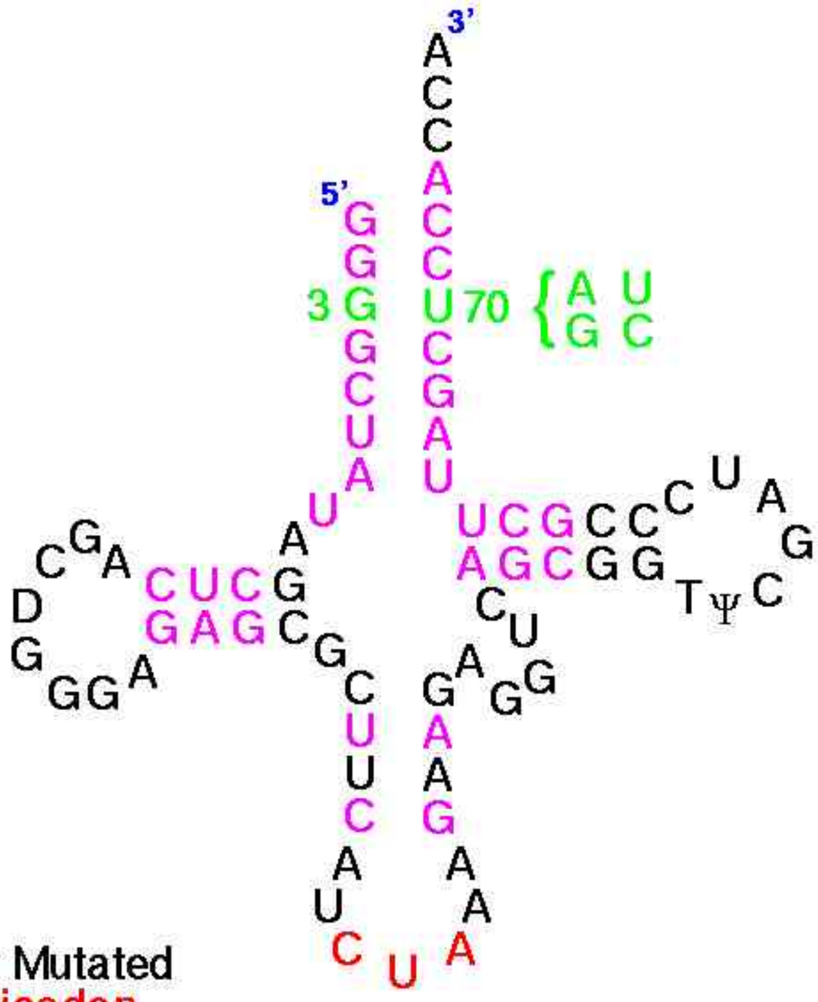
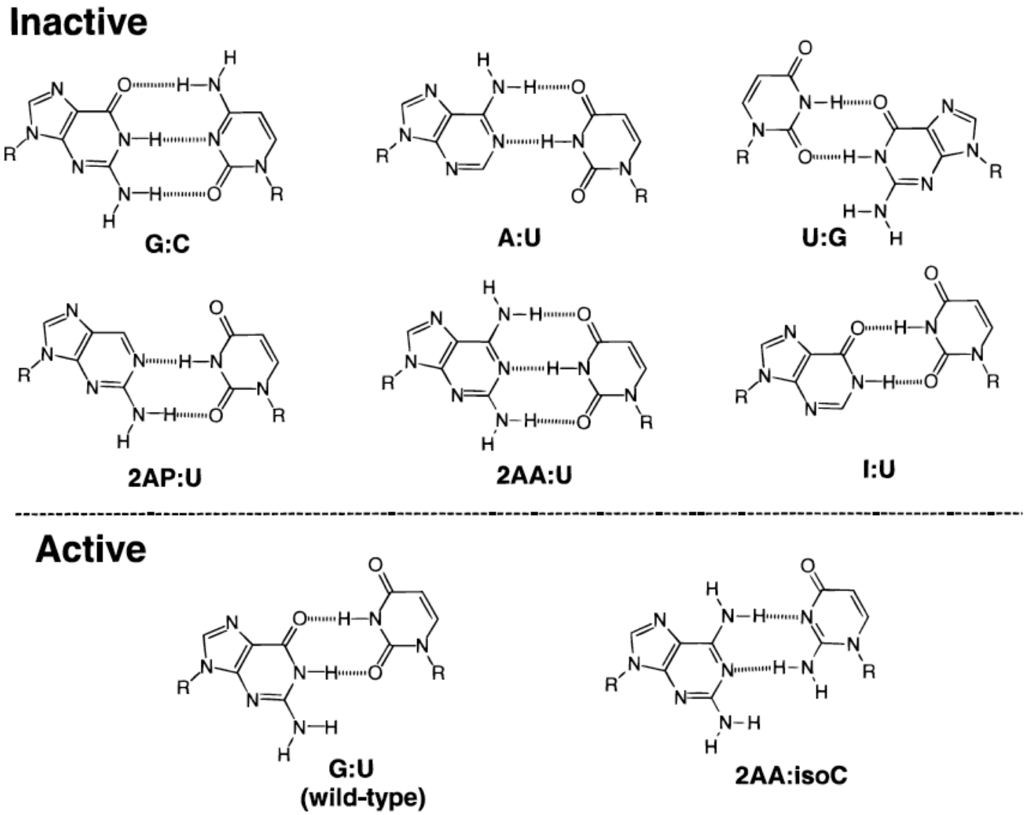
tRNA^{Ala,CUA}



Not Mutated
Anticodon
Silent Mutations
Killer Mutations

Hou & Schimmel (1988) *Nature* 333, 140

Specificity for G3•U70



Acc. Chem. Res. **1999,** *32,* 368−375

Base pair (3·70)	K_{cat}/K_m tRNA^{Ala}
G·U (wild-type)	1.0
G·A	1.1×10^{-2}
A·C	2.8×10^{-4}
C·A	1.8×10^{-4}
G·C	2.1×10^{-5}

Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 10150–10154, September 1997

AlaRS bound to tRNA^{Ala}(G•U)

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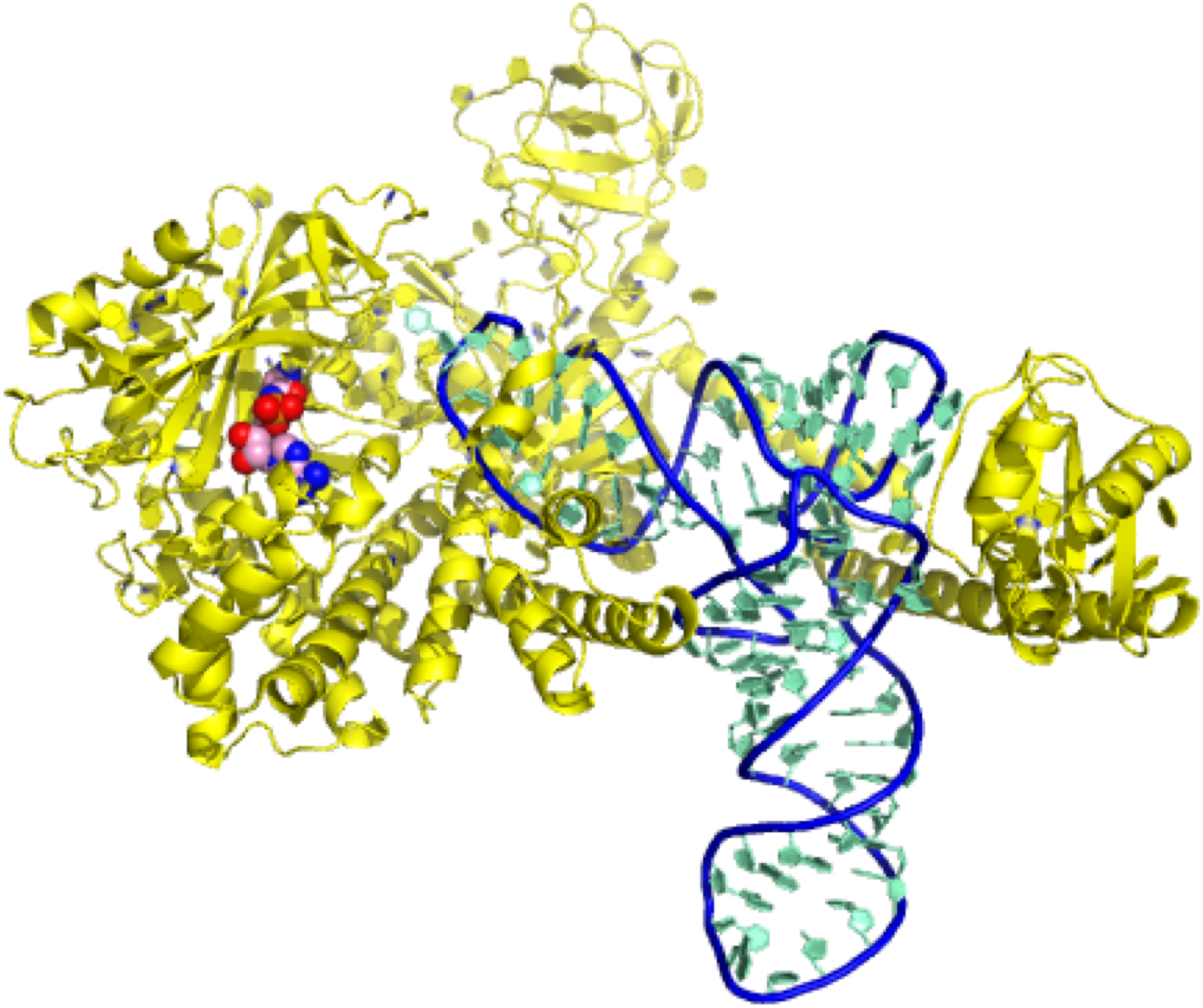
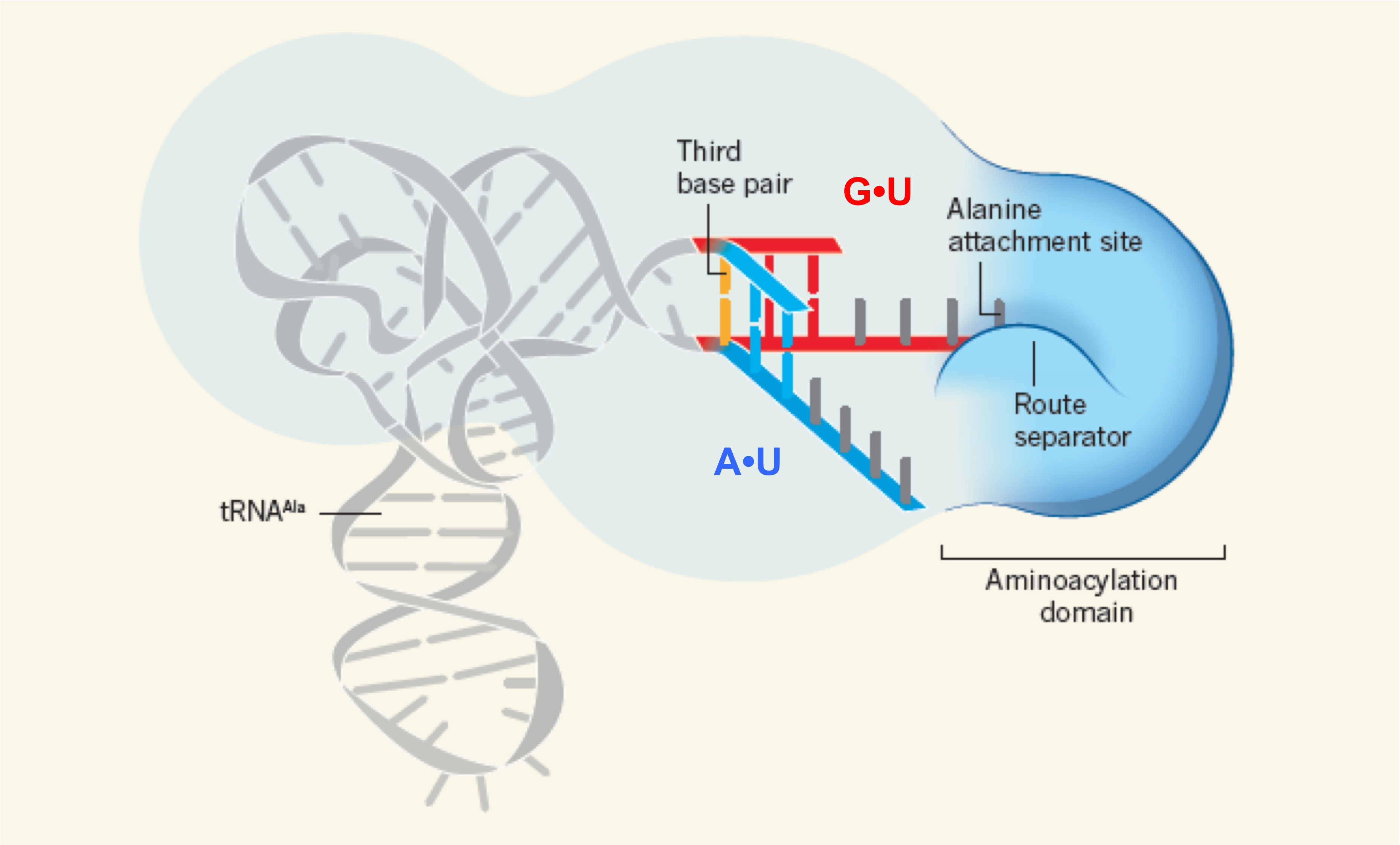
AlaRS bound to tRNA^{Ala}(A•U)



26 JUNE 2014 | VOL 510 | NATURE | 507

AlaRS bound to tRNA^{Ala}



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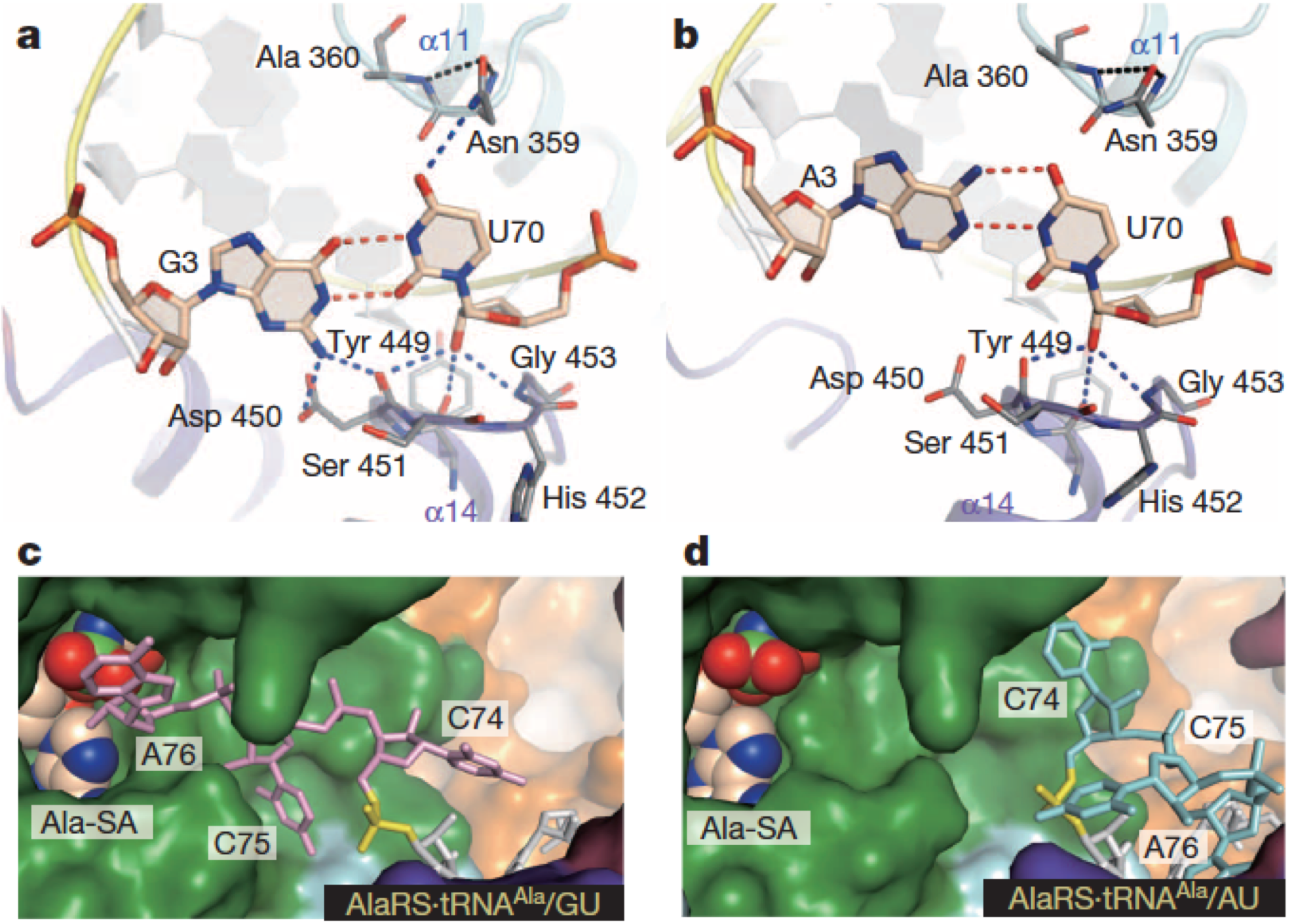
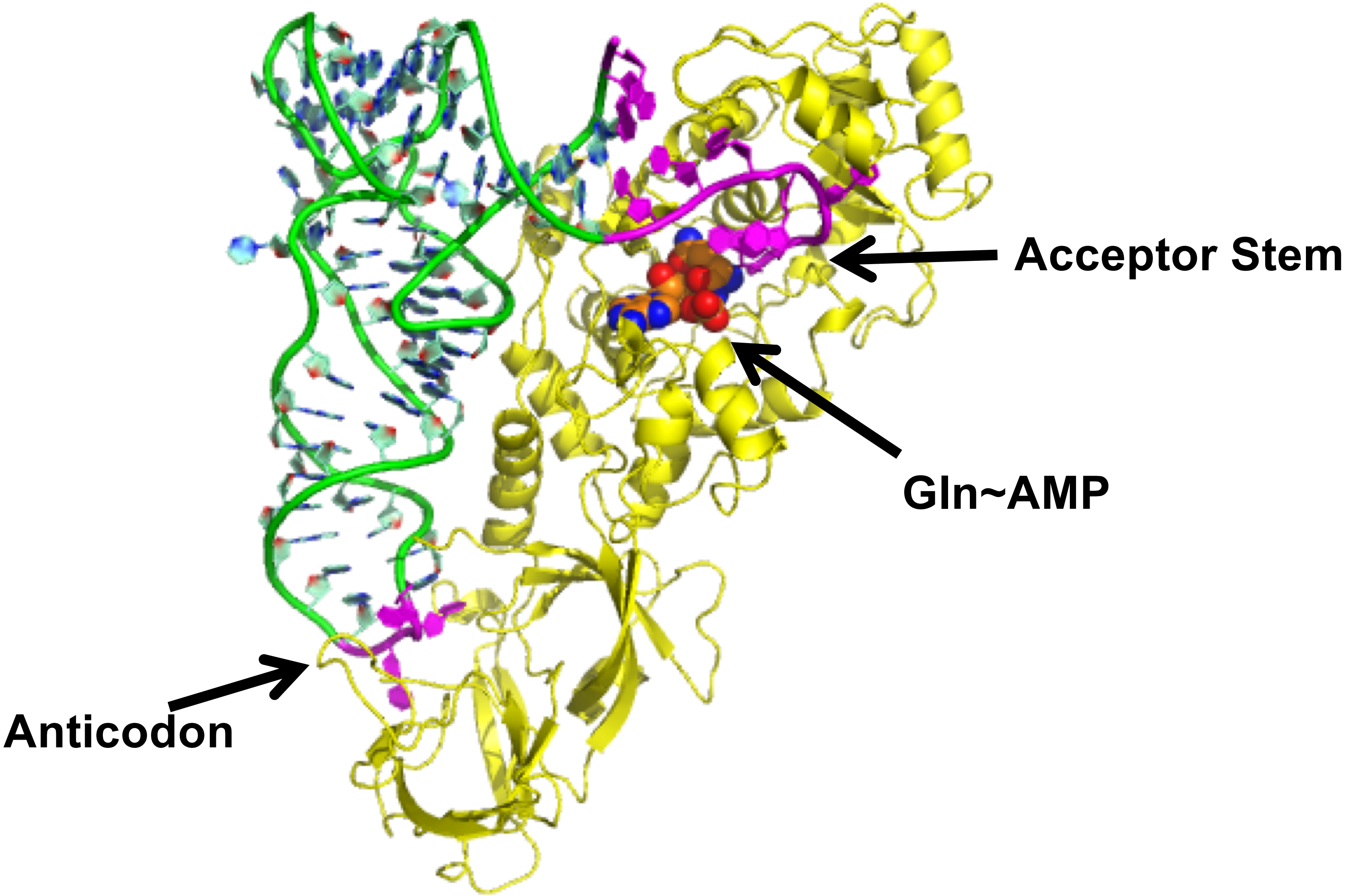
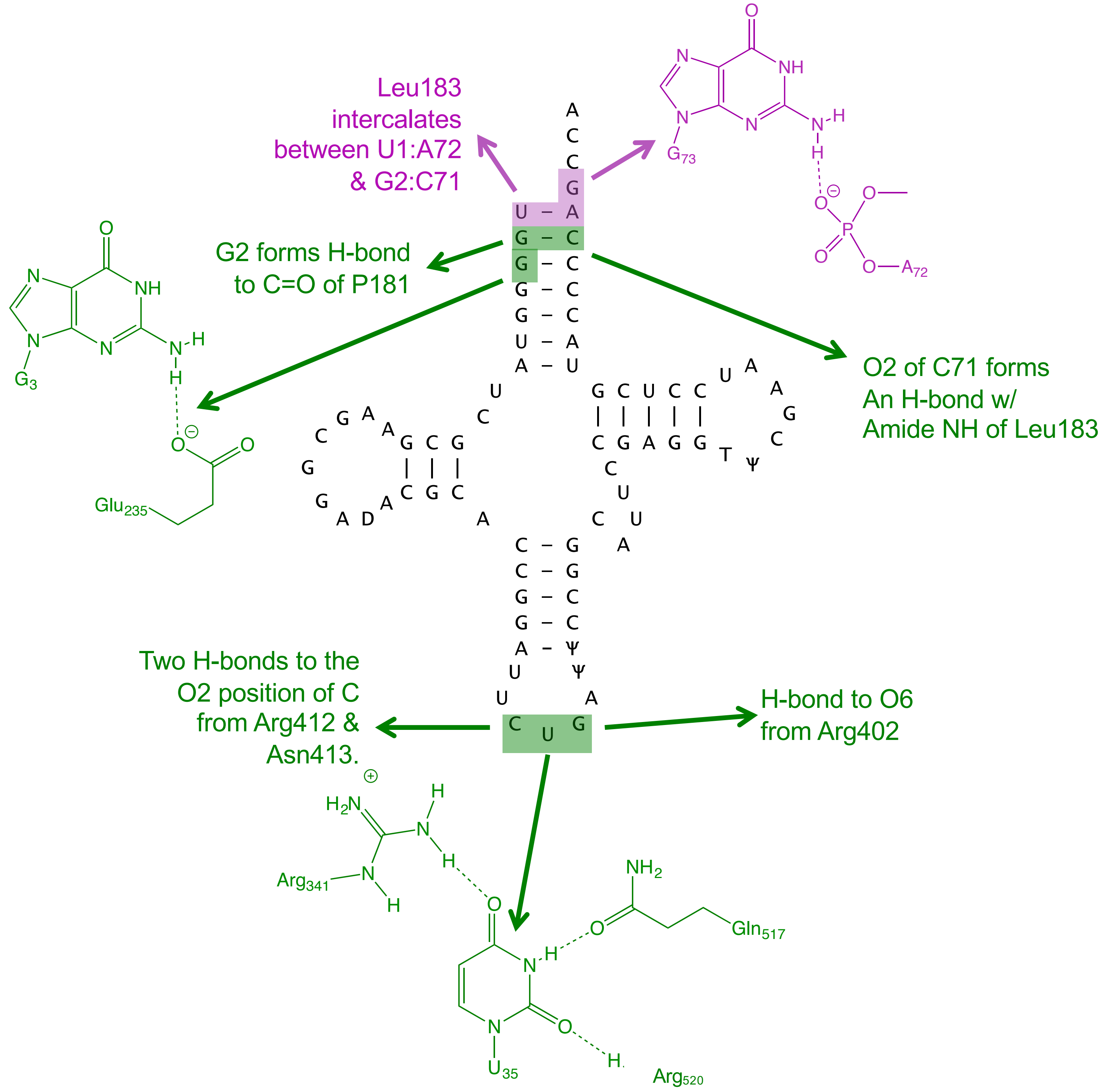

a
Ala 360
α11
Asn 359
U70
G3
Tyr 449
Gly 453
Asp 450
Ser 451
His 452
α14
b
Ala 360
α11
Asn 359
A3
U70
Tyr 449
Asp 450
Gly 453
Ser 451
His 452
α14
c
C74
A76
Ala-SA
C75
AlaRS·tRNA^{Ala}/GU
d
C74
C75
Ala-SA
A76
AlaRS·tRNA^{Ala}/AU

GlnRS•tRNA•Gln~AMP Complex



Steitz et al. (1989) *Science* 246, 1135

Direct & **Indirect** Readout Of tRNA^{Gln} Sequence



Leu183 intercalates between U1:A72 & G2:C71

G2 forms H-bond to C=O of P181

O2 of C71 forms An H-bond w/ Amide NH of Leu183

Two H-bonds to the O2 position of C from Arg412 & Asn413.

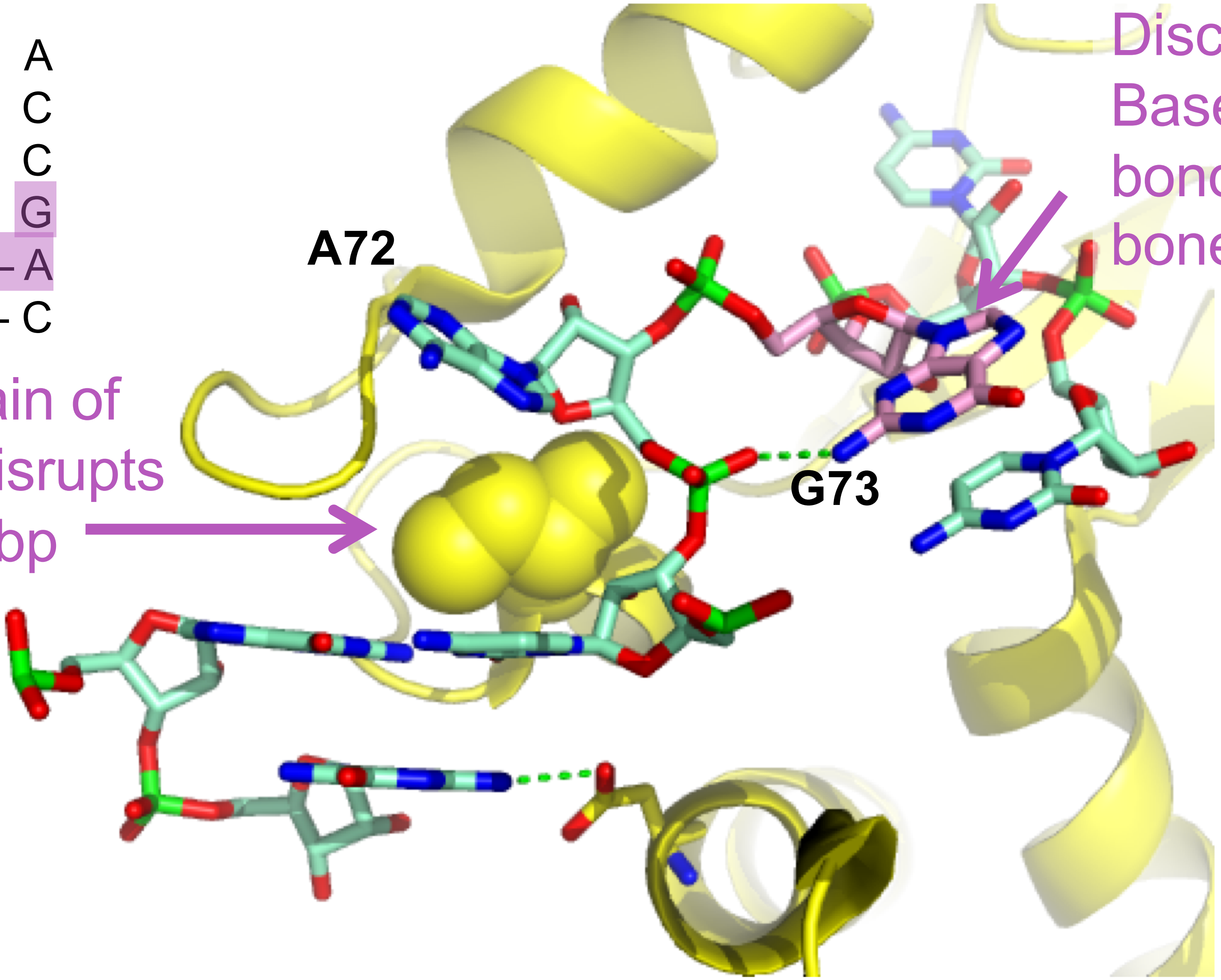
H-bond to O6 from Arg402

Kinetics of GlnRS

tRNA Variant	**kcat (s^{-1})**	**Km (μM)**	**kcat/Km ($M^{-1}s^{-1}$)**	**ΔΔG‡ (kcal/mol)**
Wild type (in vitro)*	0.2	0.15	1.3 x 10^{6}	
Discriminator Base				
G73→A73	0.14	0.2	7.0 x 10^{5}	+0.4
G73→U73	0.0068	8.0	850	+4.4
Acceptor Stem				
U1:A72→G1:A72	0.92	0.66	1.4 x 10^{6}	
U1:A72→G1:C72	0.17	1.3	1.3 x 10^{5}	+1.4
G2:C71→A2:U71	0.011	10	1.1 x 10^{3}	
G3:C70→A3:U70	0.046	3.3	1.4 x 10^{4}	+2.7
Anticodon Loop				
U/C34→A34	0.00065	2.5	260	+5.1
U35→**C35**	**0.00034**	**6.7**	**50**	**+6.1**
G36→A36	0.036	6.6	5500	+3.3

Indirect Readout in Acceptor Stem

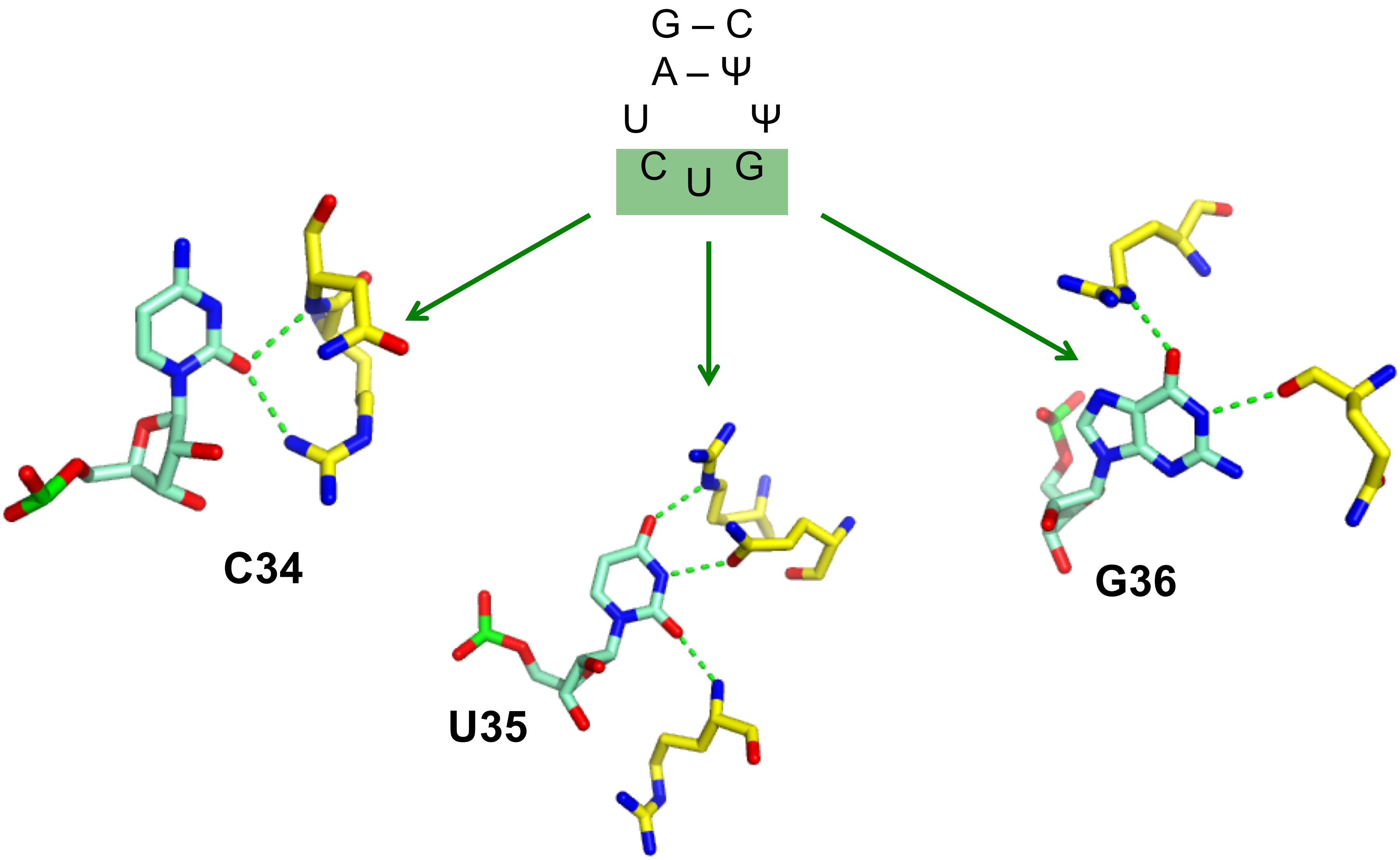
A
C
C
G
U – A
G – C



Discriminator Base, **G73**, H-bonds to back bone

Side chain of **Ile183** disrupts U1:A72 bp

Anticodon Loop – Direct Readout



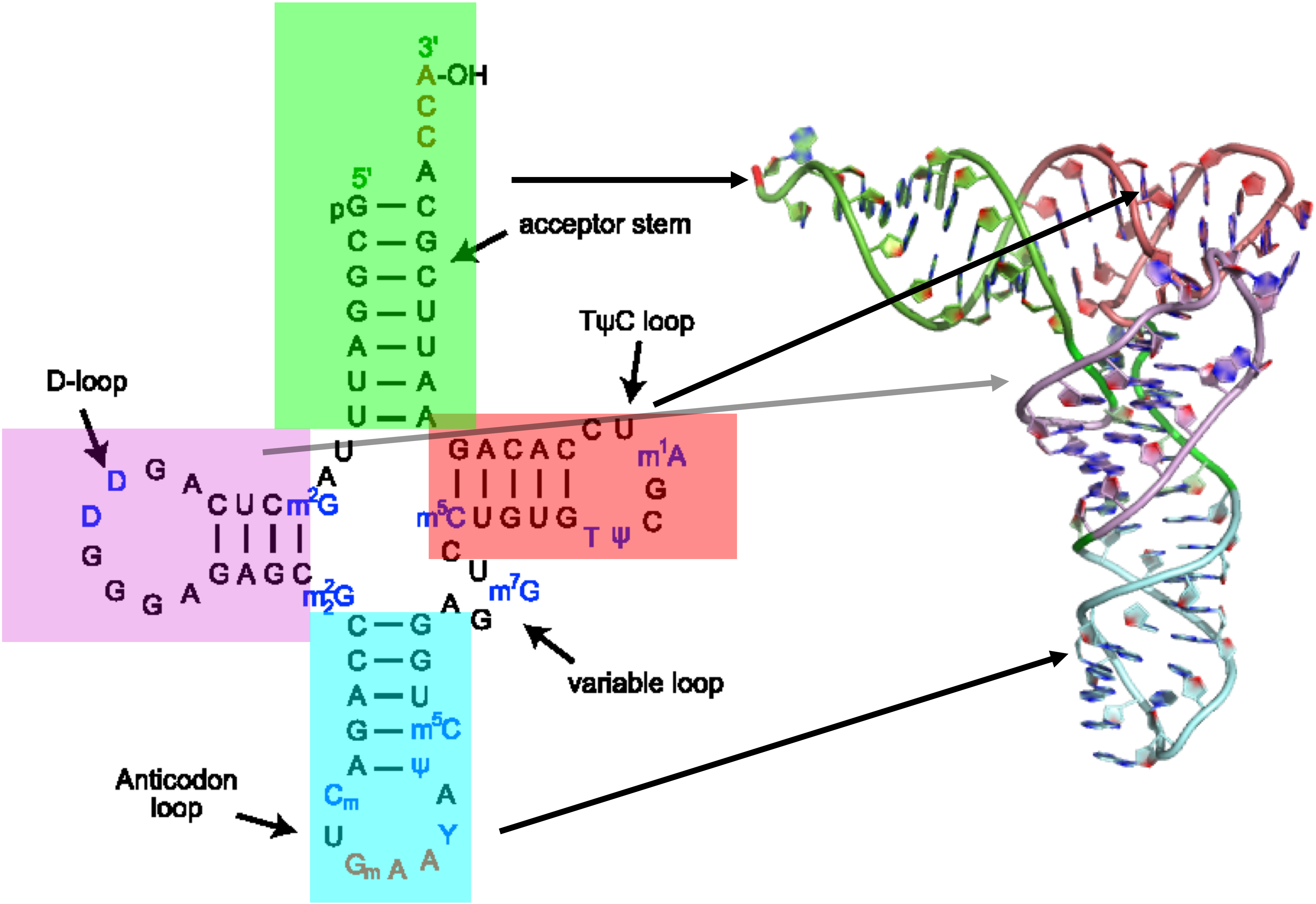
G – C
A – Ψ
U Ψ
C U G

C34

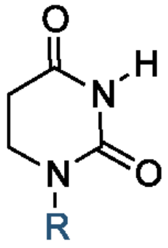
U35

G36

tRNA Structure

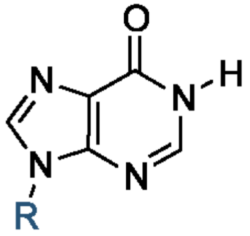


Modified Bases

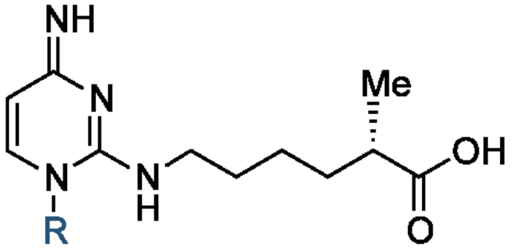


D

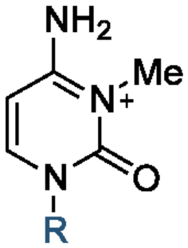
dihydrouridine



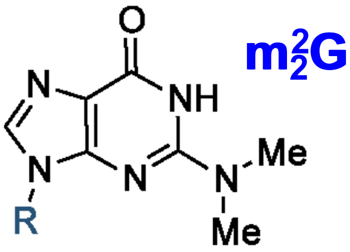
inosine



lysidine

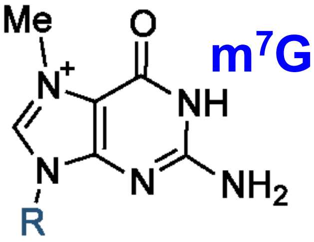


3-methylcytidine



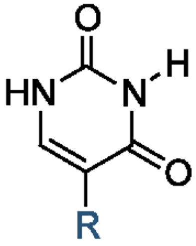
m^2_2G

N(2)-dimethylguanosine



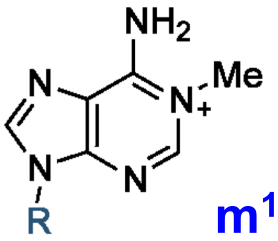
m^7G

N(7)-methylguanosine



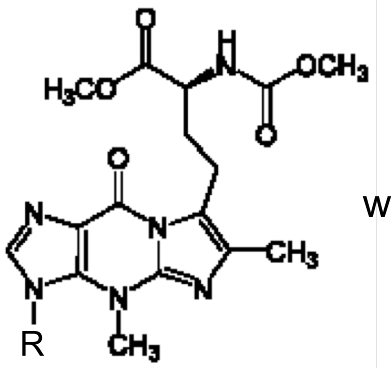
Ψ

pseudouridine



m^1A

1-methyladenosine

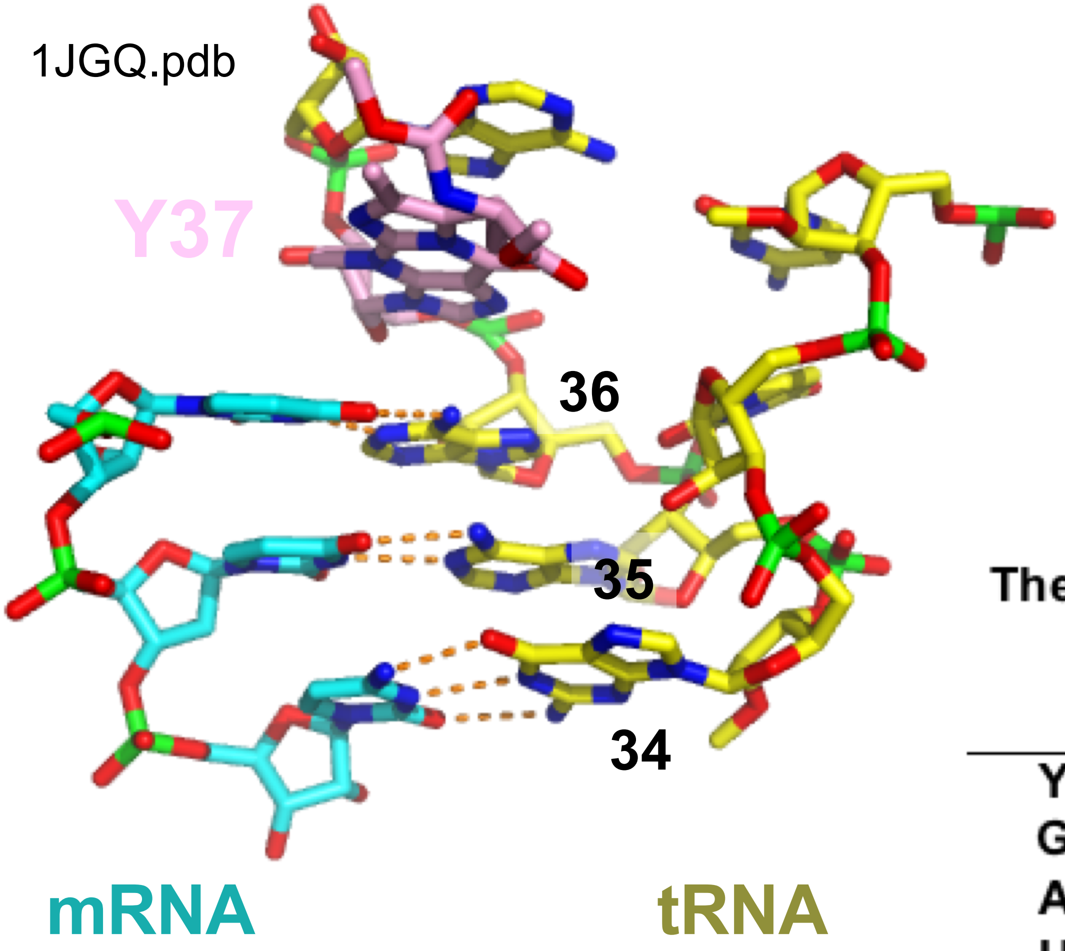


wybutosine

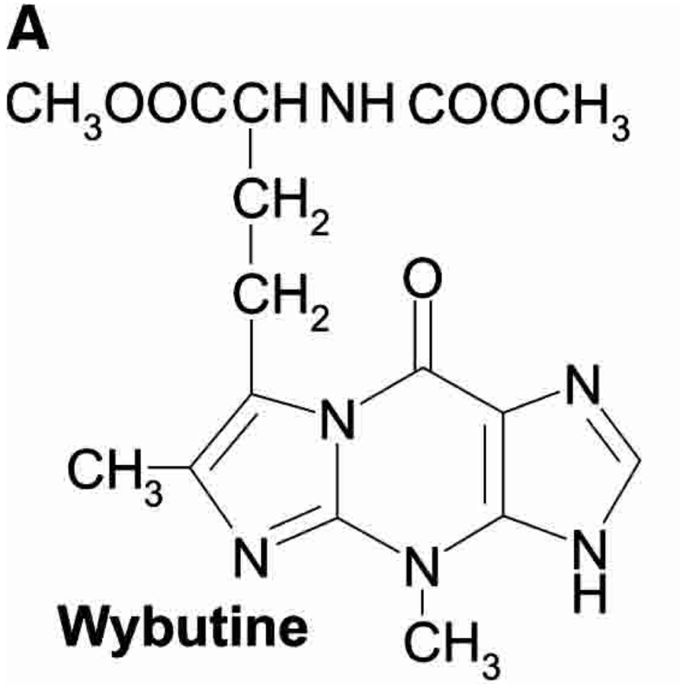
Y

http://www.atdbio.com/content/14/Transcription-Translation-and-Replication

Wybutosine Stacks



RNA (2004), 10:90–101.

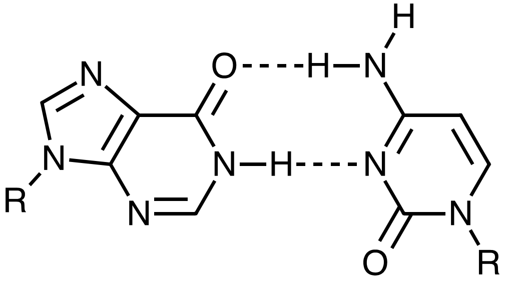


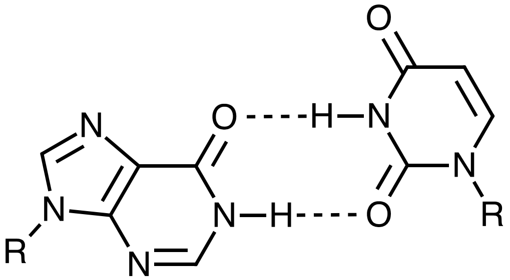
Thermodynamics of Binding to Codon

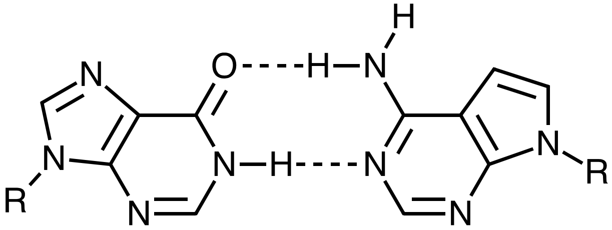
	ΔG° (kcal/mol)	ΔH° (kcal/mol)	-TΔS (kcal/mol)
Y37	-10.0	-47	37
G37	-6.9	-47	40
A37	-8.6	-42	33
U37	-8.2	-15	7
C37	-8.1	-15	7

Wobble Pairings: Anticodon Purines

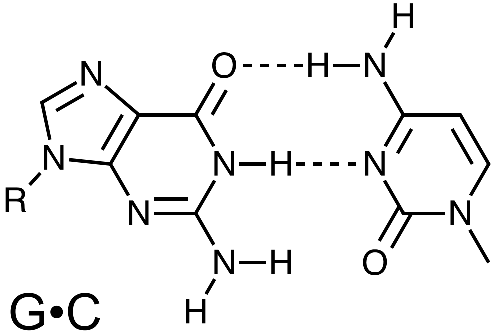
Inosine

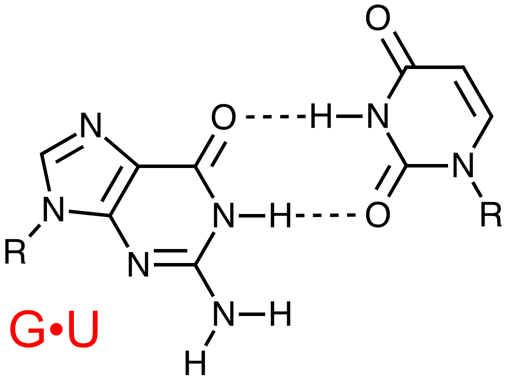

I•C


I•U

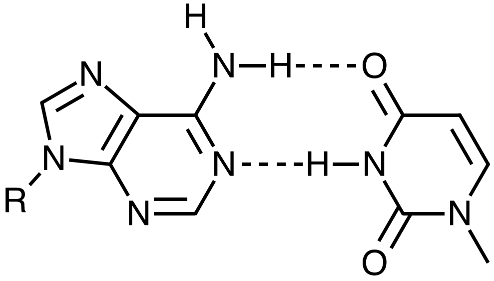

I•A

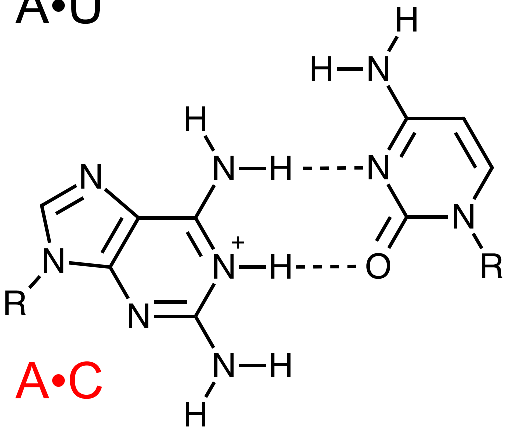
Guanine


G•C


G•U

Adenine

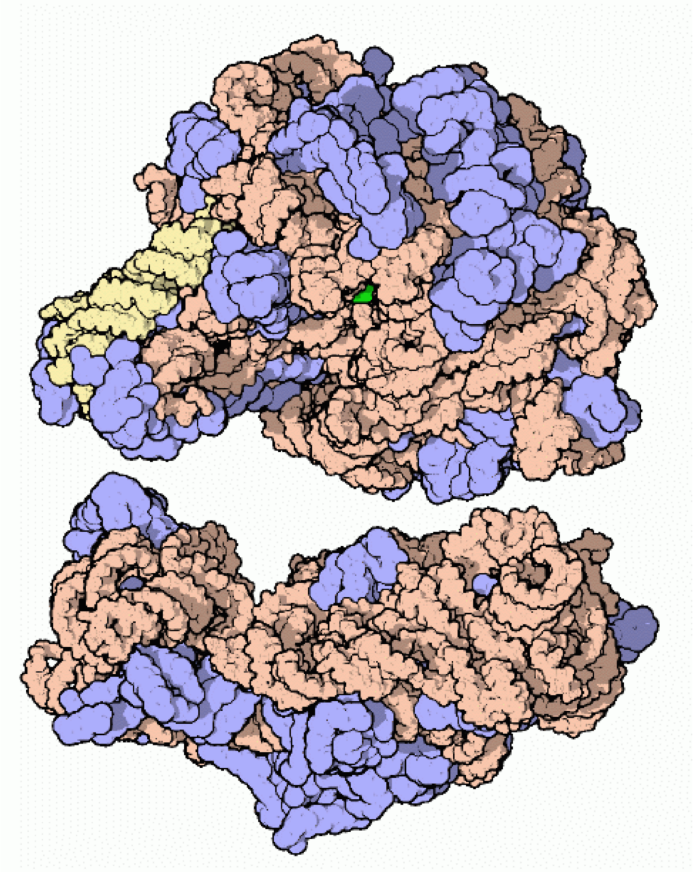

A•U


A•C

With U or C in wobble position (34) of the anticodon, the same base pairs are possible.

Cochella & Green (2004) *NSMB* **11**, 1160

The Bacterial 70 S Ribosome



Large Subunit (50 S)

1.6 MDa

2 rRNA's: 5 S (120 b), 23 S (2904 b)

31 ribosomal proteins (L1-L31)

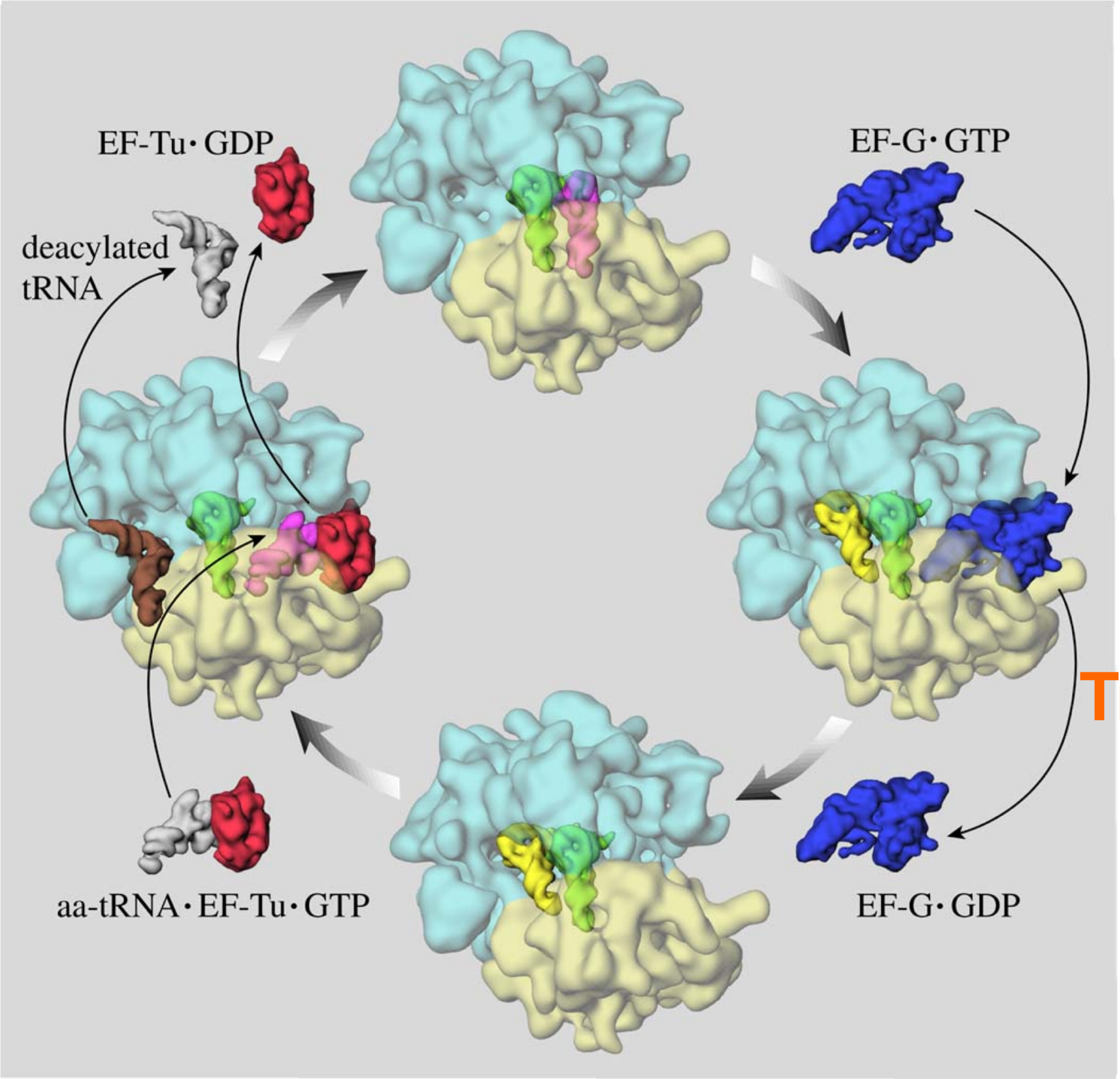
Small Subunit (30 S)

0.9 MDa

1 rRNA: 16 S (1542 b)

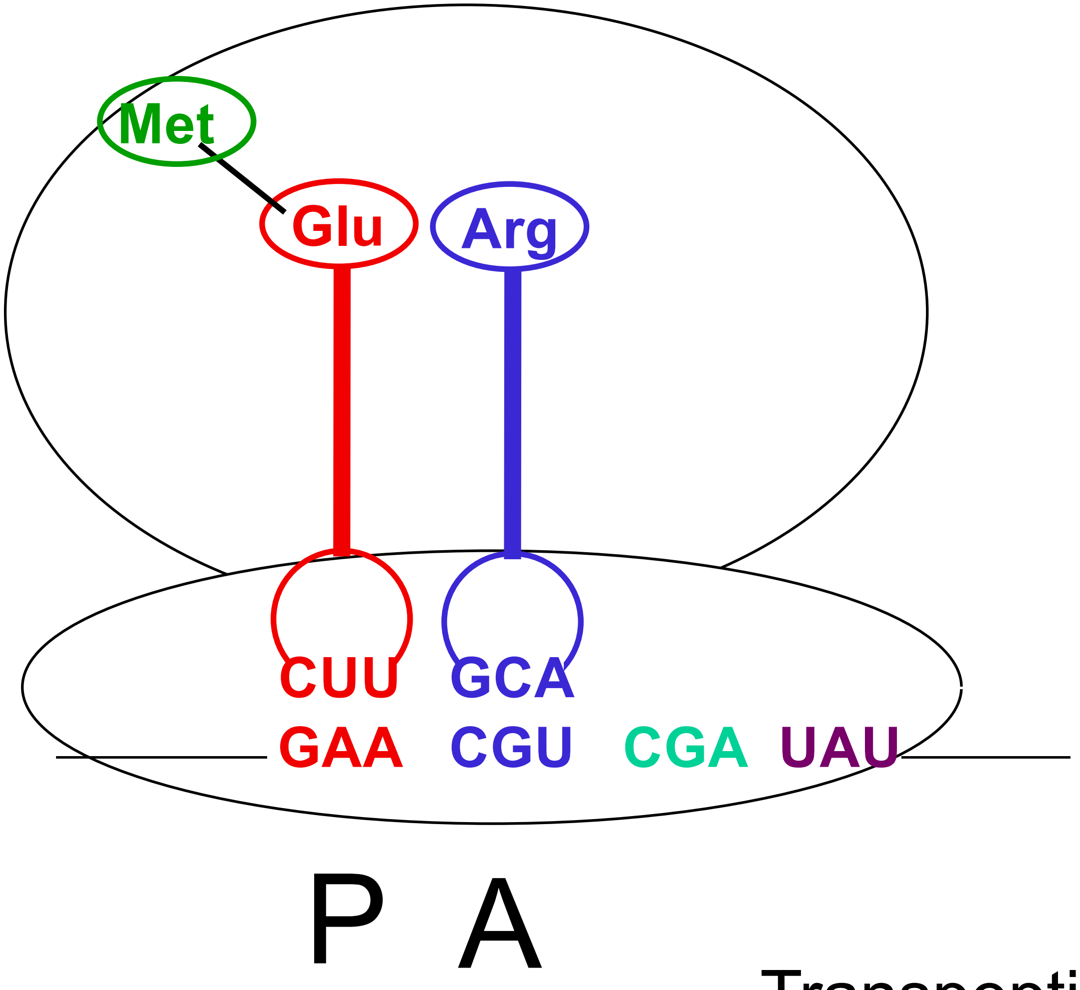
21 ribosomal proteins (S1-S21)

Elongation Cycle



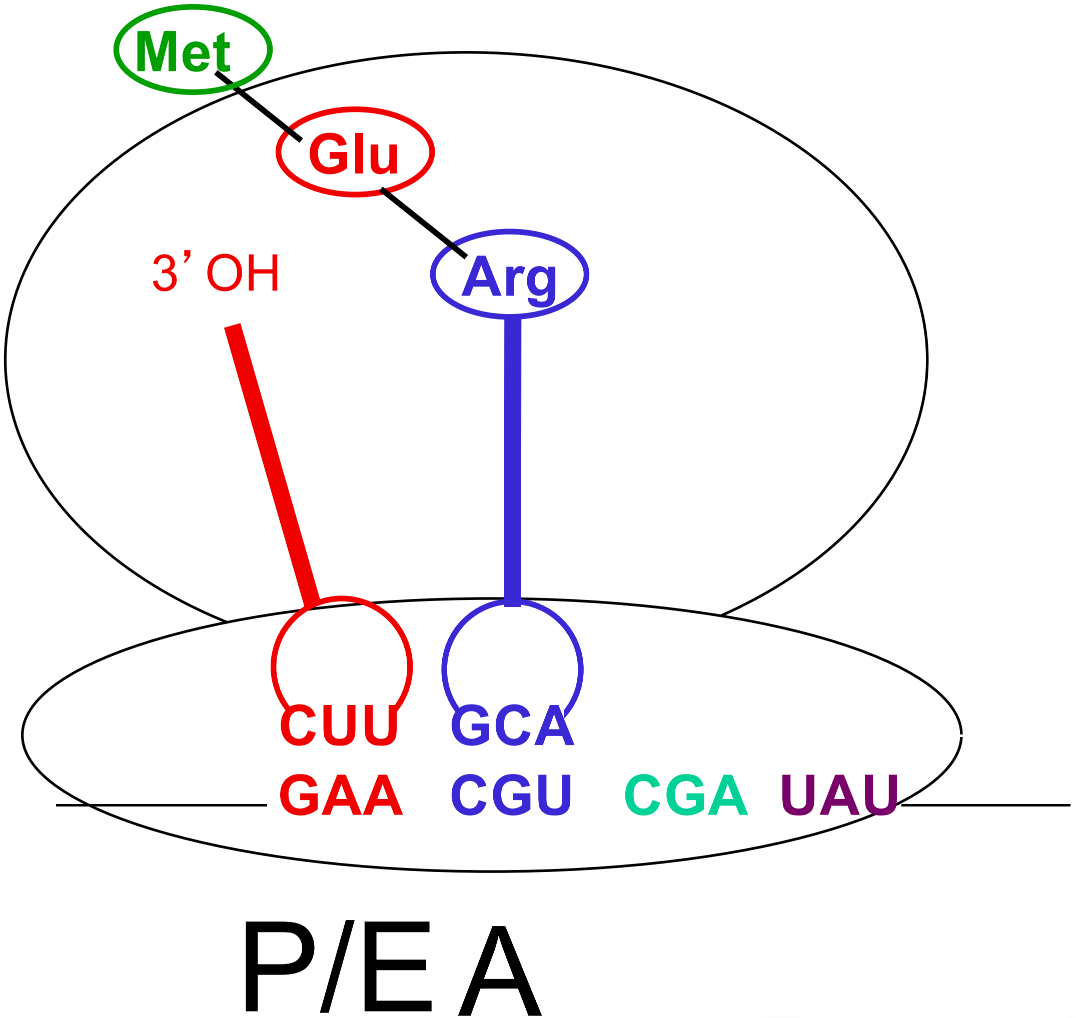
Wadsworth Center, New York State Department of Health

Peptide Synthesis Takes Place on the Ribosome



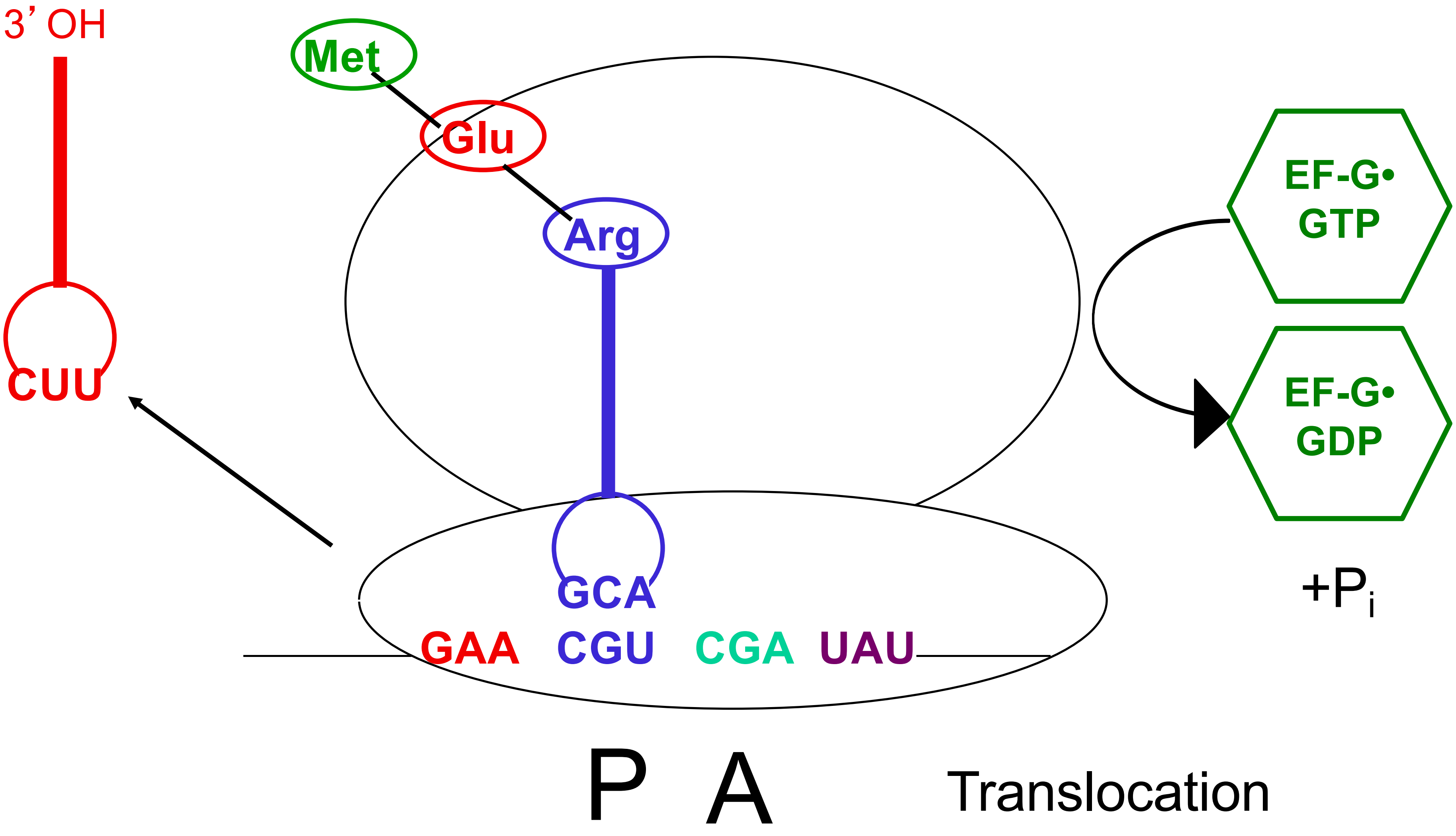
Transpeptidation

Peptide Synthesis Takes Place on the Ribosome



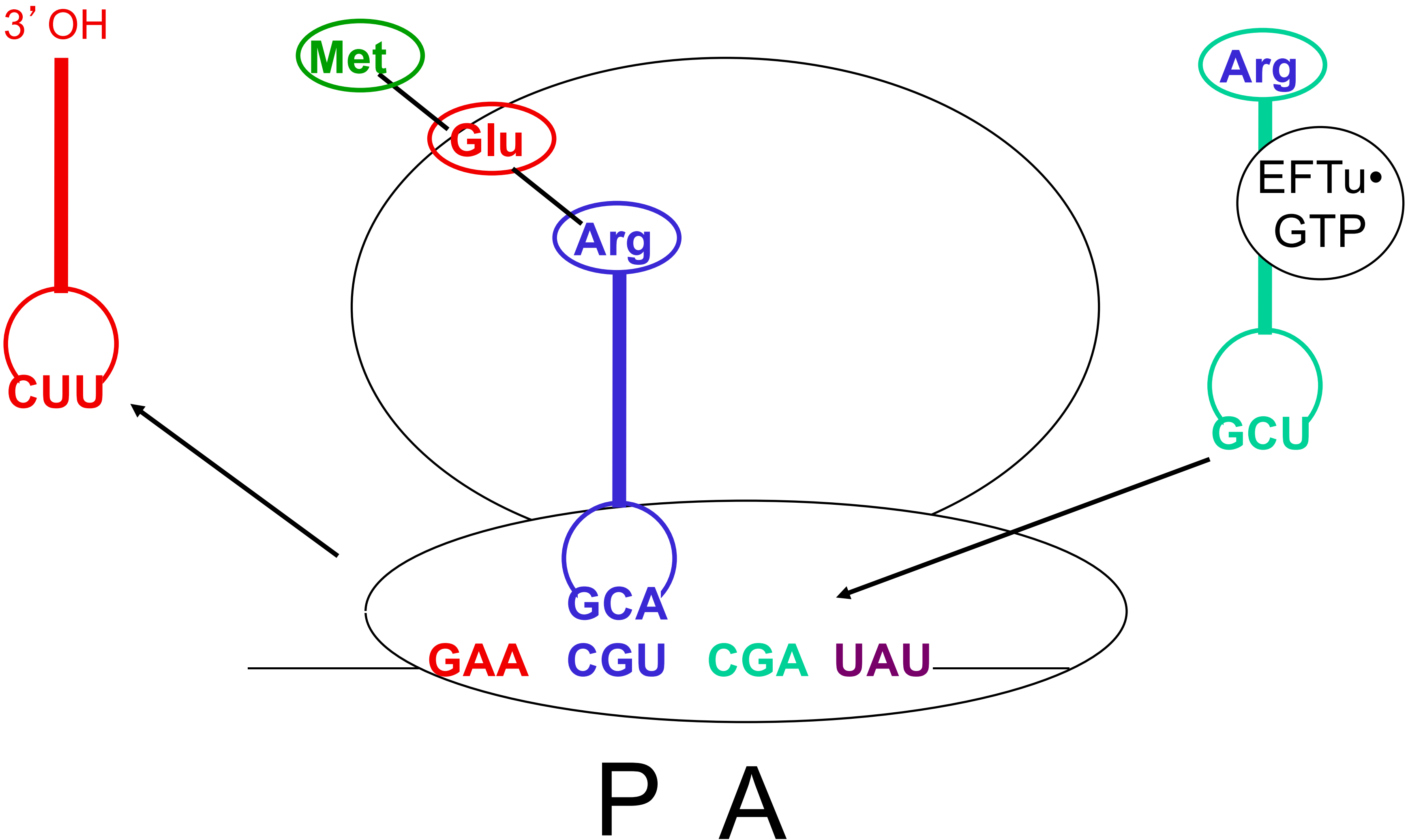
Transpeptidation

Peptide Synthesis Takes Place on the Ribosome



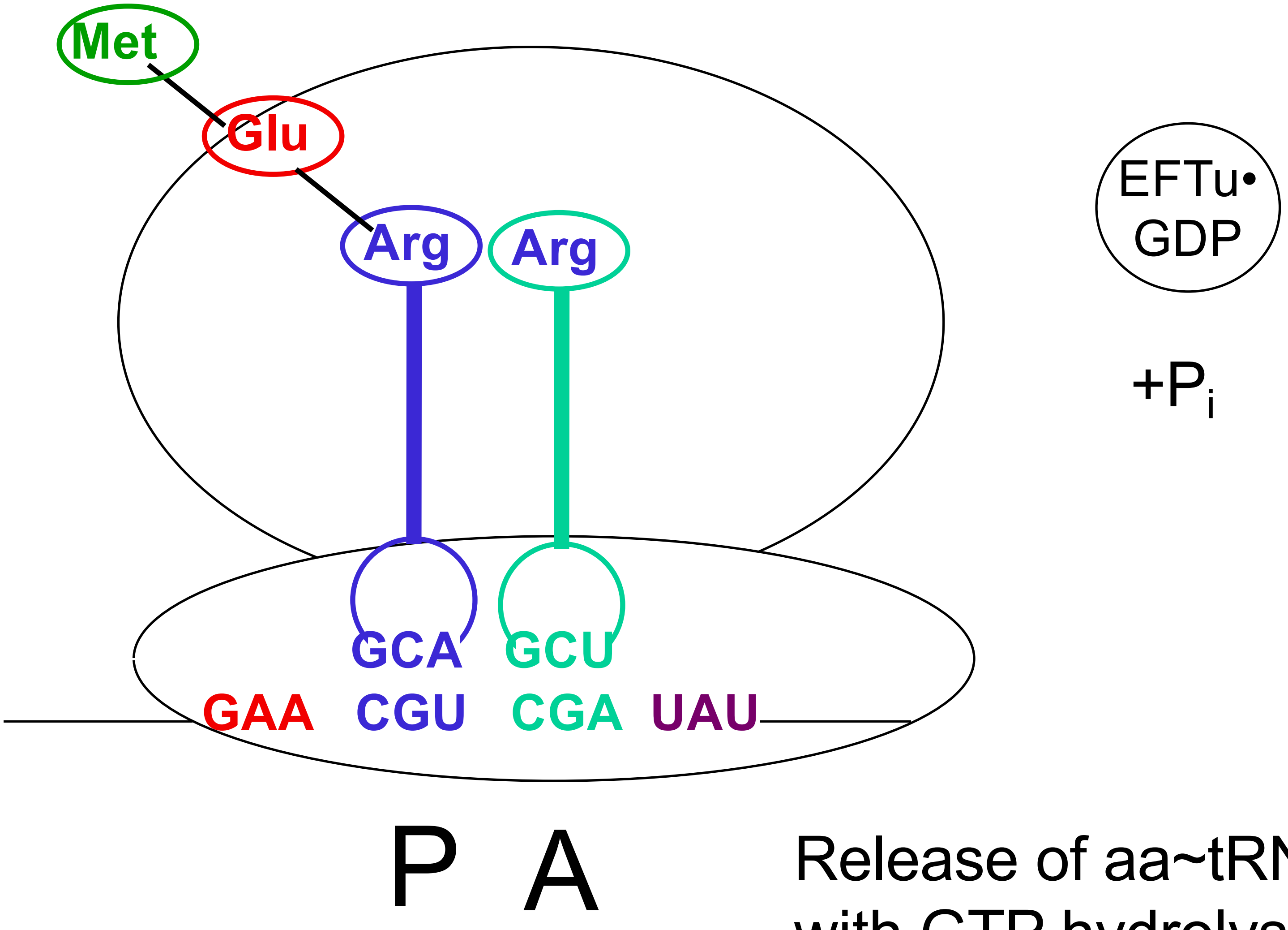
Translocation
with GTP hydrolysis

Peptide Synthesis Takes Place on the Ribosome



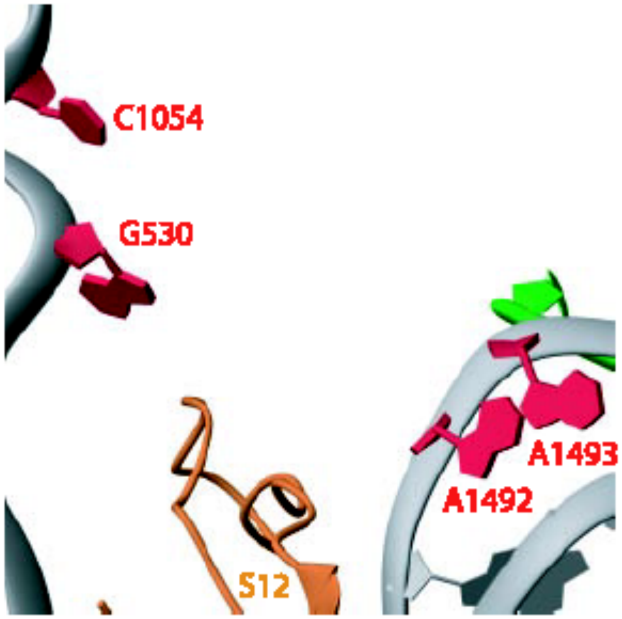
Binding EFTu•GTP•aa~tRNA

Peptide Synthesis Takes Place on the Ribosome

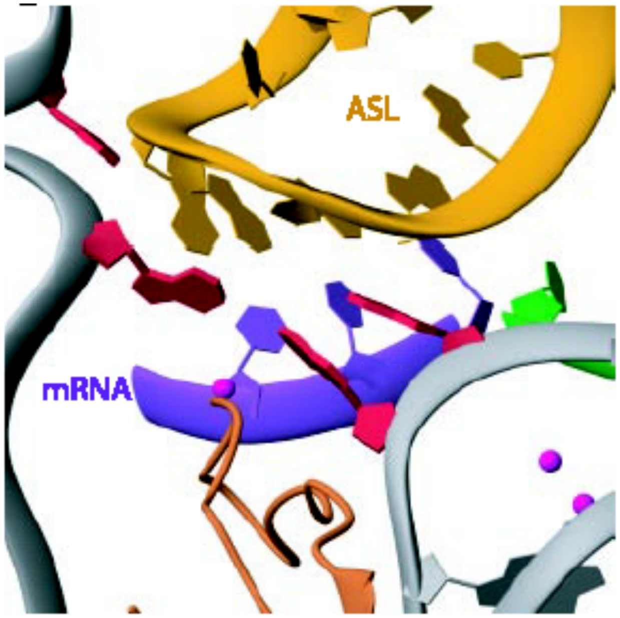


Release of aa~tRNA with GTP hydrolysis

Codon-Anticodon Recognition



Vacant A-Site

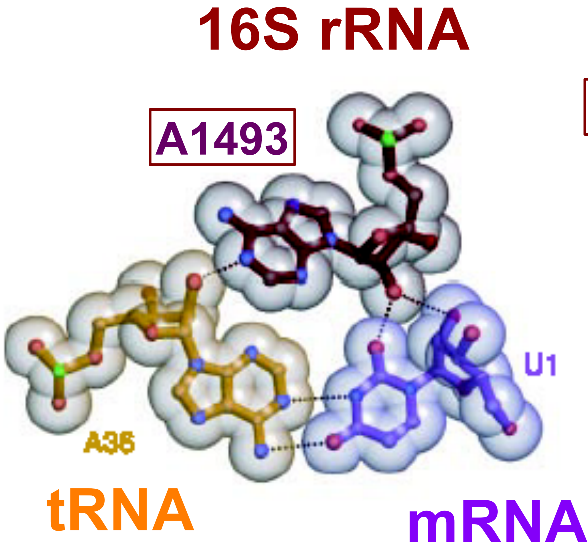


A-Site + mRNA + Anticodon

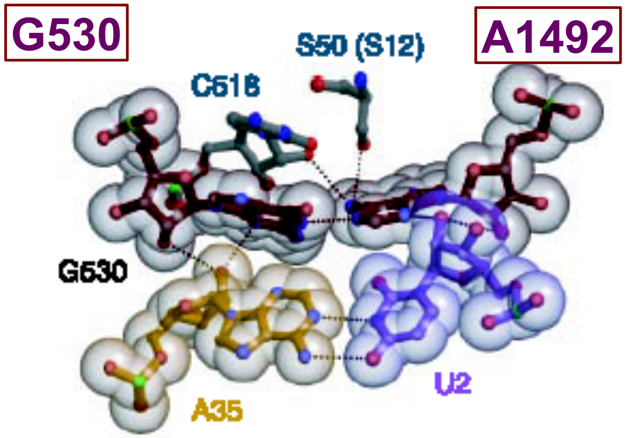
Ramakrishnan (1999) Science 292, 897-902.

16S-mRNA-tRNA Contacts

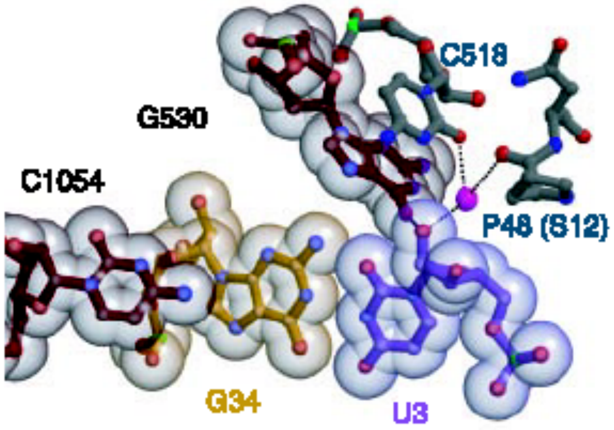
16S rRNA selectively interacts with WC base pairs at the first and second codon positions.



1st Codon Base



2nd Codon Base

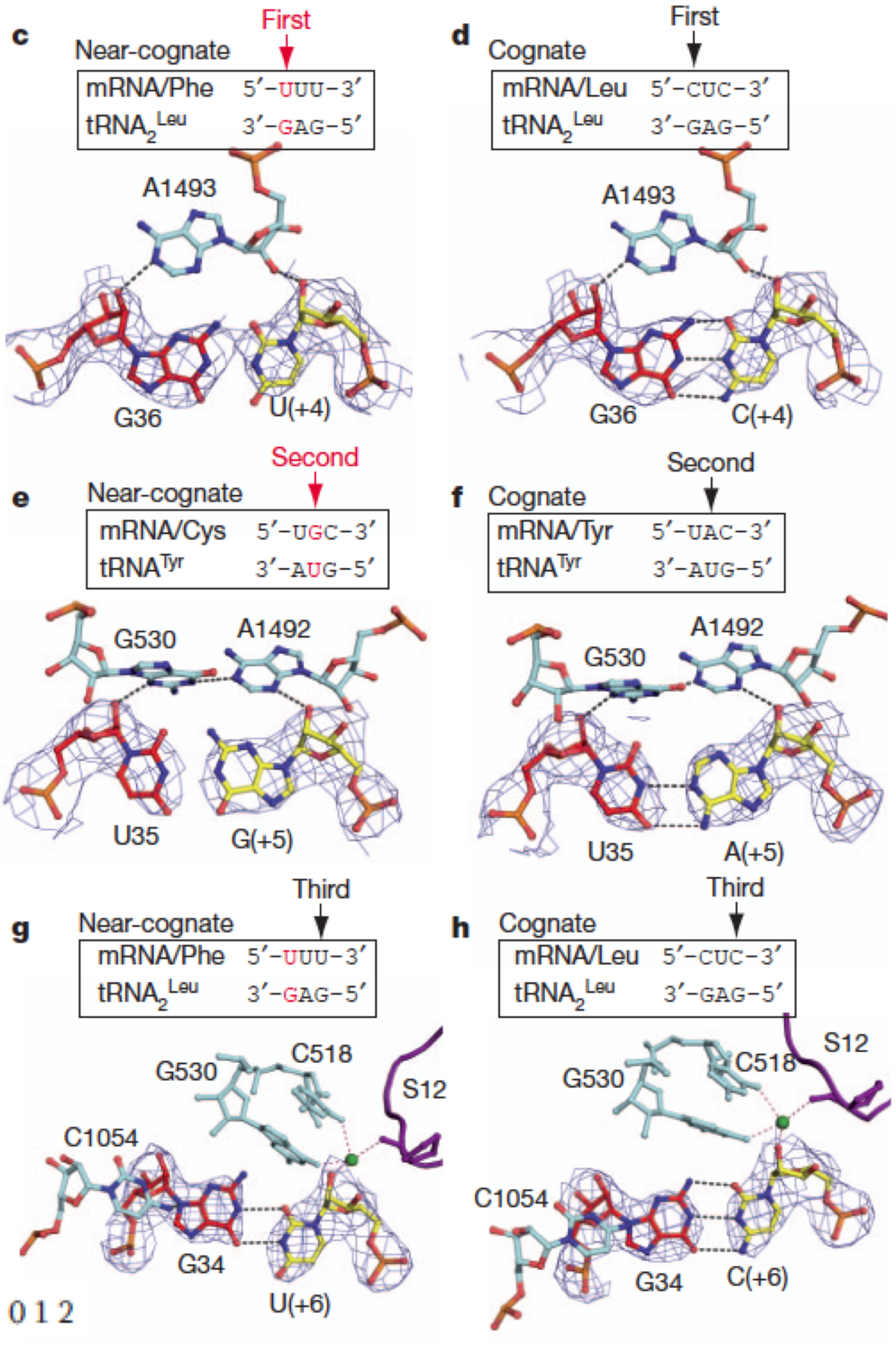


3rd Codon Base
“Wobble”

Ramakrishnan (1999) Science 292, 897-902.

16S Interactions Force WC Pairing

Repulsion Between G•U Leads to Rejection



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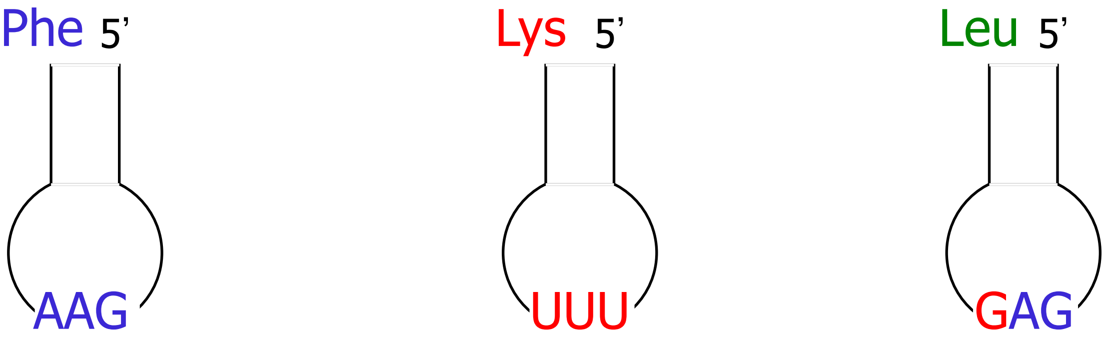
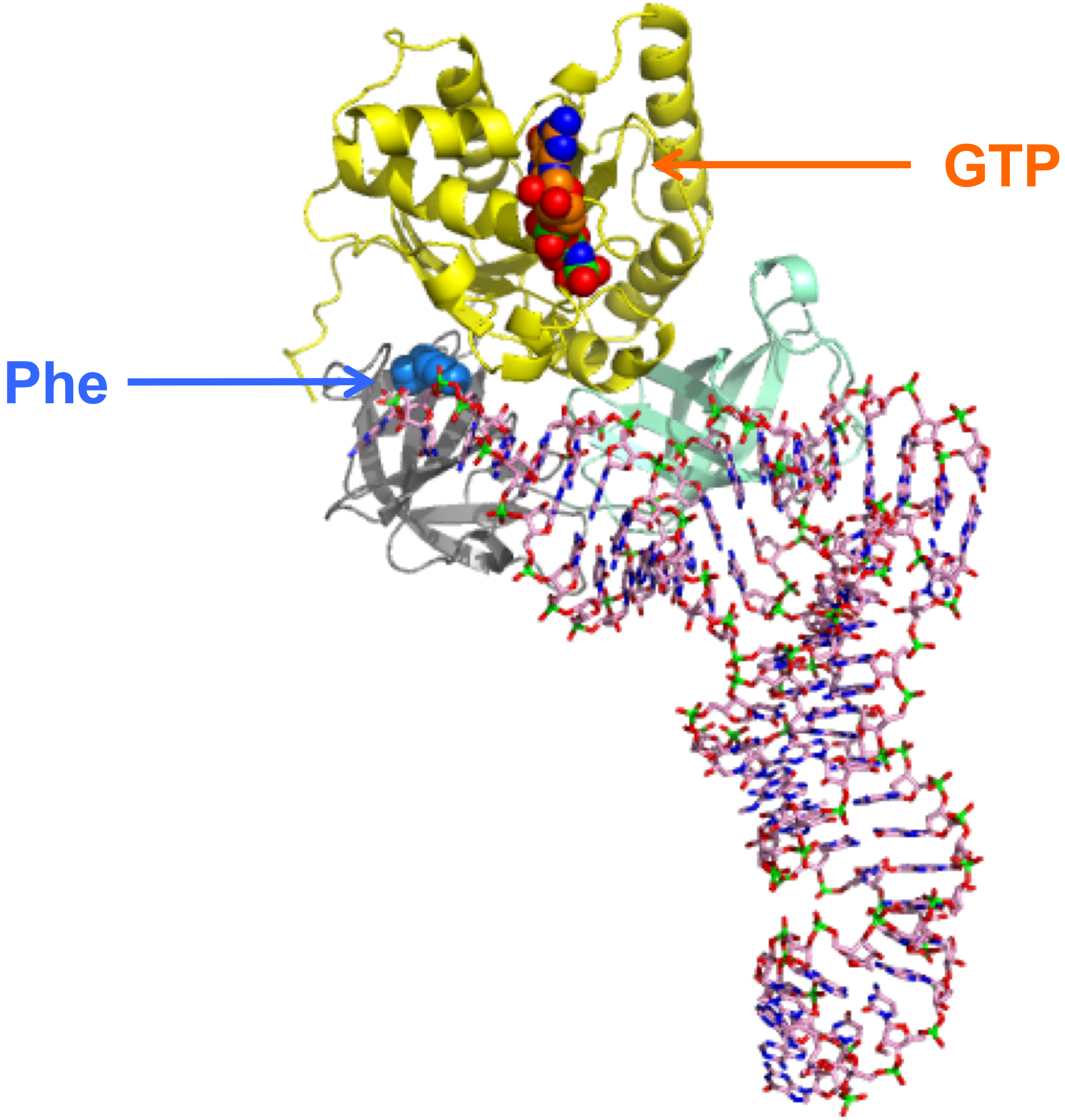
Cognate vs. Non-Cognate tRNA

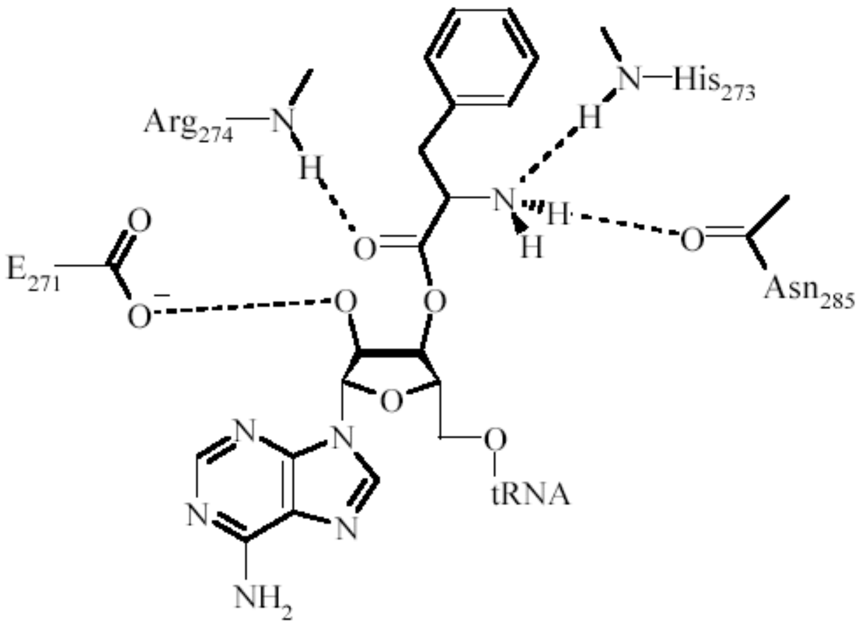


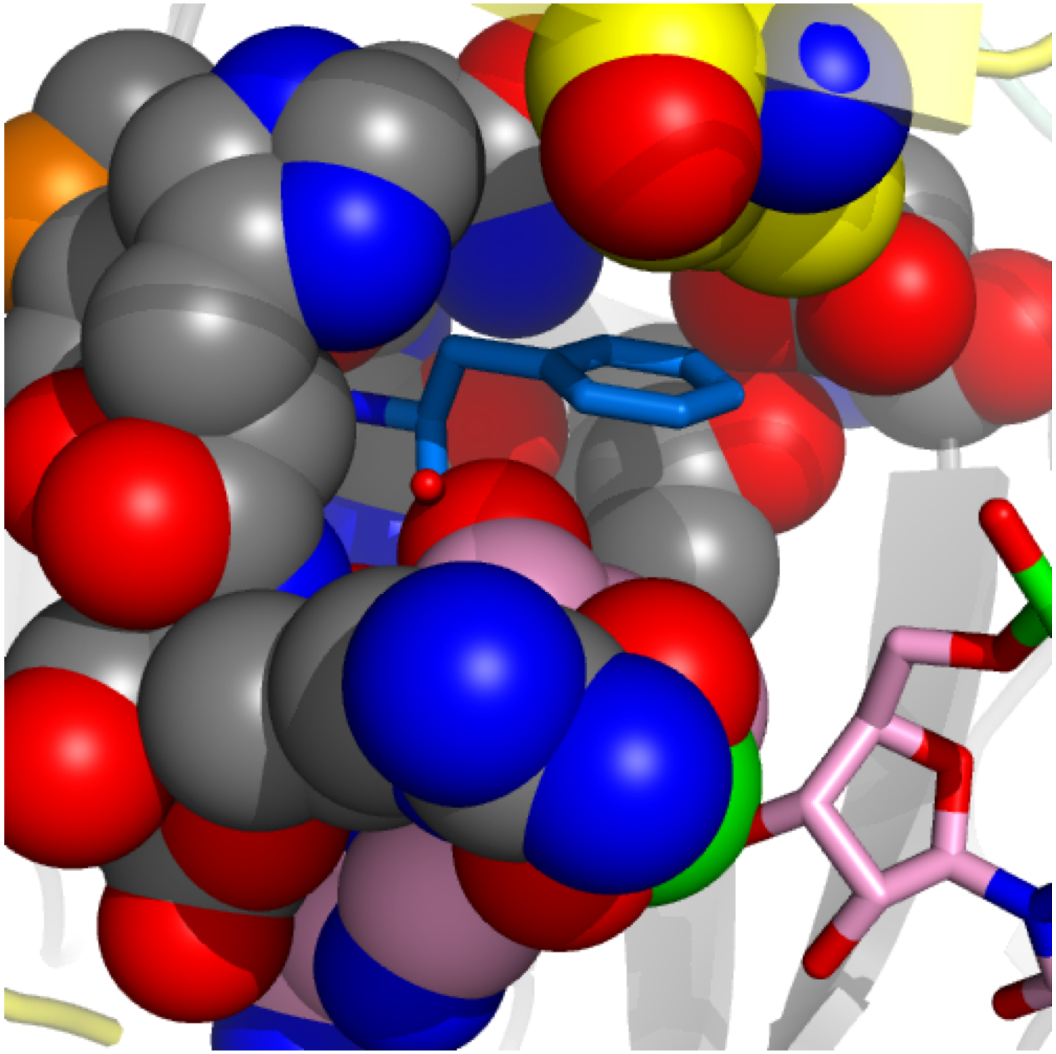
	cognate	non-cognate	near-cognate
tRNA	Phe 5’ AAG	Lys 5’ UUU	Leu 5’ GAG
mRNA	UUU	UUU	UUU
	Added to peptide 1 GDP produced	Not added 0 GDP produced	Added to peptide **8** GDP produced

EF-Tu•GTP•Phe~tRNA$^{\mathrm{Phe}}$

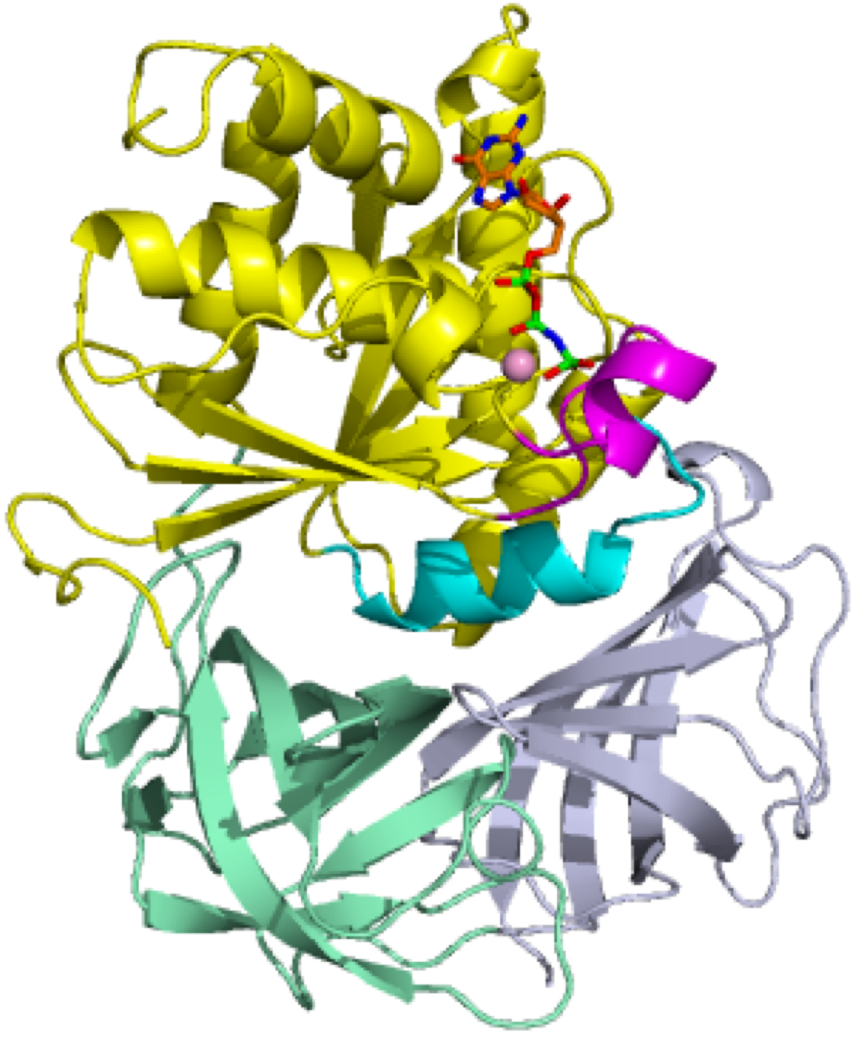


EF-Tu•Phe~tRNA Complex

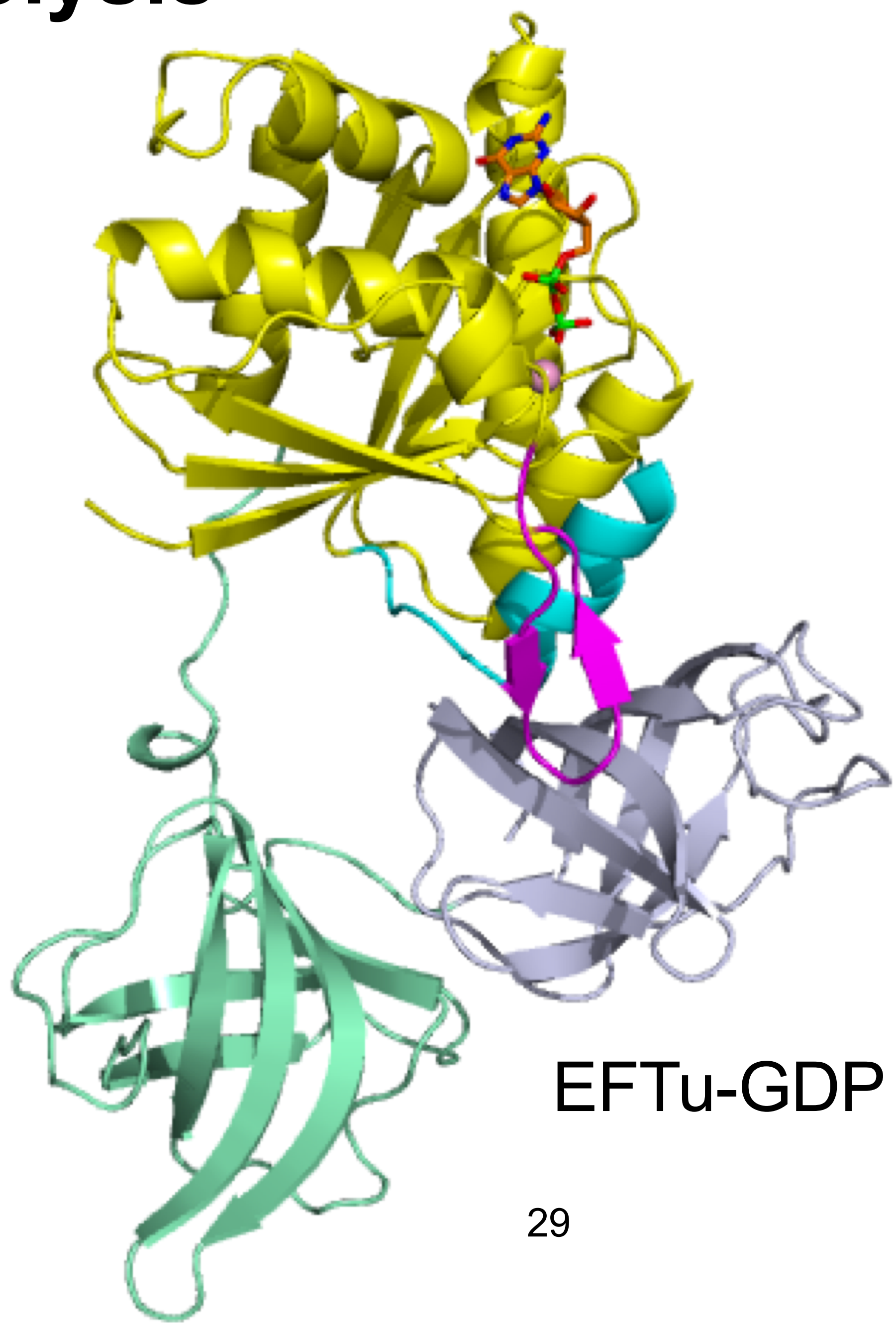




Conformational Change in EFTu Upon GTP Hydrolysis

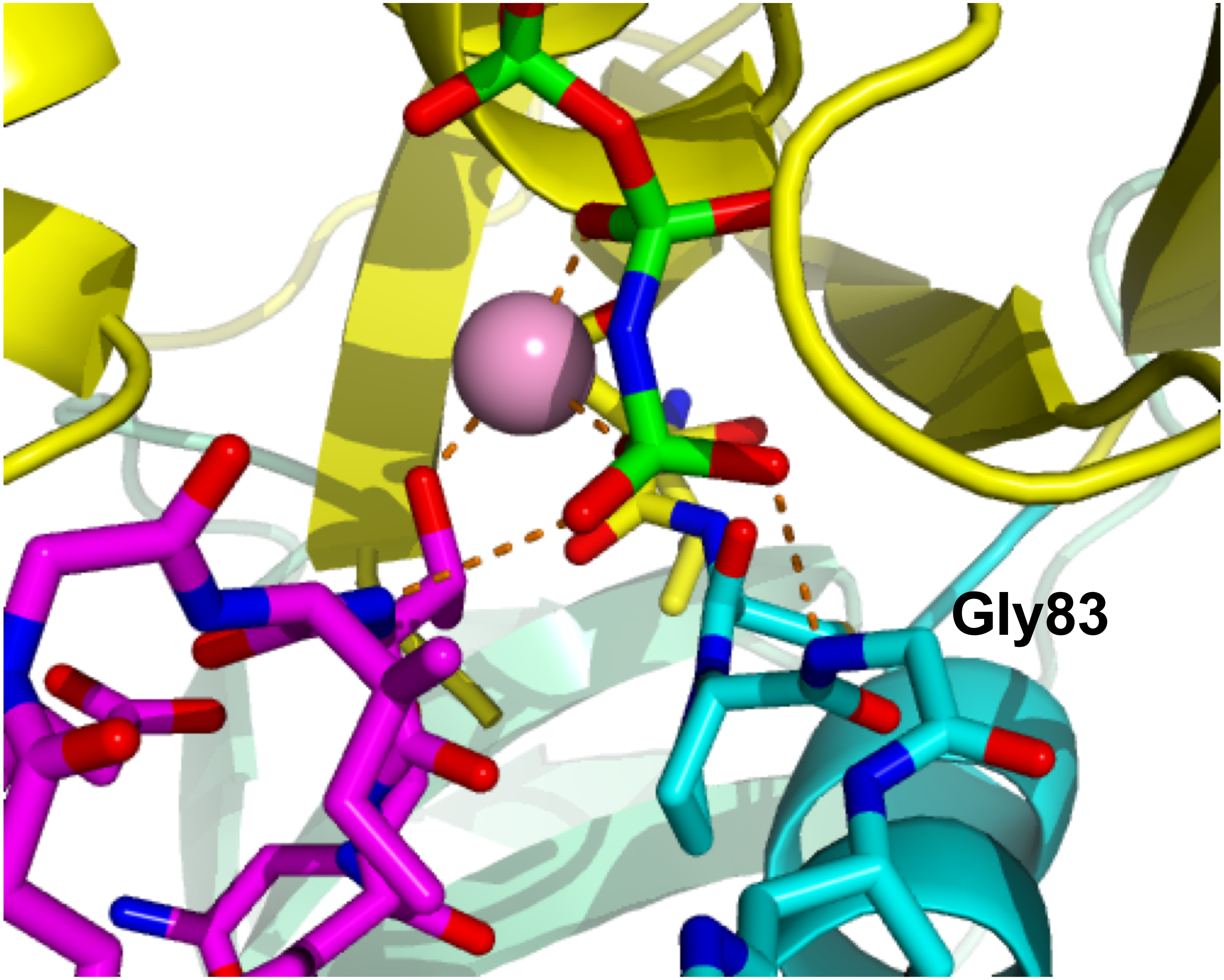


EFTu-GTP

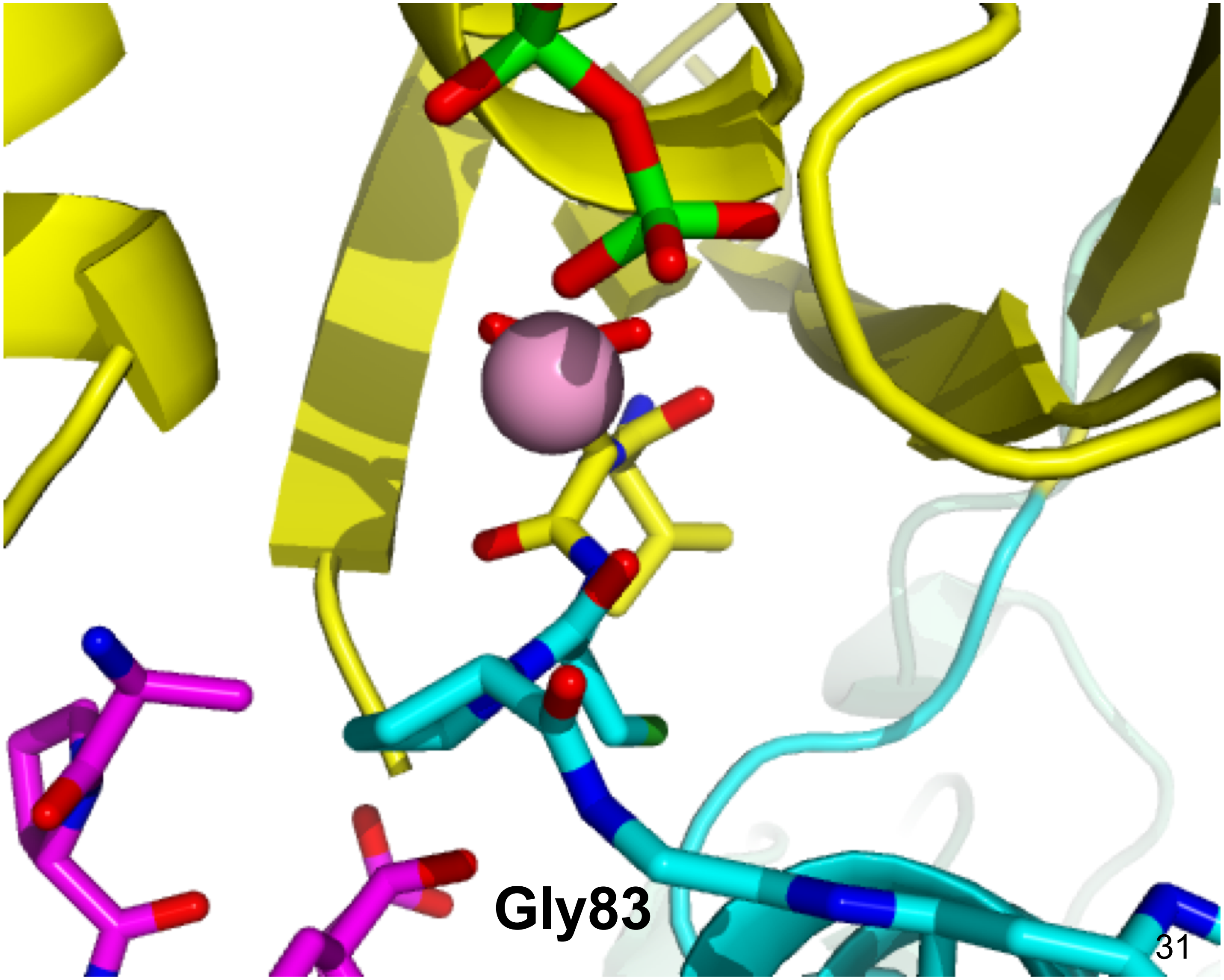


EFTu-GDP

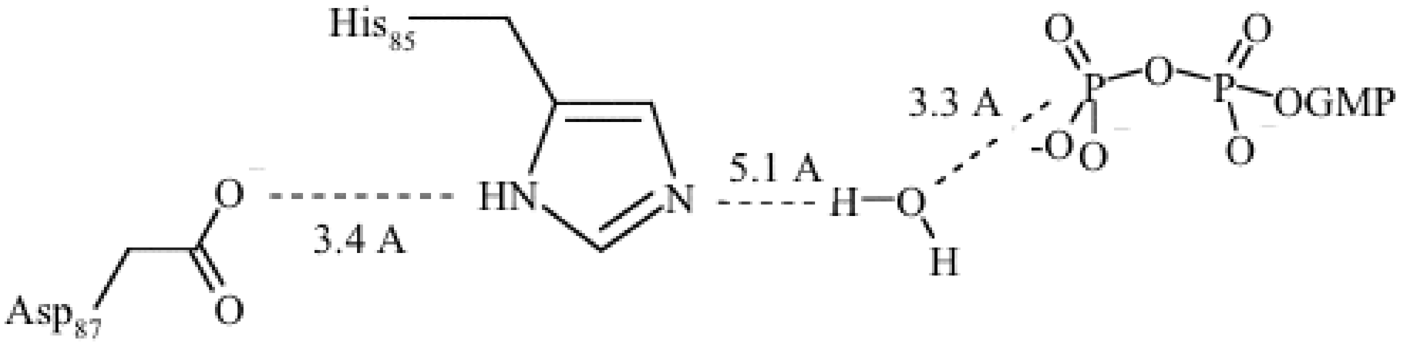
GTP-Bound



GDP-Bound



Induced Fit For GTP Hydrolysis by EF-Tu



The active site of EF-Tu for GTP hydrolysis will only assemble when codon-anticodon interactions allow for a favorable interaction between EF-Tu and the A-site of the ribosome.

Activation of EF-Tu by Binding to the Ribosome

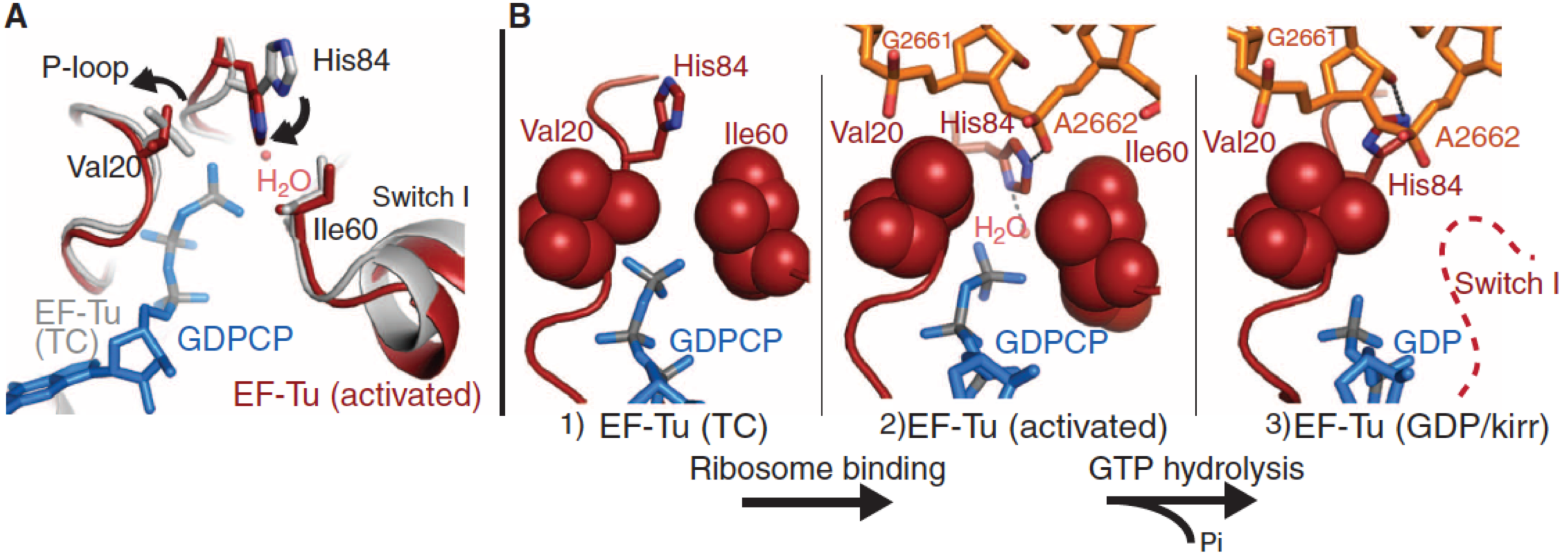
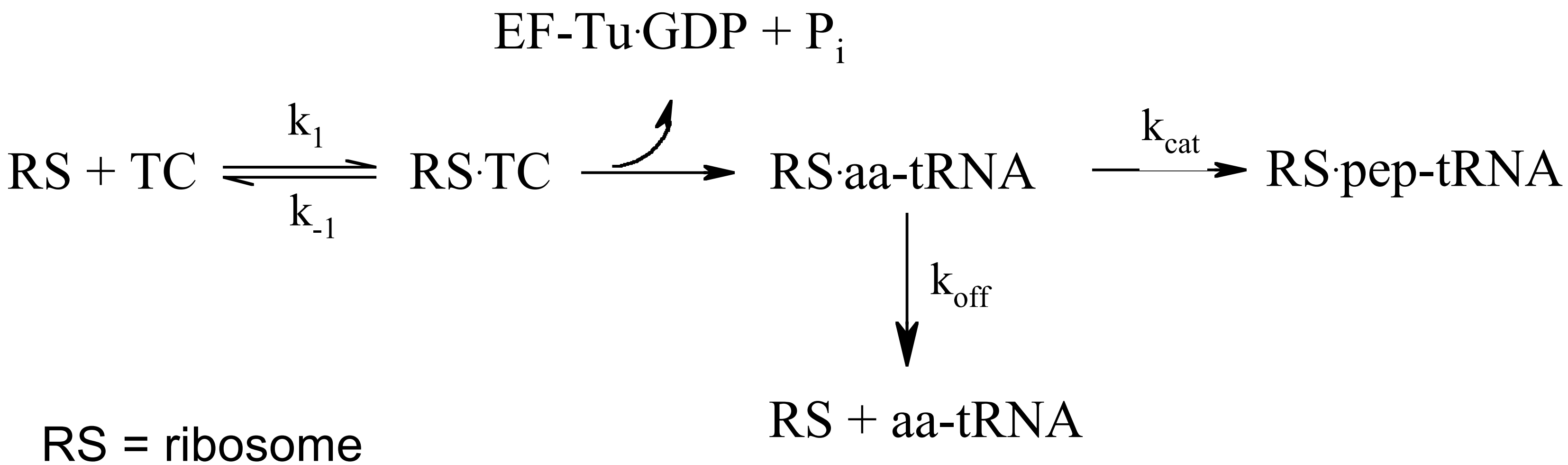


Fig. 2. The active site of EF-Tu during decoding. (**A**) Superposition of the GTPase centers from the isolated TC (gray) (*19*) and the TC-GDPCP-ribosome (red). Small movements are observed for hydrophobic gate residues Val^{20} and Ile^{60}. (**B**) GTPase activation allows the phosphate of A2662 of the SRL (orange) to position His^{84} into the active site. After GTP hydrolysis (*15*) and P_i release, switch I becomes disordered (dashed line) and His^{84} rotates away from GDP.

The Mechanism for Activation of GTP Hydrolysis on the Ribosome
Rebecca M. Voorhees, *et al.*
Science **330**, 835 (2010);
DOI: 10.1126/science.1194460

Discrimination of Near Cognates



EF-Tu·GDP + P_i

$$\mathrm{RS + TC} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{RS{\cdot}TC} \longrightarrow \mathrm{RS{\cdot}aa\text{-}tRNA} \xrightarrow{k_{cat}} \mathrm{RS{\cdot}pep\text{-}tRNA}$$

$$\mathrm{RS{\cdot}aa\text{-}tRNA} \xrightarrow{k_{off}} \mathrm{RS + aa\text{-}tRNA}$$

RS = ribosome
TC = EF-Tu•GTP•aa~tRNA

	K_m	k_{cat}	k_{cat}/K_m (rel)	k_{on} (s^{-1})	k_{off} (s^{-1})
cognate	0.5 nM	500 s^{-1}	4000	7	.3
non-cognate	large	.005 s^{-1}	<< 1		
near-cognate	200 nM	50 s^{-1}	1	.1	6