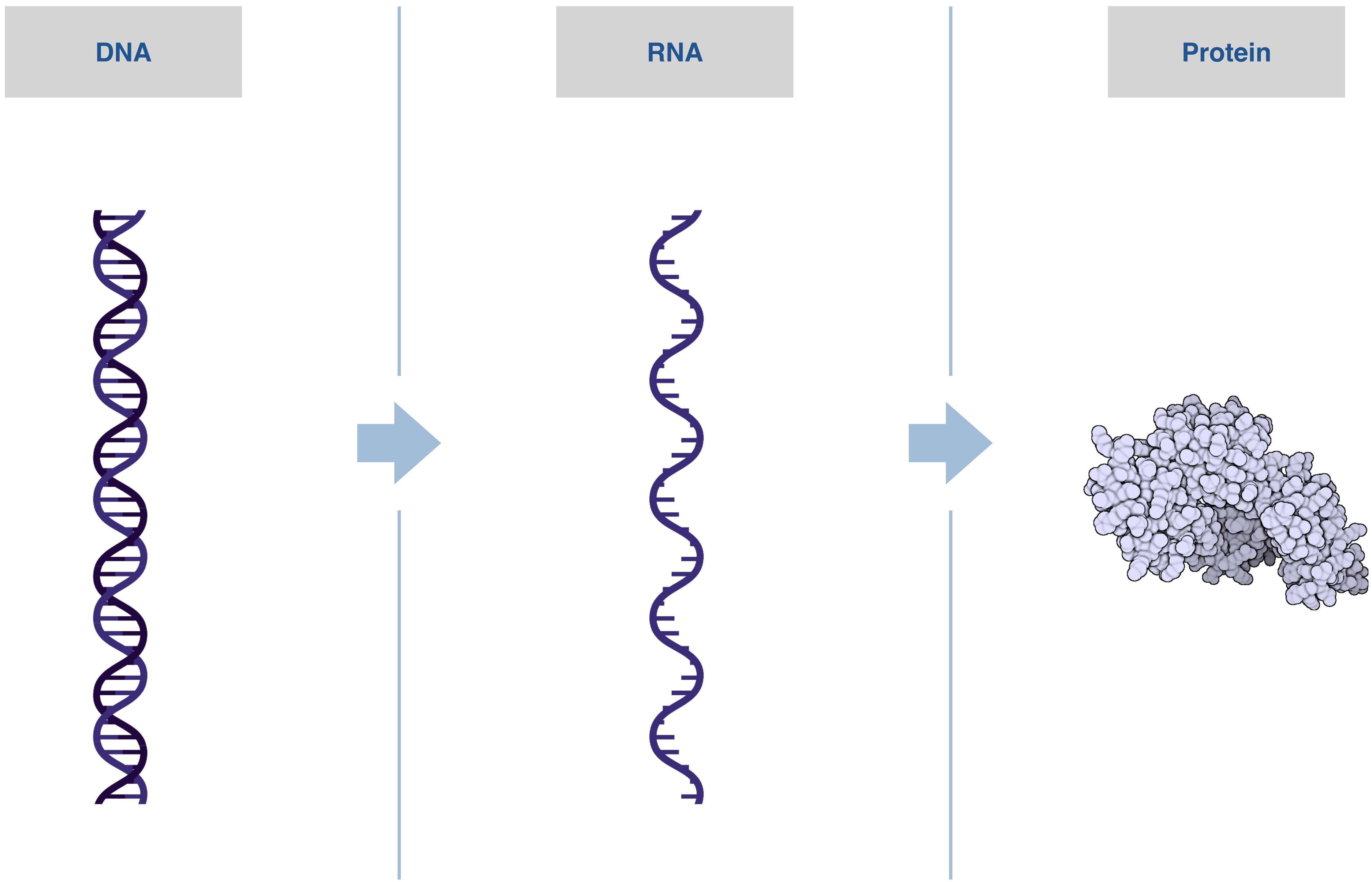
Benito Buksh

MacMillan Research Group

Group Meeting

June 7th, 2022

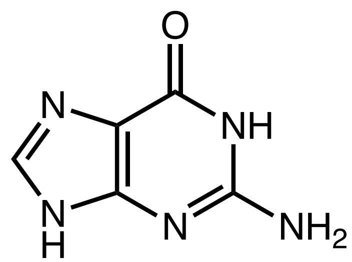
The Central Dogma of Molecular Biology

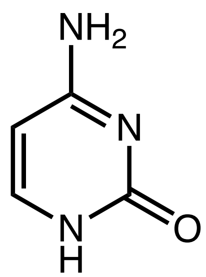


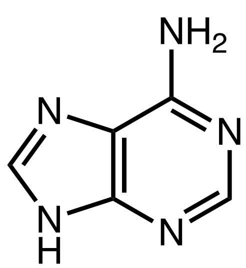
“DNA makes RNA makes protein”

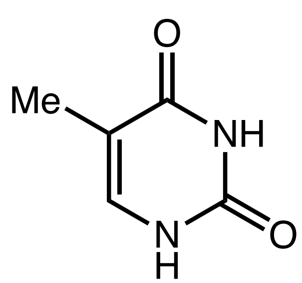
The Central Dogma of Molecular Biology

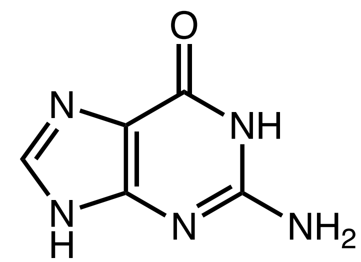
DNA	**RNA**	**Protein**

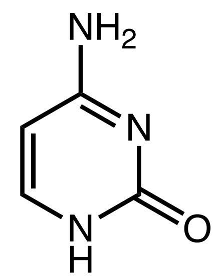
guanine 

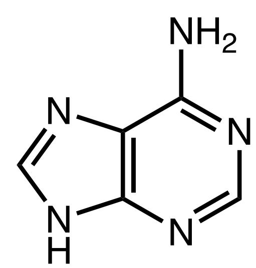
cytosine 

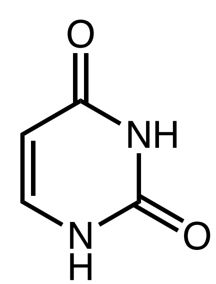
adenine 

thymine 

guanine 

cytosine 

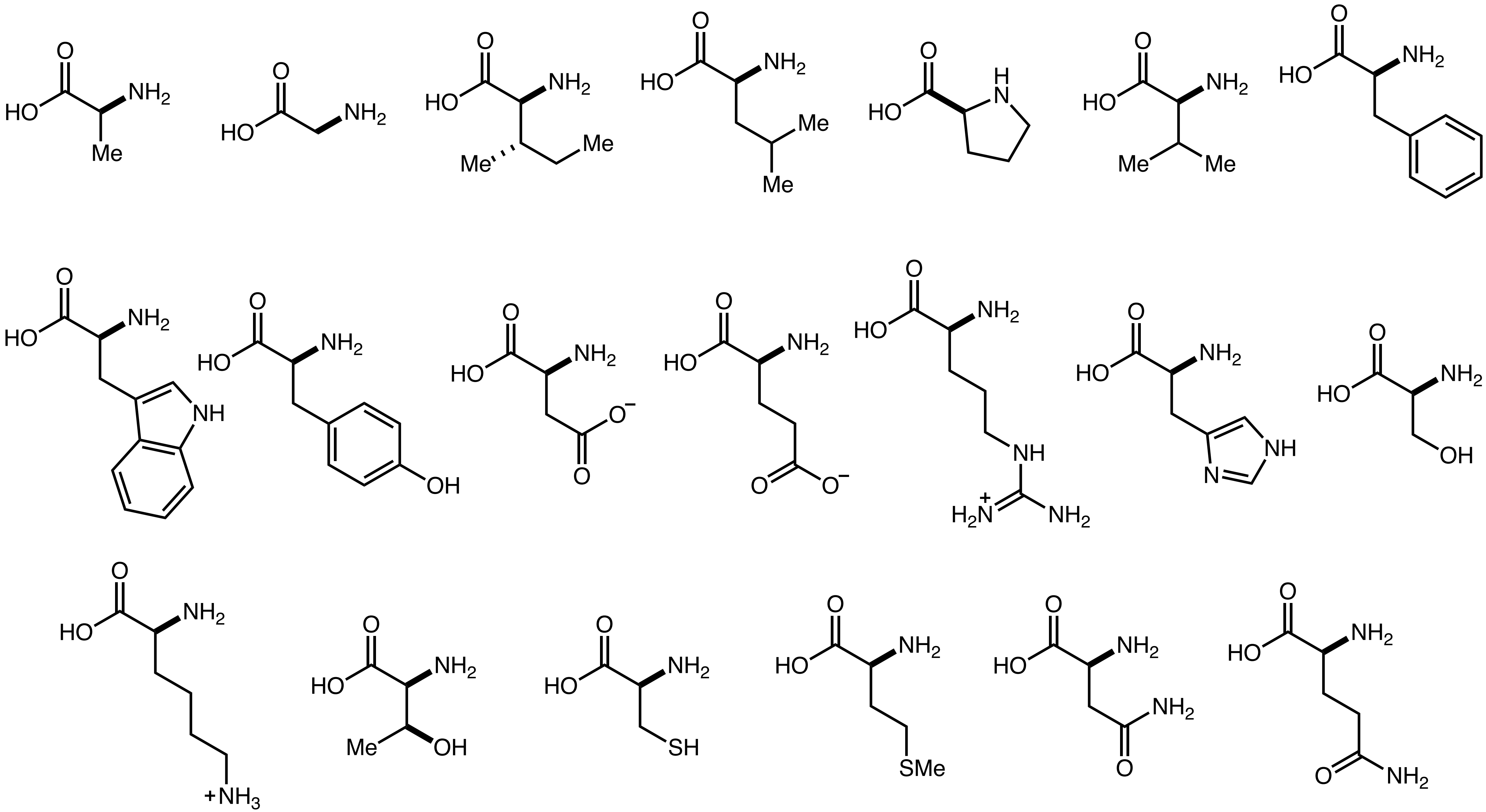
adenine 

uridine 

The 20 canonical amino acids

“DNA makes RNA makes protein”

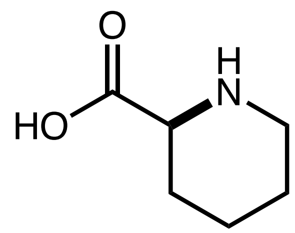
The 20 Canonical Amino Acids



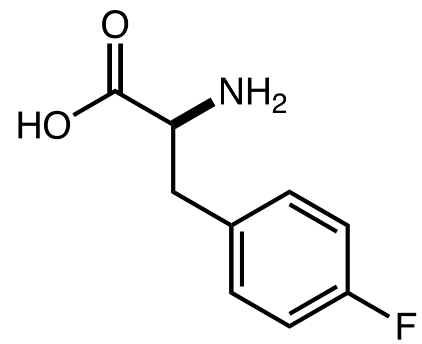
Outside of few exceptions, proteins are made up of the 20 canonical amino acids

Expanding the Genetic Code

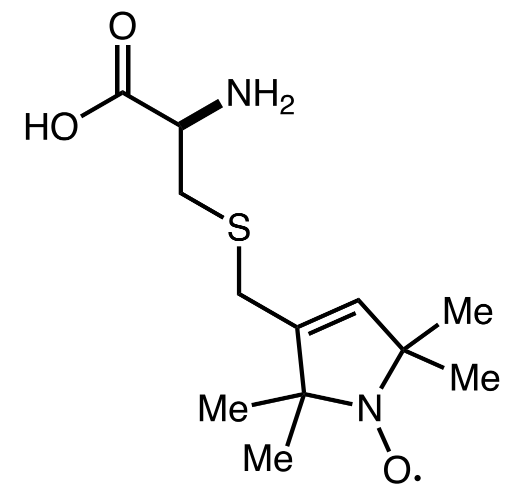
Synthetic organic chemistry allows us to make amino acids that are "noncanonical"



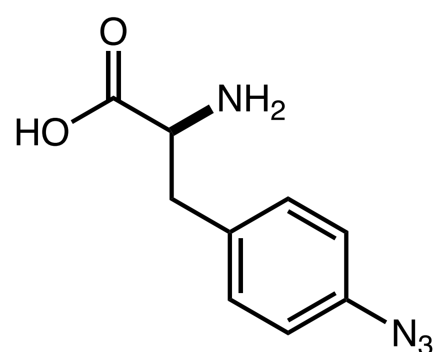
Proline analogue



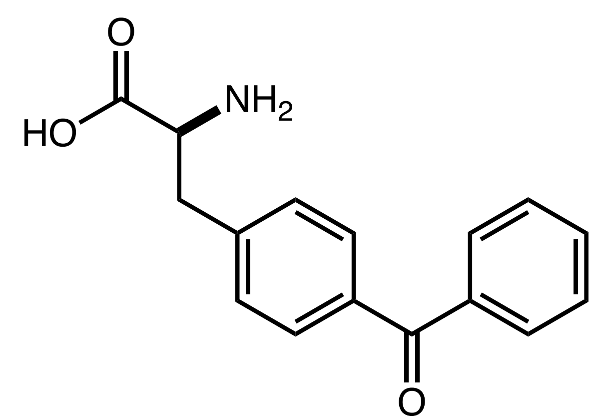
Tyrosine analogue



EPR probe



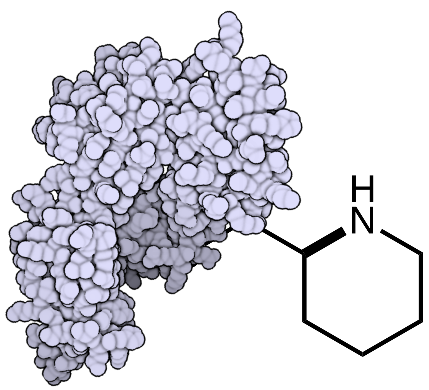
Bioorthogonal click handle



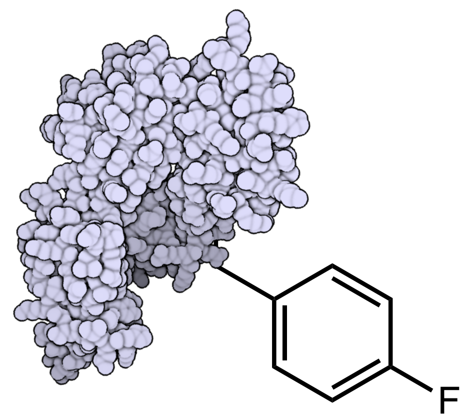
Photoaffinity label

Expanding the Genetic Code

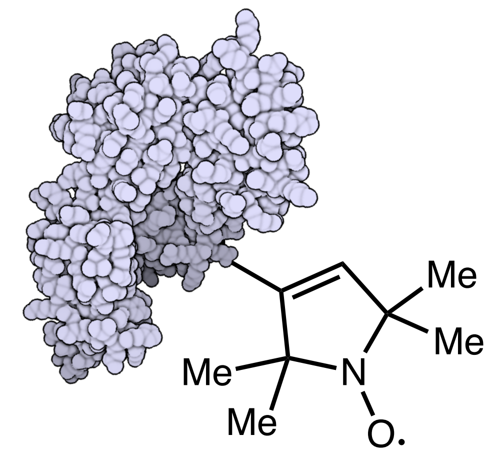
Synthetic organic chemistry allows us to make amino acids that are "noncanonical"



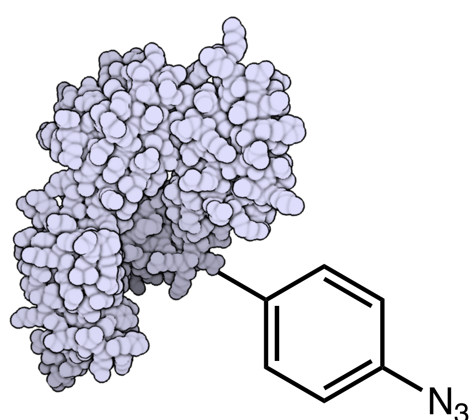
Proline analogue



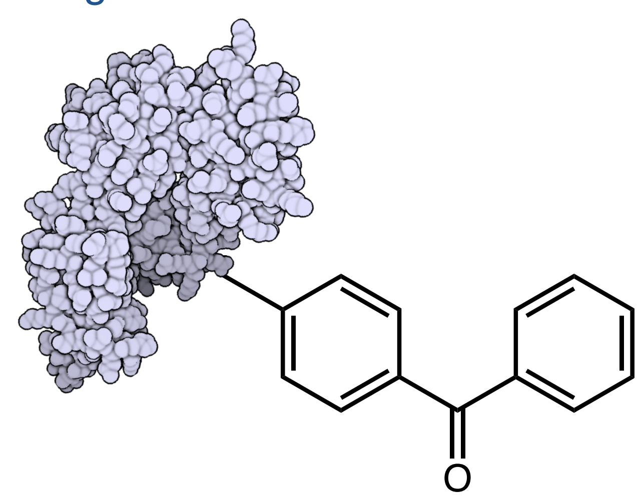
Tyrosine analogue



EPR probe



Bioorthogonal click handle

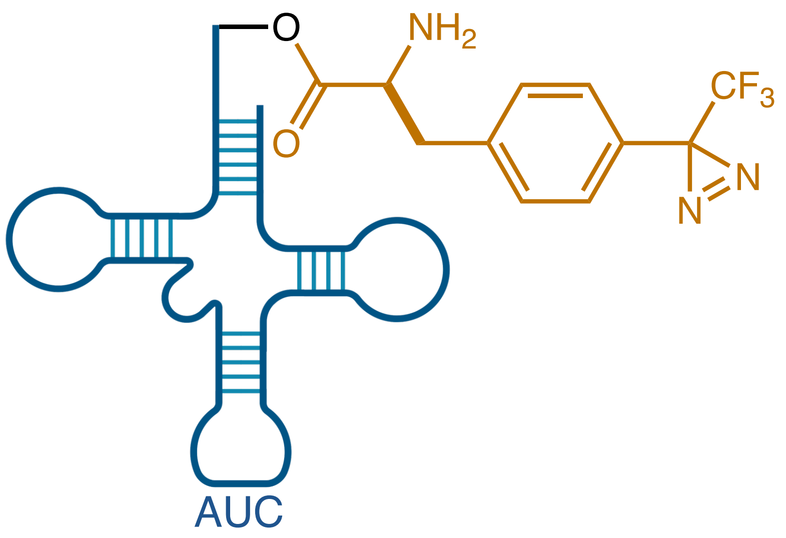


Photoaffinity label

Is it possible to expand the genetic code to incorporate noncanonical amino acids into proteins?

Expanding the Genetic Code

- The traditional genetic code
- Expanding the genetic code
    - In vitro
    - In eukaryotes, prokaryotes, and mammalian cells
- Orthogonal ribosomes
- Genetically recoded organisms and synonymous codon compression
- Outlook

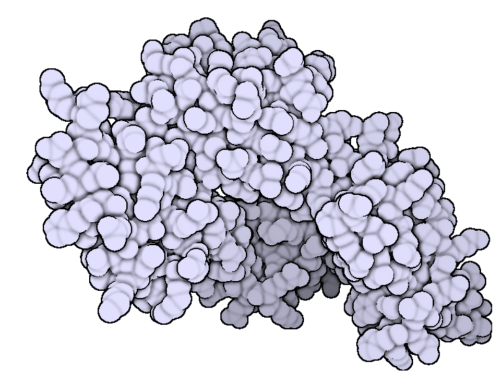


The Genetic Code

DNA

RNA

Protein



How does DNA / RNA sequence encode for protein sequence?

The Genetic Code

Codon: Sequence of three nucleotides

- 61 codons encode for amino acids
- 3 codons encode for stop codons

Second Position

First Position	T		C		A		G		Third Position
T	TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys	T
	TTC		TCC		TAC		TGC		C
	TTA	Leu	TCA		TAA	***STOP***	TGA	***STOP***	A
	TTG		TCG		TAG	***STOP***	TGG	Trp	G
C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	Gln	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	Lys	AGA	Arg	A
	ATG	Met	ACG		AAG		AGG		G
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	Glu	GGA		A
	GTG		GCG		GAG		GGG		G

Codons specify which amino acid will be added during protein synthesis

Image source: Genomenon

The Genetic Code

DNA	ATG	TAT	CGC	TCA	TAA
↓ transcription					
RNA	AUG	UAU	CGC	UCA	UAA
↓ translation					
Protein	Met	Tyr	Arg	Ser	Stop

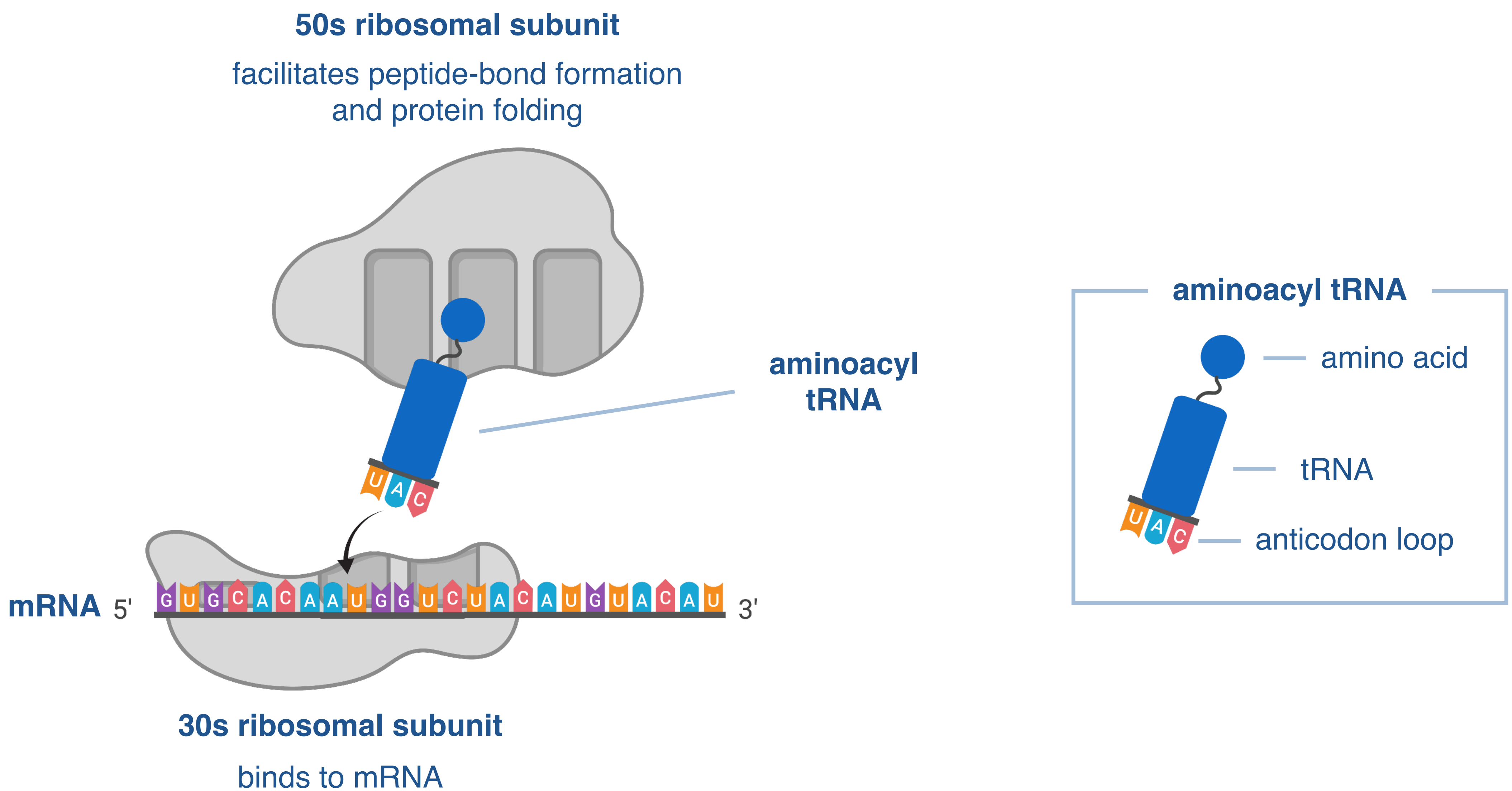
Second Position

First Position	T	C	A	G	Third Position
T	TTT, TTC: Phe TTA, TTG: Leu	TCT, TCC, TCA, TCG: Ser	TAT, TAC: Tyr TAA: ***STOP*** TAG: ***STOP***	TGT, TGC: Cys TGA: ***STOP*** TGG: Trp	T C A G
C	CTT, CTC, CTA, CTG: Leu	CCT, CCC, CCA, CCG: Pro	CAT, CAC: His CAA, CAG: Gln	CGT, CGC, CGA, CGG: Arg	T C A G
A	ATT, ATC, ATA: Ile ATG: Met	ACT, ACC, ACA, ACG: Thr	AAT, AAC: Asn AAA, AAG: Lys	AGT, AGC: Ser AGA, AGG: Arg	T C A G
G	GTT, GTC, GTA, GTG: Val	GCT, GCC, GCA, GCG: Ala	GAT, GAC: Asp GAA, GAG: Glu	GGT, GGC, GGA, GGG: Gly	T C A G

Codons specify which amino acid will be added during protein synthesis

Image source: Genomenon

Translation of RNA to Protein via the Ribosome



Aminoacyl tRNA charged with an amino acid through its anticodon loop binds to a complementary codon

Image source: Biorender

Translation of RNA to Protein via the Ribosome

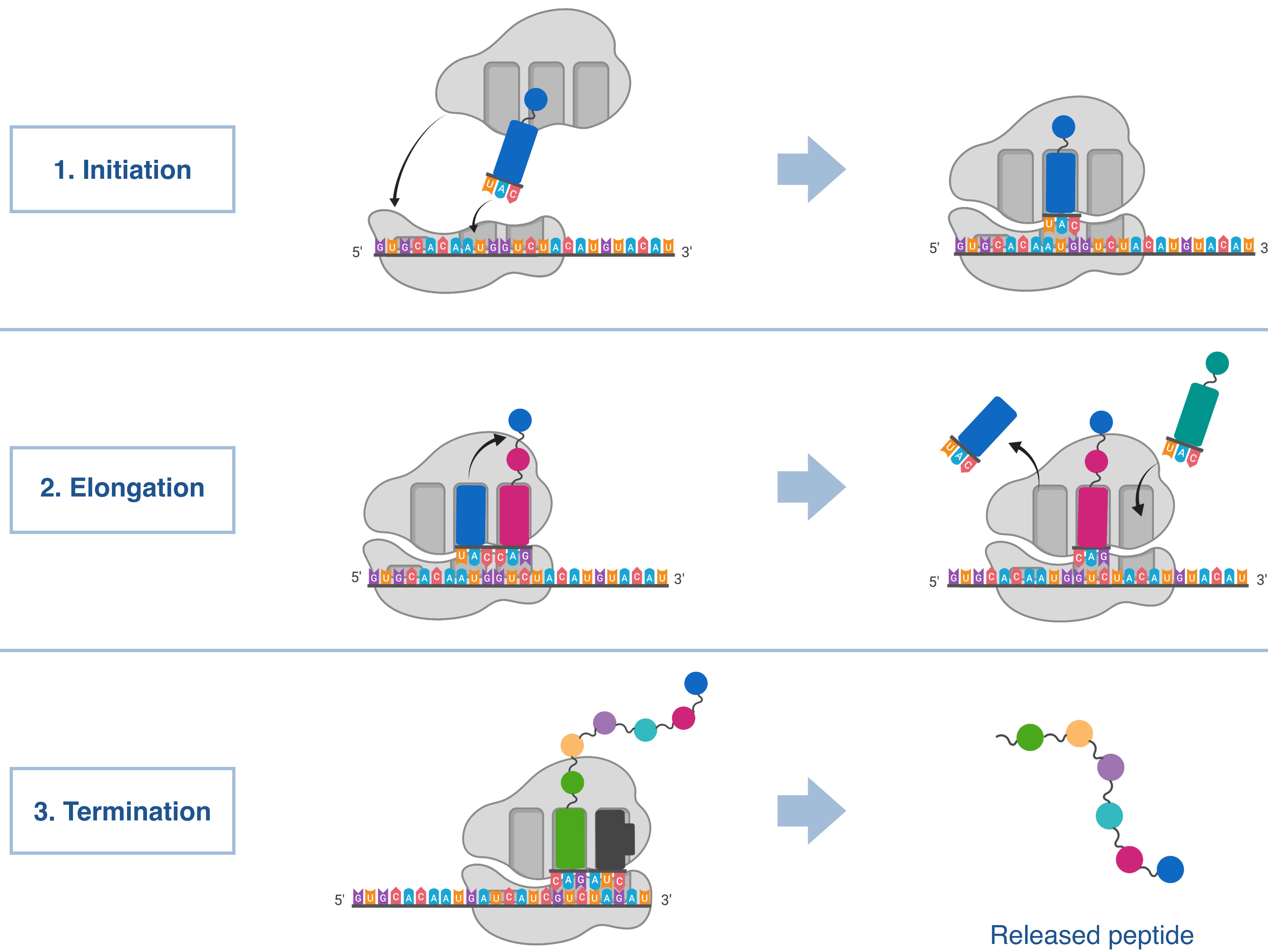
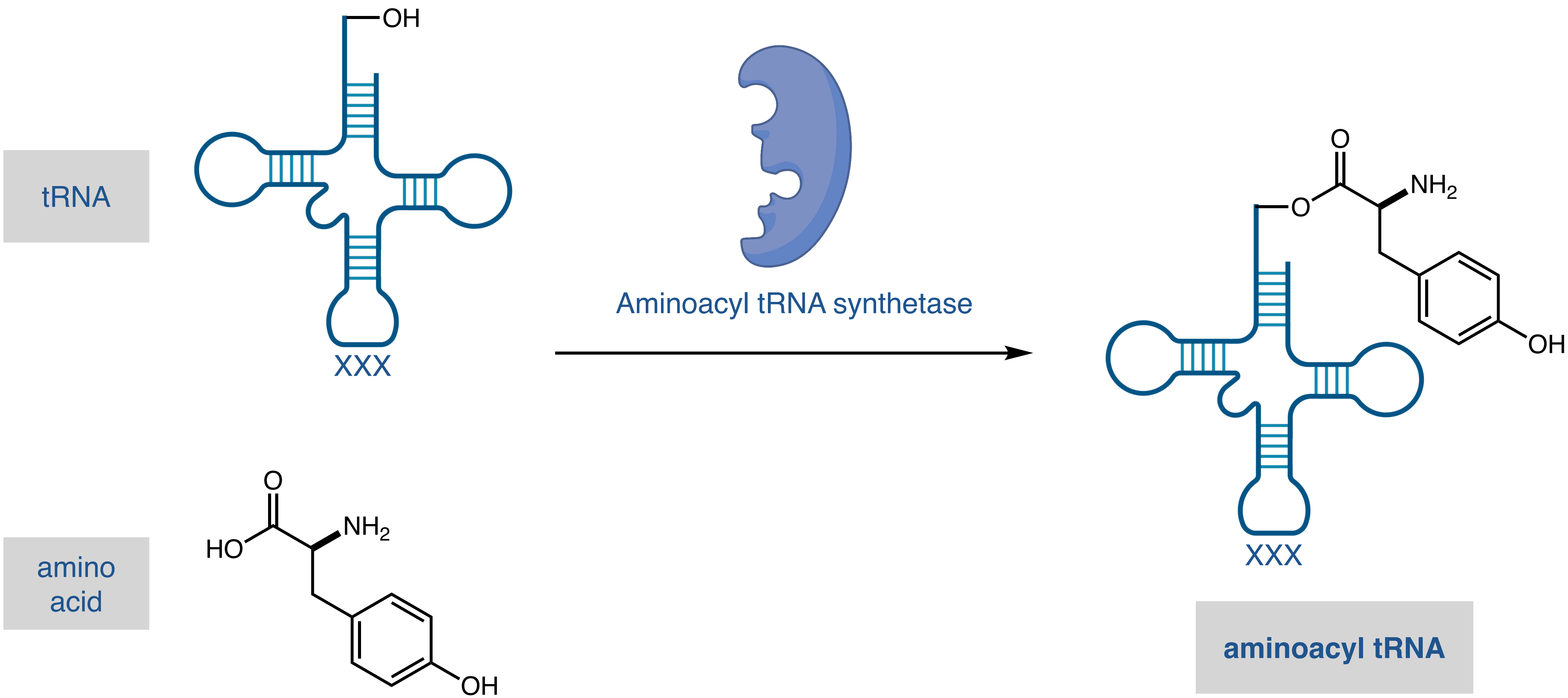


Image source: Biorender

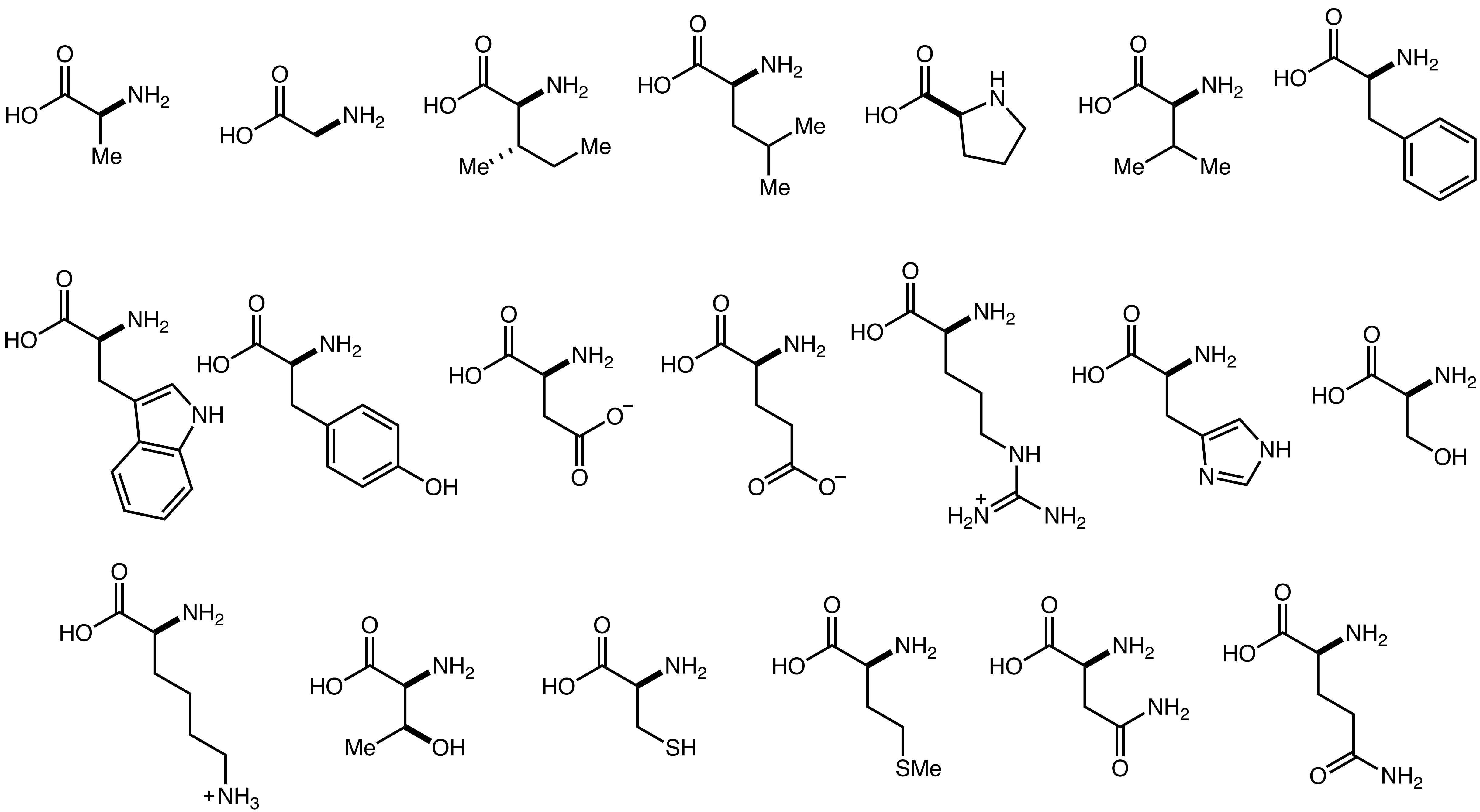
Production of Aminoacylated tRNAs



The aminoacyl tRNA synthetase is responsible for “charging” the tRNA with amino acid to form an aminoacyl tRNA

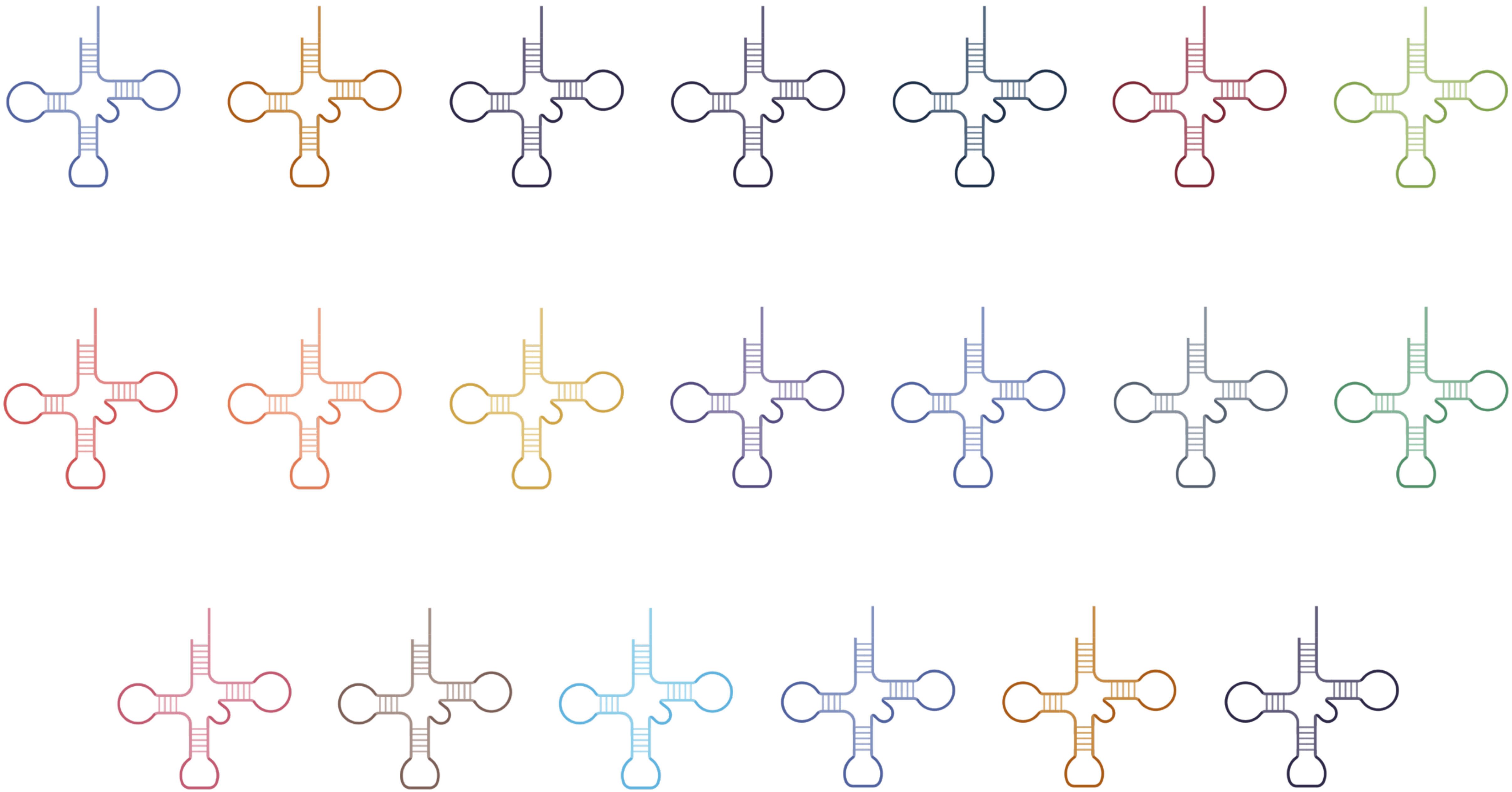
Shandell, M. A.; Tan, Z.; Cornish, V. W. et al. *Biochemistry* **2021**, *60*, 3455.

Production of Aminoacylated tRNAs



Shandell, M. A.; Tan, Z.; Cornish, V. W. et al. *Biochemistry* **2021**, *60*, 3455.

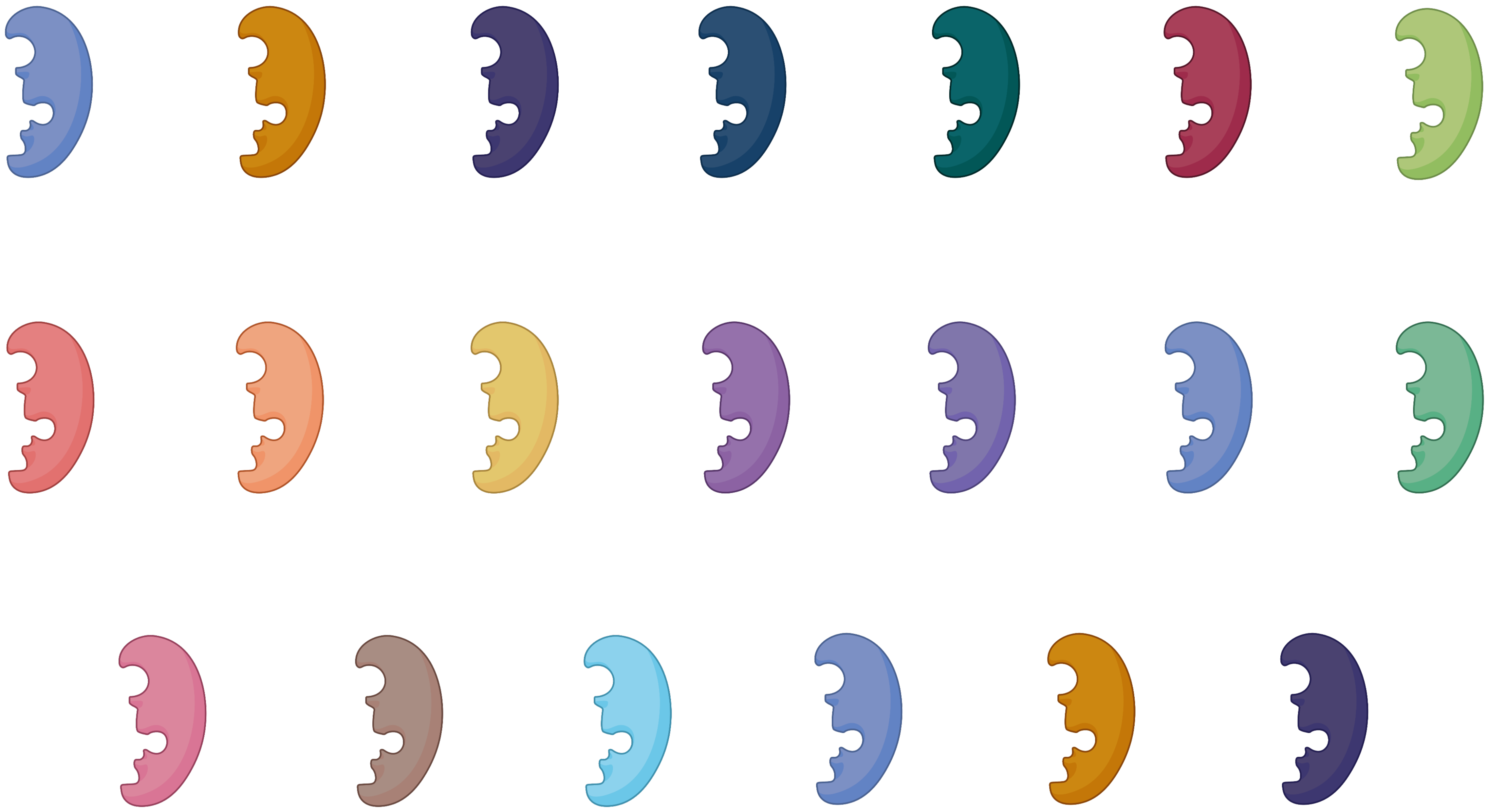
Production of Aminoacylated tRNAs



For each amino acid, there is at least one unique aminoacyl tRNA

Shandell, M. A.; Tan, Z.; Cornish, V. W. et al. *Biochemistry* **2021**, *60*, 3455.

Production of Aminoacylated tRNAs

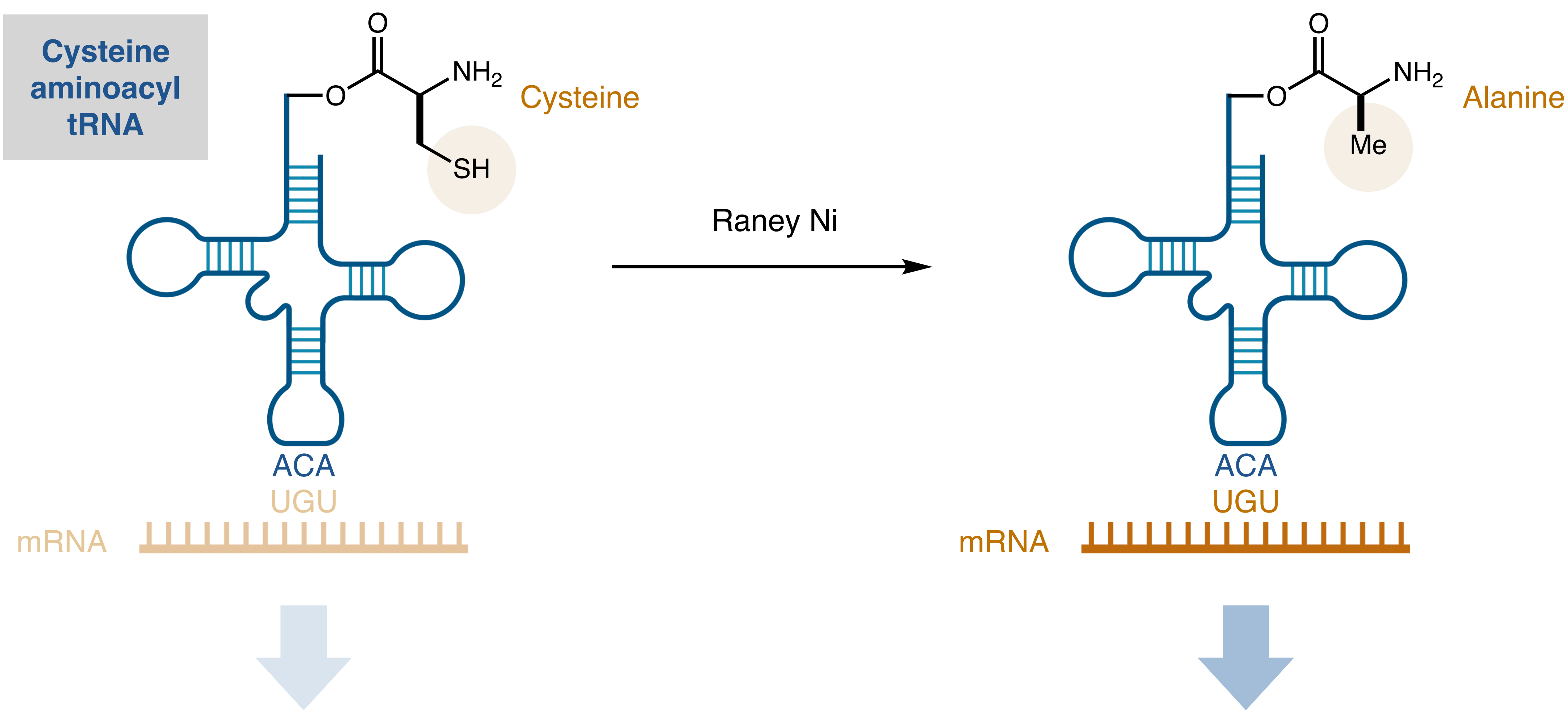


In most organisms, for each amino acid, there is a unique aminoacyl tRNA synthetase

Shandell, M. A.; Tan, Z.; Cornish, V. W. et al. *Biochemistry* **2021**, *60*, 3455.

The Adaptor Hypothesis

"Amino acid position within a protein is determined by the binding of mRNA with a tRNA carrying the amino acid"



Cysteine is incorporated into the protein

Alanine is incorporated into the protein

Chapeville, F. et al. *Proc. Natl. Acad. Sci. U. S. A.* **1962**, *48*, 1086.

Expanding the Genetic Code

- The traditional genetic code
- Expanding the genetic code
    - In vitro
    - In eukaryotes, prokaryotes, and mammalian cells
- Orthogonal ribosomes
- Genetically recoded organisms and synonymous codon compression
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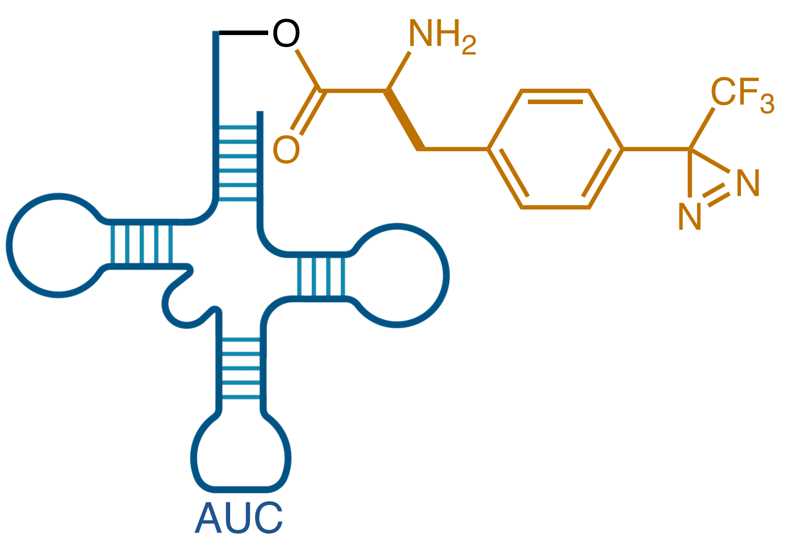
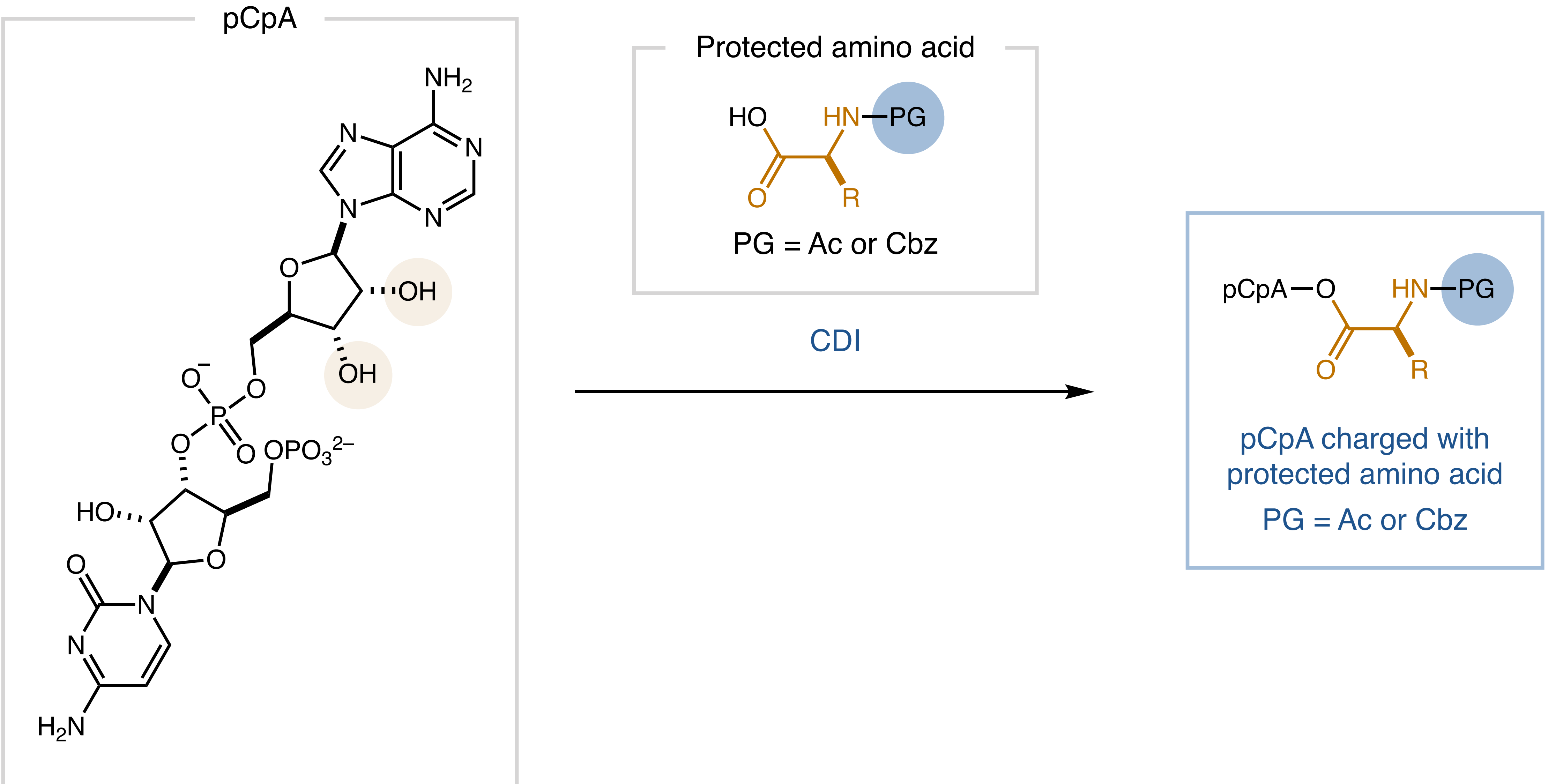
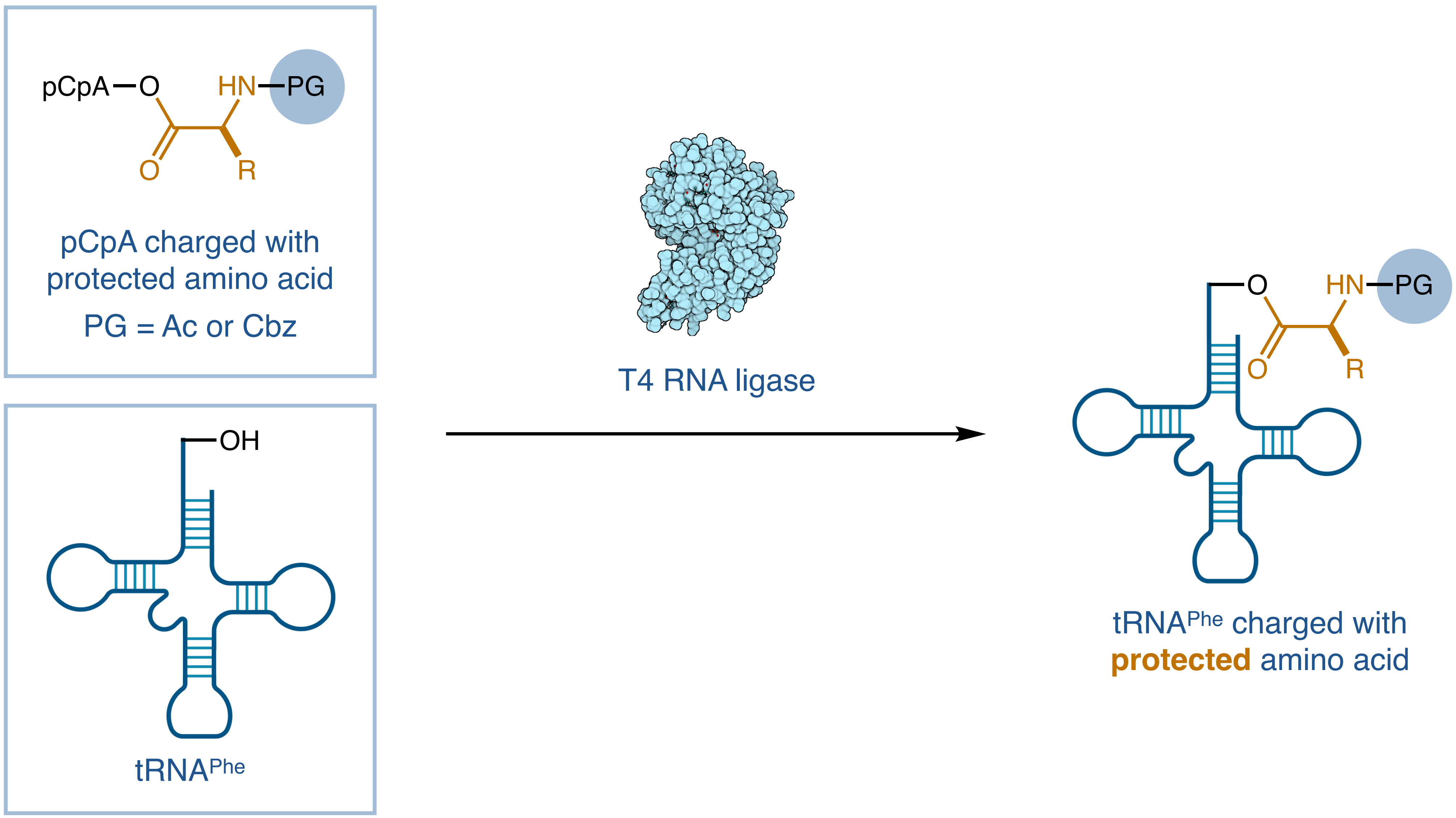


A General Method for the Synthesis of “Misacylated” tRNAs



Heckler, T. G.; Chang, L. H..; Zama, Y.; Naka, T.; Chorghade, M. S.; Hecht, S. M. *Biochemistry* **1984**, *23*, 1468.

A General Method for the Synthesis of "Misacylated" tRNAs

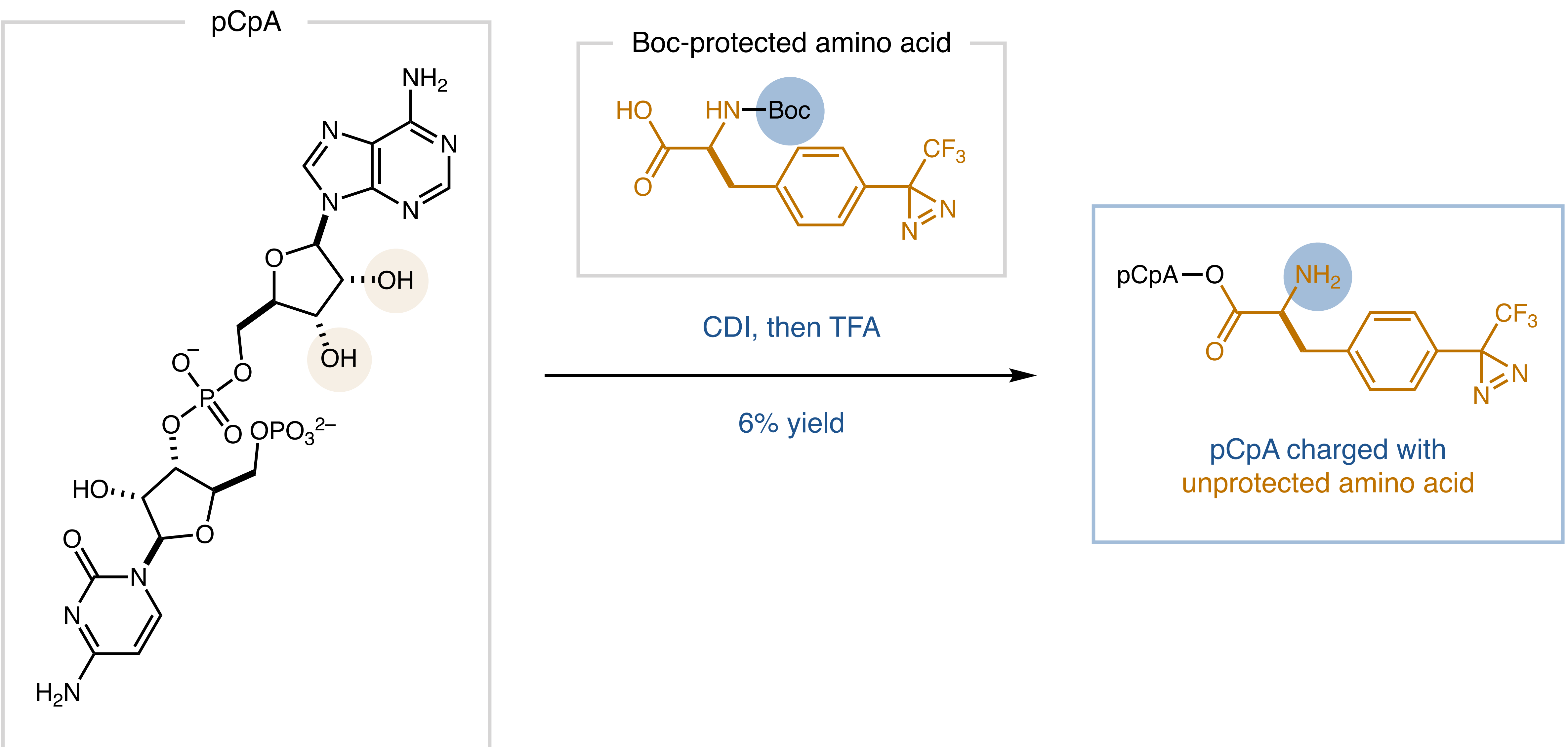


tRNA^{Phe}s can be charged with protected amino acids of choice using T4 RNA ligase

Problem: tRNA charged with protecting group cannot be accepted by the ribosome

Heckler, T. G.; Chang, L. H..; Zama, Y.; Naka, T.; Chorghade, M. S.; Hecht, S. M. *Biochemistry* **1984**, *23*, 1468.

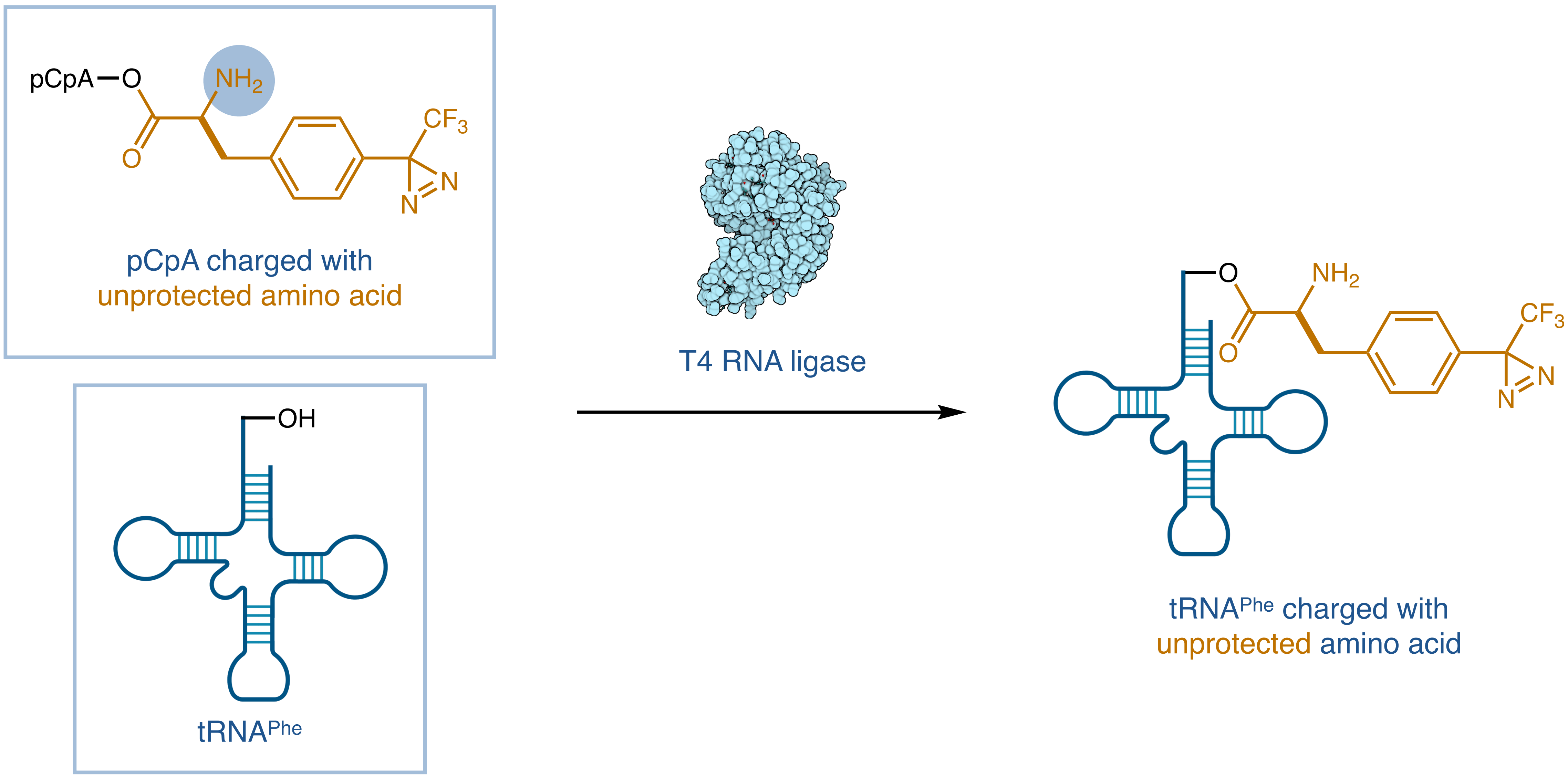
Protein Synthesis with Unprotected Aminoacyl tRNAs



Boc protection and deprotection enables synthesis of pCpA charged with unprotected amino acid

Baldini, G.; Martoglio, B.; Schachenmann, A.; Zugliani, C.; Brunner, J. *Biochemistry* **1988**, *27*, 2751.

Protein Synthesis with Unprotected Aminoacyl tRNAs



tRNA^{Phe} charged with unprotected noncanonical amino acid can be synthesized

Baldini, G.; Martoglio, B.; Schachenmann, A.; Zugliani, C.; Brunner, J. *Biochemistry* **1988**, *27*, 2751.

Stop Codons

Second Position

First Position	T	C	A	G	Third Position
T	TTT Phe TTC Phe TTA Leu TTG Leu	TCT Ser TCC Ser TCA Ser TCG Ser	TAT Tyr TAC Tyr TAA ***STOP*** TAG ***STOP***	TGT Cys TGC Cys TGA ***STOP*** TGG Trp	T C A G
C	CTT Leu CTC Leu CTA Leu CTG Leu	CCT Pro CCC Pro CCA Pro CCG Pro	CAT His CAC His CAA Gln CAG Gln	CGT Arg CGC Arg CGA Arg CGG Arg	T C A G
A	ATT Ile ATC Ile ATA Ile ATG Met	ACT Thr ACC Thr ACA Thr ACG Thr	AAT Asn AAC Asn AAA Lys AAG Lys	AGT Ser AGC Ser AGA Arg AGG Arg	T C A G
G	GTT Val GTC Val GTA Val GTG Val	GCT Ala GCC Ala GCA Ala GCG Ala	GAT Asp GAC Asp GAA Glu GAG Glu	GGT Gly GGC Gly GGA Gly GGG Gly	T C A G

Stop Codons

"Stop translating protein"

Name	Codon	*E. coli*	Yeast	Human
Amber	**UAG**	*0.3*	*0.5*	*0.5*
Ochre	UAA	*2.0*	*1.0*	*0.7*
Opal	UGA	*1.0*	*0.6*	*1.3*

Frequency / thousand codons

Of the three stop codons, the Amber stop codon is used the least in the genome

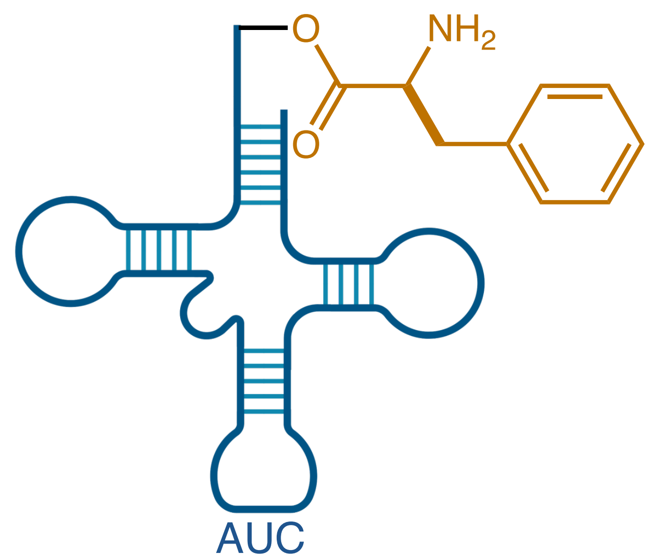
Codon frequency data: https://www.genscript.com/tools/codon-frequency-table

Bruce, A. G.; Atkins, J. F.; Wills, N.; Uhlenbeck, O.; Gesteland, R. F. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 7127.

Amber Suppression with a Amber Suppressor tRNA Charged with Phe

Amber suppression: Amber codon (UAG) has been reassigned from encoding “stop” to encoding an amino acid

Amber Suppressor
Aminoacyl tRNA
charged with phenylalanine



RNA sequence	UUU	AAC	AAC	CGG	GCG	UAG	GCG	AAC	CGG	UAA
Protein sequence	Phe	Asn	Asn	Arg	Ser	**Stop**	Ser	Asn	Arg	Stop

Traditional rules:
5 amino acid
peptide

Amber Suppression

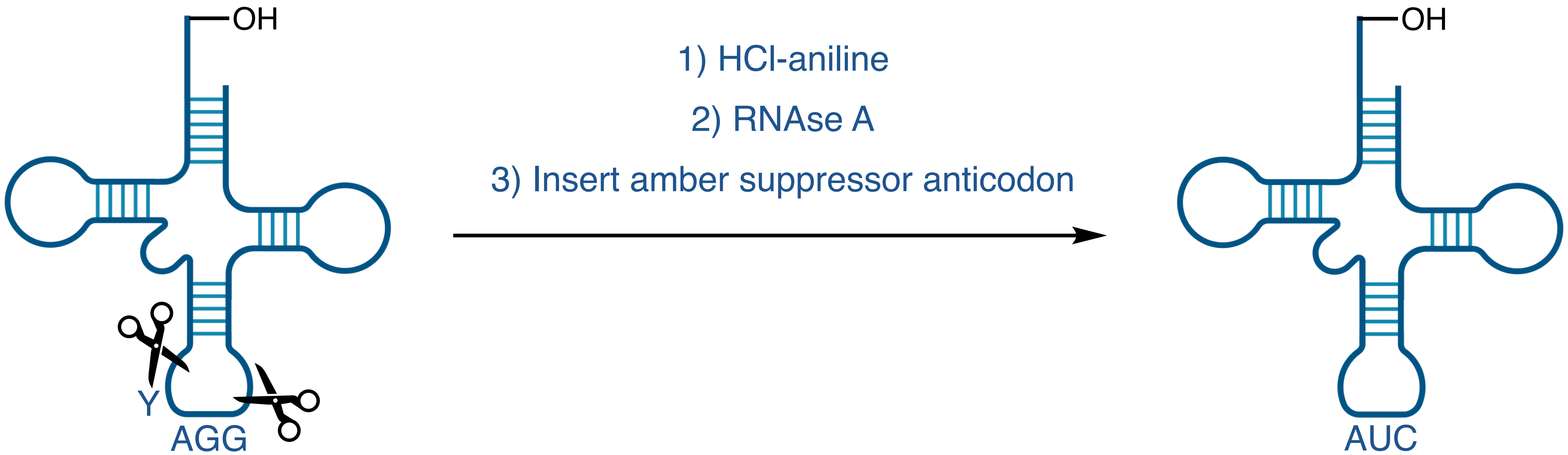
Protein sequence with Amber Suppression	Phe	Asn	Asn	Arg	Ser	**Phe**	Ser	Asn	Arg	Stop

Amber suppression:
9 amino acid
peptide

Bruce, A. G.; Atkins, J. F.; Wills, N.; Uhlenbeck, O.; Gesteland, R. F. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 7127.

Amber Suppression with a Amber Suppressor tRNA Charged with Phe

Step 1: Preparation of $tRNA^{CUA}$ (amber suppressor tRNA) via anticodon loop replacement

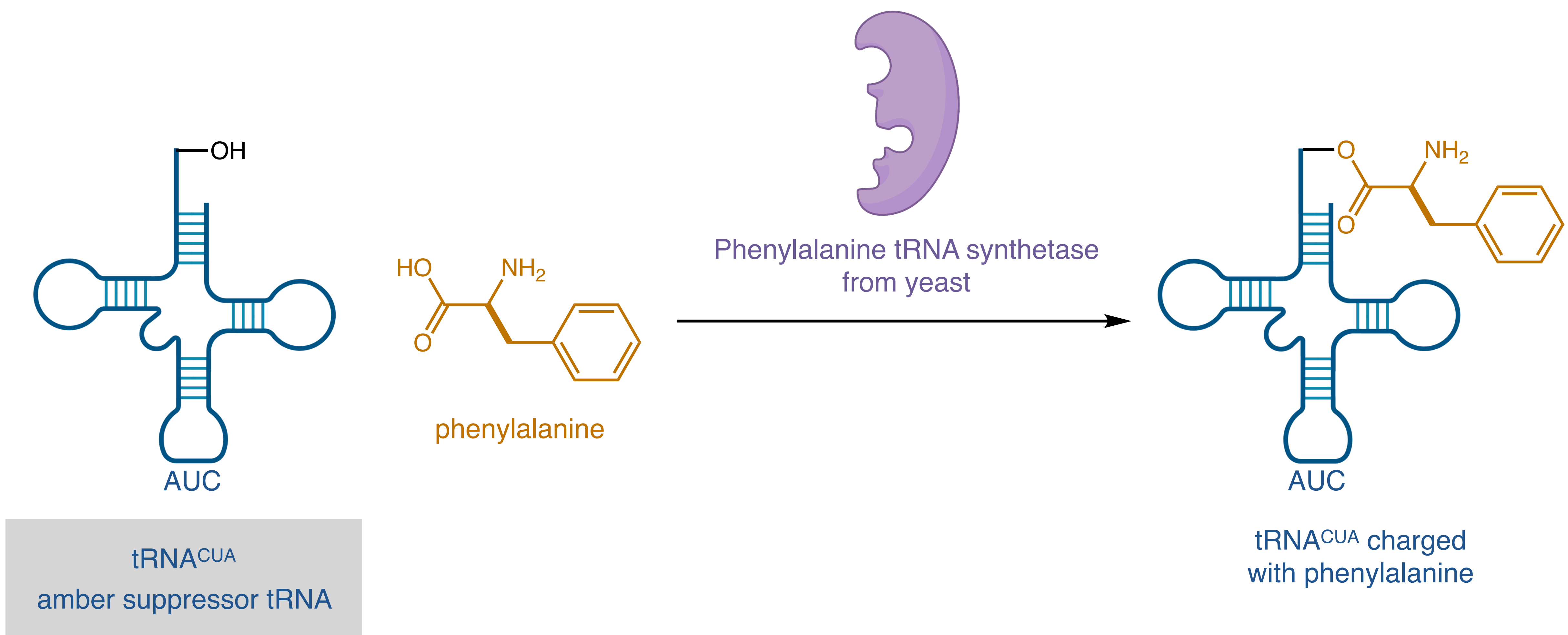


tRNA^CUA
amber suppressor tRNA

Bruce, A. G.; Atkins, J. F.; Wills, N.; Uhlenbeck, O.; Gesteland, R. F. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 7127.

Amber Suppression with a Amber Suppressor tRNA Charged with Phe

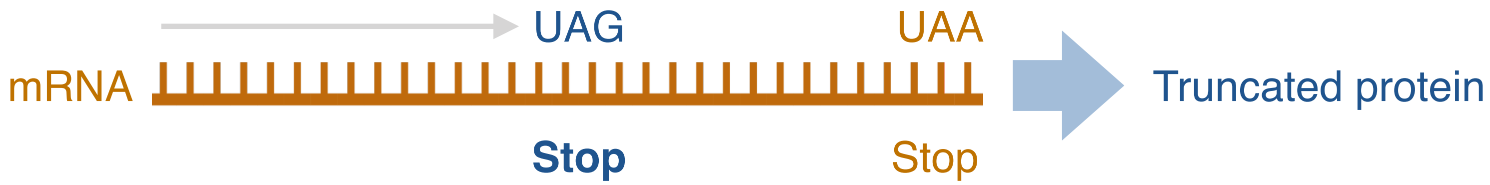
Step 2: Preparation of tRNA^{CUA} charged with phenylalanine



Phenylalanine tRNA synthetase accepts tRNA^{CUA}

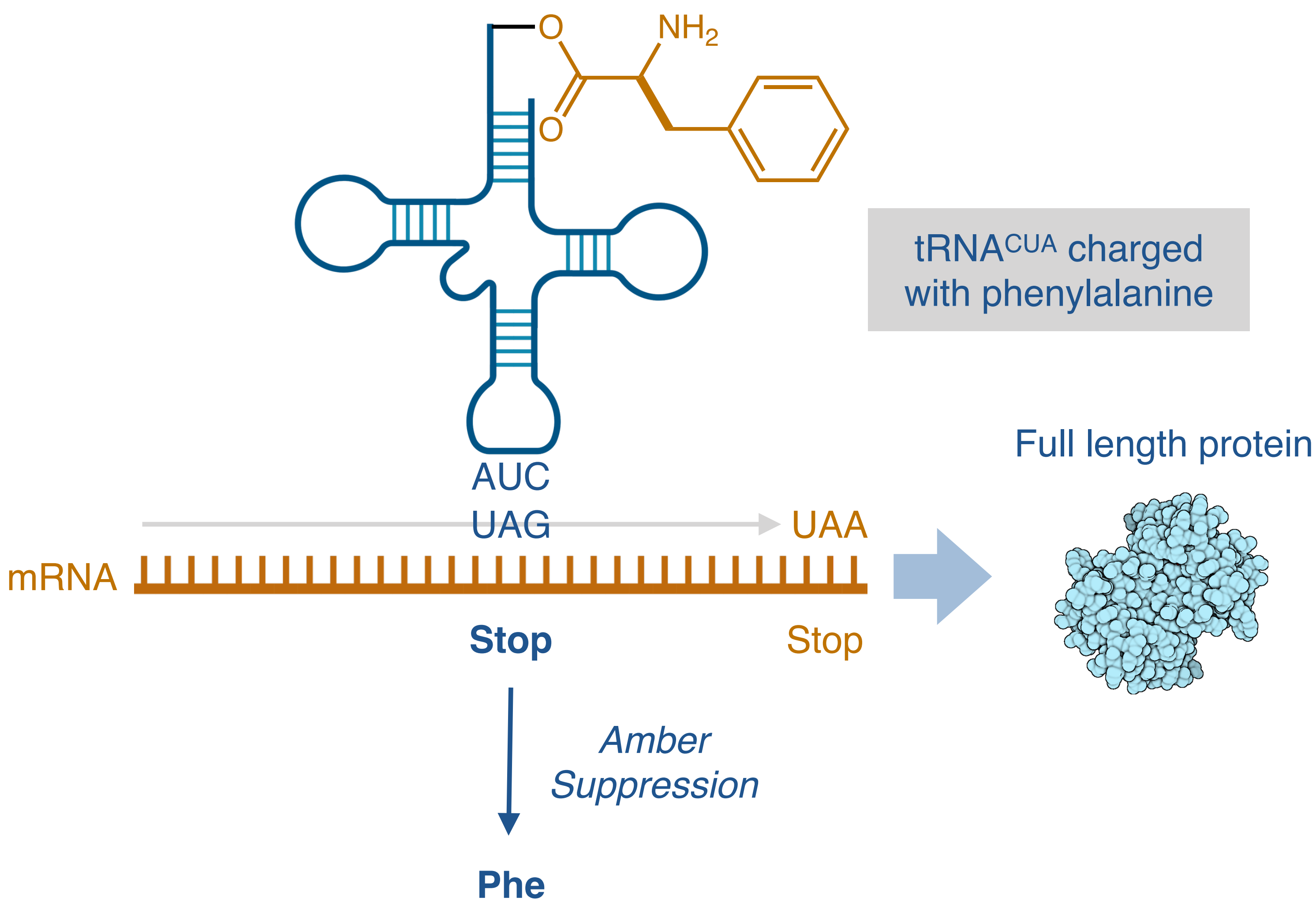
Bruce, A. G.; Atkins, J. F.; Wills, N.; Uhlenbeck, O.; Gesteland, R. F. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 7127.

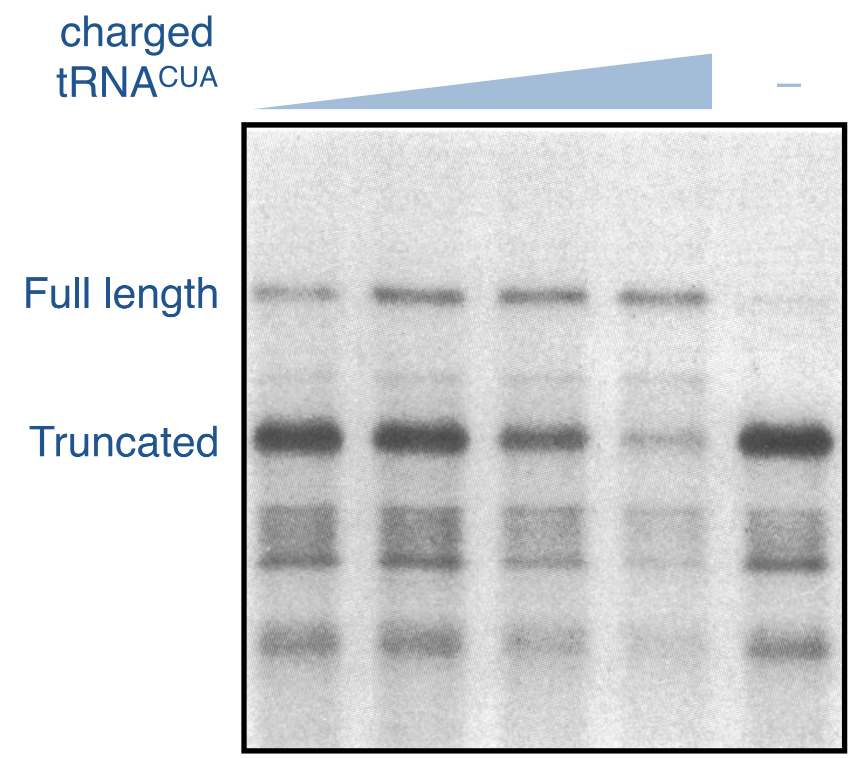
Amber Suppression with a Amber Suppressor tRNA Charged with Phe



Bruce, A. G.; Atkins, J. F.; Wills, N.; Uhlenbeck, O.; Gesteland, R. F. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 7127.

Amber Suppression with a Amber Suppressor tRNA Charged with Phe



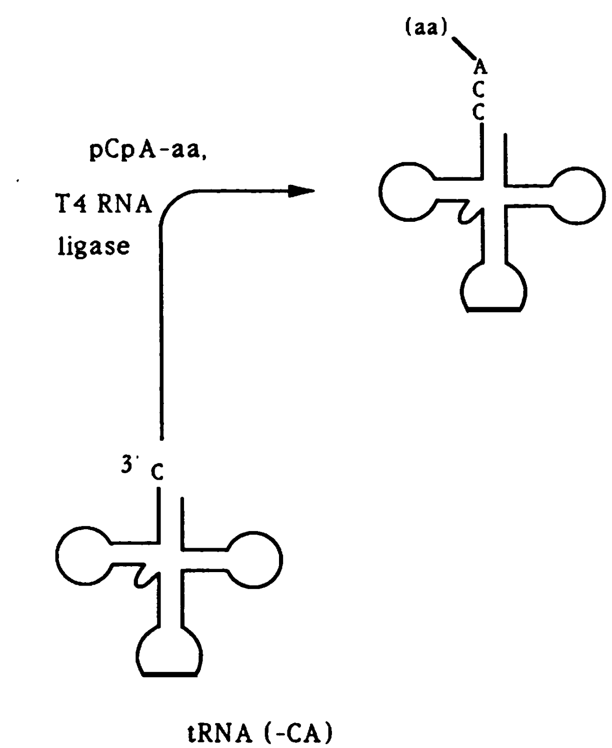


Full length protein expression is dependent upon presence of tRNA charged with phenylalanine

Bruce, A. G.; Atkins, J. F.; Wills, N.; Uhlenbeck, O.; Gesteland, R. F. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 7127.

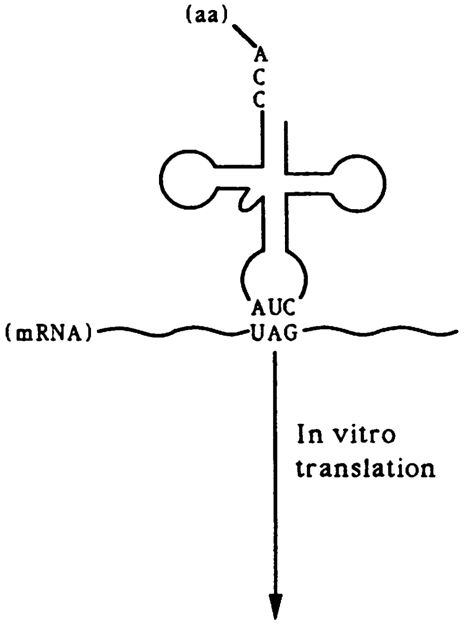
The Precedent for the Breakthrough

Precedent 1:
tRNAs can be charged
with any amino acid
using T4 ligase and pCpA-aa



Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

The Precedent for the Breakthrough



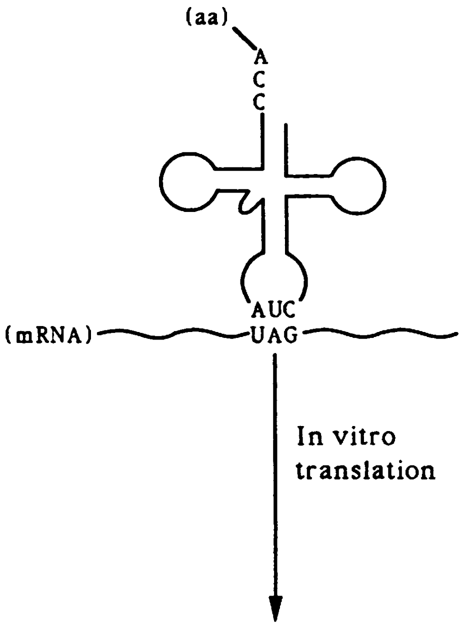
Precedent 2:
Phe can be incorporated
at amber codon

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids

Peter Schultz

Current Institution: Scripps Research



Precedent 2:
Phe can be incorporated at amber codon

"aa" can be an unnatural amino acid

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids

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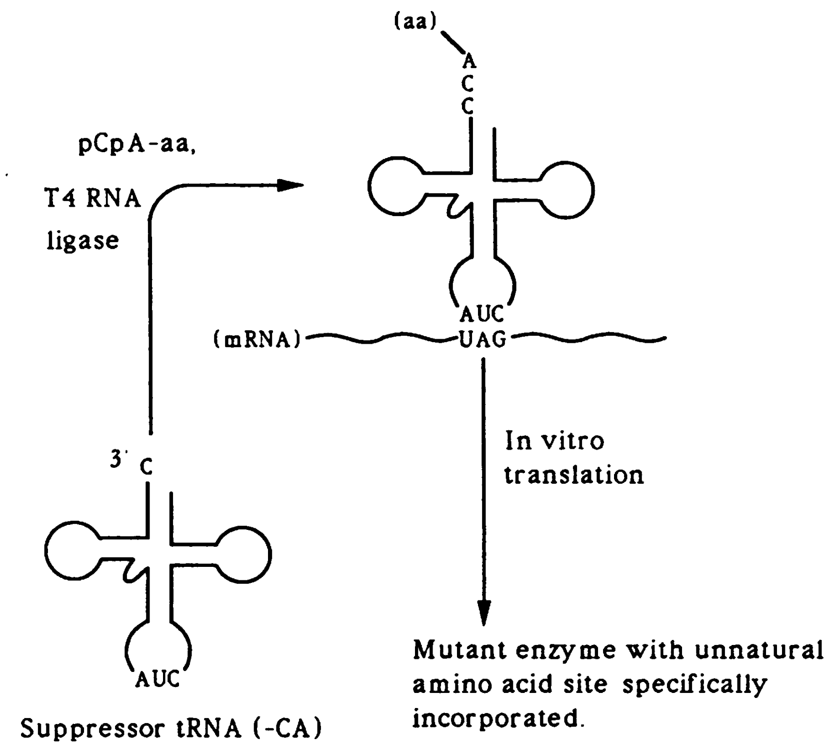


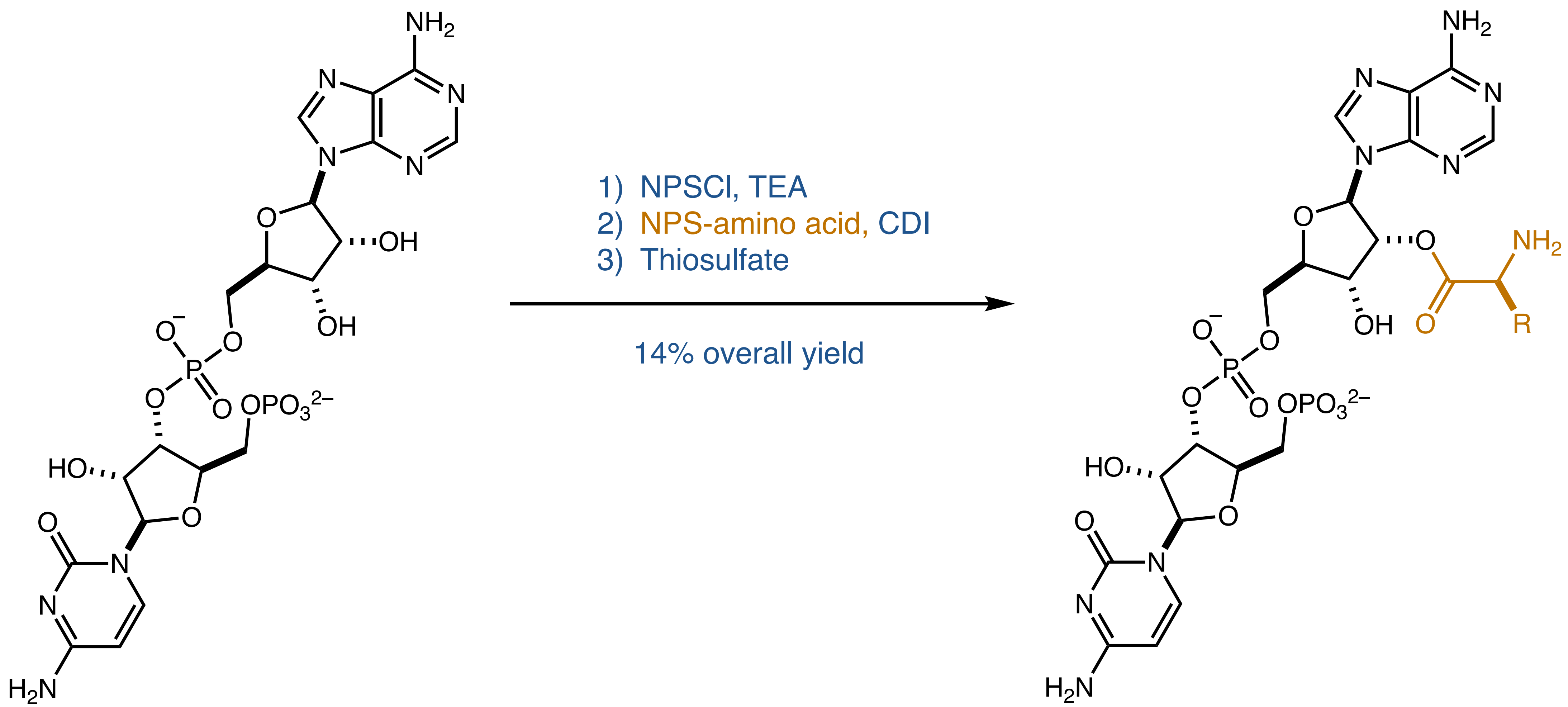
“aa” can be an unnatural amino acid

Can noncanonical amino acids be incorporated into proteins using this method?

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids

Preparation of pCpA charged with noncanonical amino acid

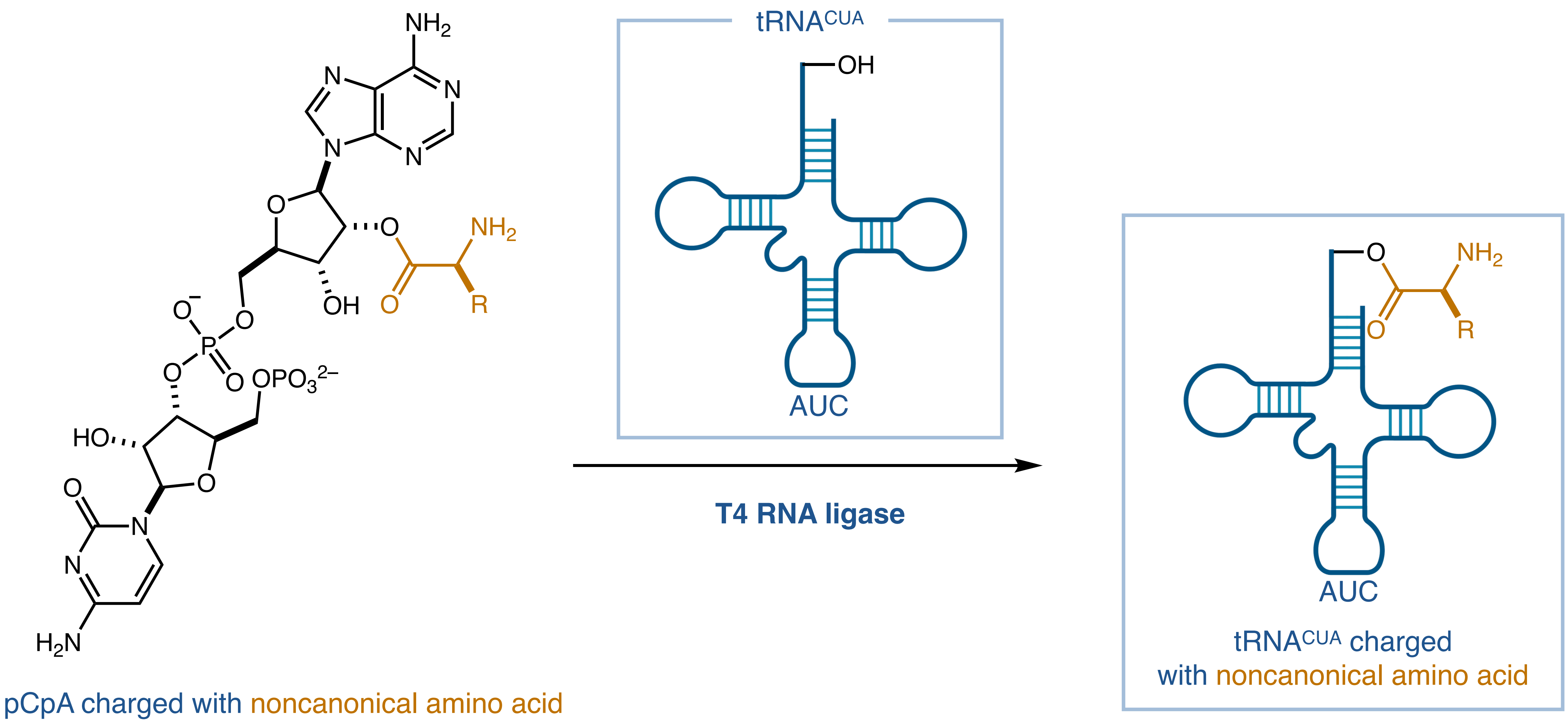


pCpA

pCpA charged with noncanonical amino acid

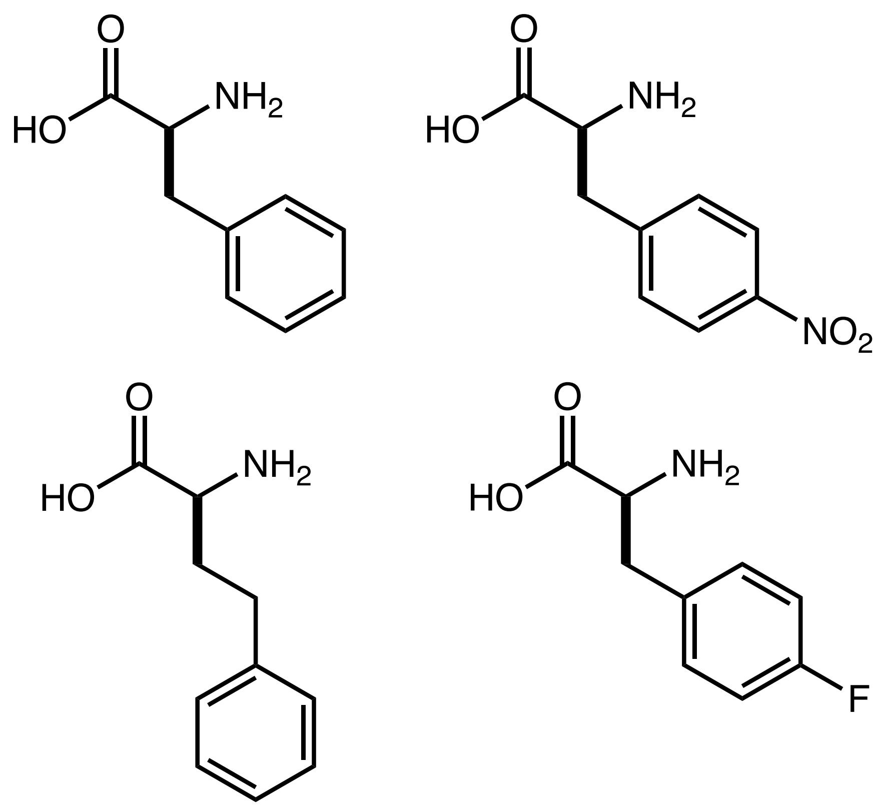
Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

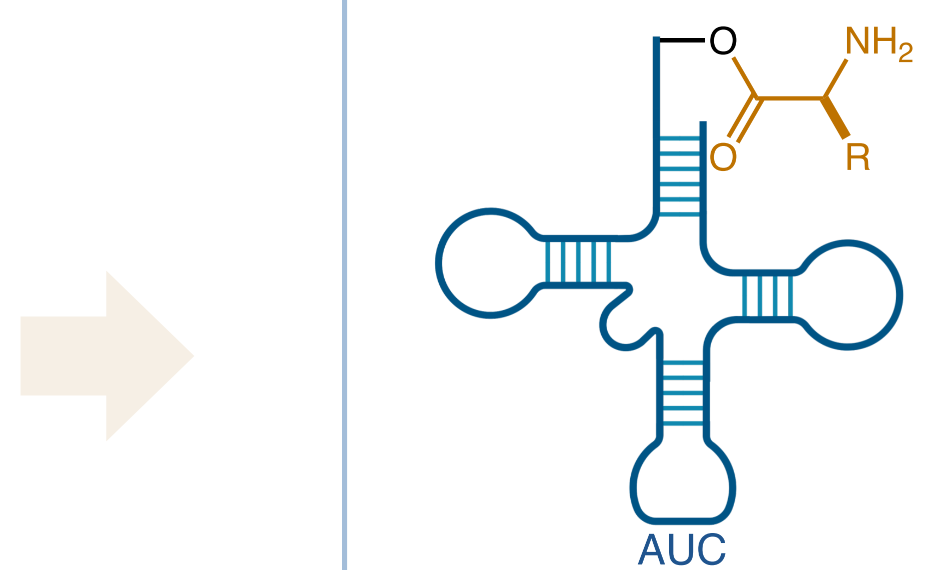
The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids



Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids

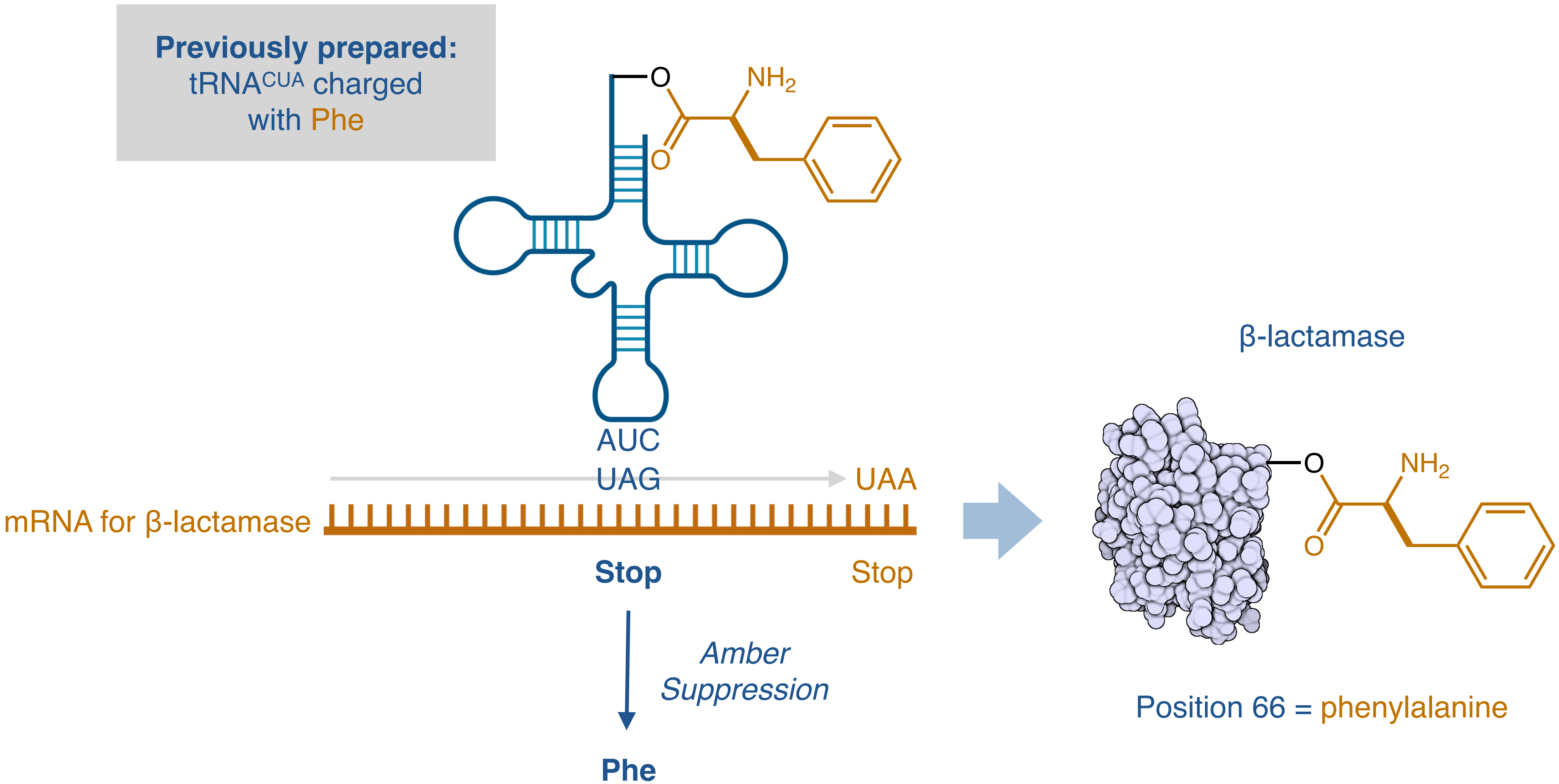




Method can be used to prepare tRNA^{CUA} with a variety of noncanonical amino acids

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

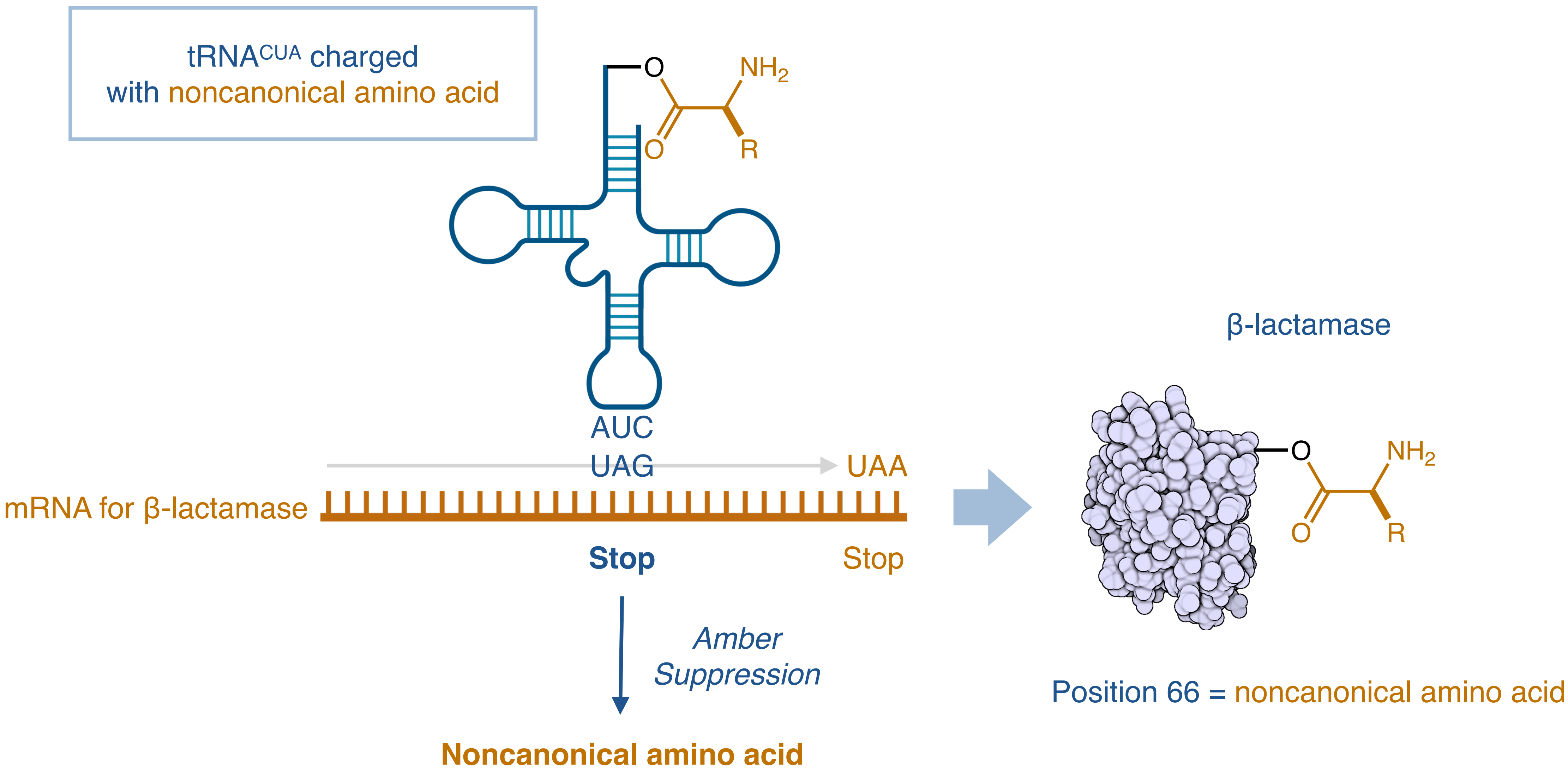
The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids



Native β-lactamase can be expressed in E. coli lysate using amber suppression

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

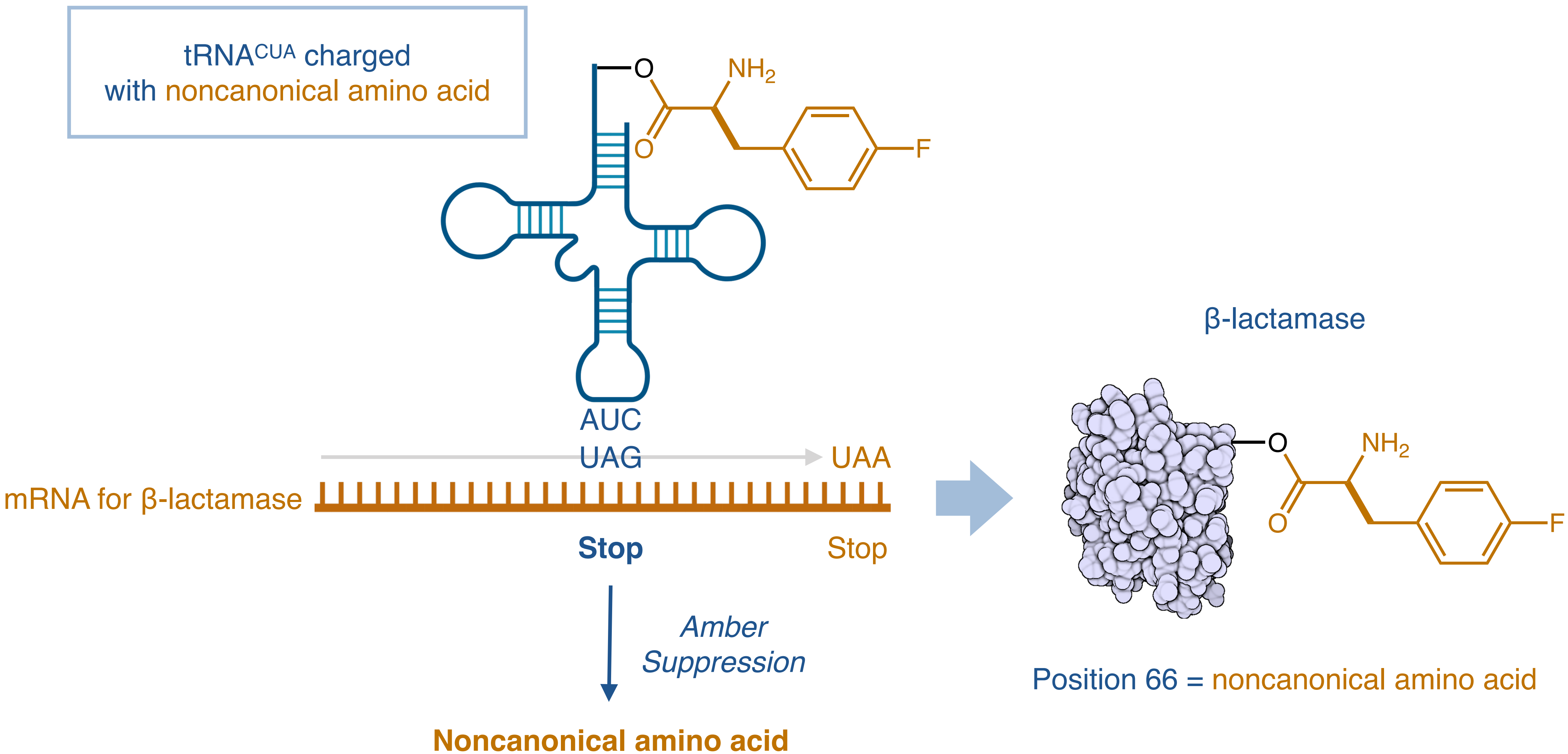
The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids



tRNA^{CUA} charged with noncanonical amino acid incorporates the noncanonical amino acid at position 66 in the protein

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

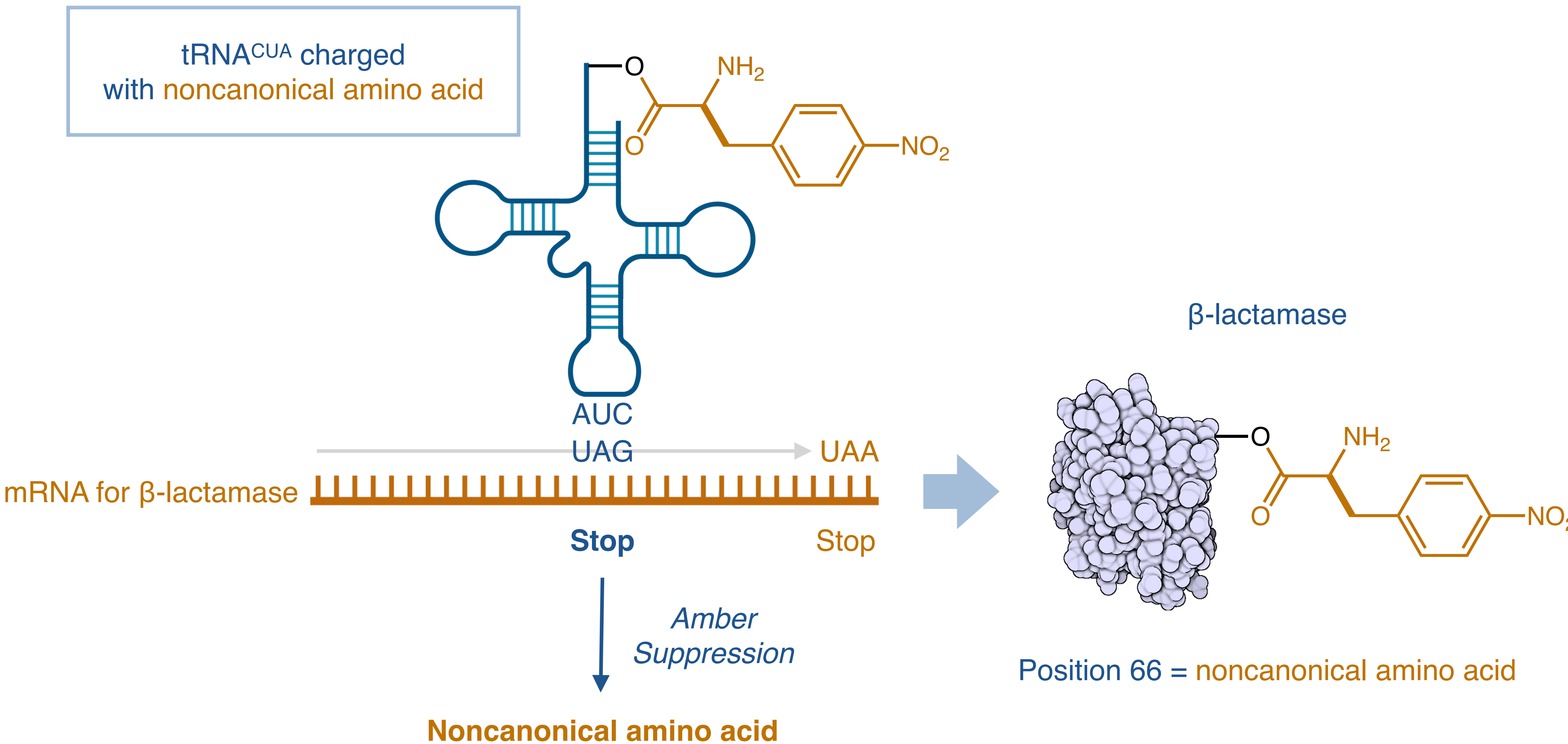
The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids



tRNA^{CUA} charged with noncanonical amino acid incorporates the noncanonical amino acid at position 66 in the protein

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

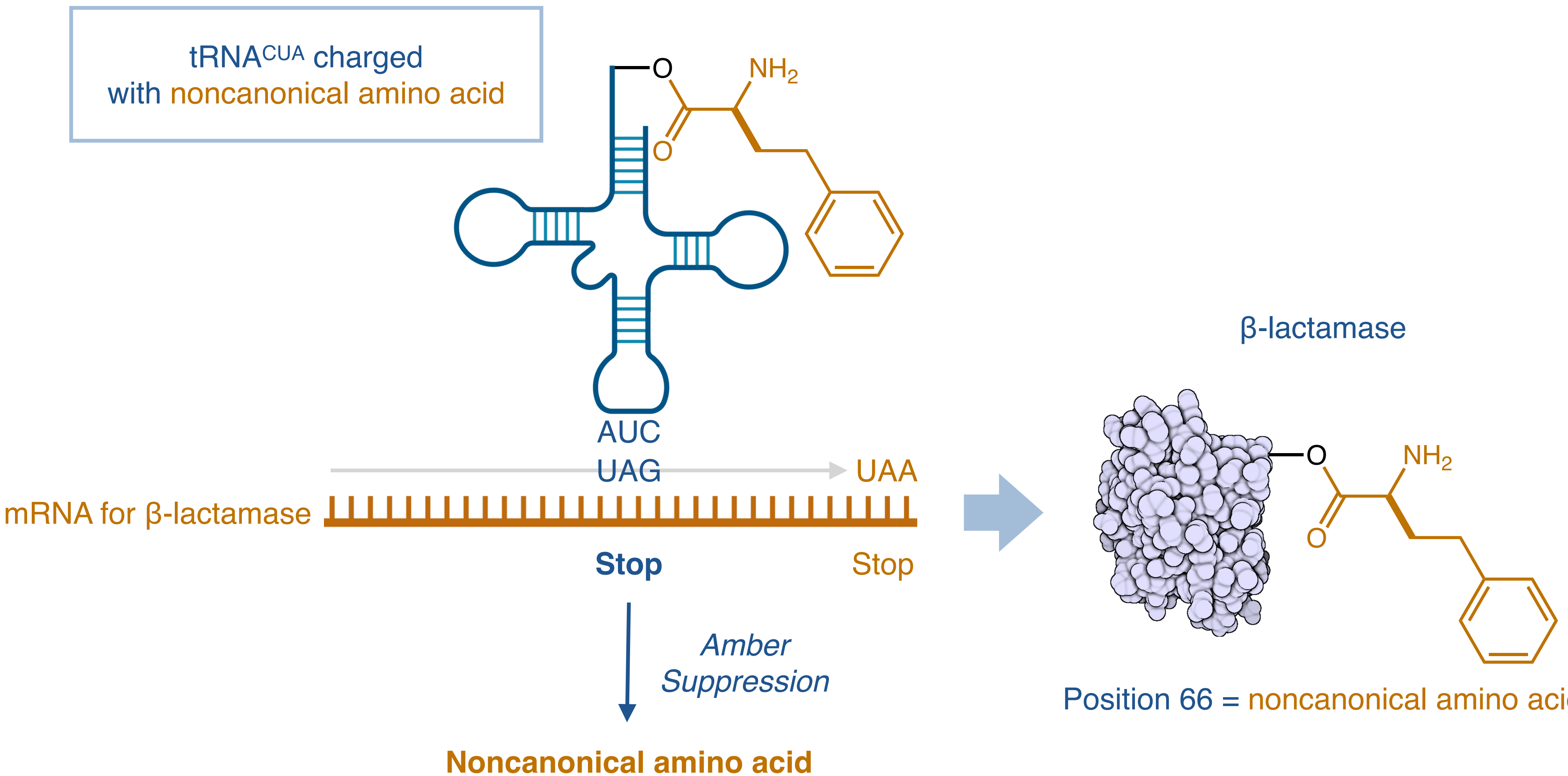
The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids



tRNA^{CUA} charged with noncanonical amino acid incorporates the noncanonical amino acid at position 66 in the protein

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids

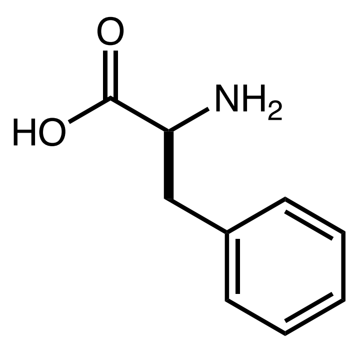
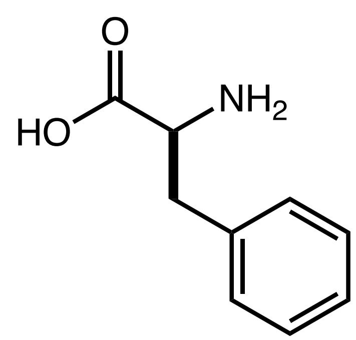
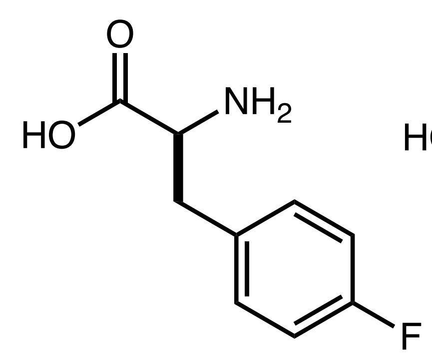
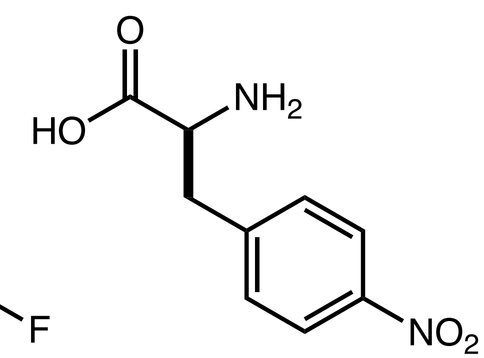
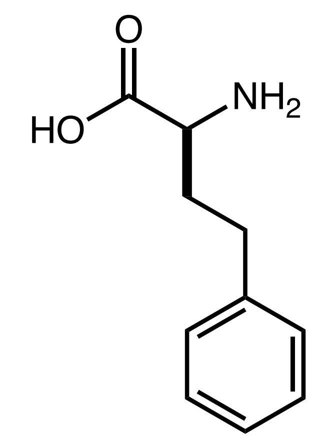


tRNA^{CUA} charged with noncanonical amino acid incorporates the noncanonical amino acid at position 66 in the protein

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

The Breakthrough: Amber Suppression to Incorporate Noncanonical Amino Acids

Properties of β-lactamase mutants with noncanonical amino acids

	wild type β-lactamase	β-lactamase prepared using $tRNA^{CUA}$ with Phe or noncanonical amino acid			
Amino acid at position 66 of β-lactamase					
K_m (substrate binding)	55 ± 5	59 ± 6	59 ± 2	57 ± 4	72 ± 14
k_{cat} (turnover #)	880 ± 10	870	1120 ± 290	370 ± 70	150 ± 60

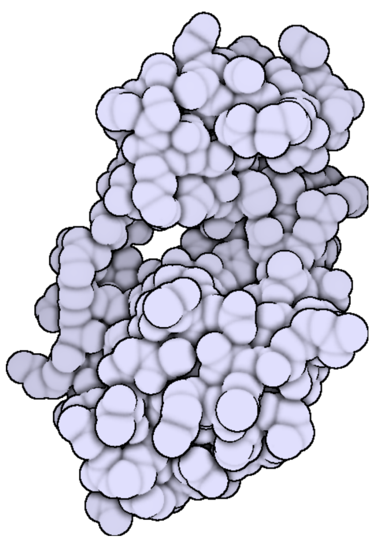
para-fluorophenylalanine increases enzyme turnover number

Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schutz, P. G. *Science* **1989**, *244*, 182.

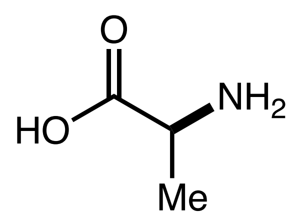
Expanding the Repertoire of Noncanonical Amino Acids

Expression efficiency (vs. wild type expression)

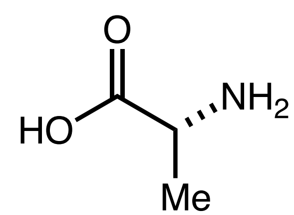
T4 Lysozyme



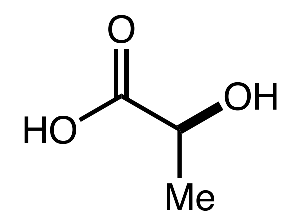
Position 82 =
noncanonical amino acid



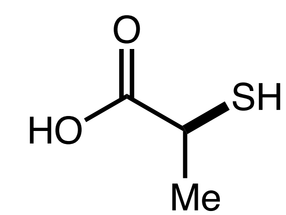
wild type



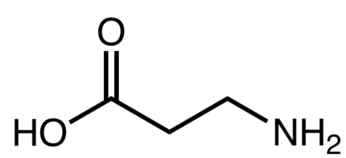
< 5%



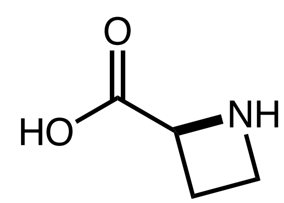
30%



incorporated, but efficiency not reported



< 5%



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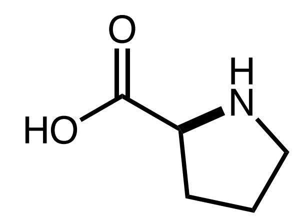
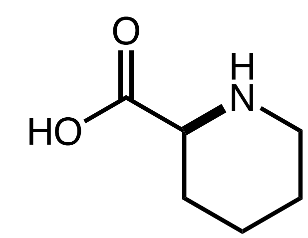
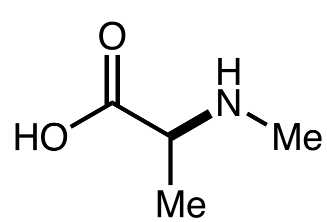


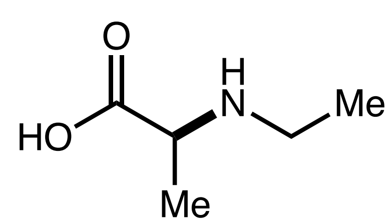
46%



43%



24%



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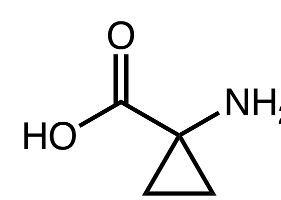
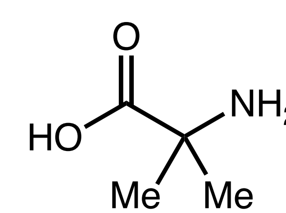


28%



23%

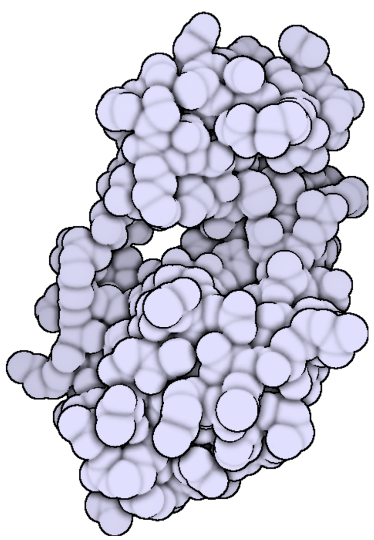
A diverse range of non canonical amino acids can be incorporated via amber suppression

Ellman, J. A.; Mendel, D.; Schultz, P. G. *Science* **1992**, *255*, 197.

Expanding the Repertoire of Noncanonical Amino Acids

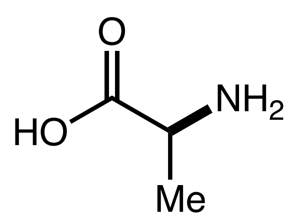
Thermodynamic stability (T_m)

T4 Lysozyme



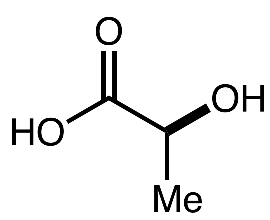
Position 82 =
noncanonical amino acid

Wild type

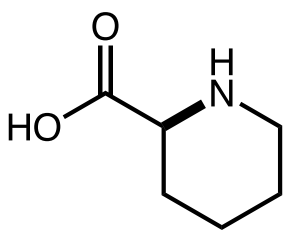


43.4 ± 0.25 °C

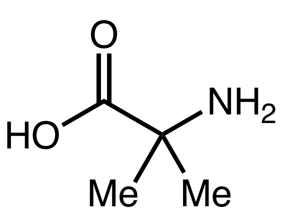
Noncanonical amino acids



39.7 ± 0.25 °C



41.3 ± 0.25 °C

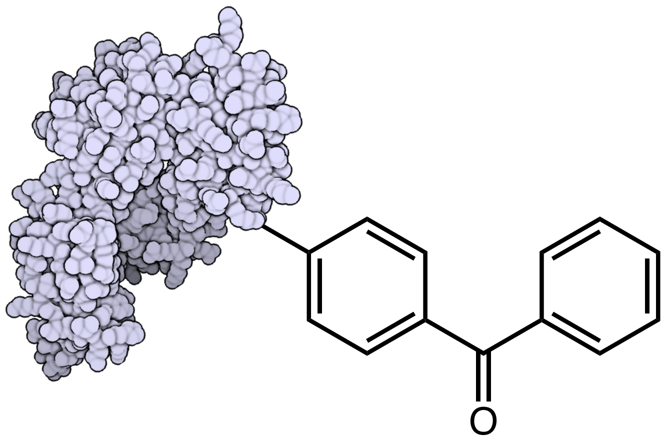


44.6 ± 0.25 °C

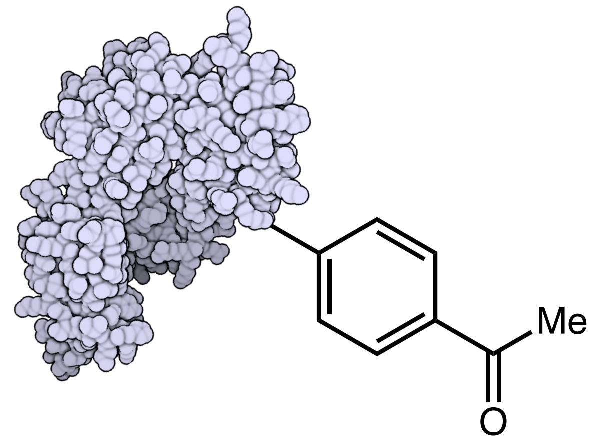
Changes in amino acid substitution at Ala82 affect thermal stability of the protein

Ellman, J. A.; Mendel, D.; Schultz, P. G. *Science* **1992**, *255*, 197.

Expanding the Repertoire of Noncanonical Amino Acids

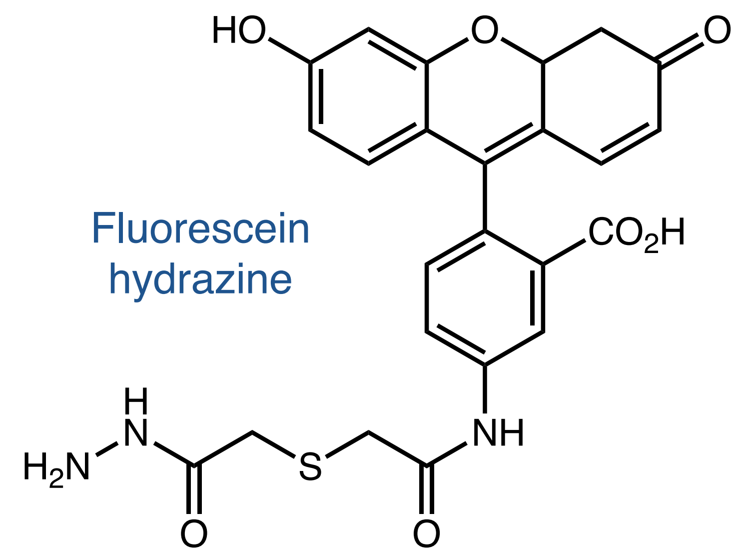


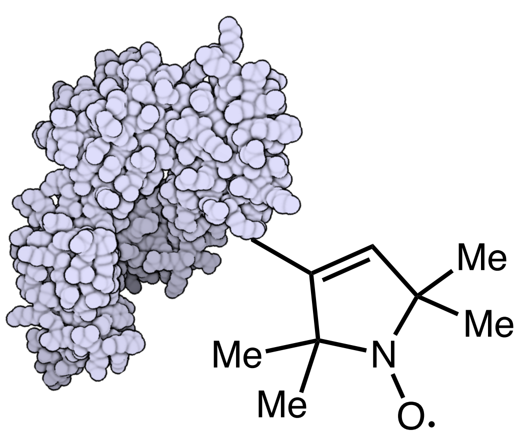
Photoaffinity label



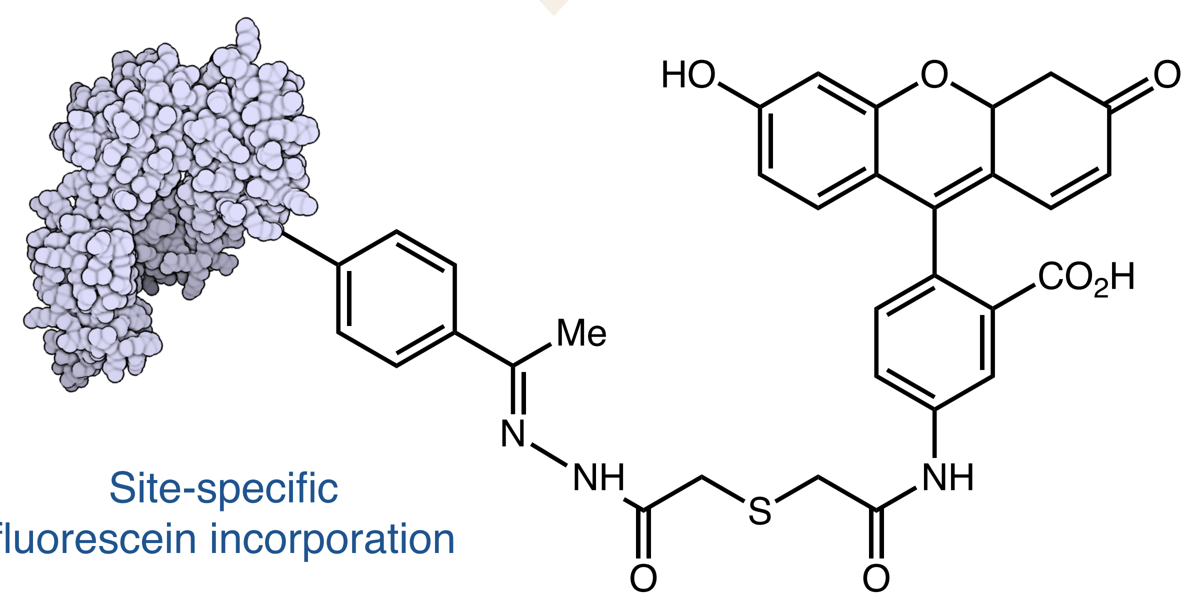
Ketone handle

Fluorescein hydrazine





EPR probe



Site-specific fluorescein incorporation

Cornish, V. W.; Hahn, K.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 8150.

Cornish, V. W. et al. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 2910.

Alternate Codons for UAA Incorporation: Quadruplet Codons

Stop Codons

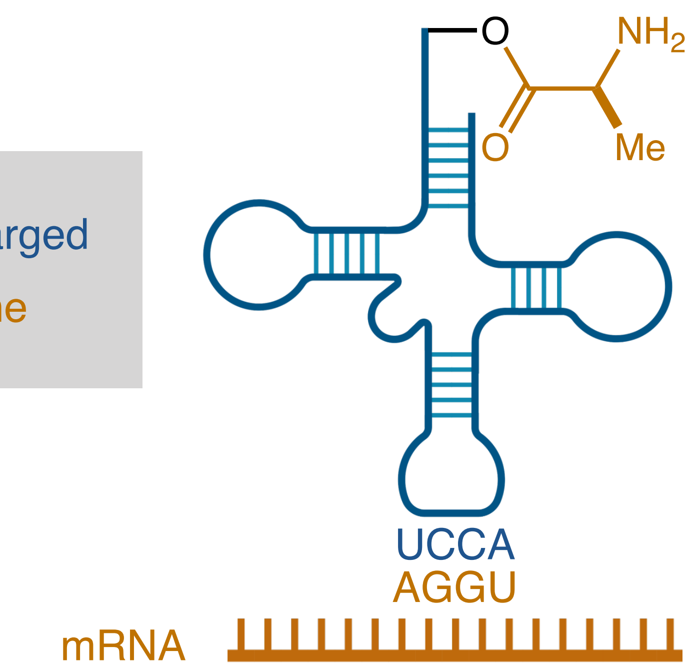
Amber	**UAG**
Ochre	UAA
Opal	UGA

Problem: limited range (3) of endogenous codons to suppress

Solution: quadruplet codons

ACCU	CCCU	CUAU
AGGU	CUCU	CCUA
CGGU	GGGU	

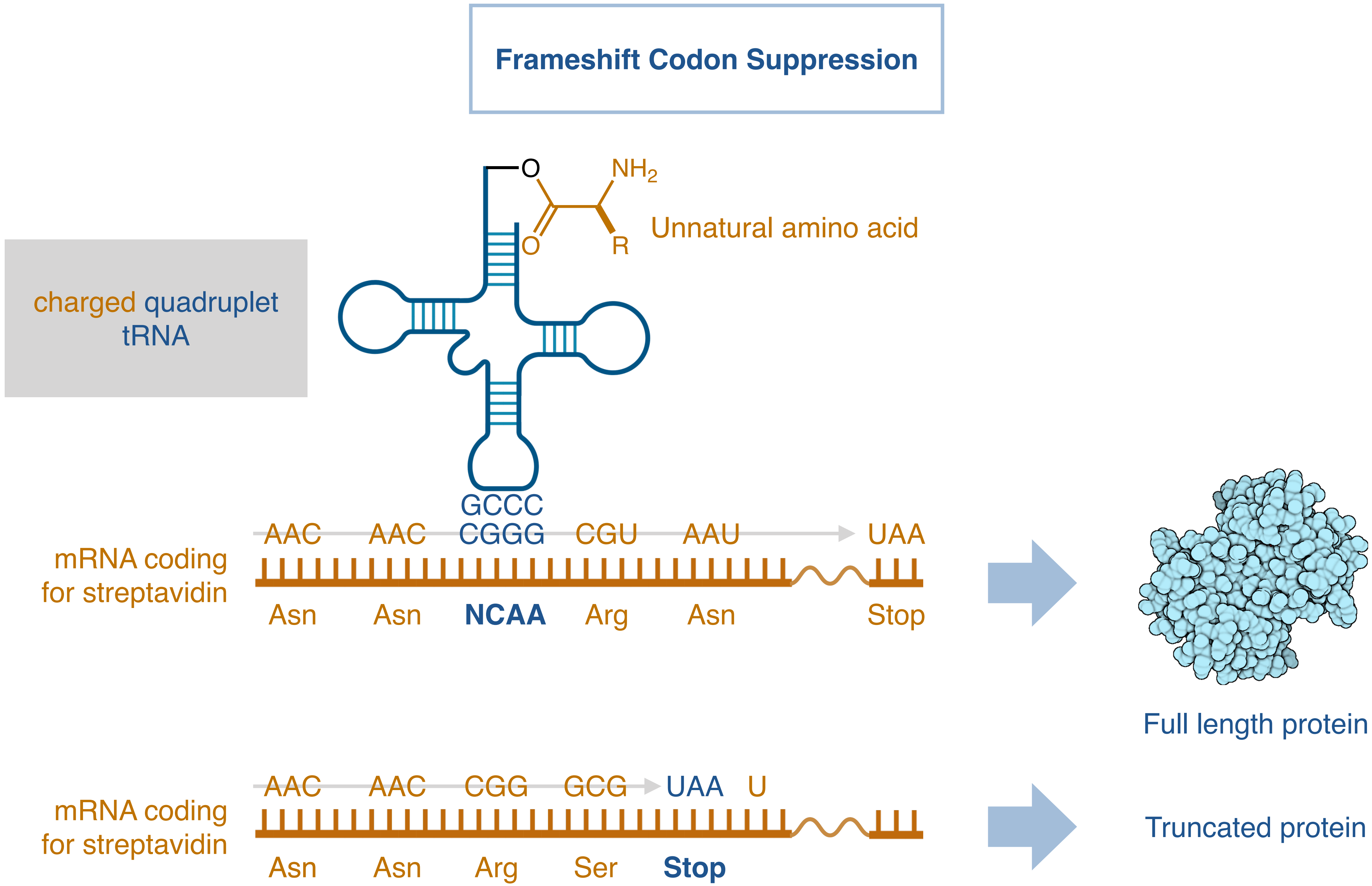
$tRNA^{ACCU}$ charged with alanine



Alanine is incorporated at UCCA codon

Ma, C.; Kudlicki, W.; Odom, O. W.; Kramer, G.; Hardesty, B. *Biochemistry* **1993**, *32*, 7939.

Alternate Codons for UAA Incorporation: Quadruplet Codons

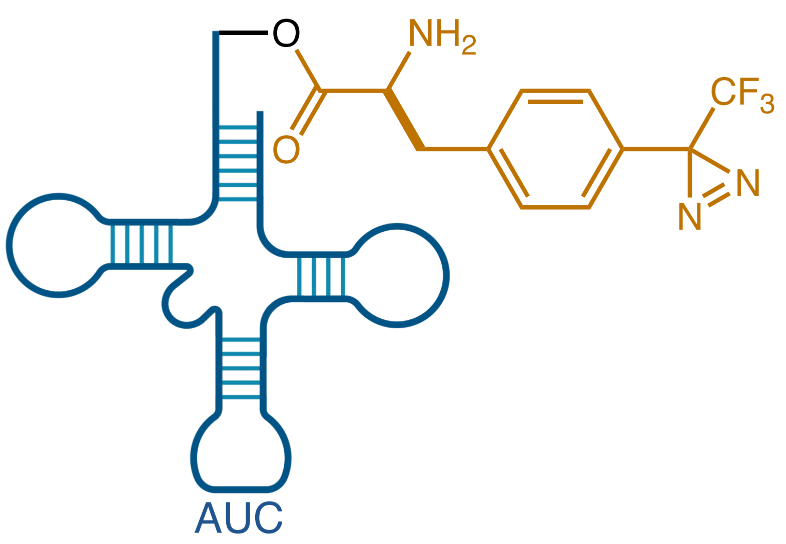


When the frameshift does not happen, a termination codon (UAA) appears, forming the truncated protein

Hohsaka, T.; Ashizuka, Y.; Taira, H.; Murakami, H.; Sisido, M. *Biochemistry* **2001**, *40*, 11060.

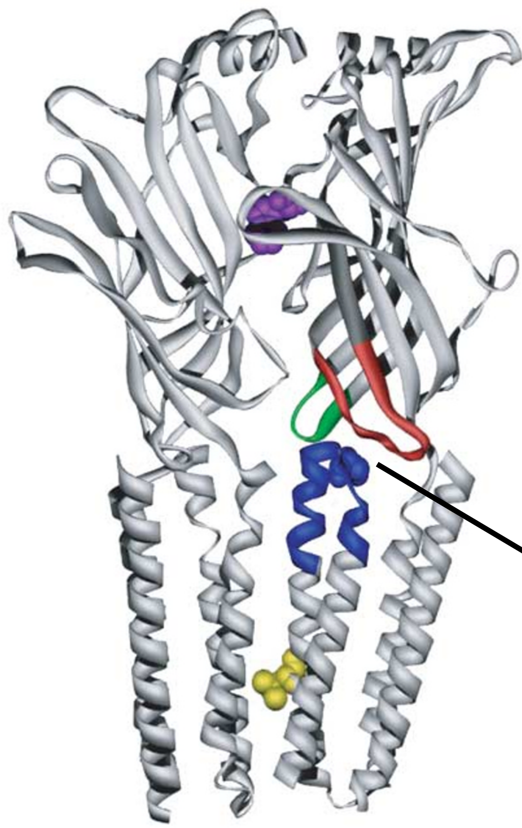
Expanding the Genetic Code

- The traditional genetic code
- Expanding the genetic code
    - In vitro
    - In eukaryotes, prokaryotes, and mammalian cells
- Orthogonal ribosomes
- Genetically recoded organisms and synonymous codon compression
- Outlook

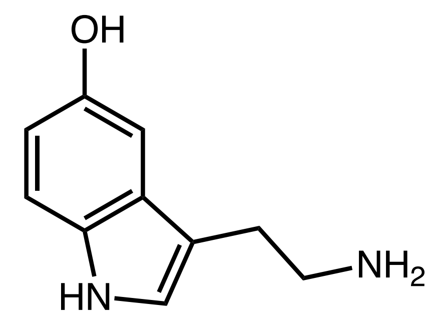


Incorporation of UAAs in Eukaryotic Systems

5-hydroxytryptamine type 3 receptor (5-HT₃)



serotonin binding leads to ion channel opening



5-hydroxytryptamine (serotonin)

Proline 8

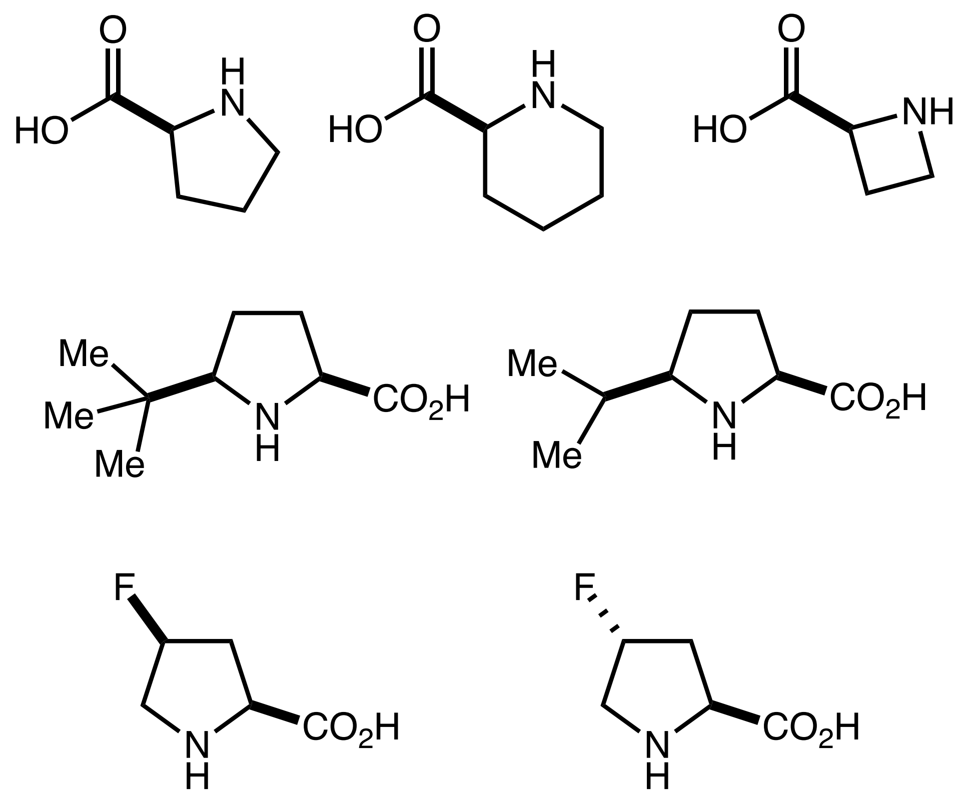
Hypothesis: Proline 8 plays an important role linking serotonin binding to channel opening through proline cis-trans isomerization

Question: How does cis-trans isomerization of proline 8 influence ion channel opening?

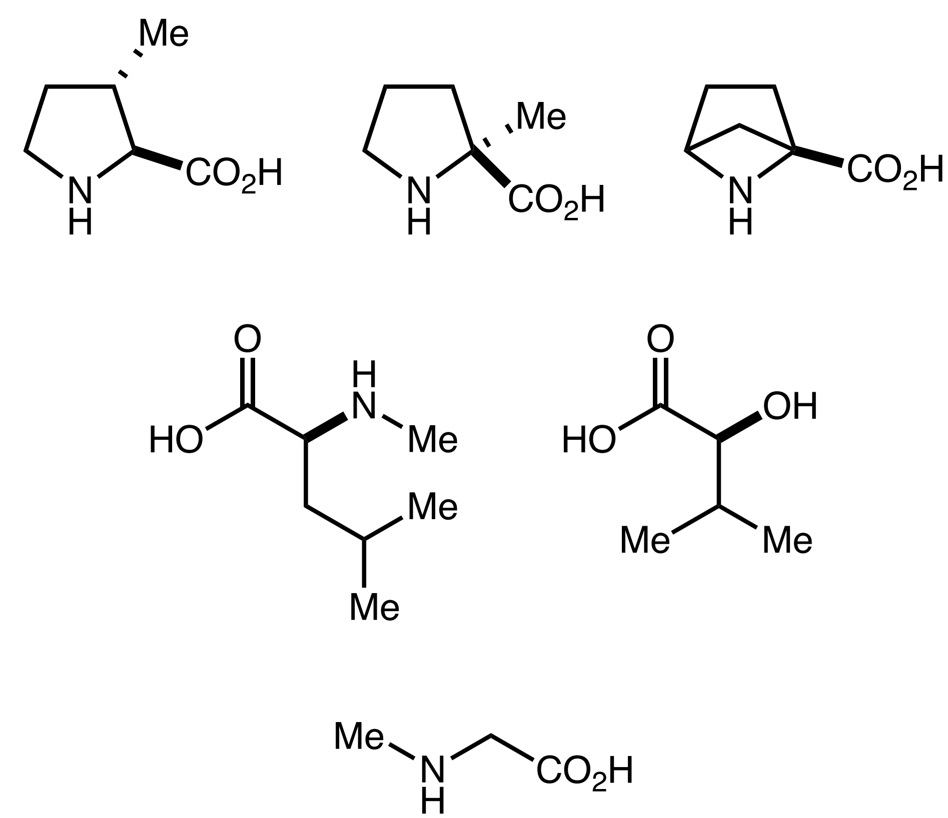
Lumis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; Broadhurst, R. W.; Dougherty, D. A. *Nature* **2005**, *438*, 248.

Incorporation of UAAs in Eukaryotic Systems

Proline analogues favoring the cis conformer

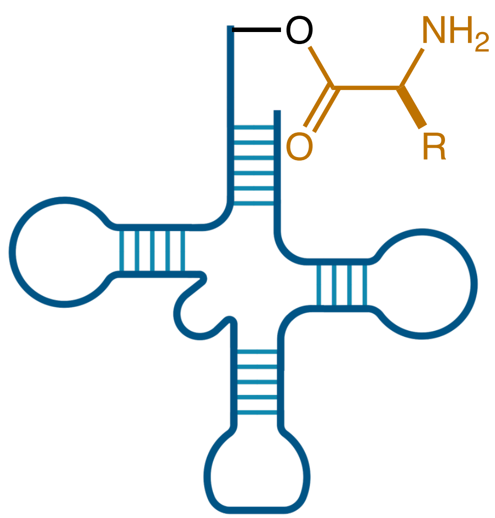


Proline analogues favoring the trans conformer

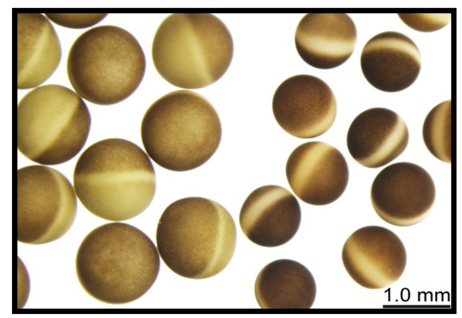


Lumis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; Broadhurst, R. W.; Dougherty, D. A. *Nature* **2005**, *438*, 248.

Incorporation of UAAs in Eukaryotic Systems



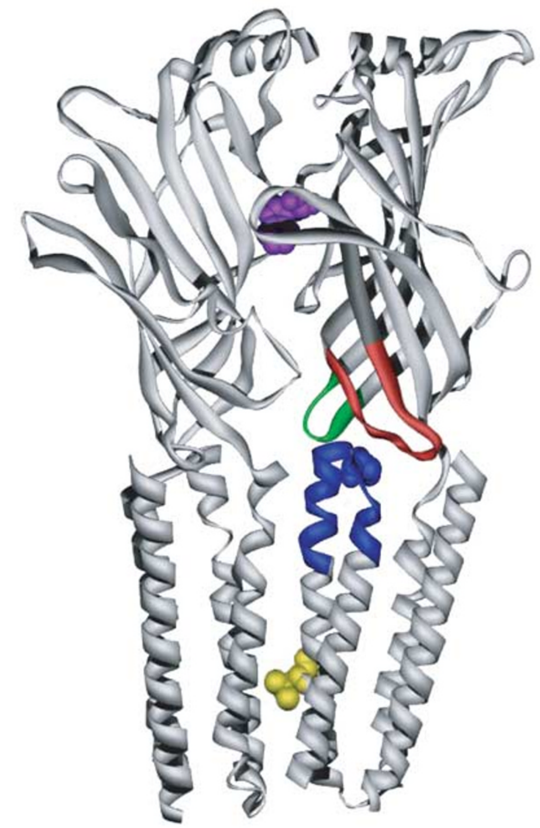
tRNA^CUA charged with proline analogue



Xenopus oocytes
(Frog egg)

5-HT$_3$ mRNA with amber codon at position 8

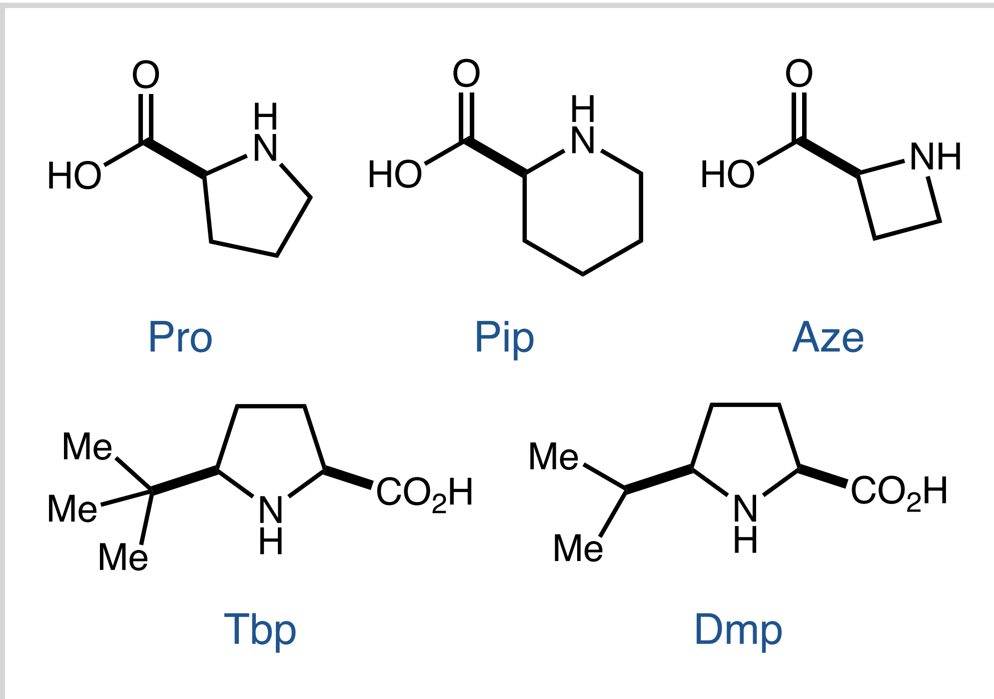
5-hydroxytryptamine type 3 receptor (5-HT$_3$)



5-HT$_3$ with proline analogue at position 8

Lumis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; Broadhurst, R. W.; Dougherty, D. A. *Nature* **2005**, *438*, 248.

Incorporation of UAAs in Eukaryotic Systems



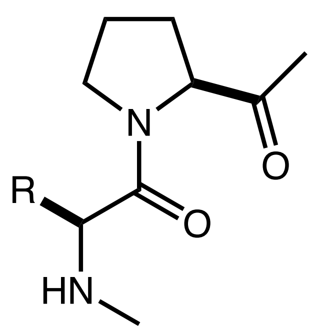
Residue	Per cent *cis**	EC50 (μM)†
Pro	5	1.29 ± 0.07
Pip	12	0.75 ± 0.06
Aze	18	0.42 ± 0.03
Tbp	55	0.030 ± 0.024
Dmp	71	0.021 ± 0.009

Percent cis of proline isomer and EC_{50} for serotonin dependent ion channel opening are correlated

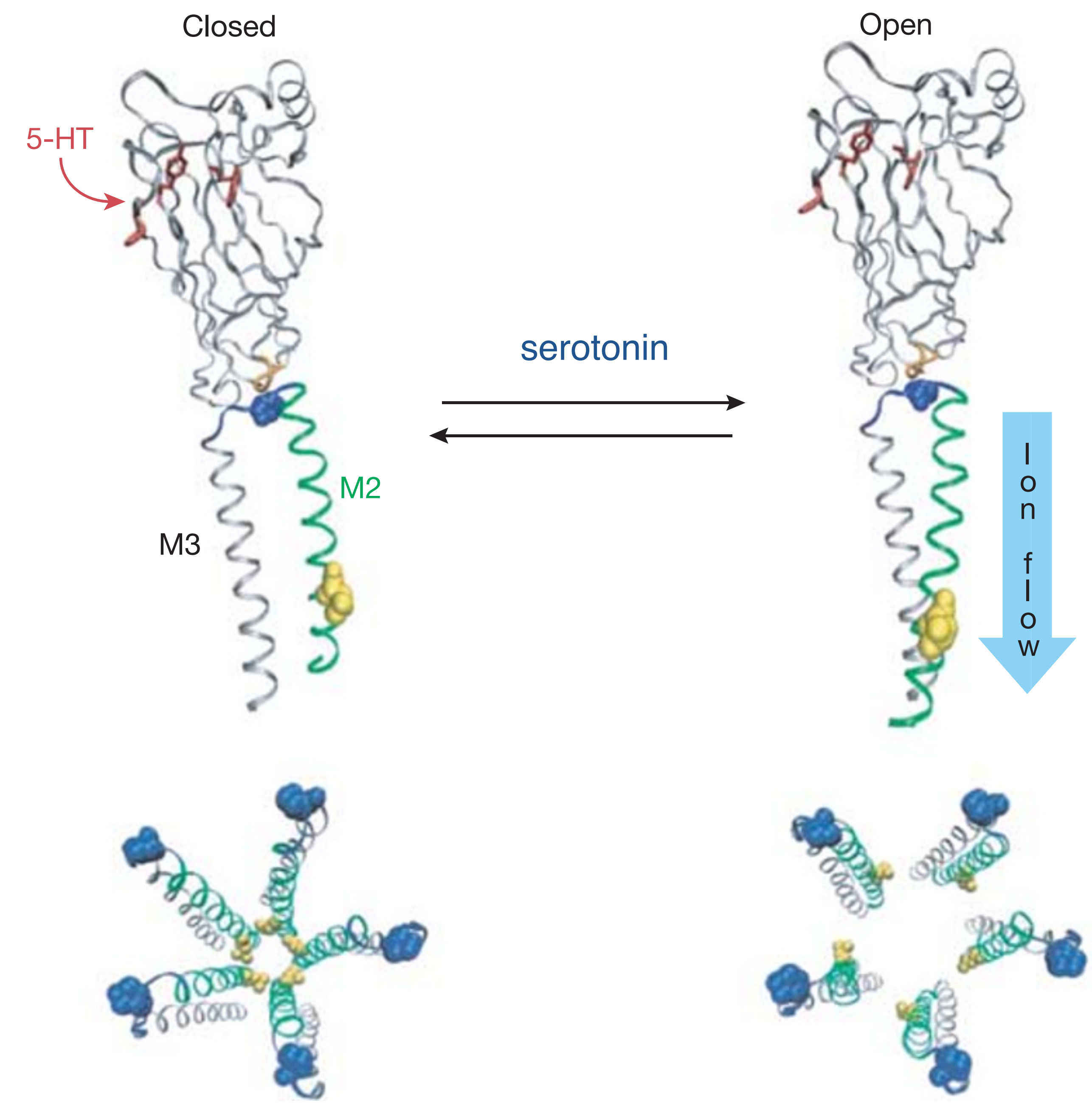
Proline analogues favoring the cis isomer produce an ion channel highly dependent on serotonin binding

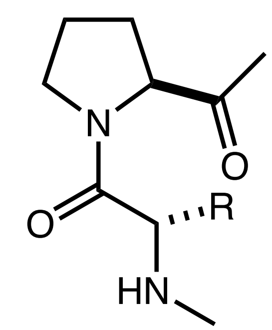
Lumis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; Broadhurst, R. W.; Dougherty, D. A. *Nature* **2005**, *438*, 248.

Incorporation of UAAs in Eukaryotic Systems



trans proline



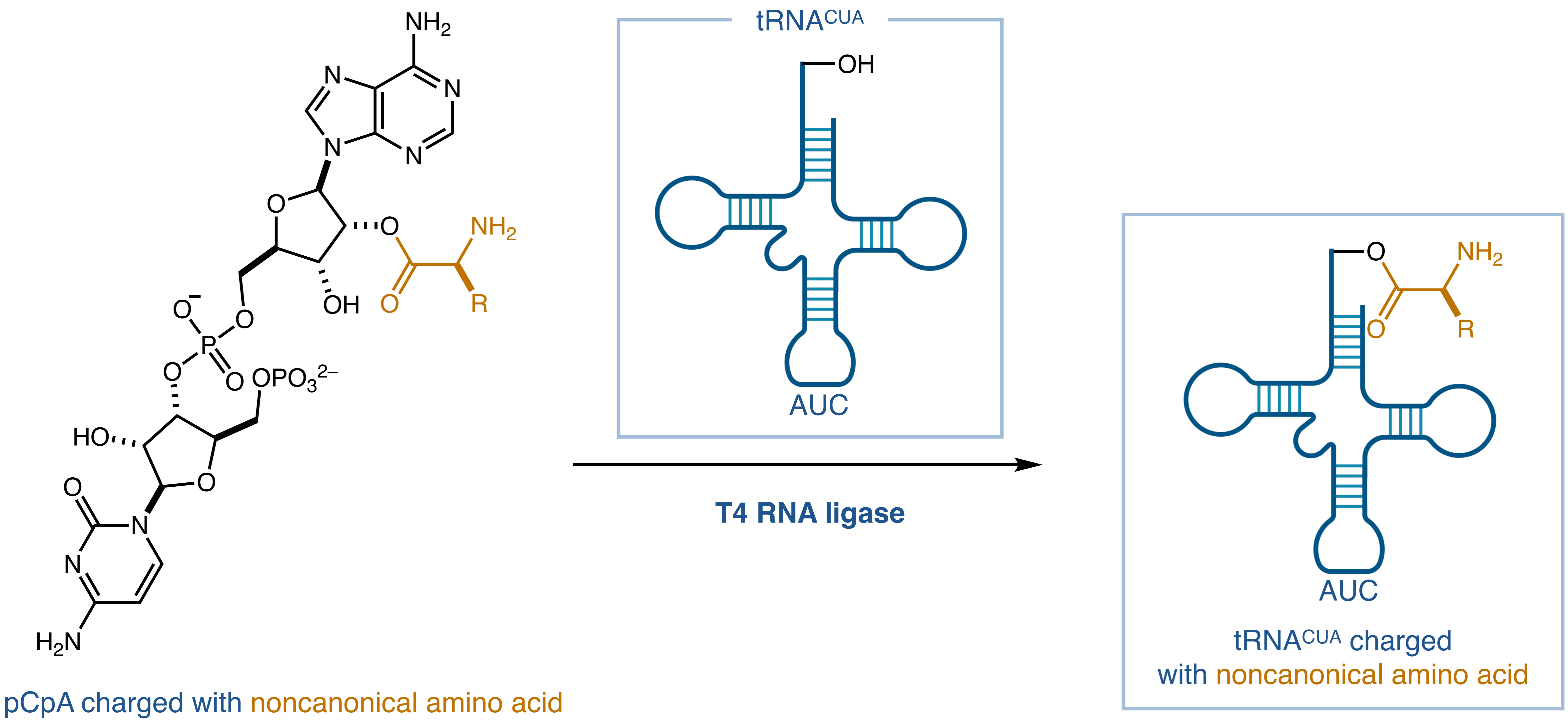


cis proline

Results suggest cis-trans isomerization of proline 8 interconverts the open and closed states of the ion channel

Lumis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; Broadhurst, R. W.; Dougherty, D. A. *Nature* **2005**, *438*, 248.

Noncanonical Amino Acid Incorporation in Live Cells

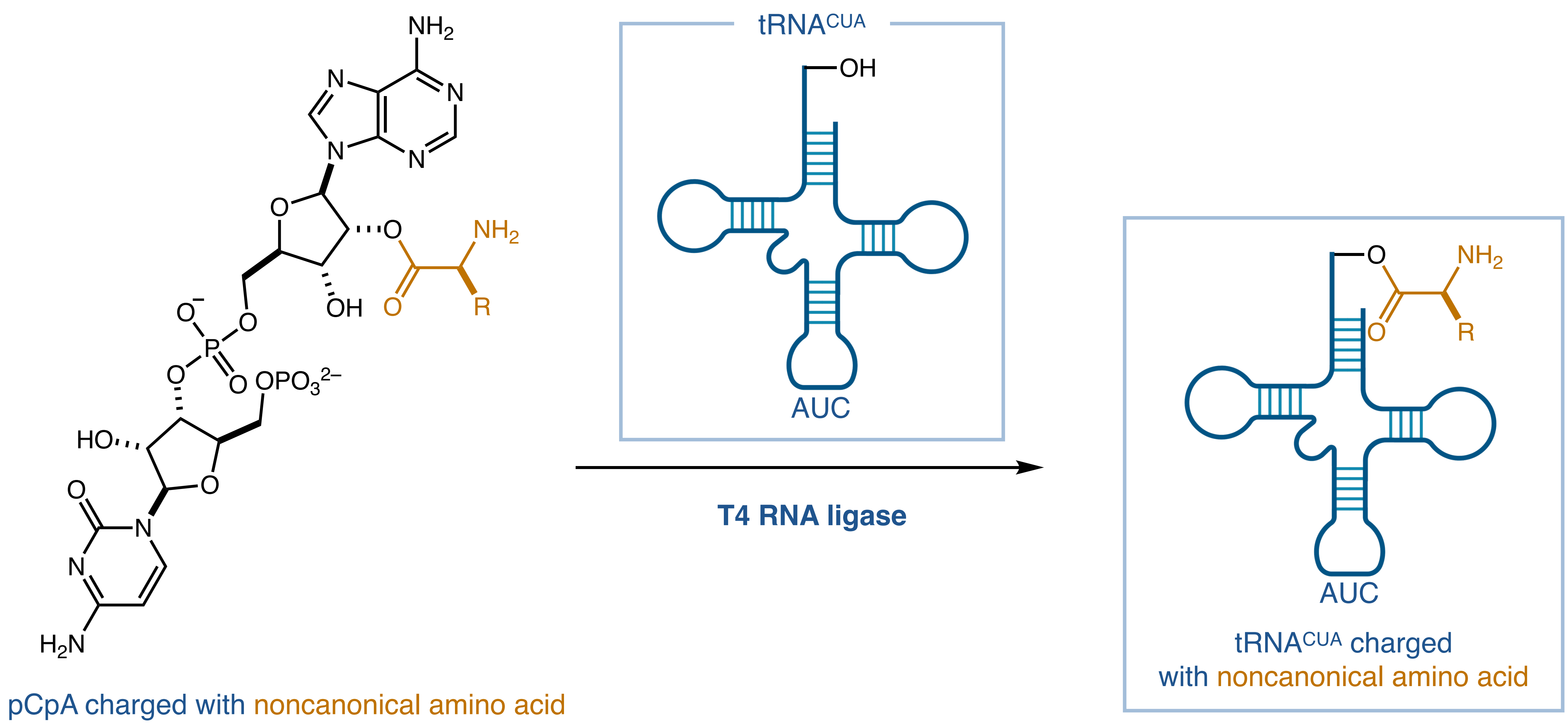


All works mentioned thus far prepared tRNA^{CUA} charged with non canonical amino acid via T4 RNA ligase

Question: Can the tRNA^{CUA} charged with the non canonical amino acid be generated directly in cells?

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

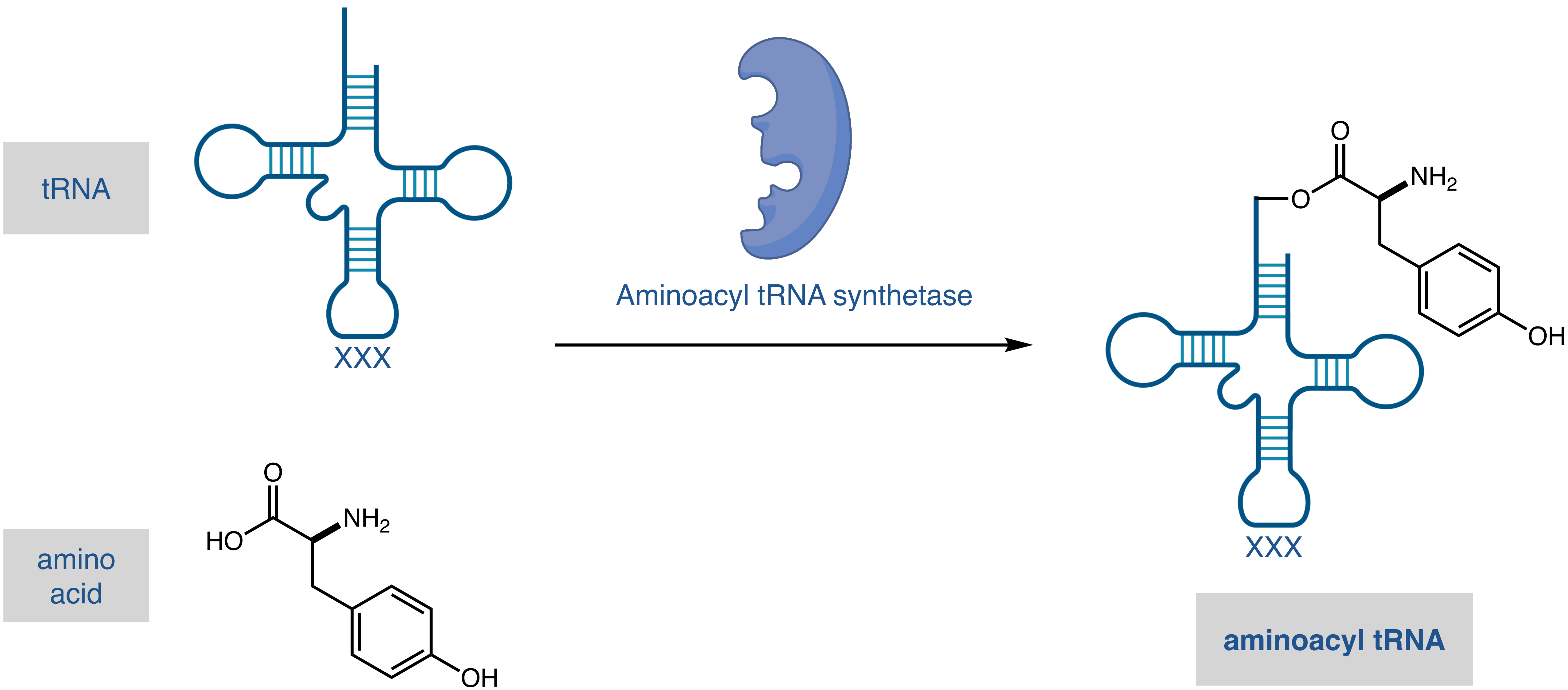
Noncanonical Amino Acid Incorporation in Live Cells



Solution: Express enzymatic machinery to generate aminoacylated tRNA directly E. coli

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

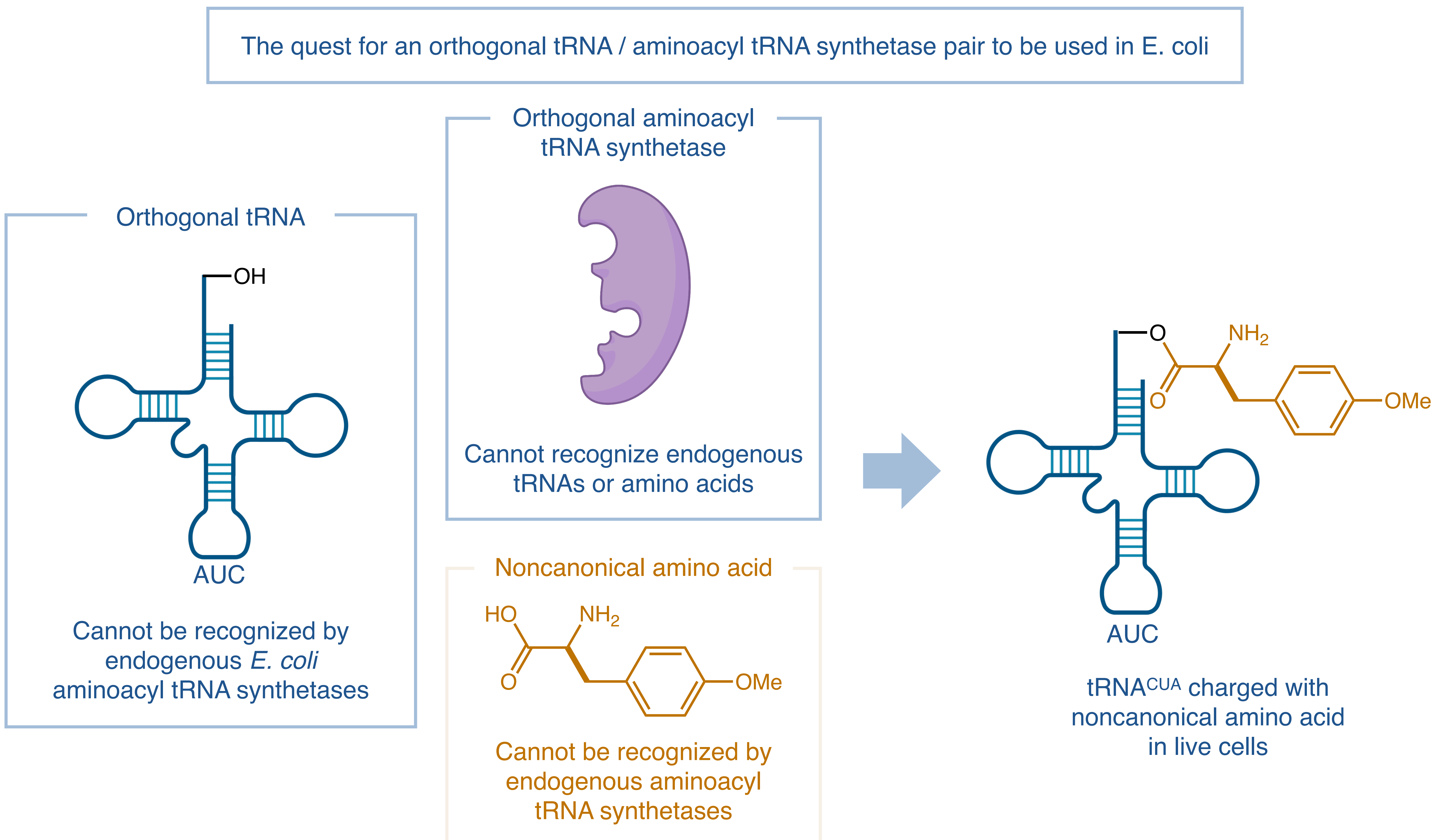


The aminoacyl tRNA synthetase is responsible for “charging” the tRNA with amino acid to form an aminoacyl tRNA

For the 20 canonical amino acids, there are at least 20 different aminoacyl tRNAs charged by 20 different aminoacyl tRNA synthetases

Shandell, M. A.; Tan, Z.; Cornish, V. W. et al. *Biochemistry* **2021**, *60*, 3455.

Noncanonical Amino Acid Incorporation in Live Cells

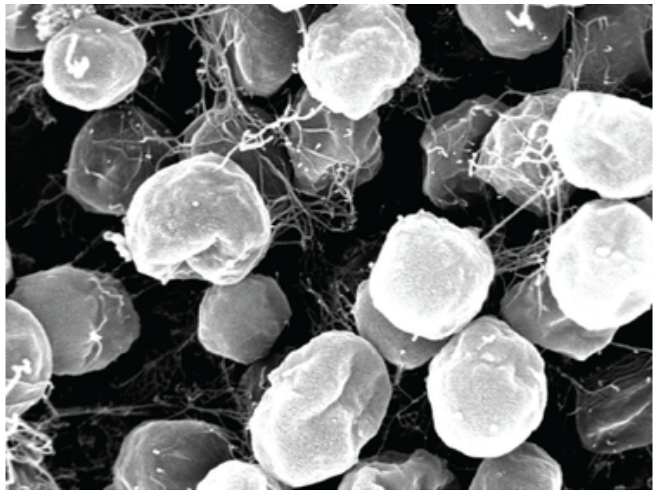


Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

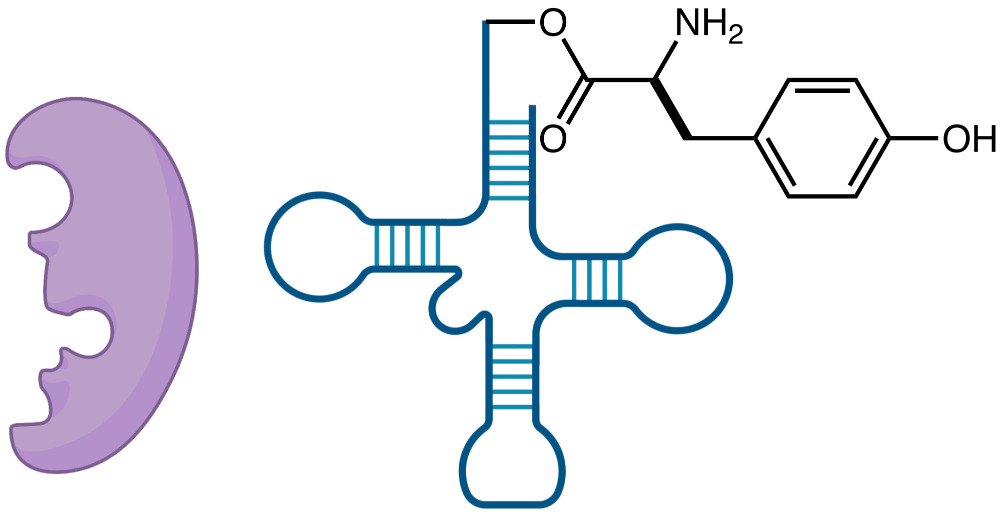
The quest for an orthogonal tRNA / aminoacyl tRNA synthetase pair to be used in E. coli

Methanococcus jannaschii



Hyperthermophilic organism belonging to the kingdom Archaea

The tyrosyl tRNA / aminoacyl tRNA synthetase pair from M. jannaschii



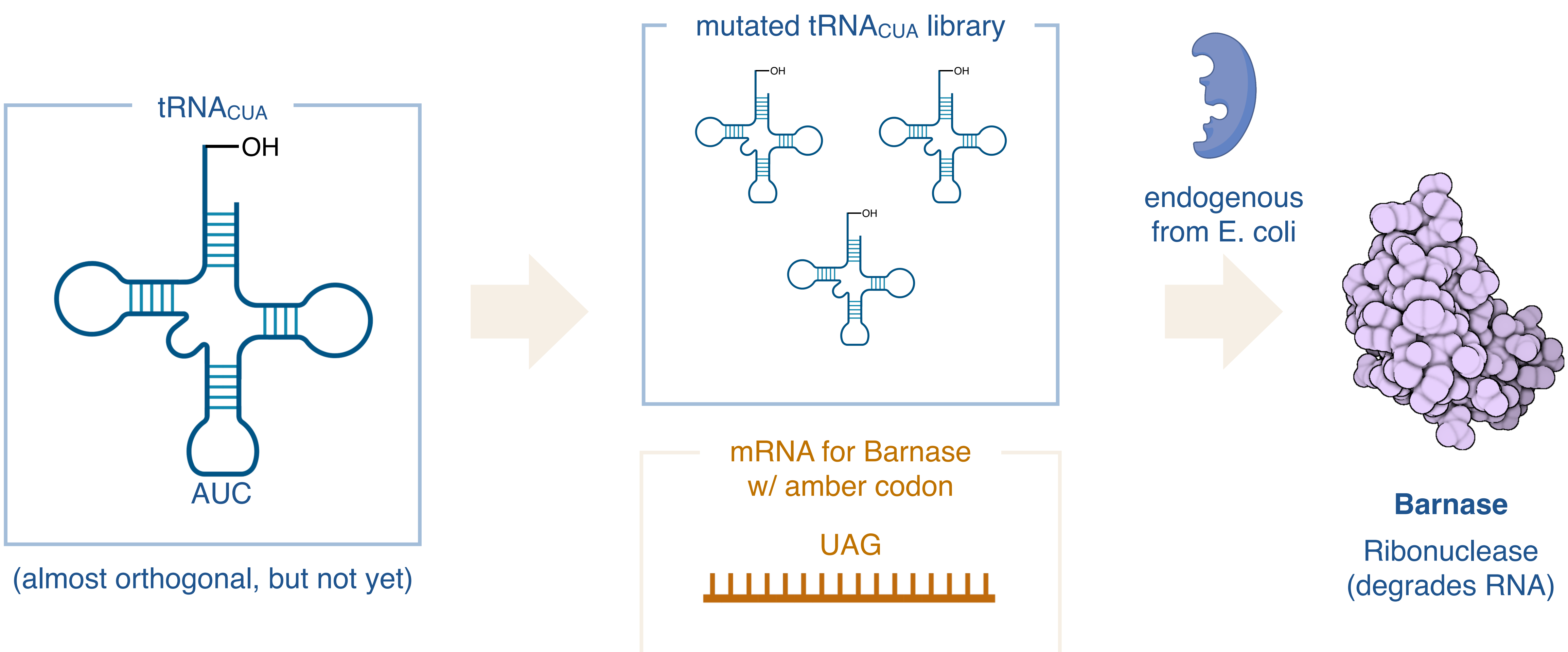
- tRNA synthetase not recognize E. coli tRNAs
- Previously shown to charge $tRNA^{CUA}$ with Tyr
- Can perform amber suppression in E. coli

Can the tyrosyl tRNA / aminoacyl tRNA synthetase pair from *Methanococcus* be evolved to be orthogonal?

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

Problem: orthogonal tRNA is still recognized by E. Coli aminoacyl tRNA synthetases



Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

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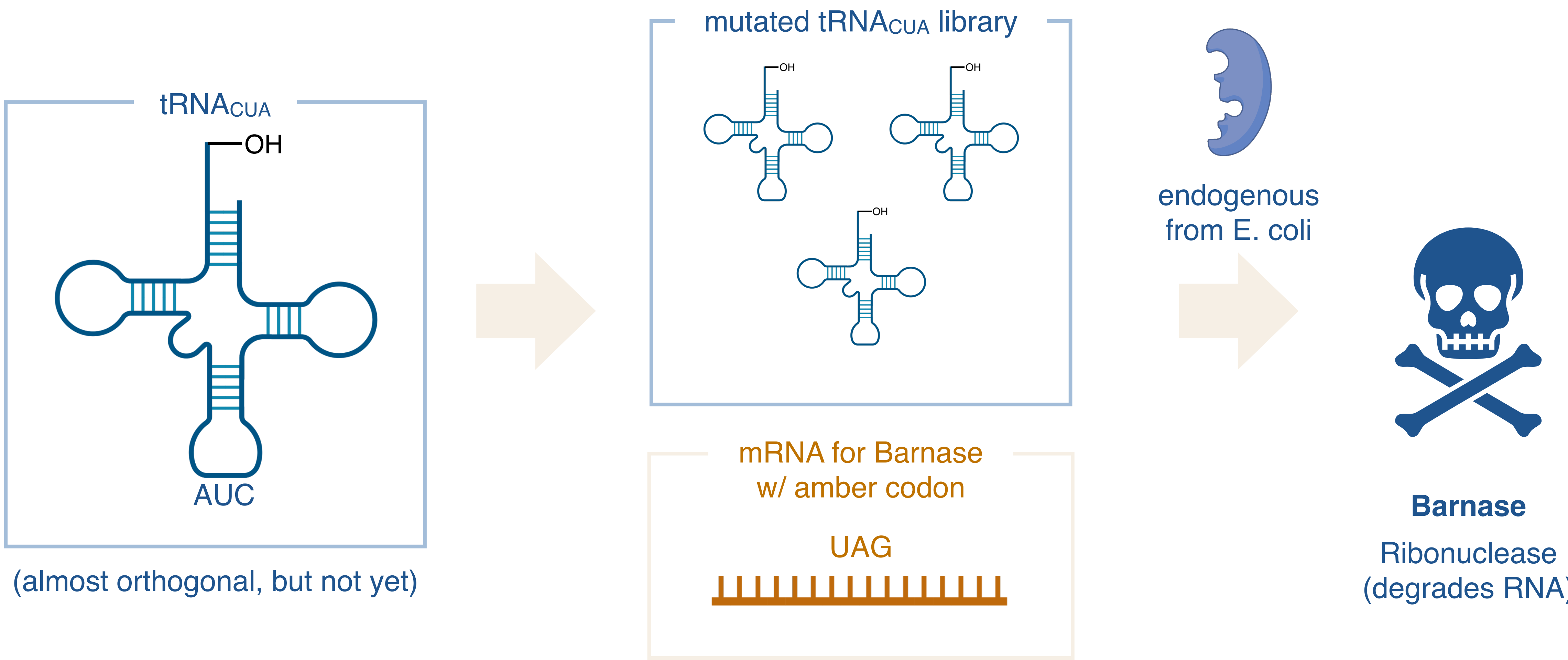


Negative selection: If endogenous aminoacyl tRNA synthetase charges **tRNA$_{CUA}$,** barnase is produced, leading to cell death

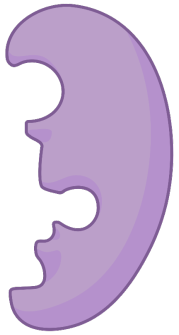
Result: Orthogonal tRNA^{CUA} that cannot be recognized by E. coli aminoacyl tRNA synthetases

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

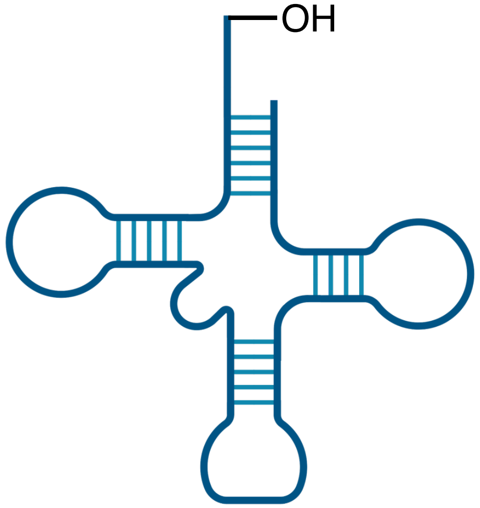
Noncanonical Amino Acid Incorporation in Live Cells

Goal: Evolve an orthogonal aminoacyl tRNA synthetase

aminoacyl tRNA synthetase from M. jannaschii



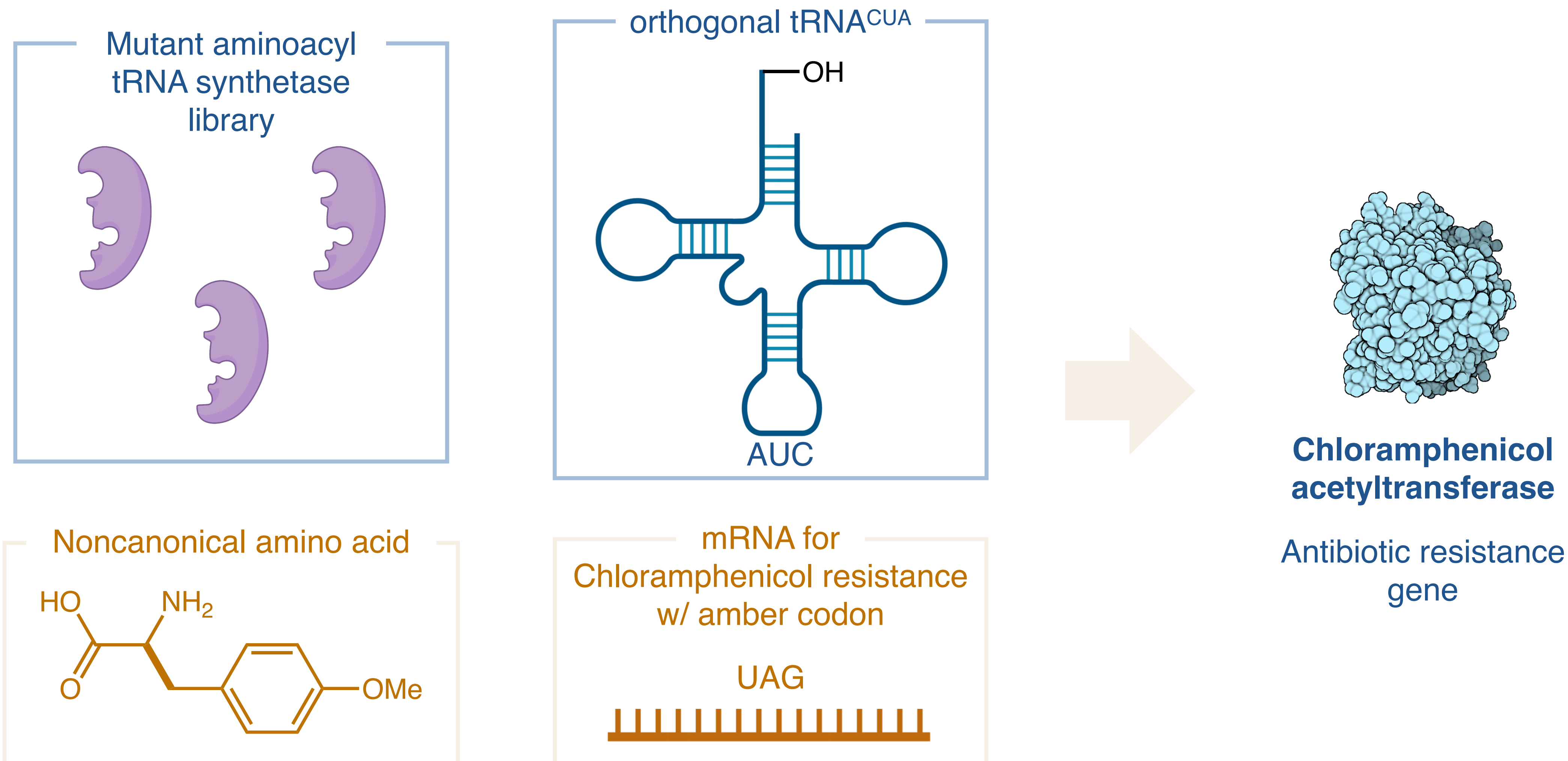
orthogonal $tRNA^{CUA}$



Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

Goal: Evolve an orthogonal aminoacyl tRNA synthetase



Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

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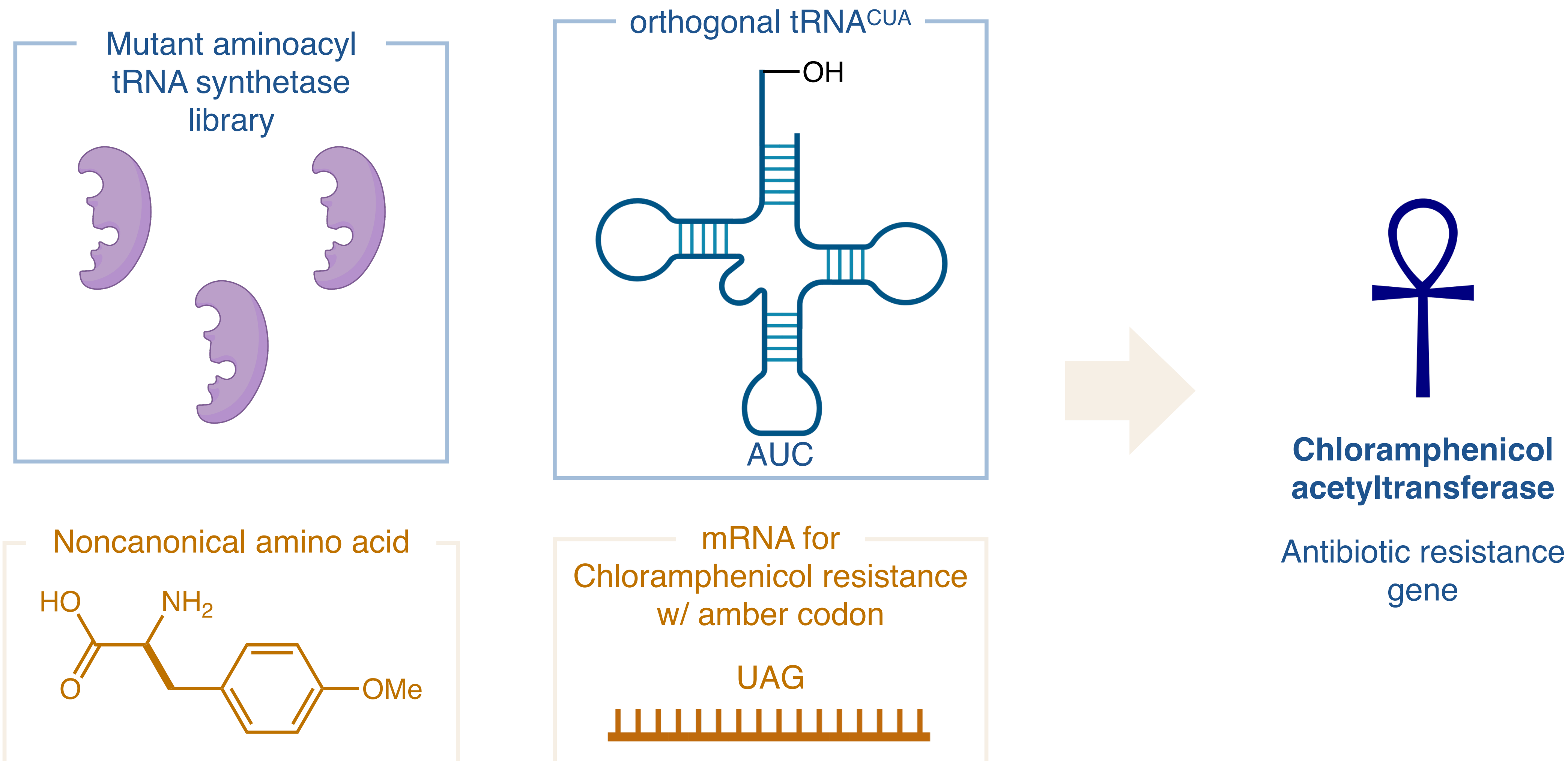
Goal: Evolve an orthogonal aminoacyl tRNA synthetase

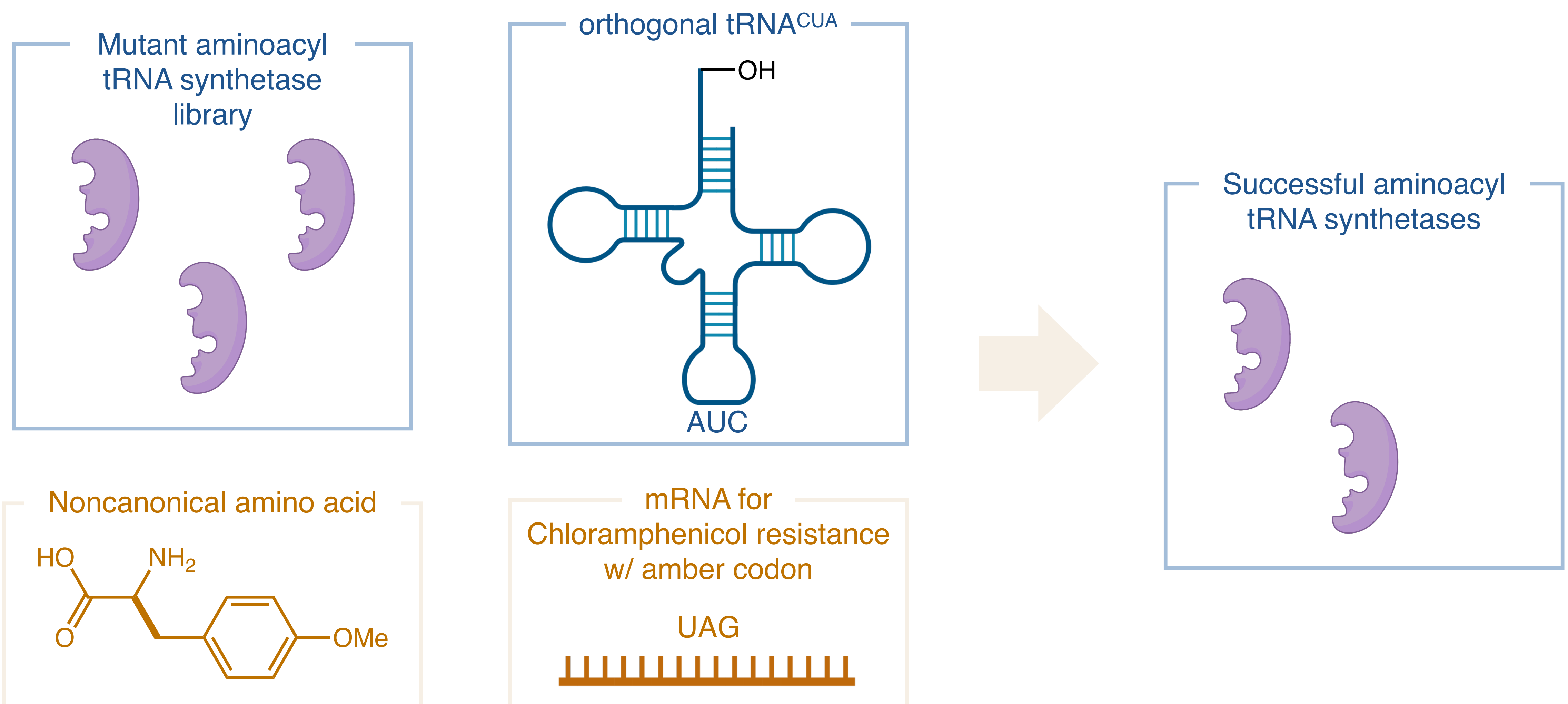


Positive selection: If the mutant aminoacyl tRNA synthetase can charge $tRNA^{CUA}$ with any amino acid, the cells are antibiotic resistant

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

Goal: Evolve an orthogonal aminoacyl tRNA synthetase



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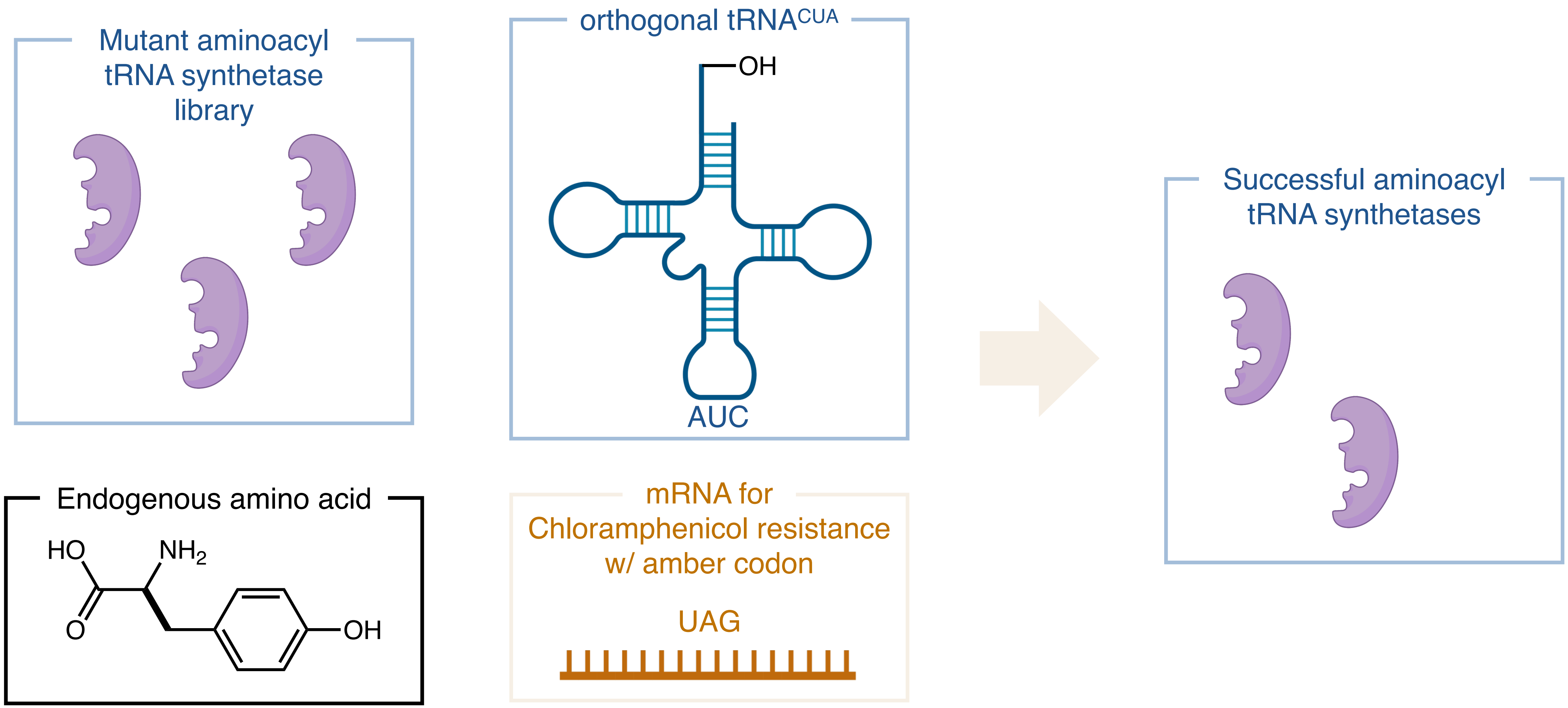
Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

Goal: Evolve an orthogonal aminoacyl tRNA synthetase

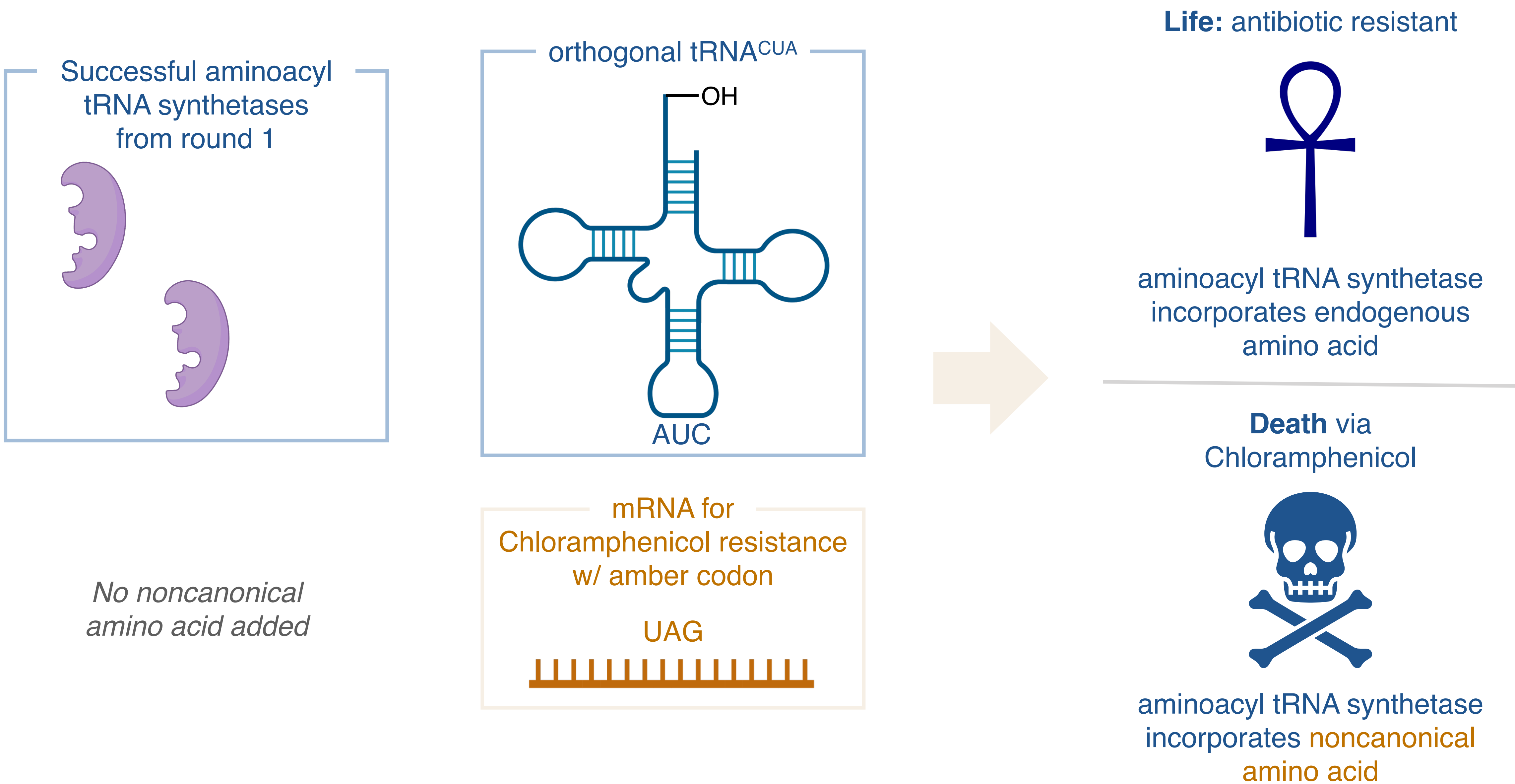


Problem: The mutant aminoacyl tRNA synthetase can charge tRNA^{CUA} with an endogenous amino acid and still survive

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells

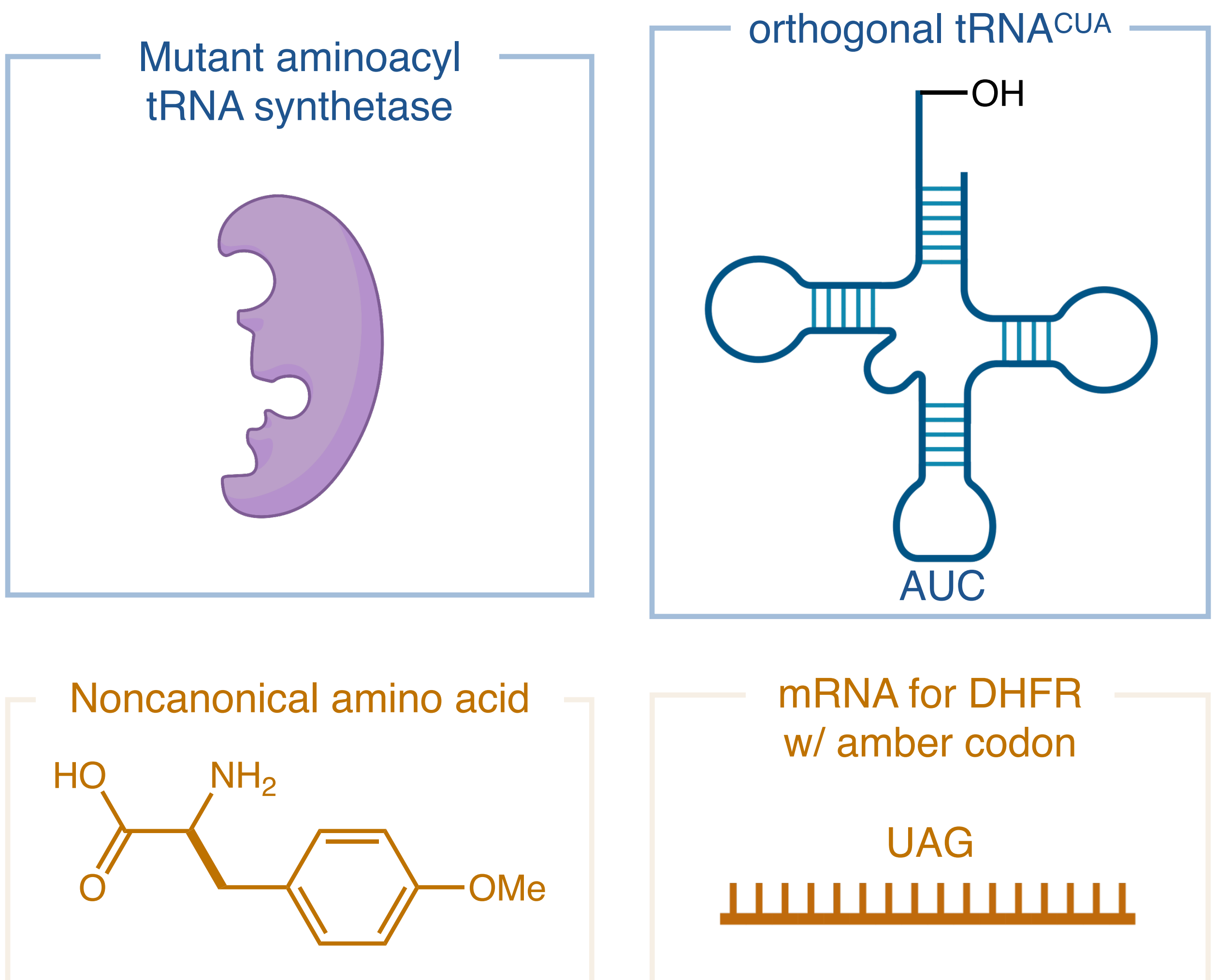
Goal: Evolve an orthogonal aminoacyl tRNA synthetase

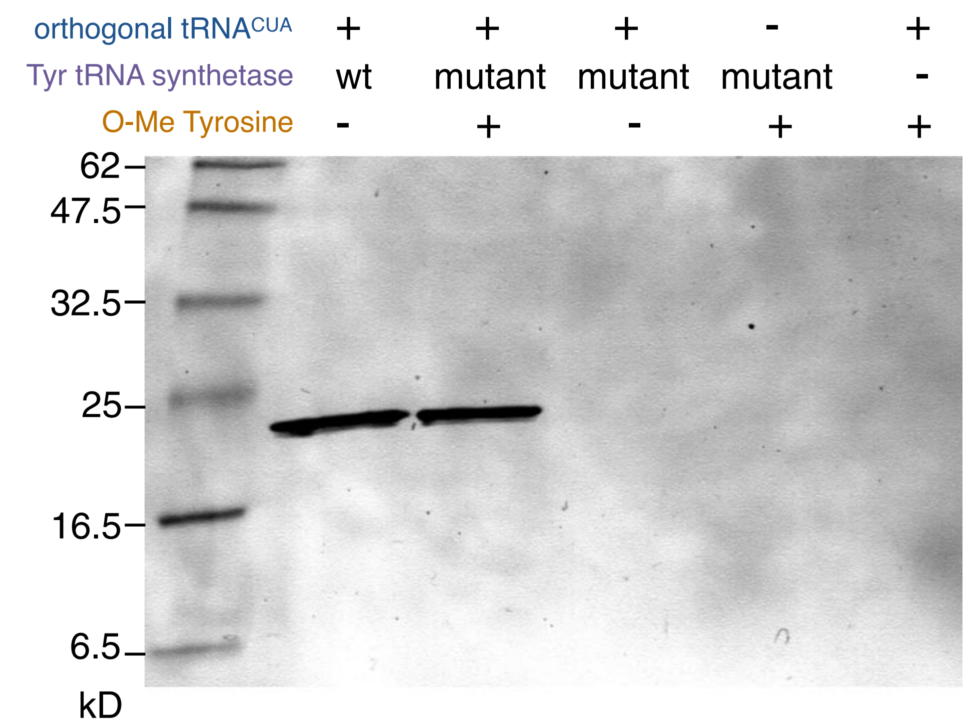


Isolate the cells that died from an identical plate supplemented with the unnatural amino acid

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

Noncanonical Amino Acid Incorporation in Live Cells





Orthogonal tRNA synthetase / tRNA pair incorporates O-Me tyrosine into DHFR

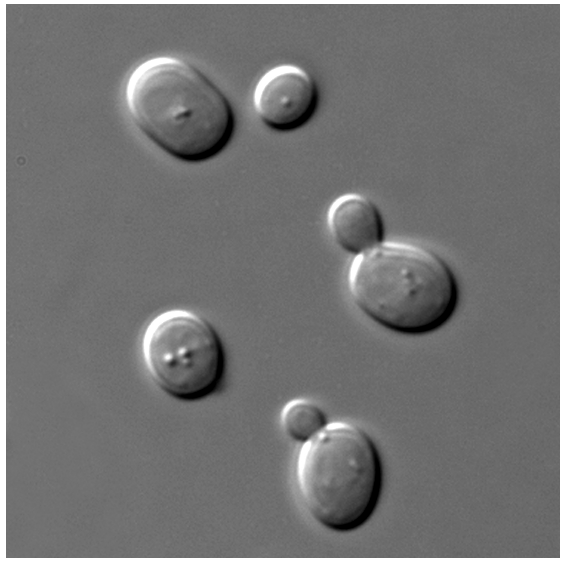
All components required for expression of DHFR with noncanonical amino acid

Wang, L.; Brock. A.; Herberich, B.; Schultz, P. G. *Science* **2001**, *292*, 498.

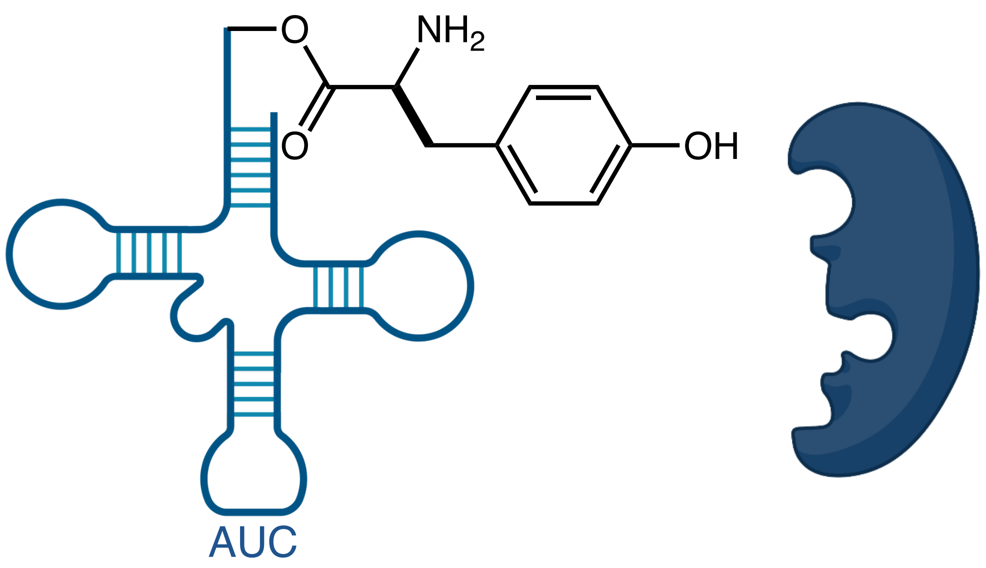
Noncanonical Amino Acid Incorporation in Yeast

Goal: Evolve an orthogonal aminoacyl tRNA synthetase for use in yeast

Yeast



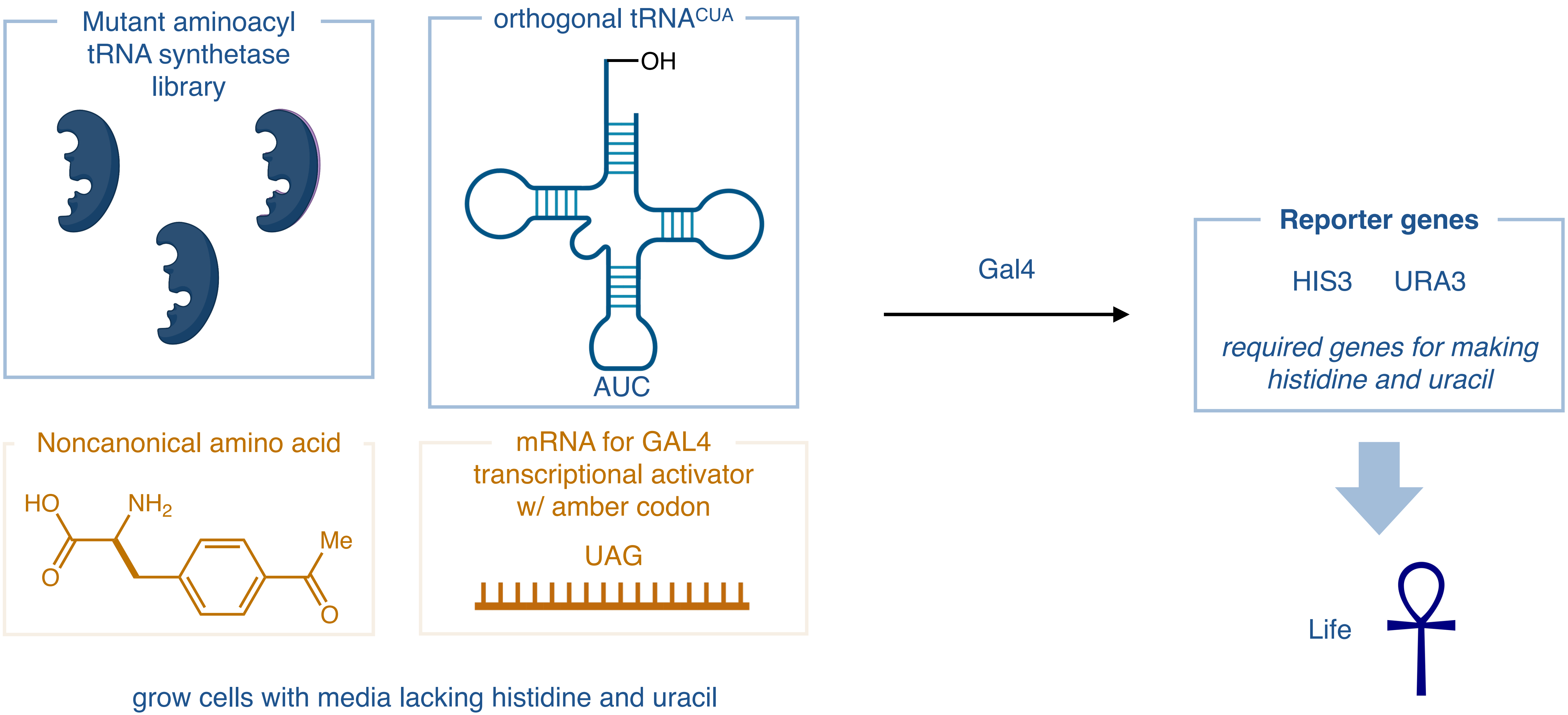
The tyrosyl tRNA / aminoacyl tRNA synthetase pair from E. coli



Chin, J. W. et al. *Science* **2003**, *301*, 964.

Noncanonical Amino Acid Incorporation in Yeast

Goal: Evolve an orthogonal aminoacyl tRNA synthetase for use in yeast

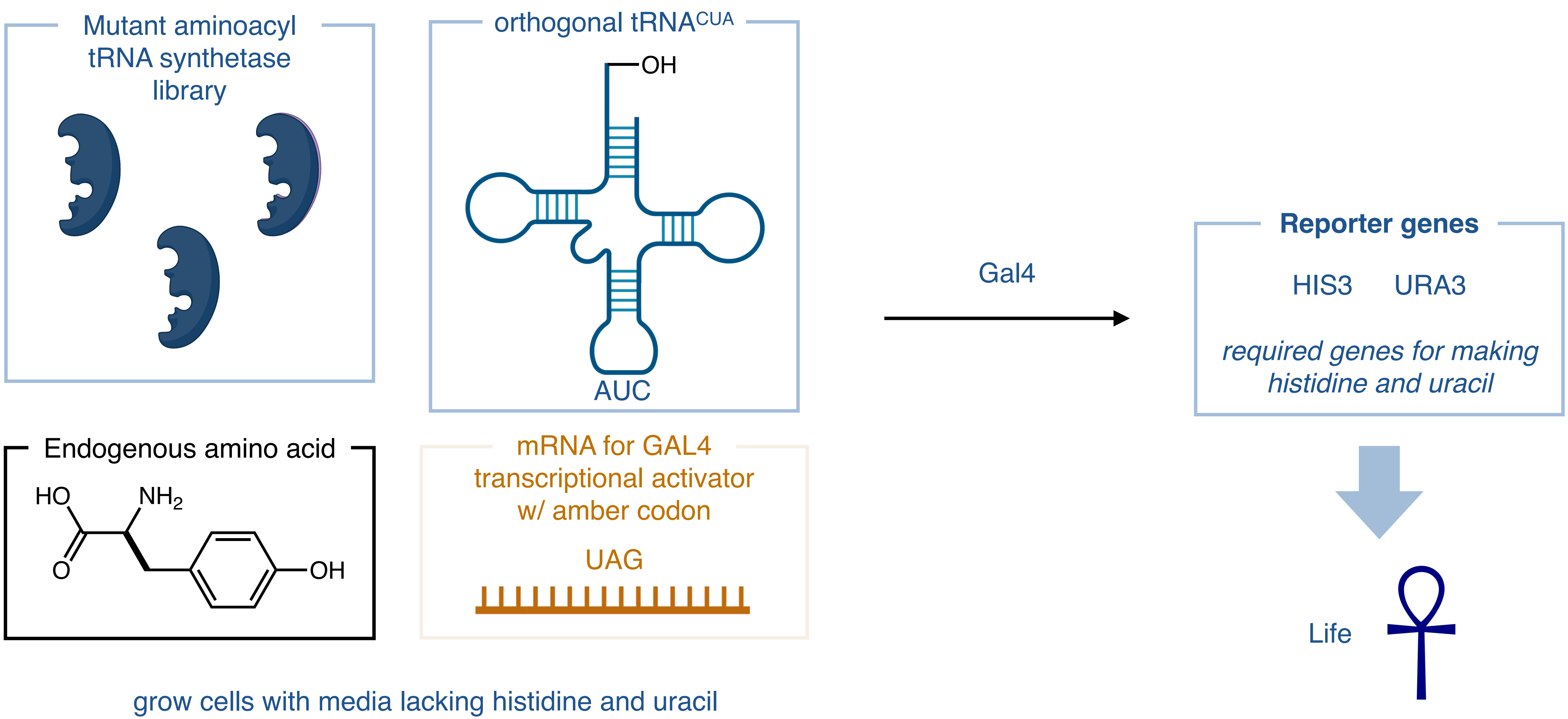


Positive selection: If the mutant aminoacyl tRNA synthetase can charge tRNA^{CUA} with any amino acid, the cells produce histidine and uracil and live

Chin, J. W. et al. *Science* **2003**, *301*, 964.

Noncanonical Amino Acid Incorporation in Yeast

Goal: Evolve an orthogonal aminoacyl tRNA synthetase for use in yeast



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Chin, J. W. et al. *Science* **2003**, *301*, 964.

Noncanonical Amino Acid Incorporation in Yeast

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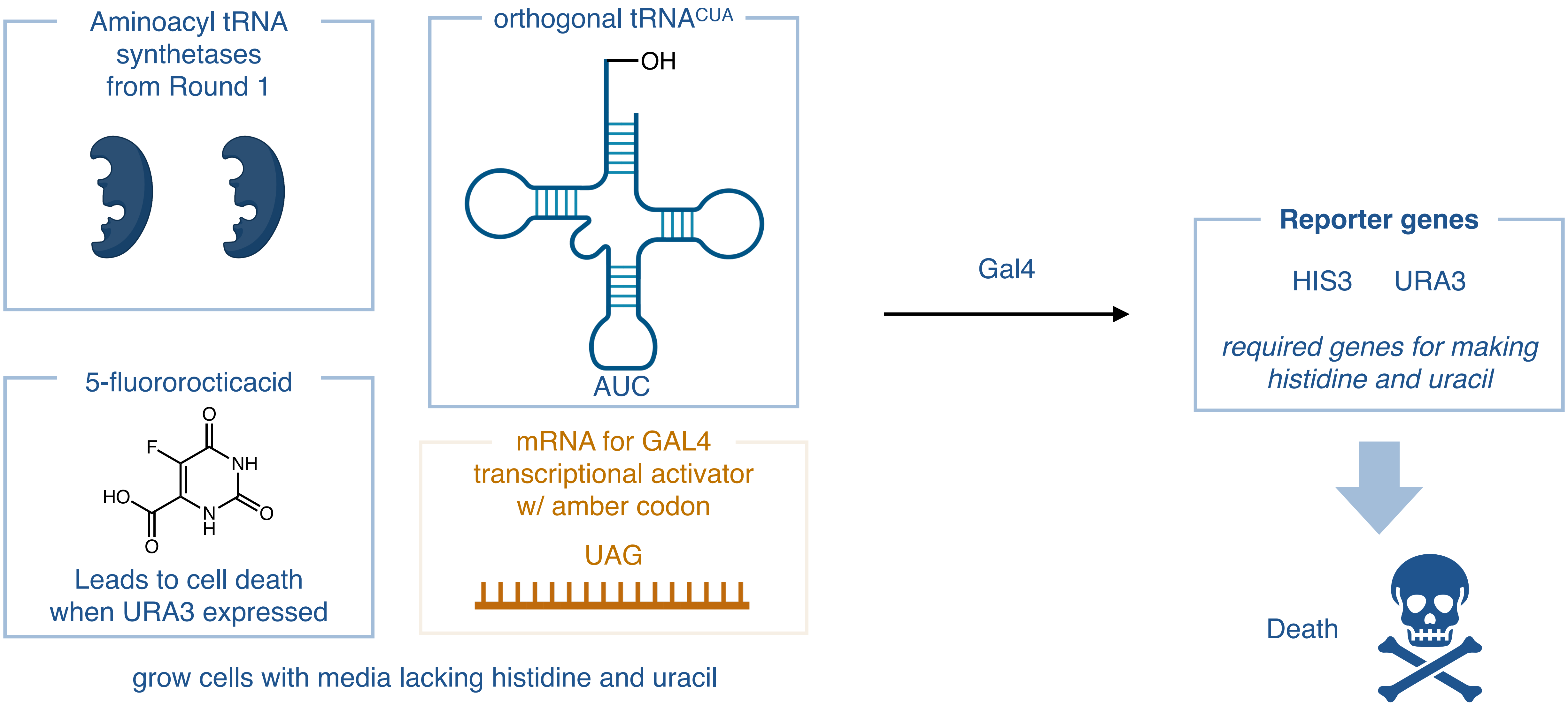
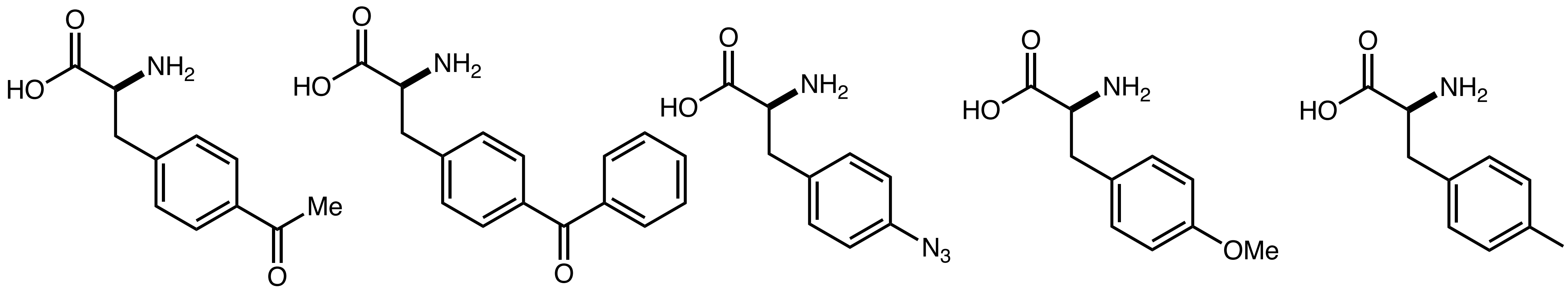


Negative selection: If the mutant aminoacyl tRNA synthetase can charge tRNA^{CUA} with an endogenous amino acid, URA3 will be expressed, and the cell will die via 5-fluorooctic acid

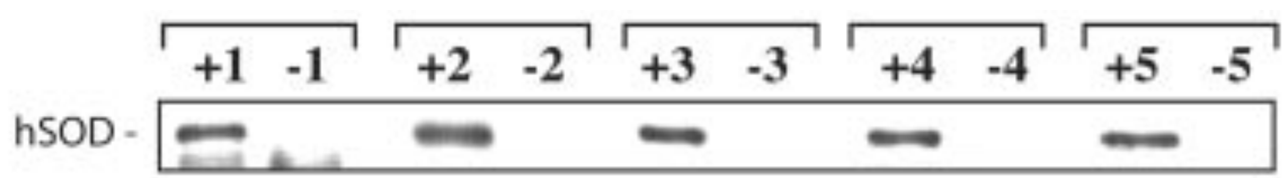
Chin, J. W. et al. *Science* **2003**, *301*, 964.

Noncanonical Amino Acid Incorporation in Yeast



1 2 3 4 5

Unique aminoacyl tRNA synthetase evolved for each noncanonical amino acid

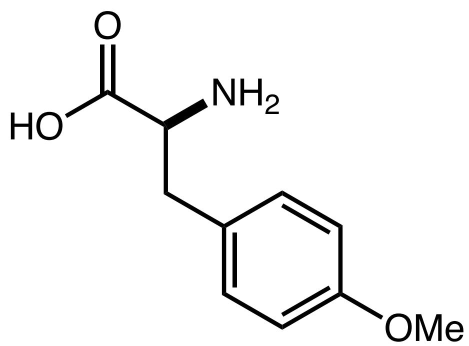


Noncanonical amino acids are successfully incorporated into human superoxide dismutase (hSOD) in yeast

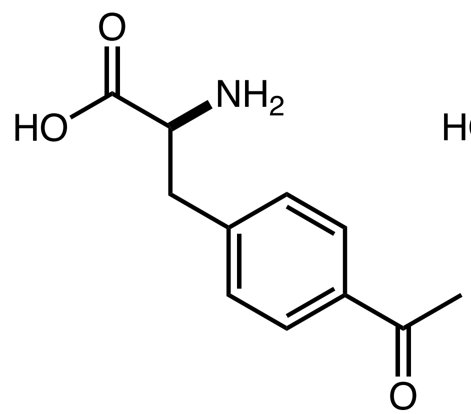
Chin, J. W. et al. *Science* **2003**, *301*, 964.

Noncanonical Amino Acid Incorporation in Mammalian Cells

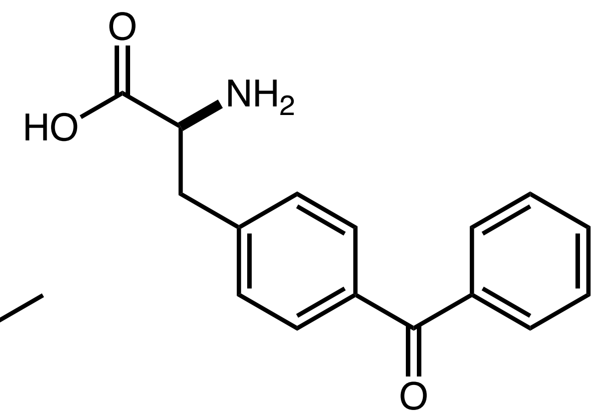
Noncanonical amino acid incorporation in CHO and 293T cells



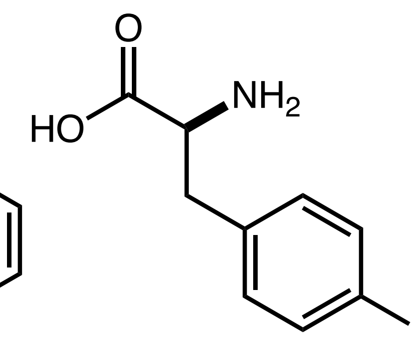
pMpa



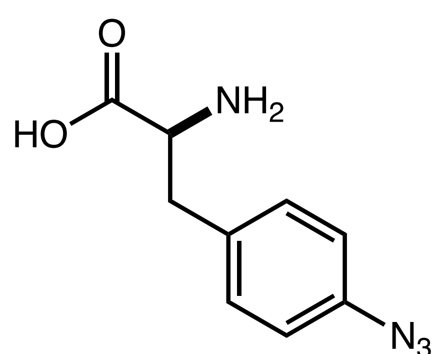
pApa



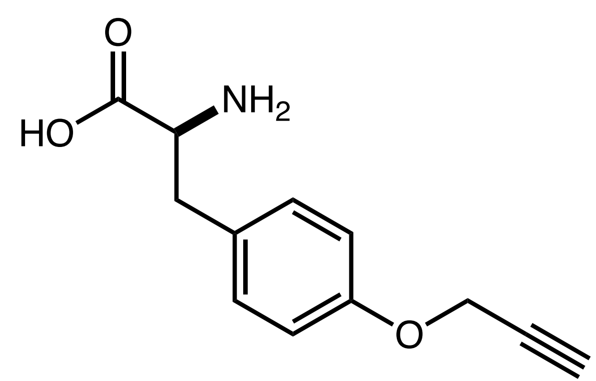
pBpa



pIpa



pAzpa



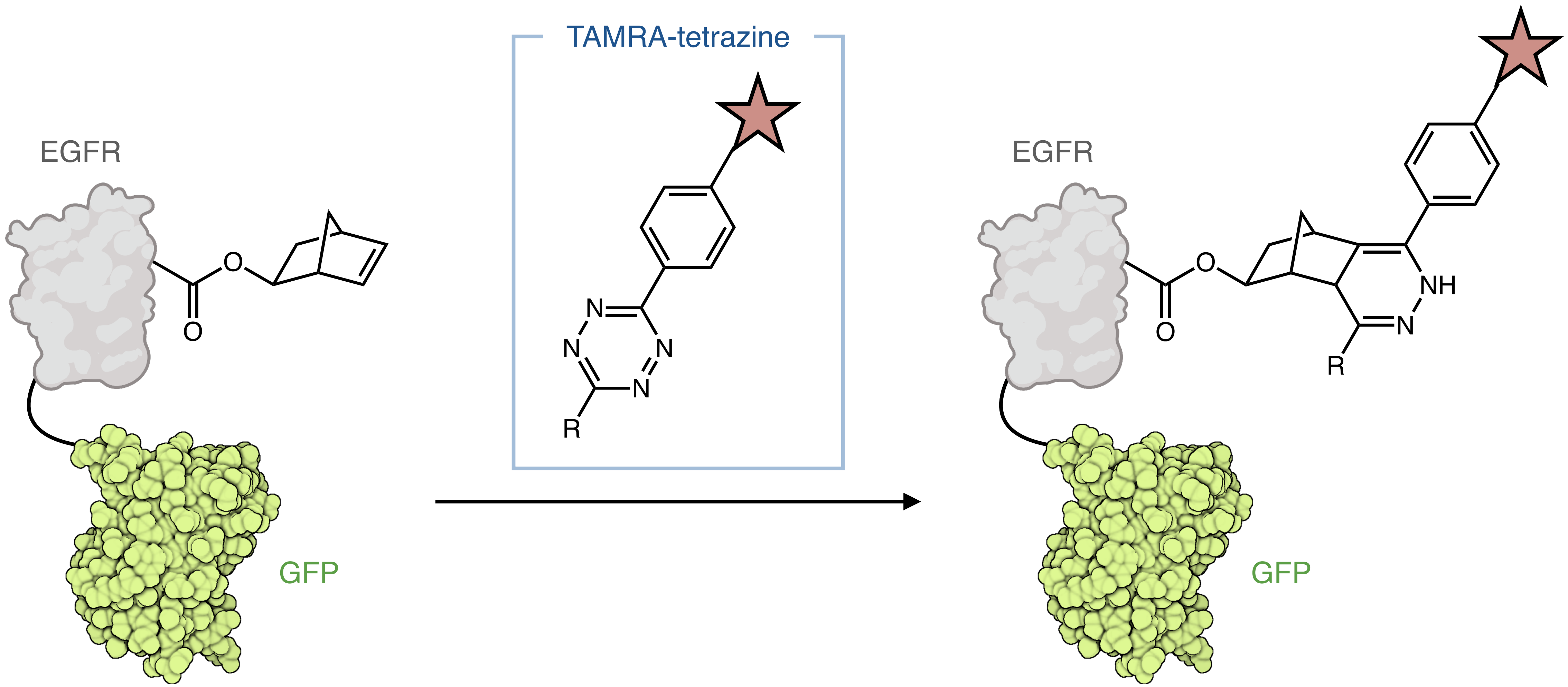
pPpa

A unique aminoacyl tRNA synthetase was evolved for each amino acid

Liu, W.; Brock, A.; Chen, S.; Chen, S.; Schultz, P. G. *Nat. Biotechnol.* **2007**, *4*, 239.

Noncanonical Amino Acid Incorporation in Mammalian Cells for Imaging

Imaging cell surface proteins via bioorthogonal click chemistry



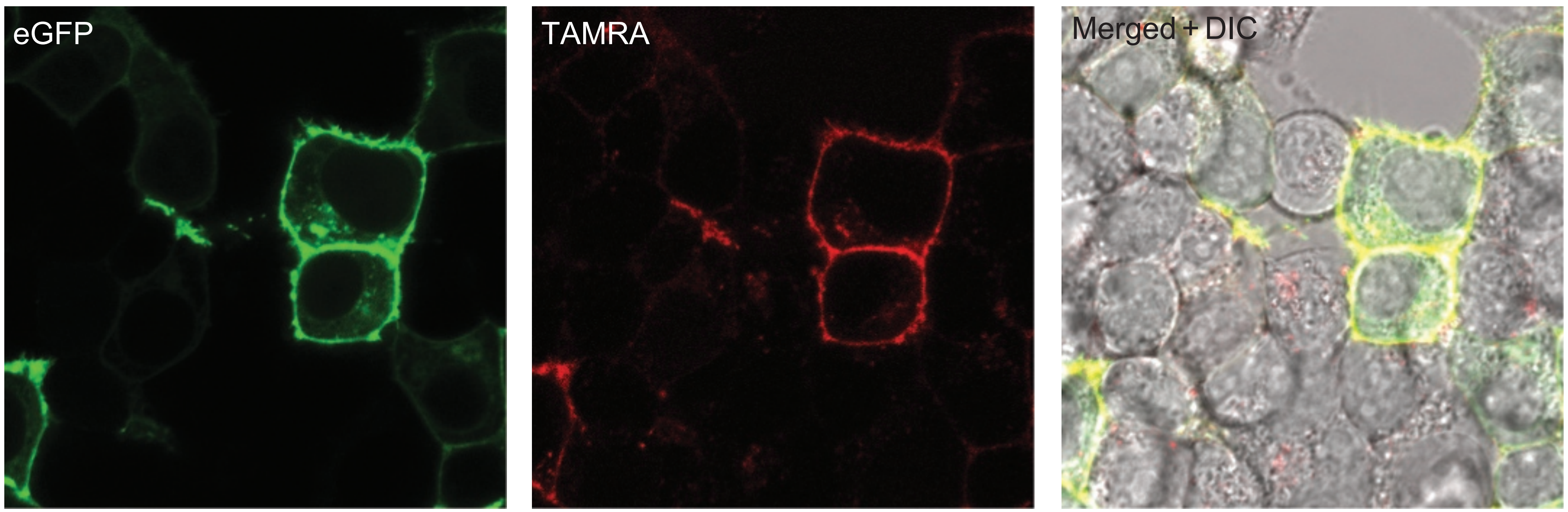
EGFR-GFP with amber codon at position 128

EGFR-GFP labeled with TAMRA fluorophore

Lang, K. et al. *Nat. Chem.* **2012**, *4*, 298.

Noncanonical Amino Acid Incorporation in Mammalian Cells for Imaging

Imaging cell surface proteins via bioorthogonal click chemistry

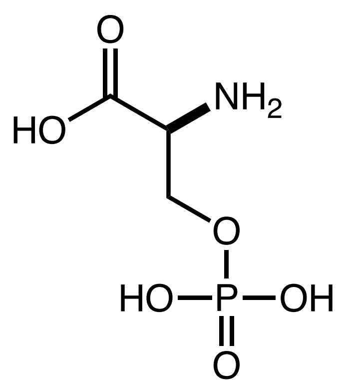


eGFP and TAMRA signal colocalize

Lang, K. et al. *Nat. Chem.* **2012**, *4*, 298.

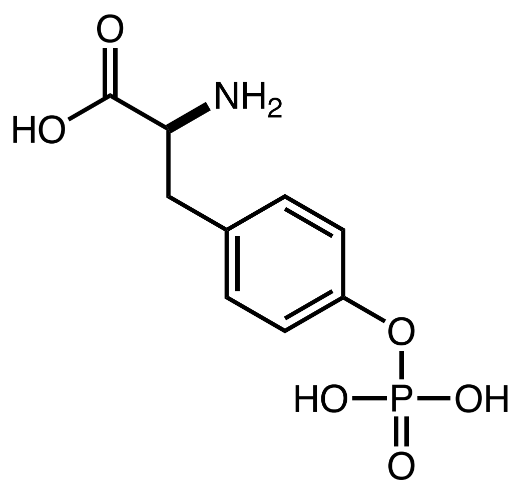
Genetic Encoding of Traceless Post-Translational Modifications

Phosphorylation of a protein can lead to activation, deactivation, degradation, or membrane transport



Phosphoserine

Science 2011



Phosphotyrosine

Nat. Chem. Biol. 2017

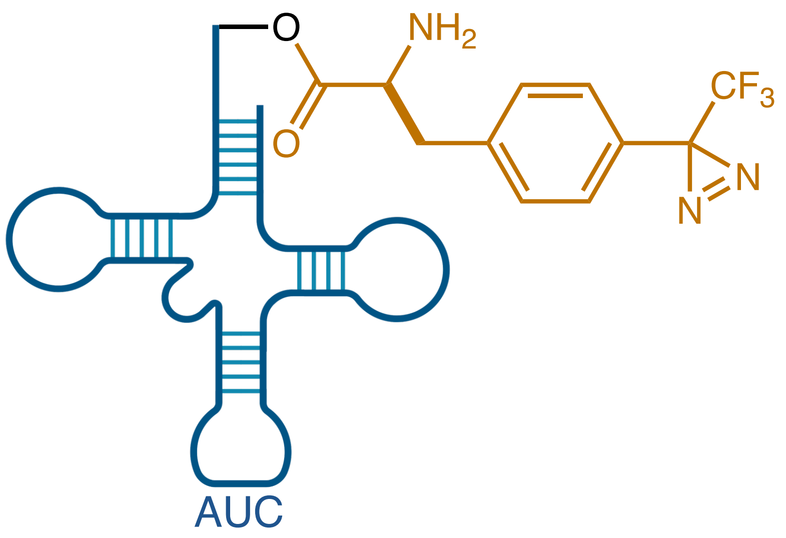
Phosphorylated serine and tyrosine can be installed at specific positions via noncanonical amino acid incorporation

Hoppmann, C. et al. *Nat. Chem. Biol.* **2017**, *13*, 842.

Park, H-S. et al. *Science* **2011**, *333*, 1151.

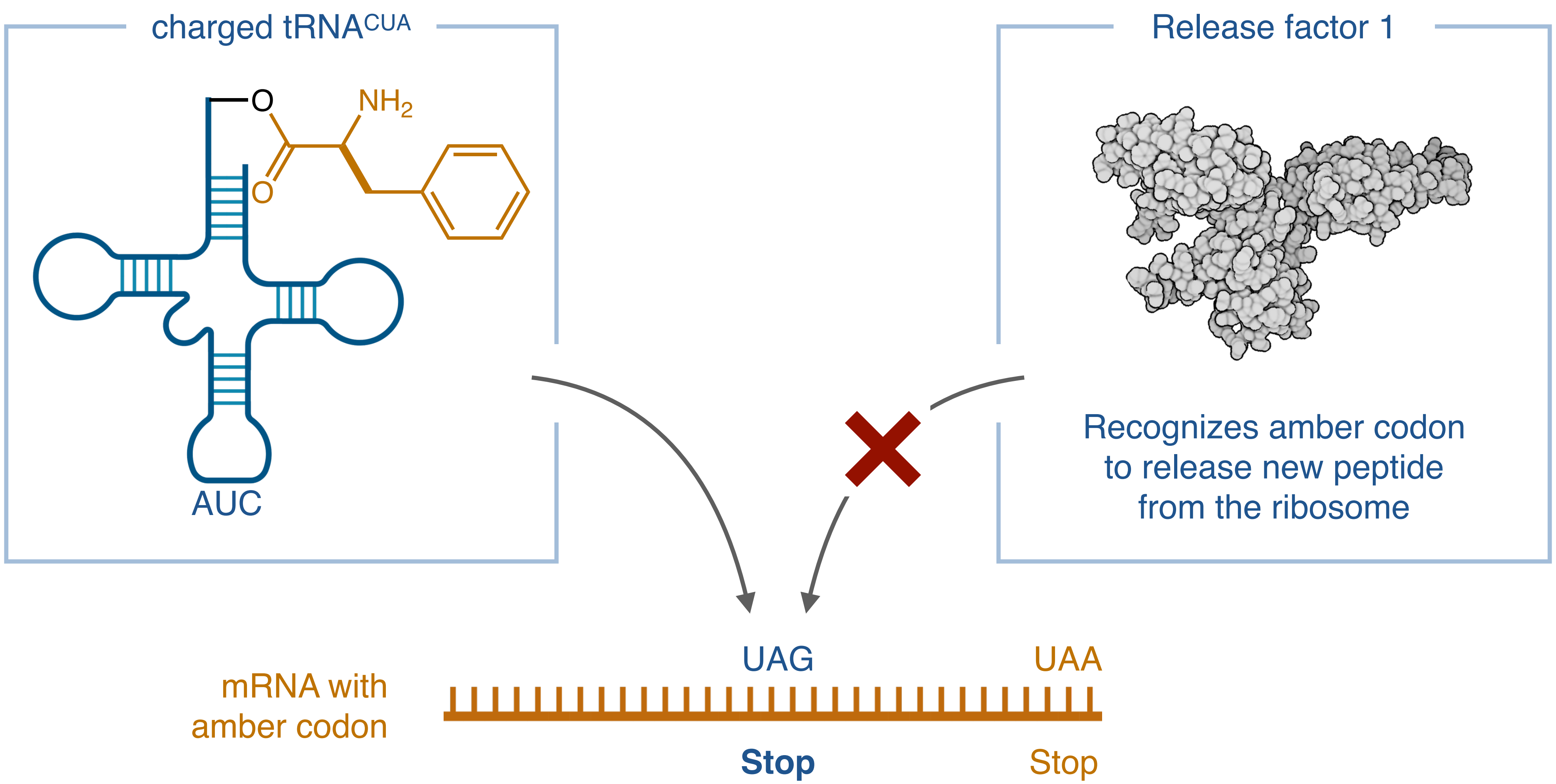
Expanding the Genetic Code

- The traditional genetic code
- Expanding the genetic code
  - In vitro
  - In eukaryotes, prokaryotes, and mammalian cells
- Orthogonal ribosomes
- Genetically recoded organisms and synonymous codon compression
- Outlook



Orthogonal Ribosomes

Problem: Release factors compete with tRNA^{CUA}, diminishing amber suppression efficiency



Decreased interaction of release factor 1 would lead to increased efficiency of amber suppression

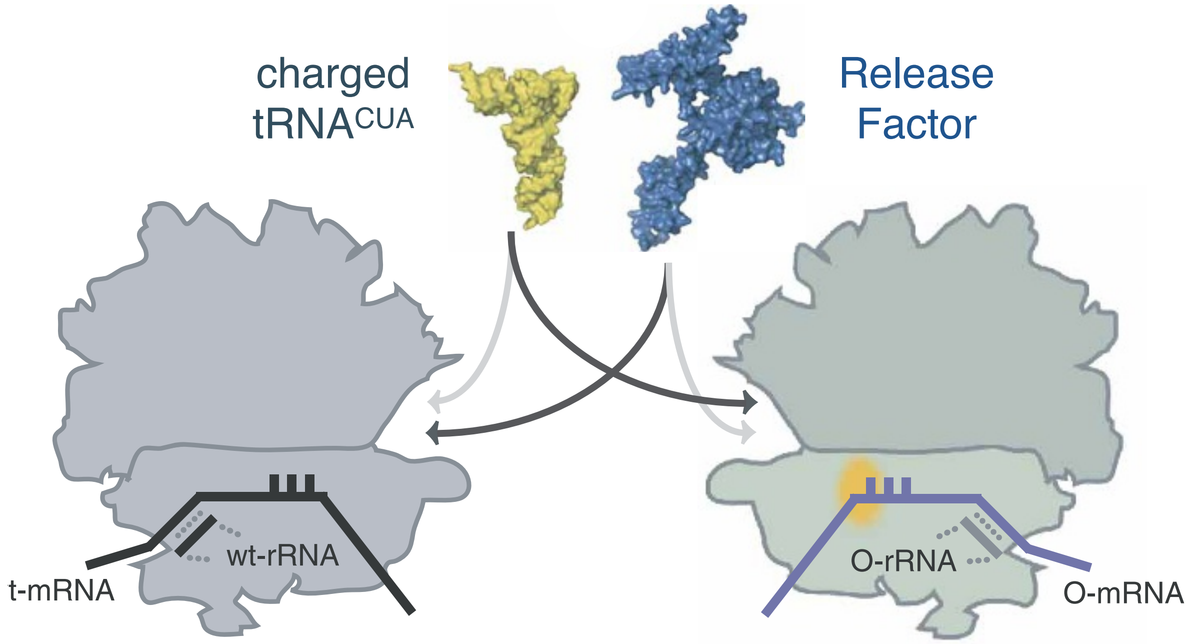
Rackham, O.; Chin, J. W. *Nat. Chem. Biol.* **2005**, *1*, 159.

Orthogonal Ribosomes

Problem: Release factors compete with $tRNA^{CUA}$, diminishing amber suppression efficiency

Jason W. Chin

Current Institution:
MRC Laboratory of Molecular Biology

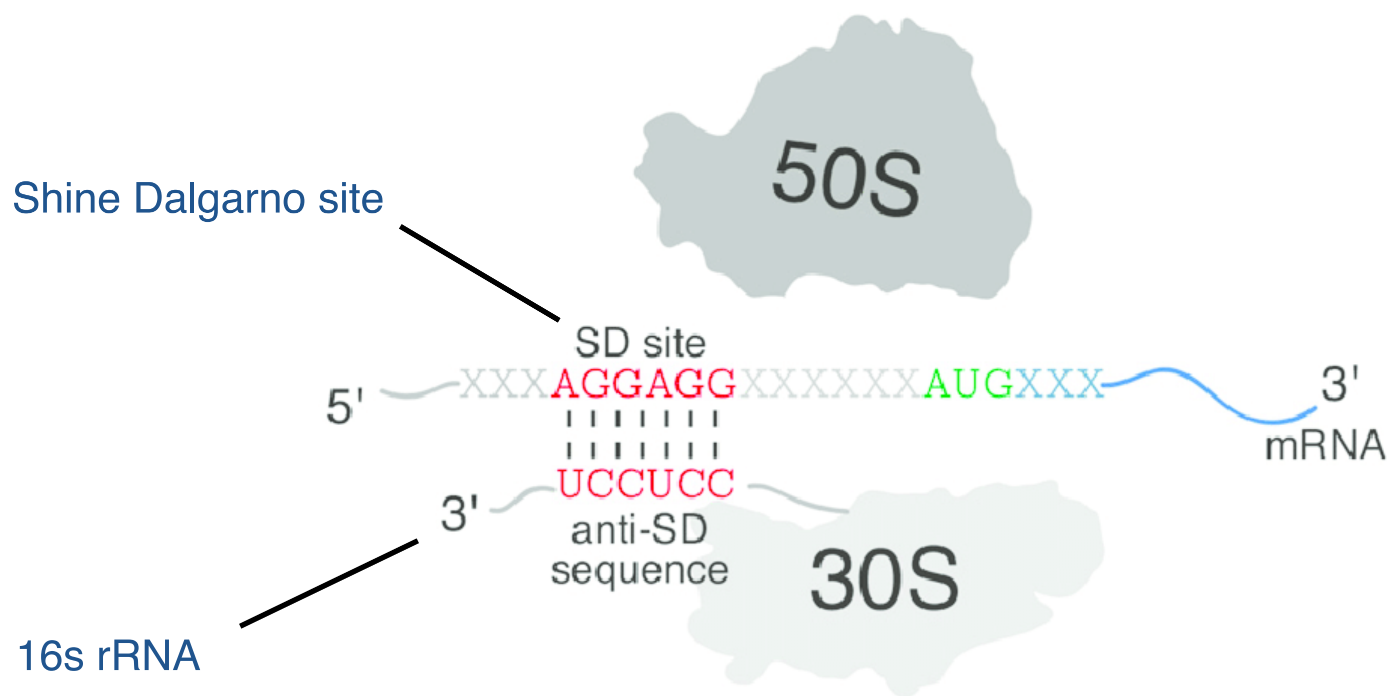


Can an orthogonal ribosome be engineered to specifically translate an orthogonal mRNA and increase amber suppression?

Wang, K.; Neumann, H.; Peak-Chew, S. Y.; Chin, J. W. *Nat. Biotechnol.* **2007**, *25*, 770.

Orthogonal Ribosomes

16S rRNA of 30s ribosome recognizes the Shine-Dalgarno (SD) site upstream of the start codon



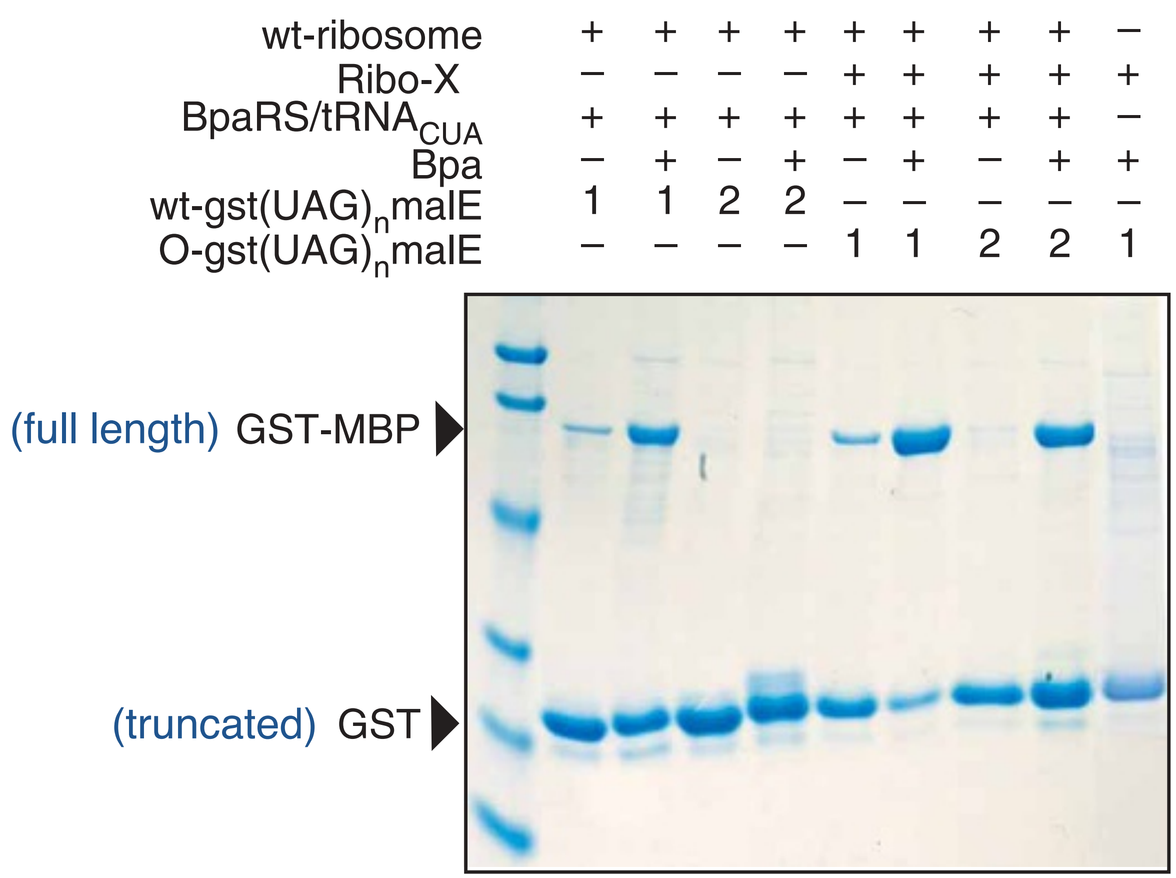
Solution an orthogonal ribosome: Alternate Shine-Dalgarno site and anti-SD sequence

Image source: Researchgate

Rackham, O.; Chin, J. W. *Nat. Chem. Biol.* **2005**, *1*, 159.

Orthogonal Ribosomes

Ribo-X

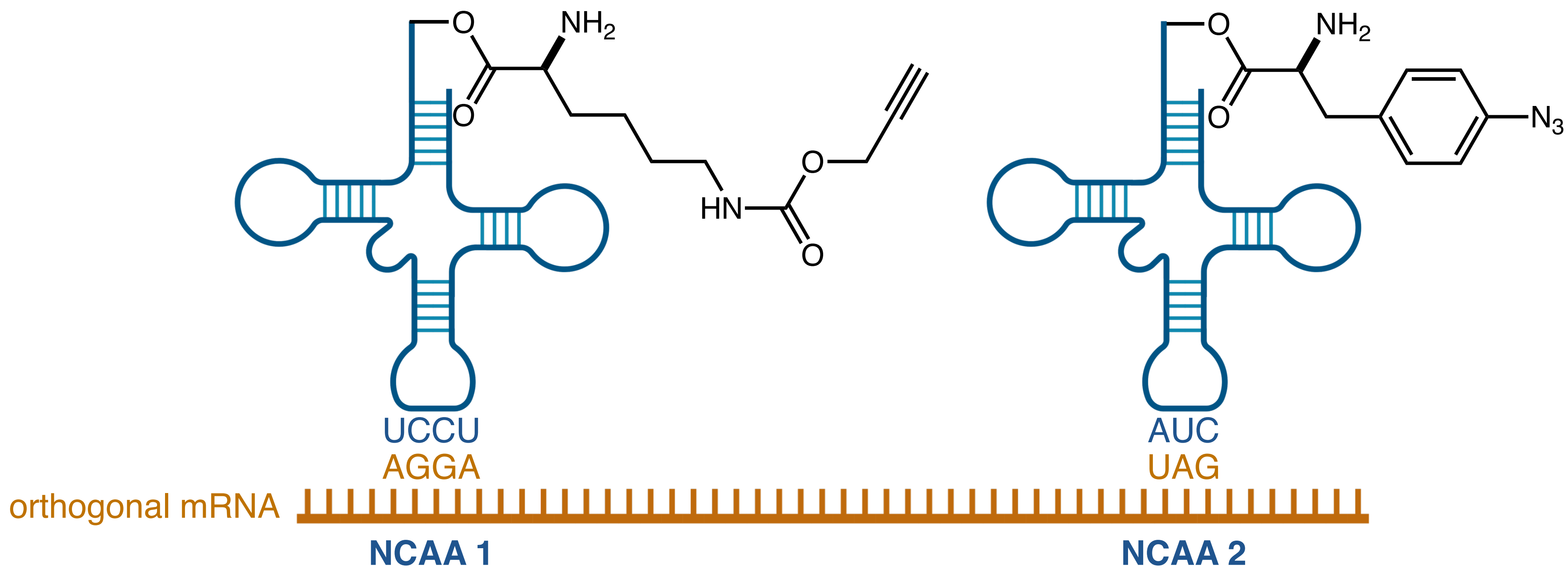


Ribo-X improves noncanonical amino acid incorporation and is hypothesized to decrease ribosomal interaction with Release Factor 1

Wang, K.; Neumann, H.; Peak-Chew, S. Y.; Chin, J. W. *Nat. Biotechnol.* **2007**, *25*, 770.

Orthogonal Ribosomes

Ribo-Q1

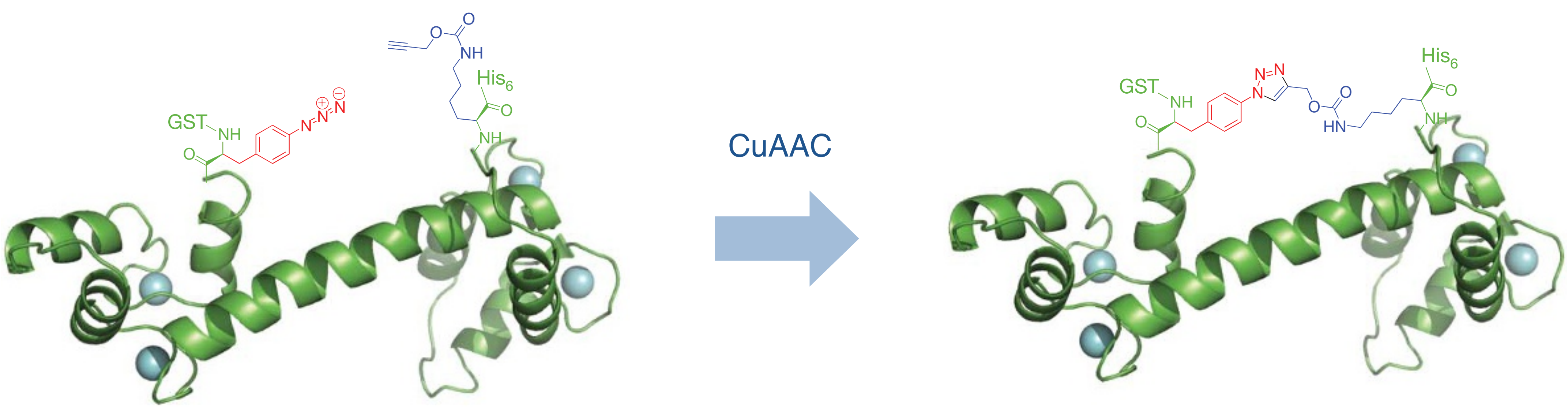


The orthogonal ribosome Ribo-Q1 efficiently decodes quadruplet codons and amber codons

Neumann, H.; Wang, K.; Davis, L.; Garcia-Alai, M.; Chin, J. W. *Nature* **2010**, *464*, 441.

Orthogonal Ribosomes

Ribo-Q1



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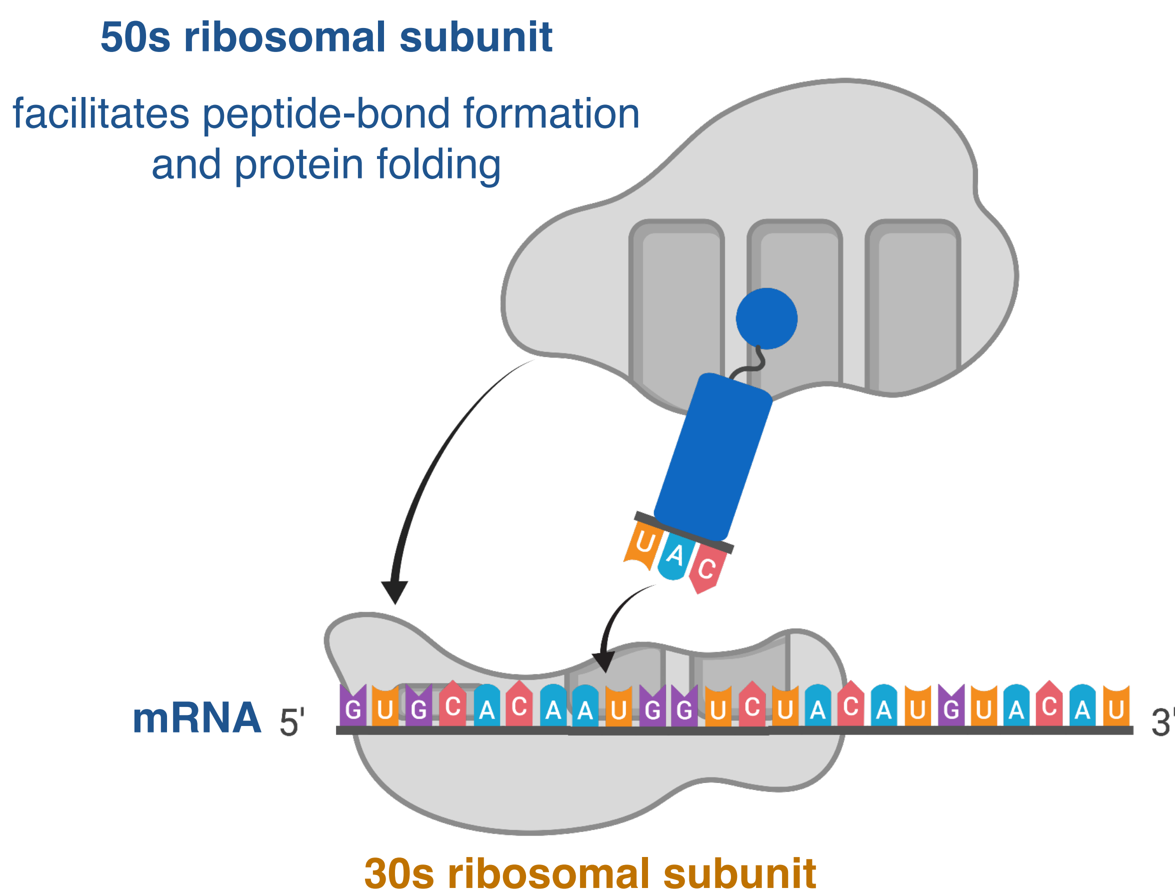
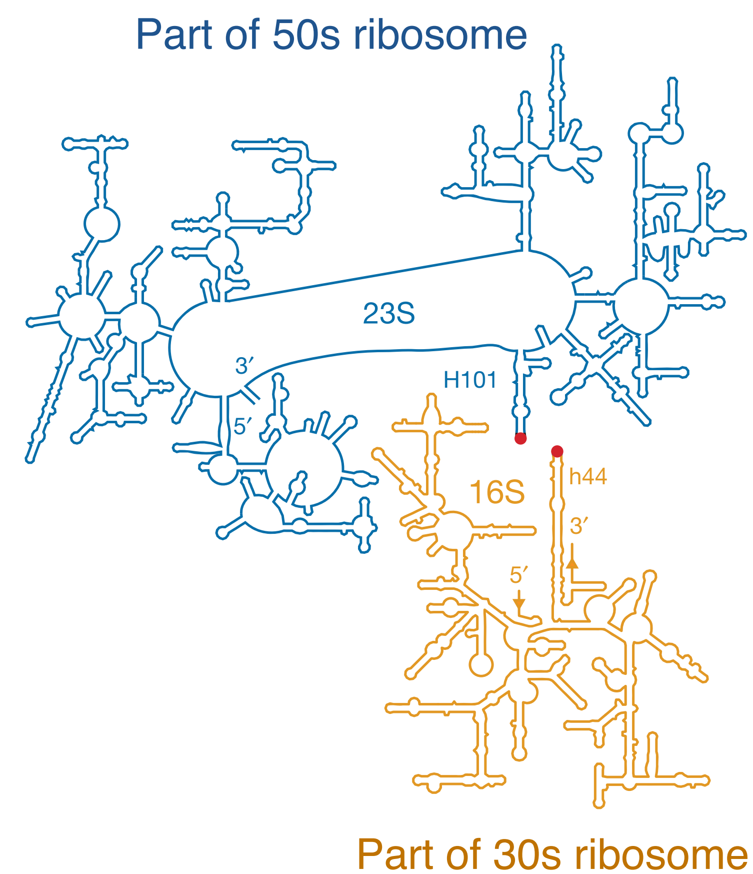
Neumann, H.; Wang, K.; Davis, L.; Garcia-Alai, M.; Chin, J. W. *Nature* **2010**, *464*, 441.

Orthogonal Ribosomes

oRibo-T





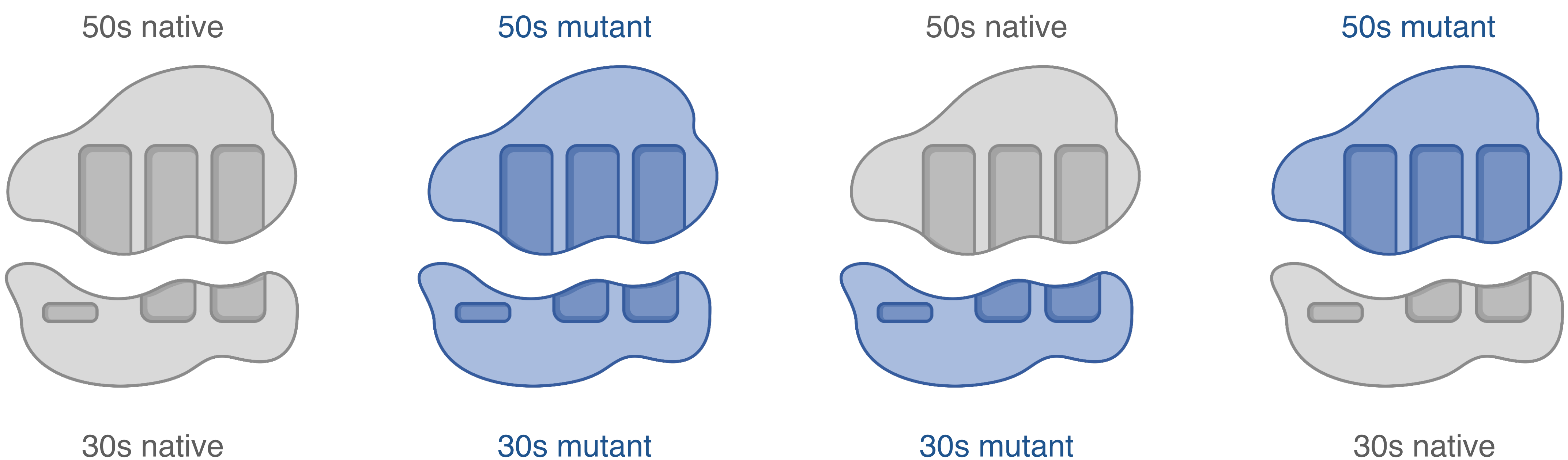
Previous work: Engineering of 16s rRNA of the 30s ribosomal subunit

Can the 23s rRNA of the 50s ribosomal subunit be engineered as well to improve noncanonical amino acid incorporation?

Orelle, C.; Carlson, E. D.; Szal, T.; Florin, T.; Jewett, M. C.; Mankin, A. S. *Nature* **2015**, *524*, 119.

Orthogonal Ribosomes

oRibo-T

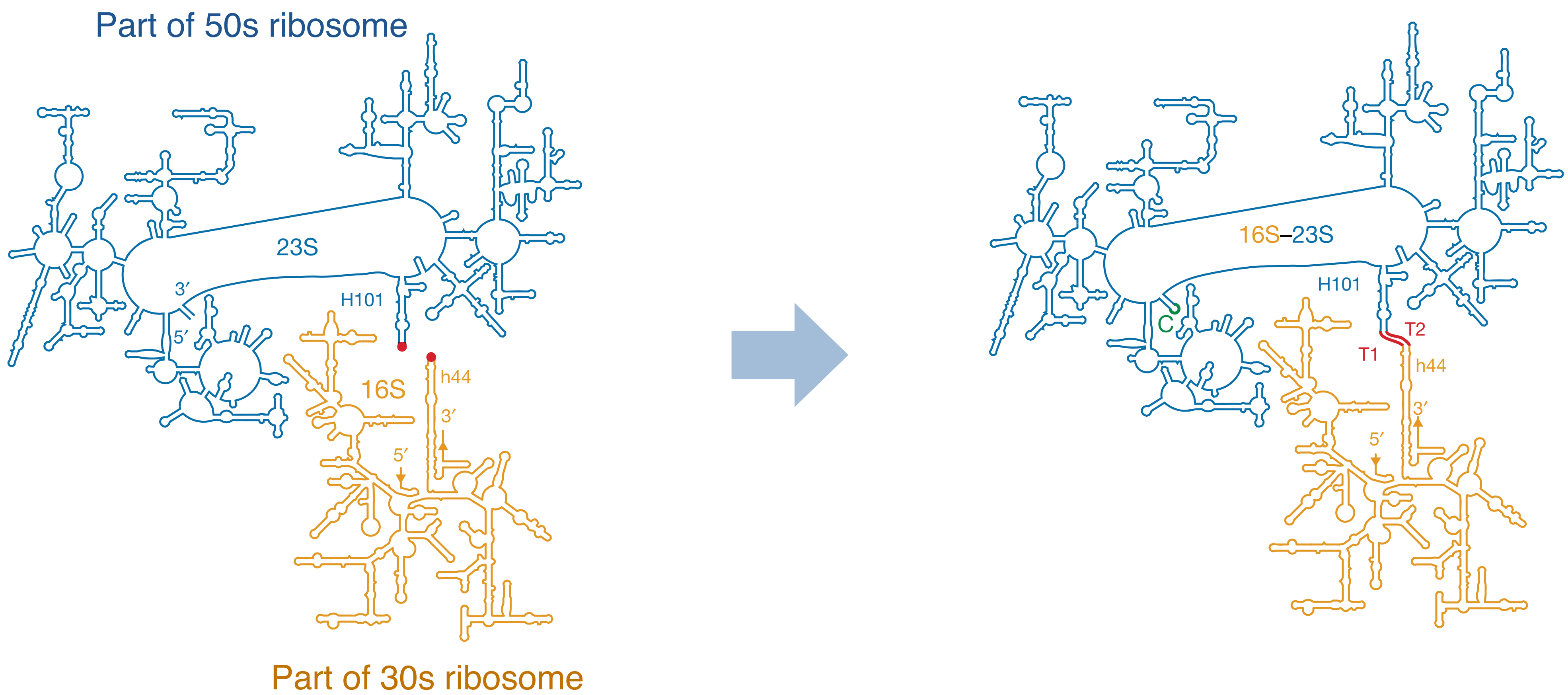


Problem: Engineered rRNA of the 50s / 30s ribosomal subunits can interact with endogenous 30s / 50s subunits

Orelle, C.; Carlson, E. D.; Szal, T.; Florin, T.; Jewett, M. C.; Mankin, A. S. *Nature* **2015**, *524*, 119.

Orthogonal Ribosomes

oRibo-T



Solution: ‘Stapled ribosomes’ link 16S and 23S rRNA together

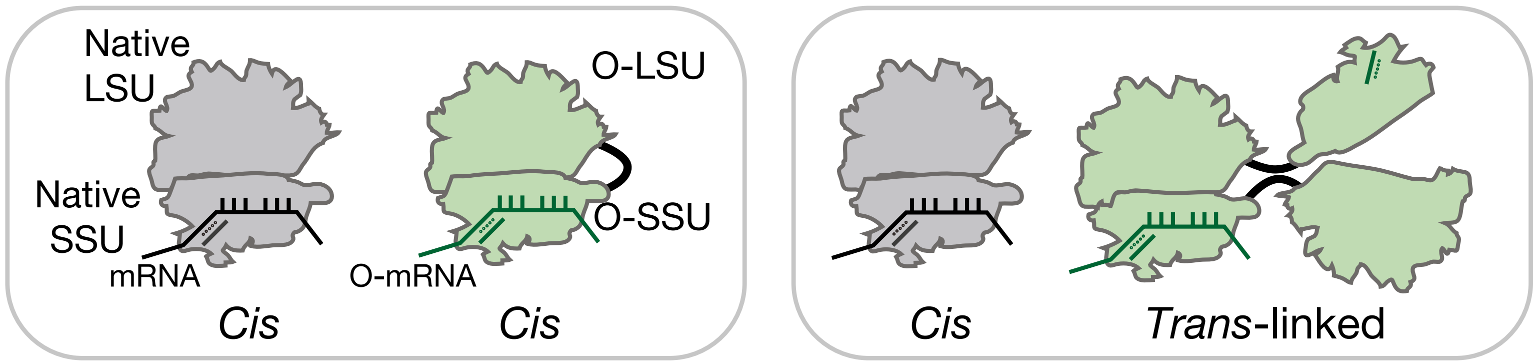
This work: The first fully orthogonal ribosome (both subunits)

Problem: Low activity

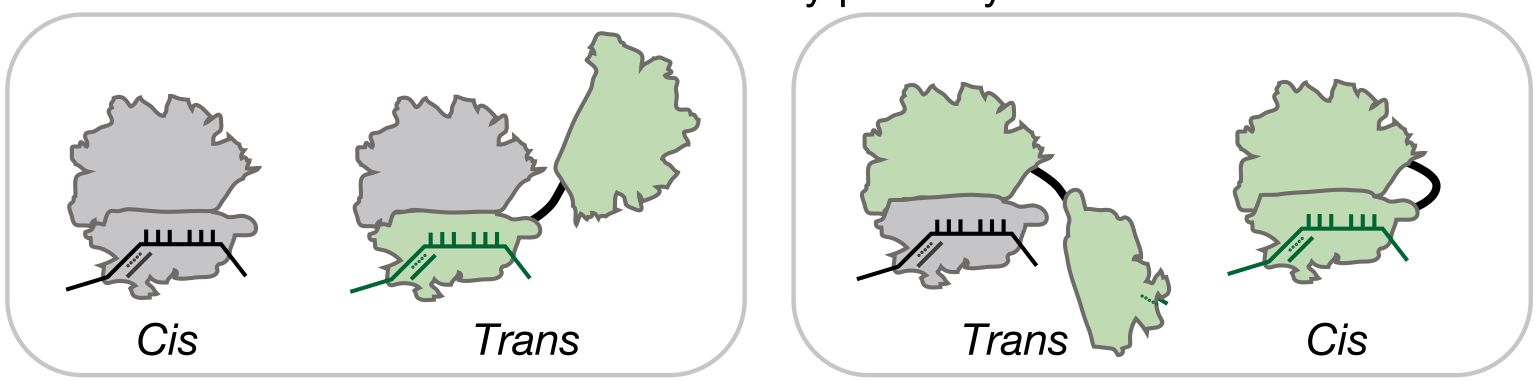
Orelle, C.; Carlson, E. D.; Szal, T.; Florin, T.; Jewett, M. C.; Mankin, A. S. *Nature* **2015**, *524*, 119.

Orthogonal Ribosomes

Orthogonal translation pathways



Cross-assembly pathways



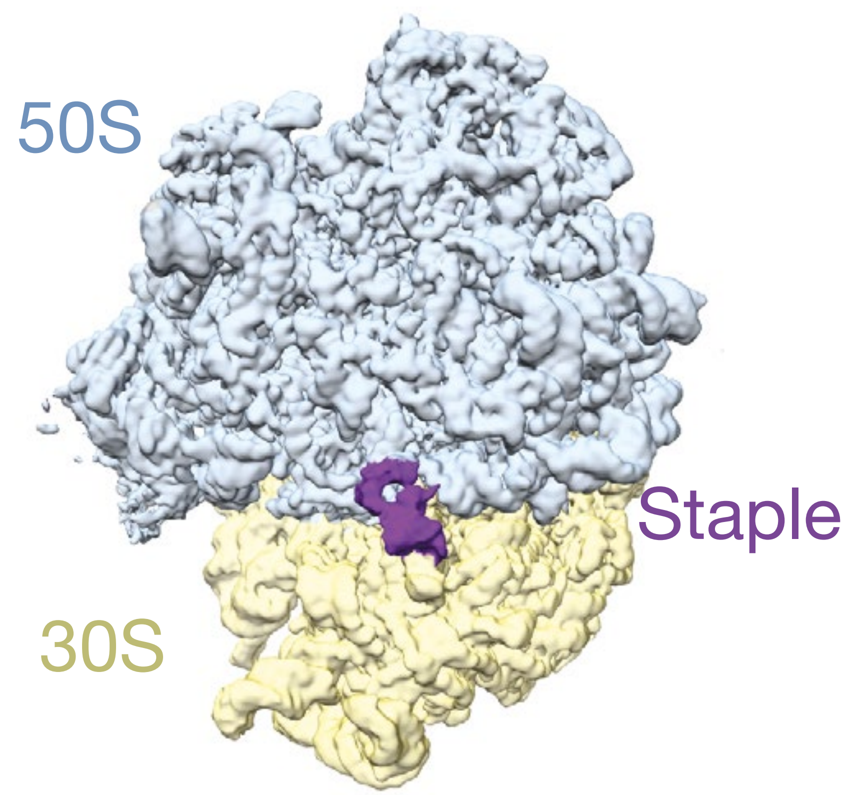
'Stapled ribosomes' can still interact with endogenous 50s and 30s subunits

Schmied, W. H.; Tnimov, Z.; Uttapmapinant, C.; Rae, C. D.; Fried, S. D.; Chin, J. W. *Nature* **2018**, *564*, 444.

Orthogonal Ribosomes

o-d2d8

Cryo-EM structure of o-d2d8



This work: Engineer a 'stapled ribosome' that does not associate with endogenous subunits

Orthogonal 'stapled' ribosomes have activities comparable to that of the parent orthogonal ribosome

Schmied, W. H.; Tnimov, Z.; Uttapmapinant, C.; Rae, C. D.; Fried, S. D.; Chin, J. W. *Nature* **2018**, *564*, 444.

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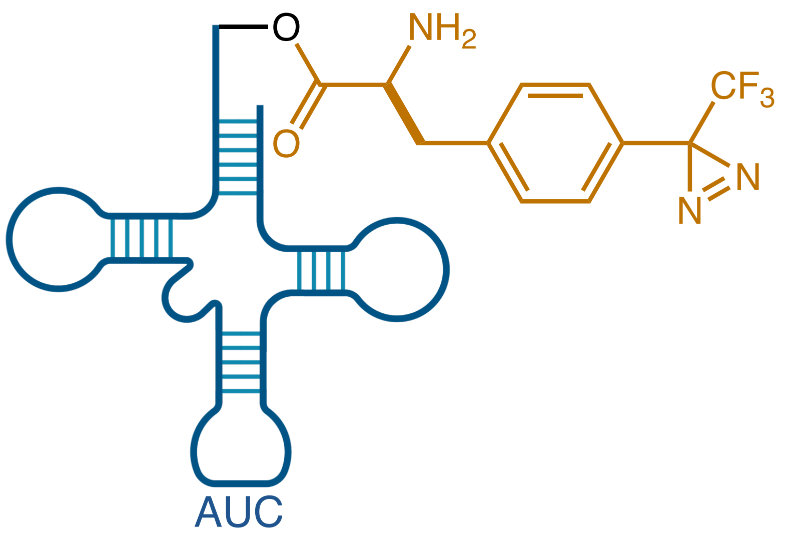
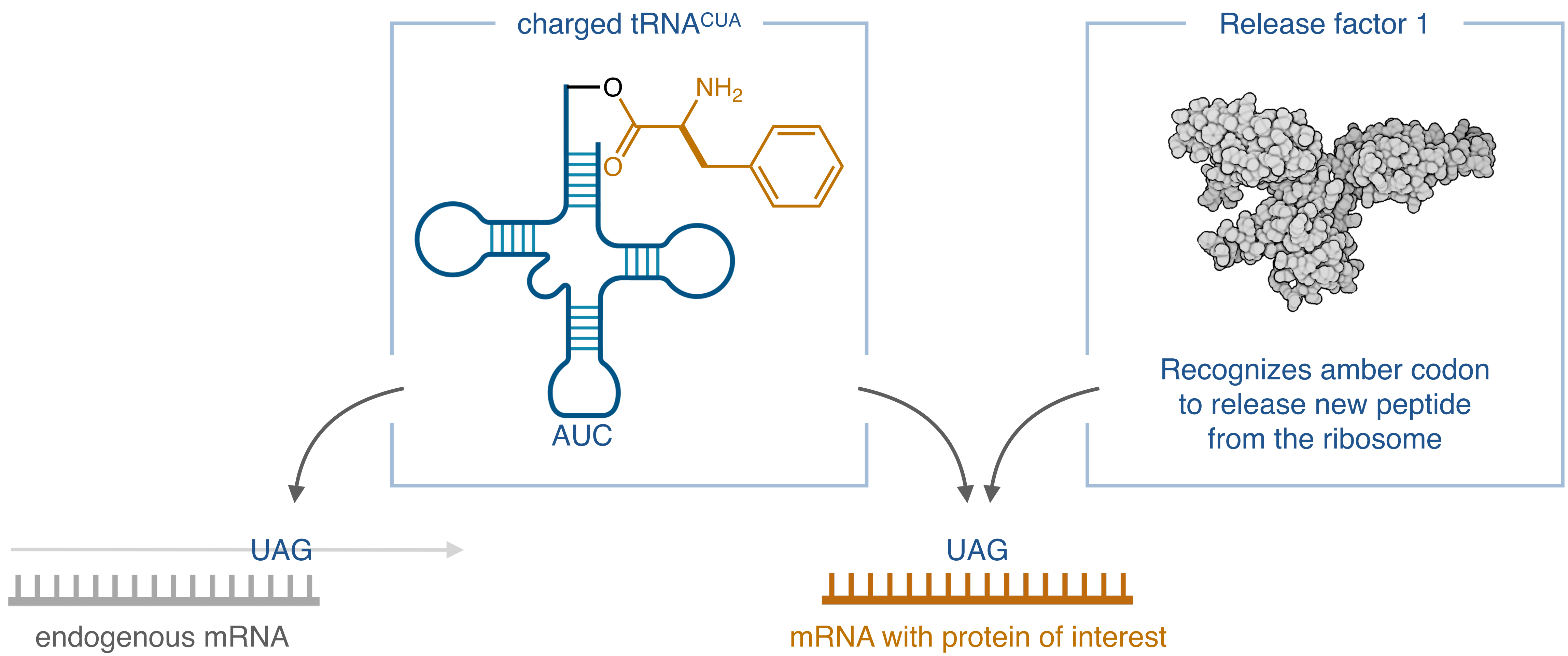


Genomically Recoded Organisms



Problems with amber suppression

Release factors compete with tRNA^{CUA}, diminishing amber suppression efficiency

Charged tRNA^{CUA} suppress endogenous UAG stop codons

Solution: Recode endogenous UAG (amber codon) to UAA and delete Release Factor 1

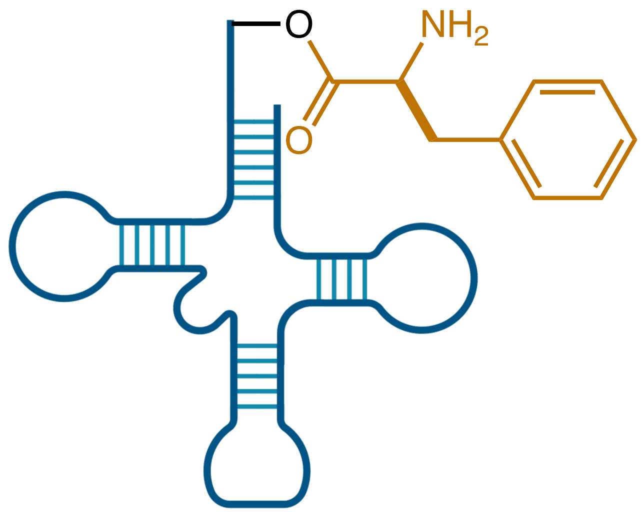
Lajoie, M. J. et al. *Science* **2013**, *342*, 357.

Genomically Recoded Organisms

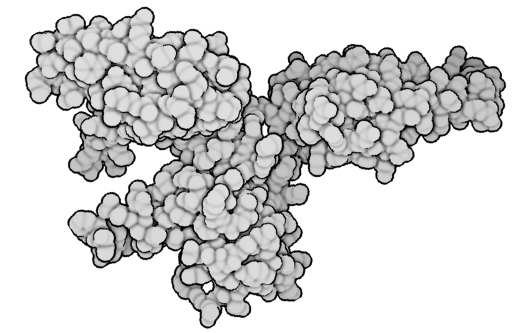
Stop Codons

Amber	**UAG**
Ochre	UAA
Opal	UGA

charged tRNA^{CUA}



Release factor 1



Recognizes amber codon to release new peptide from the ribosome

UAA

endogenous mRNA

UAG

mRNA with protein of interest

Problems with amber suppression

Release factors compete with tRNA^{CUA}, diminishing amber suppression efficiency

Charged tRNA^{CUA} suppress endogenous UAG stop codons

Solution: Recode endogenous UAG (amber codon) to UAA and delete Release Factor 1

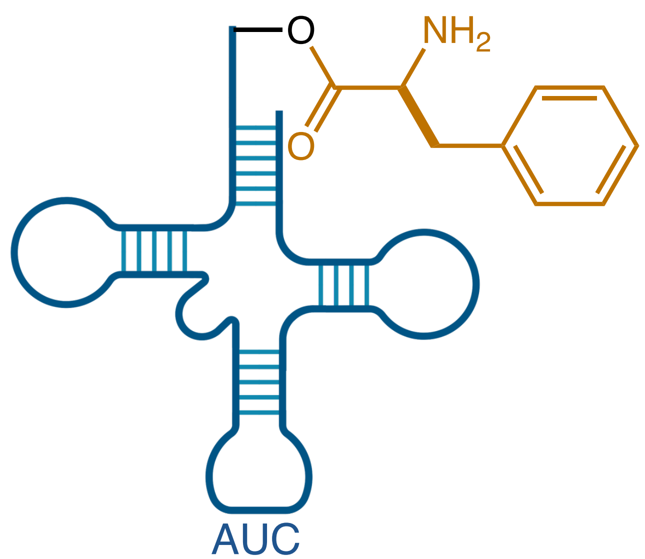
Lajoie, M. J. et al. *Science* **2013**, *342*, 357.

Genomically Recoded Organisms

Stop Codons

Amber	**UAG**
Ochre	UAA
Opal	UGA

charged tRNA^{CUA}



endogenous mRNA

mRNA with protein of interest

Problems with amber suppression

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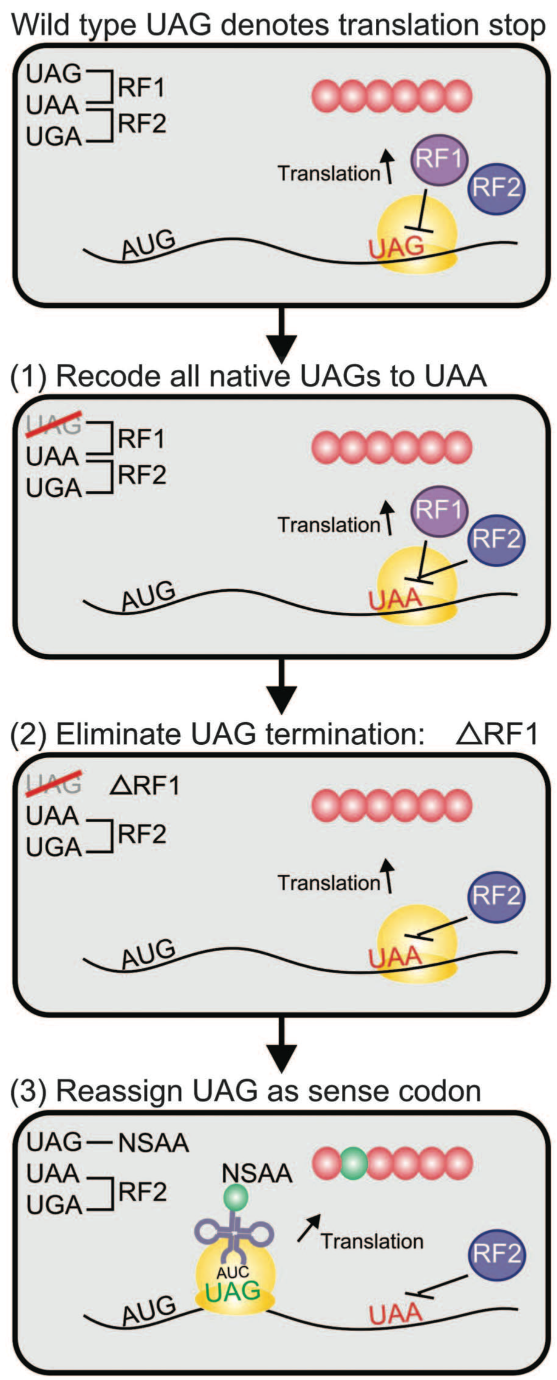
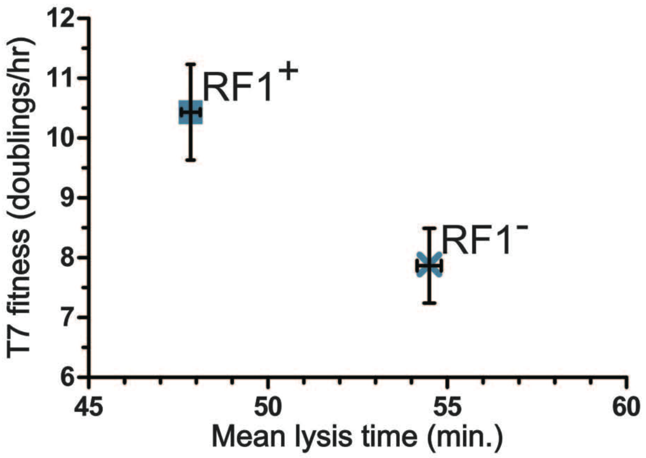
Genomically Recoded Organisms



Project resulted in the E. coli species **C321.ΔA**

Phages rely on host to express proteins necessary for propagation



T7 virus fitness is reduced when release factor 1 is removed

Lajoie, M. J. et al. *Science* **2013**, *342*, 357.

ARTICLE

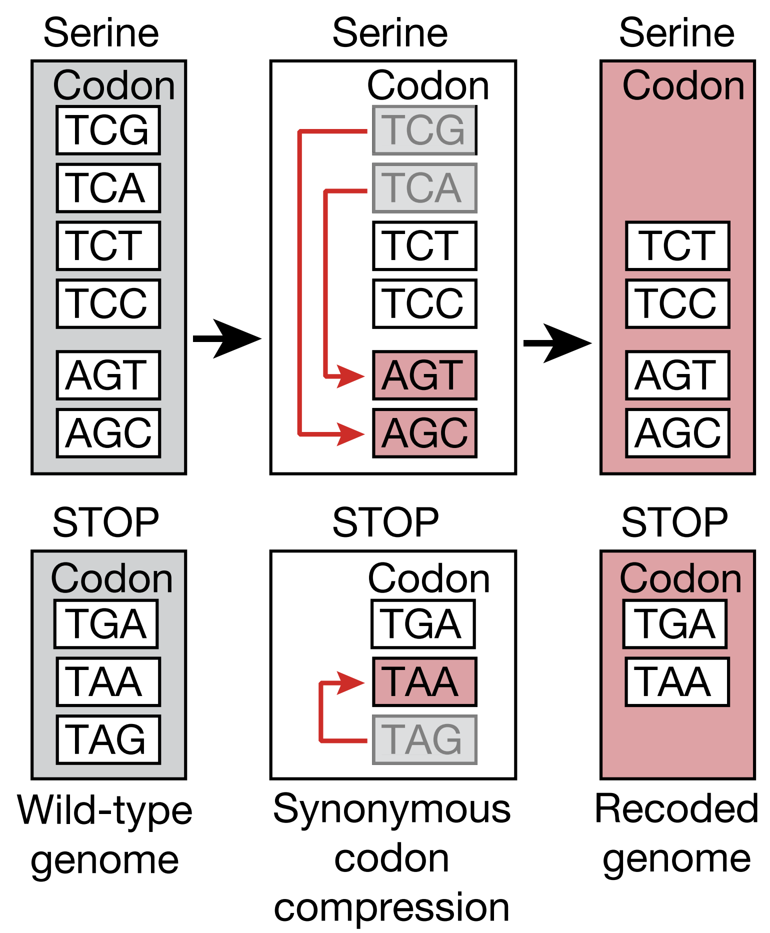
https://doi.org/10.1038/s41586-019-1192-5

Total synthesis of *Escherichia coli* with a recoded genome

Julius Fredens[1,4], Kaihang Wang[1,2,4], Daniel de la Torre[1,4], Louise F. H. Funke[1,4], Wesley E. Robertson[1,4], Yonka Christova[1], Tiongsun Chia[1], Wolfgang H. Schmied[1], Daniel L. Dunkelmann[1], Václav Beránek[1], Chayasith Uttamapinant[1,3], Andres Gonzalez Llamazares[1], Thomas S. Elliott[1] & Jason W. Chin[1]*

Fredens, J. et al. *Nature* **2019**, *569*, 514.

Total Synthesis of E. coli with a Recoded Genome

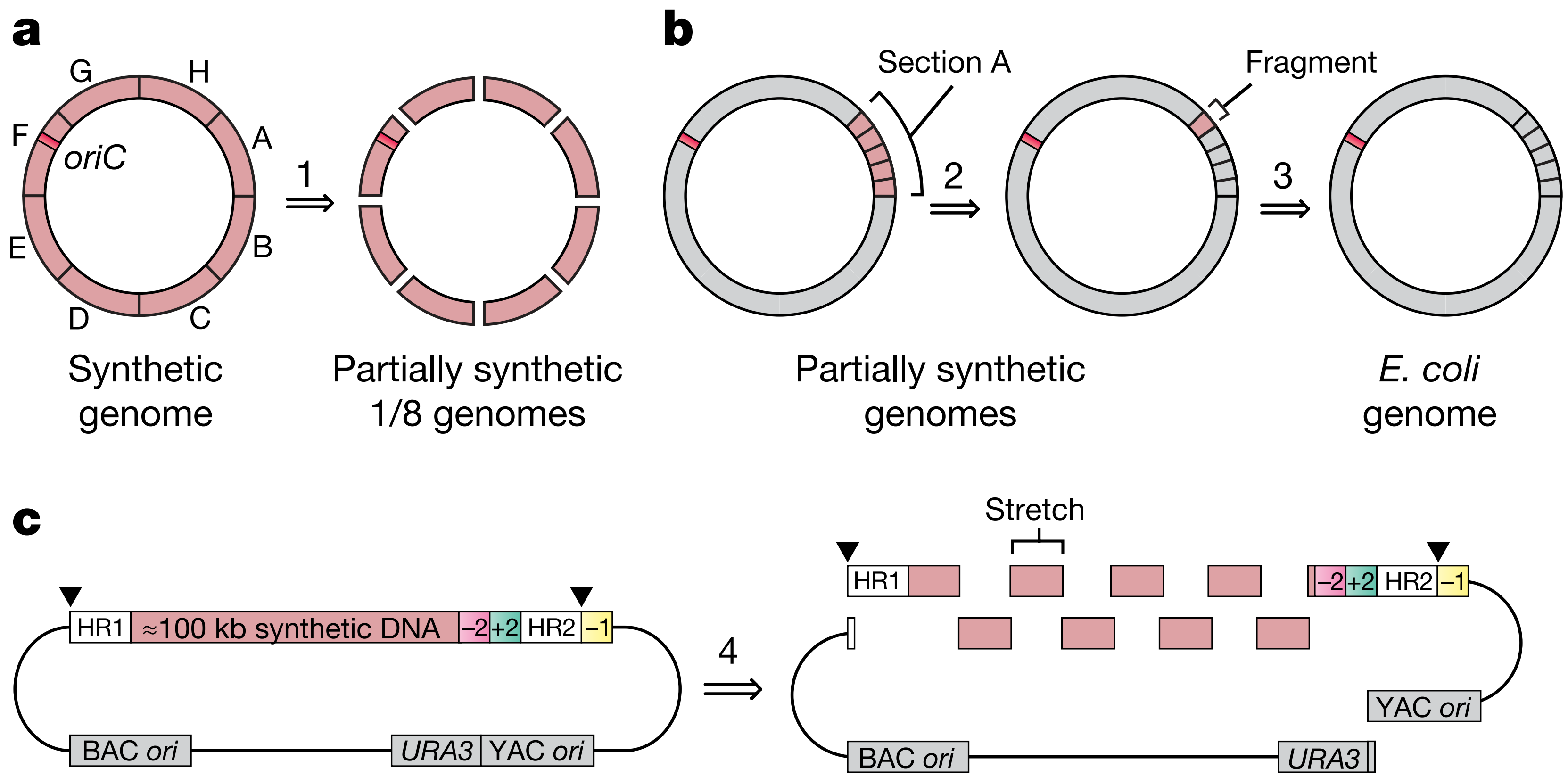


Serine: TCA = AGT and TCG = AGC

Stop codon: TAG = TAA

Fredens, J. et al. *Nature* **2019**, *569*, 514.

Total Synthesis of E. coli with a Recoded Genome



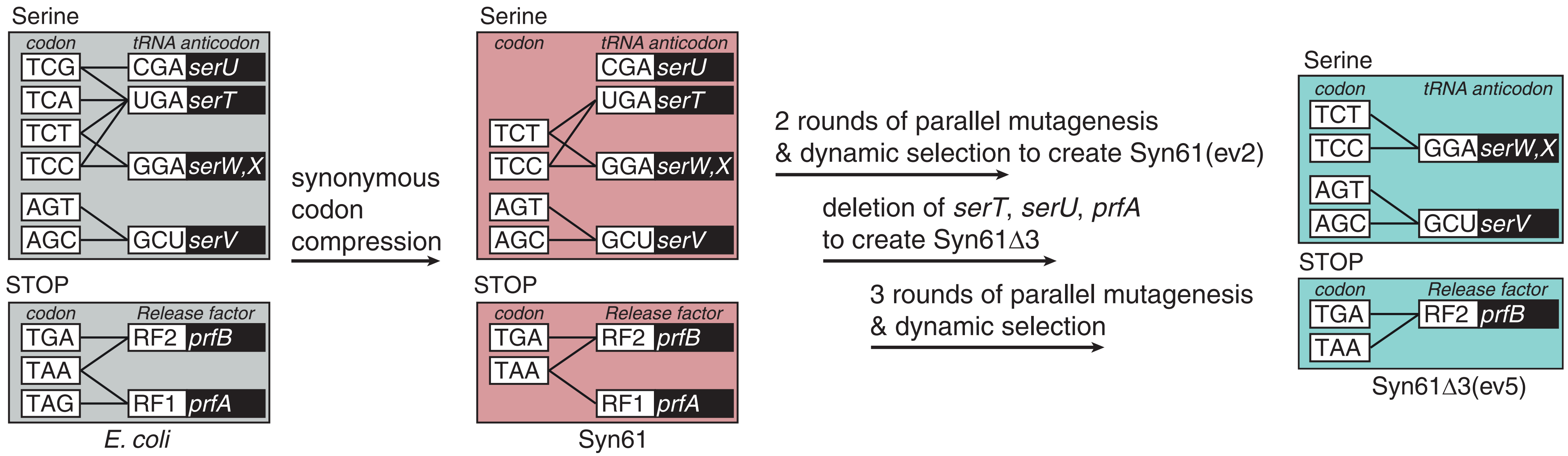
E. coli genome was divided into 8 fragments which were created using REXER

Project resulted in the successful creation of the *E. coli* species **Syn61**

Fredens, J. et al. *Nature* **2019**, *569*, 514.

Total Synthesis of E. coli with a Recoded Genome

Syn61Δ3

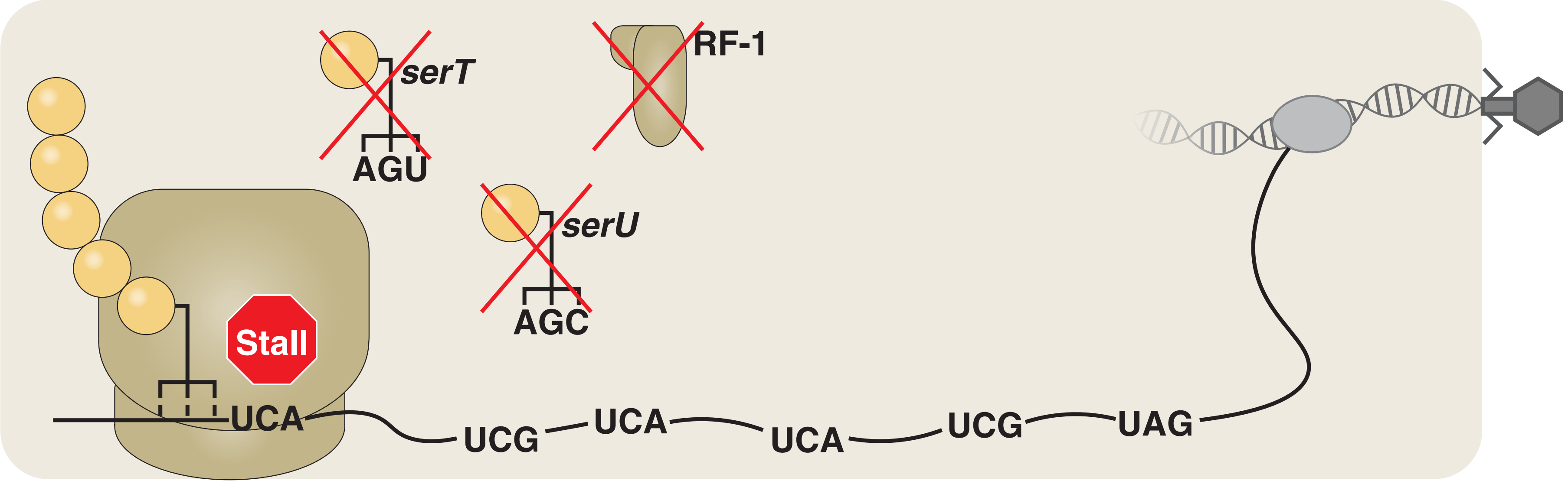


Syn61 can be evolved to remove serine tRNAs and release factor 1

Robertson, W. E. et al. *Science* **2021**, *372*, 1057.

Total Synthesis of E. coli with a Recoded Genome

Syn61Δ3



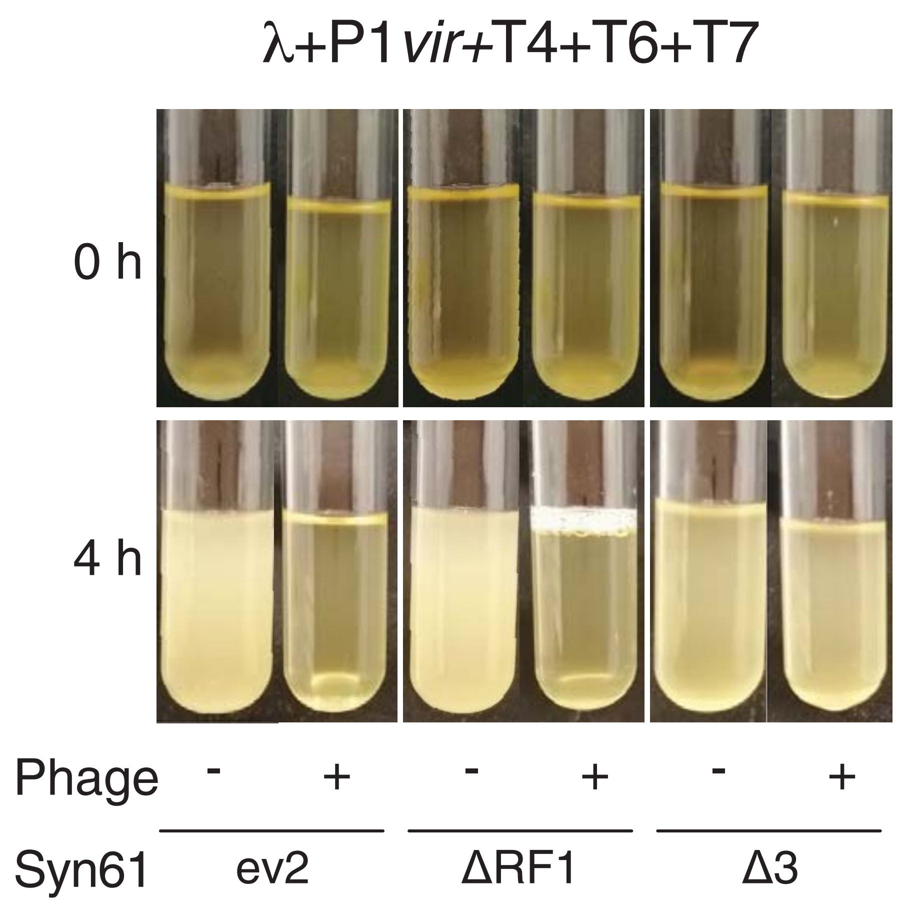
Phages use endogenous translation machinery to reproduce

What happens when phages try to infect Syn61Δ3?

Robertson, W. E. et al. *Science* **2021**, *372*, 1057.

Total Synthesis of E. coli with a Recoded Genome

Syn61Δ3

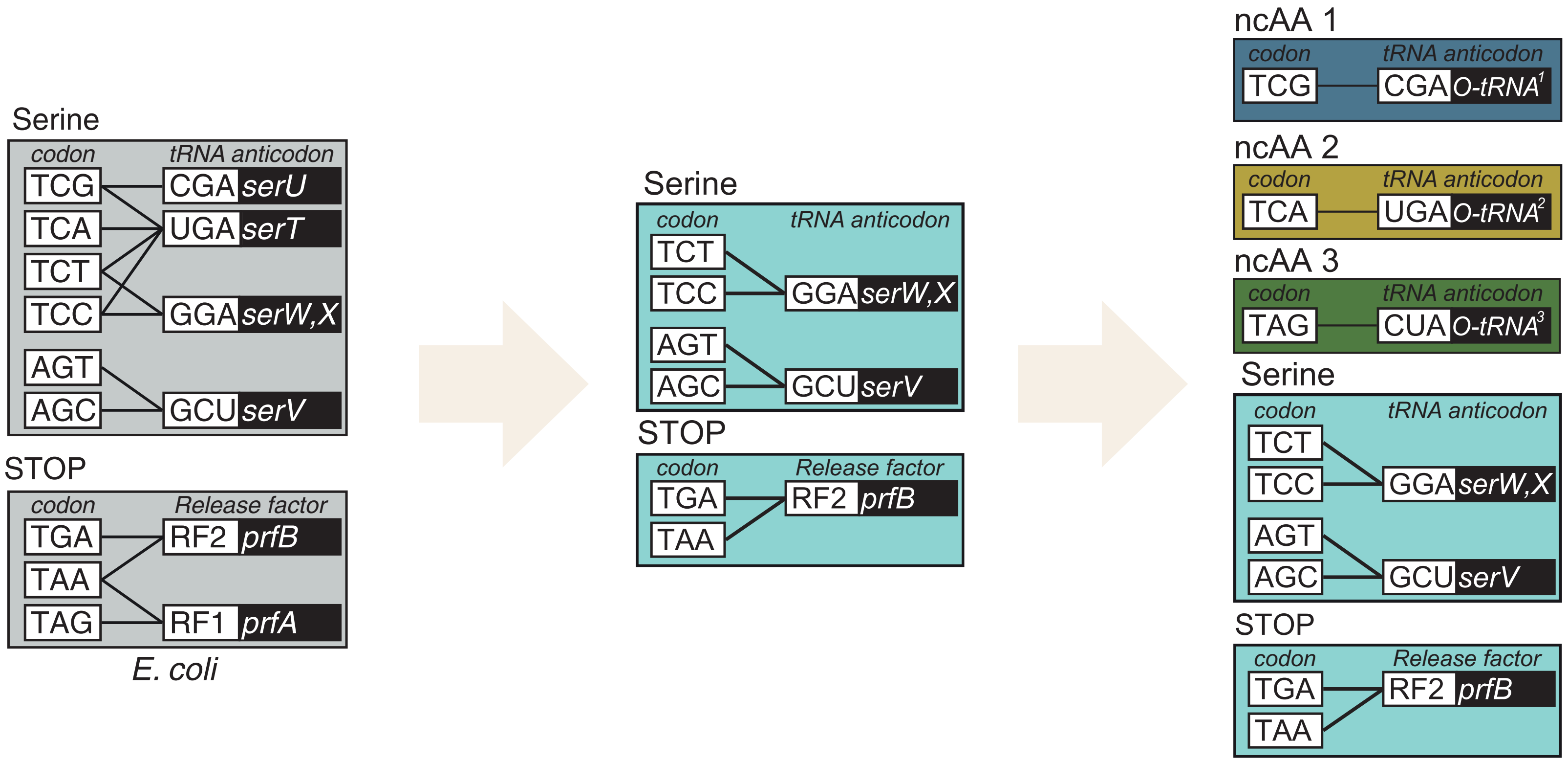


Syn61Δ3 is more resistant to phage infection

Robertson, W. E. et al. *Science* **2021**, *372*, 1057.

Total Synthesis of E. coli with a Recoded Genome

Syn61Δ3



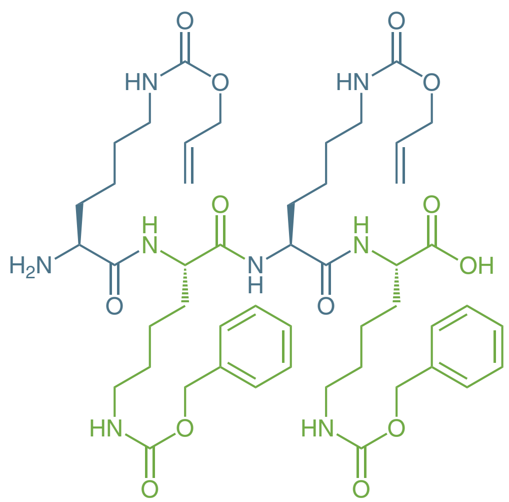
The codons TCG, TCA, and TAG are no longer present in the genome

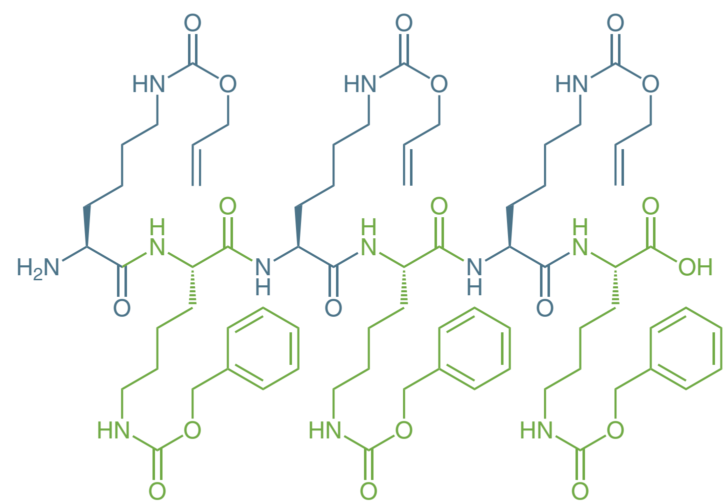
TCG, TCA, and TAG can be recoded to incorporate three different noncanonical amino acids

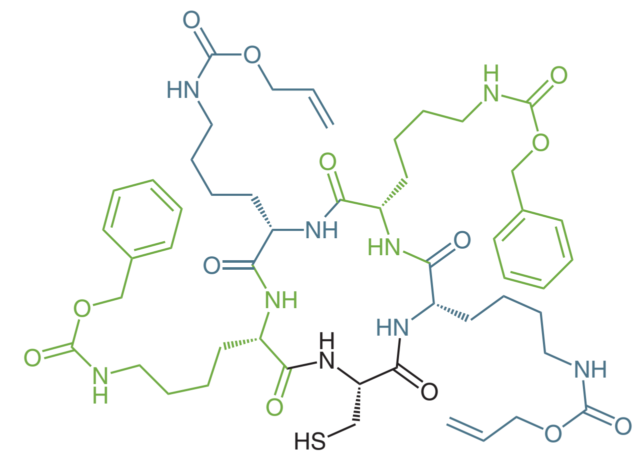
Robertson, W. E. et al. *Science* **2021**, *372*, 1057.

Total Synthesis of E. coli with a Recoded Genome

Syn61Δ3



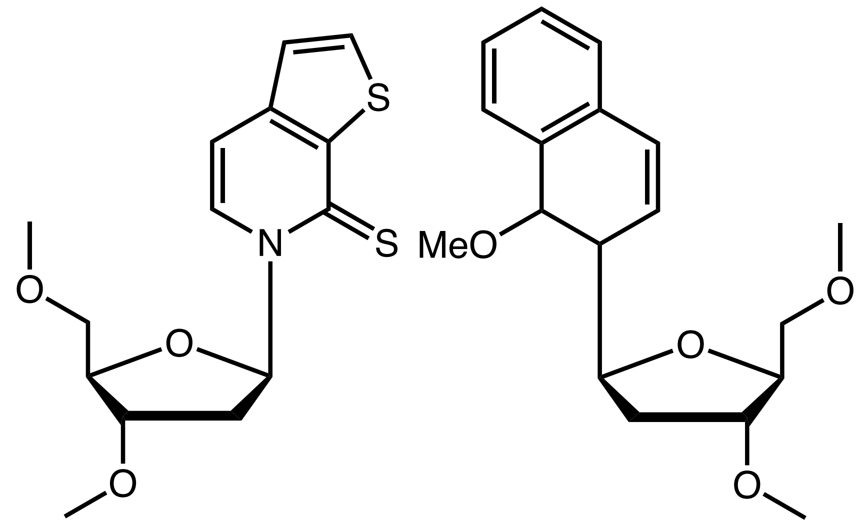




Syn61Δ3 can be used to synthesize noncanonical heteropolymers and macrocycles

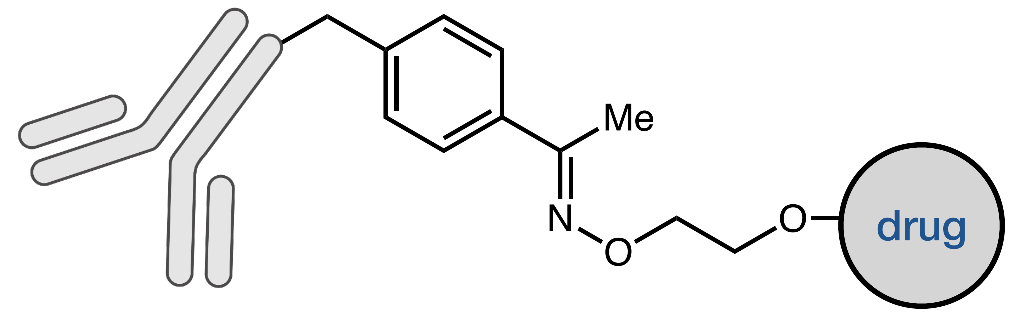
Robertson, W. E. et al. *Science* **2021**, *372*, 1057.

New Conceptual Applications and the Future of Genetic Code Expansion



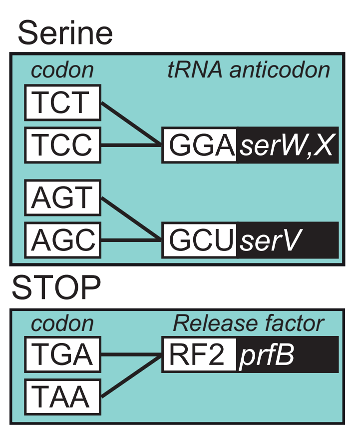
Non-natural nucleic acid base pairs

Nature **2014**, *509*, 385.

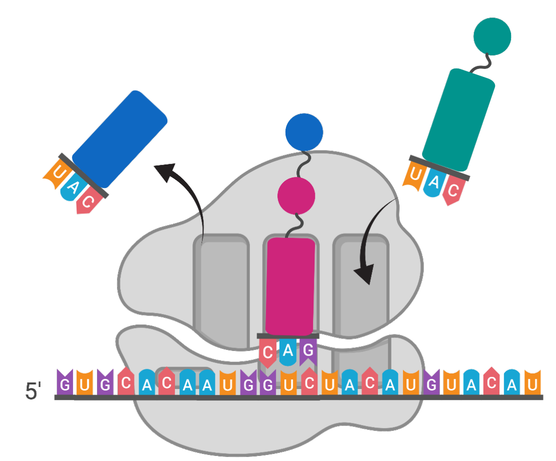


antibody-drug conjugate generation

Proc. Natl. Acad. Sci **2014**, *111*, 1766.



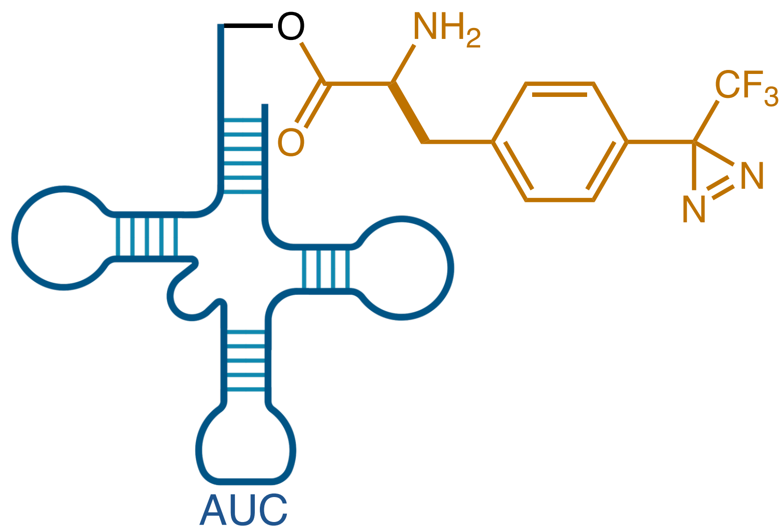
Genomically recoded mammalian cells?



Additional mechanistic exploration to improve noncanonical amino acid incorporation

Shandell, M. A.; Tan, Z.; Cornish, V. W. et al. *Biochemistry* **2021**, *60*, 3455.

Questions?



Shandell, M. A.; Tan, Z.; Cornish, V. W. et al. *Biochemistry* **2021**, *60*, 3455.