



Aminoacyl-tRNA synthetases



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Problem: Antibiotics resistance

Why are Aa-tRNA synthetases a good drug target?

1. **Essential** role in cells
2. **Divergence** between prokaryotic and eukaryotic AARS
3. Strong **conservation** of gene sequences that suggest that prokaryotic-specific drugs might be of broad spectrum
4. 20 different AARS that can be **targeted** individually or in combination
5. **Soluble**, readily **purifiable**, **express** well, and can be assayed in a high- throughput regime proteins

A failure to address the problem of antibiotic resistance could result in:



10m
deaths
per year
by 2050

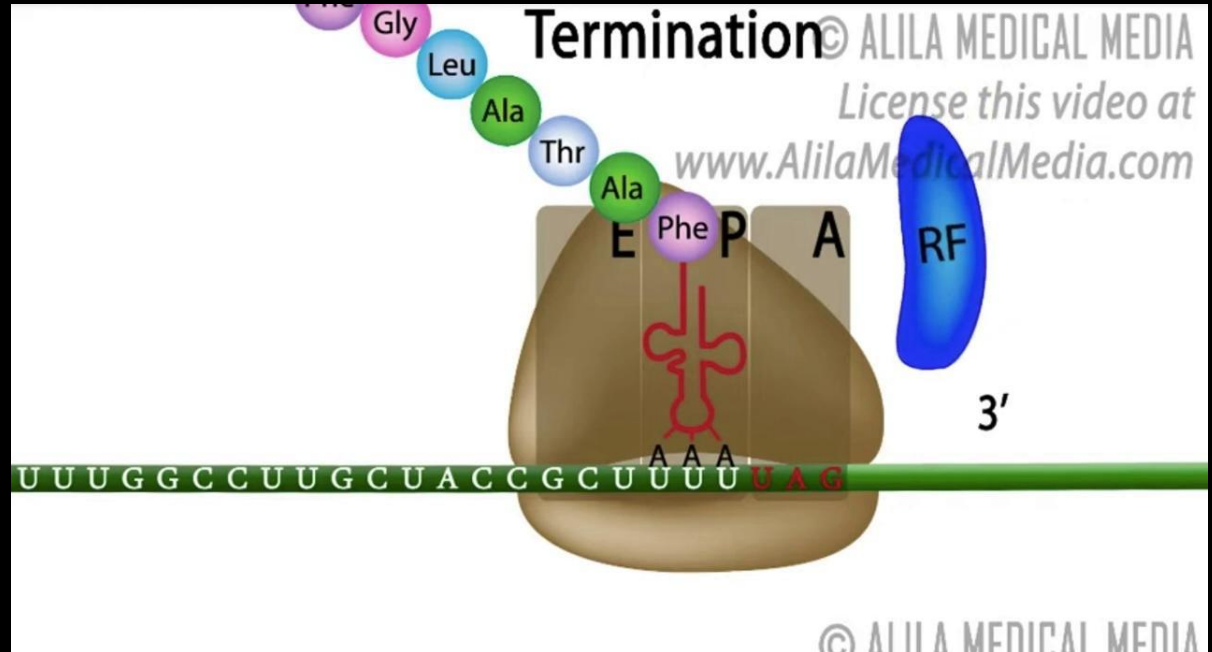
Health matters: antimicrobial resistance [Internet]. GOV.UK. 2021 [cited 9 March 2021]. Available from: <https://www.gov.uk/government/publications/health-matters-antimicrobial-resistance/health-matters-antimicrobial-resistance#:~:text=We%20also%20need%20them%20to.every%20year%20globally%20by%202050>

Background: Basic concepts into translation

Translation is the process of translating the sequence of a messenger RNA (mRNA) molecule to a sequence of **amino acids** during **protein synthesis**.

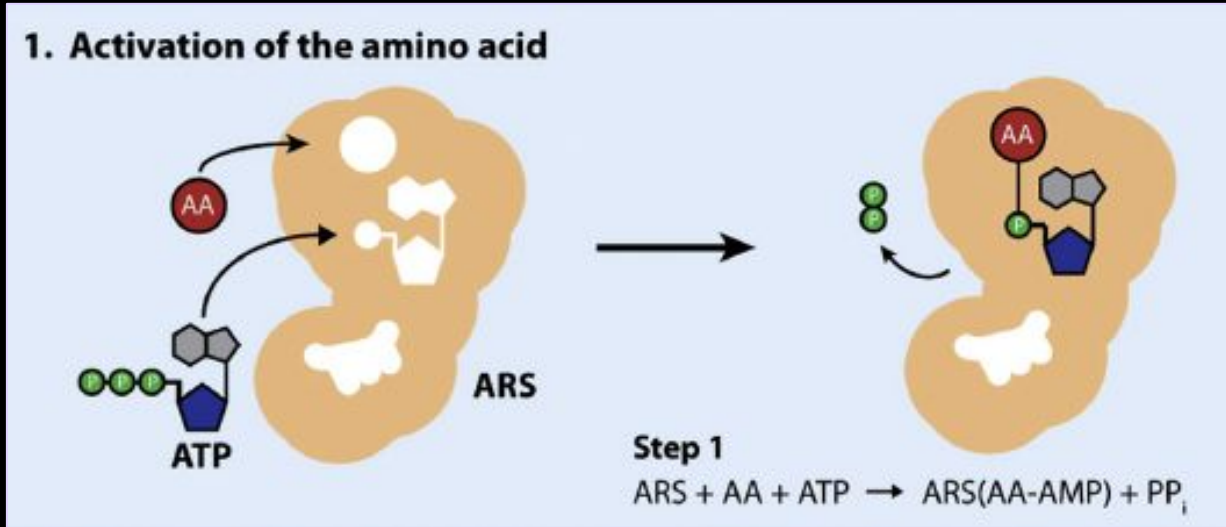
It involves 3 steps:

1. **Initiation**
2. **Elongation**
3. **Termination.**



Aa-tRNA synthetases structure and function

tRNA synthetase function at a glance

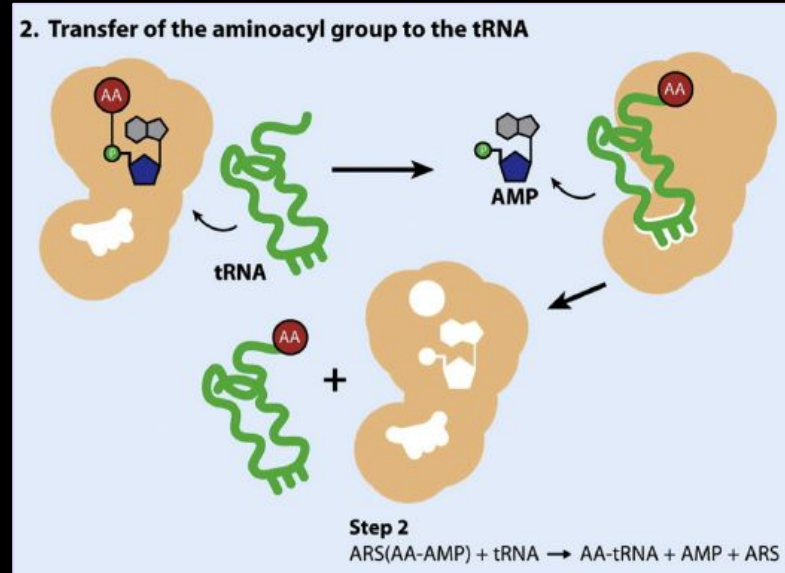


Rajendran V, Kalita P, Shukla H, Kumar A, Tripathi T. Aminoacyl-tRNA synthetases: Structure, function, and drug discovery. *Int J Biol Macromol.* 2018 May;111:400-414.

First step: A specific AARS recognizes its cognate AA in the presence of ATP.

As a consequence an enzyme-amino acid-AMP complex is formed by releasing a pyrophosphate.

tRNA synthetase function at a glance

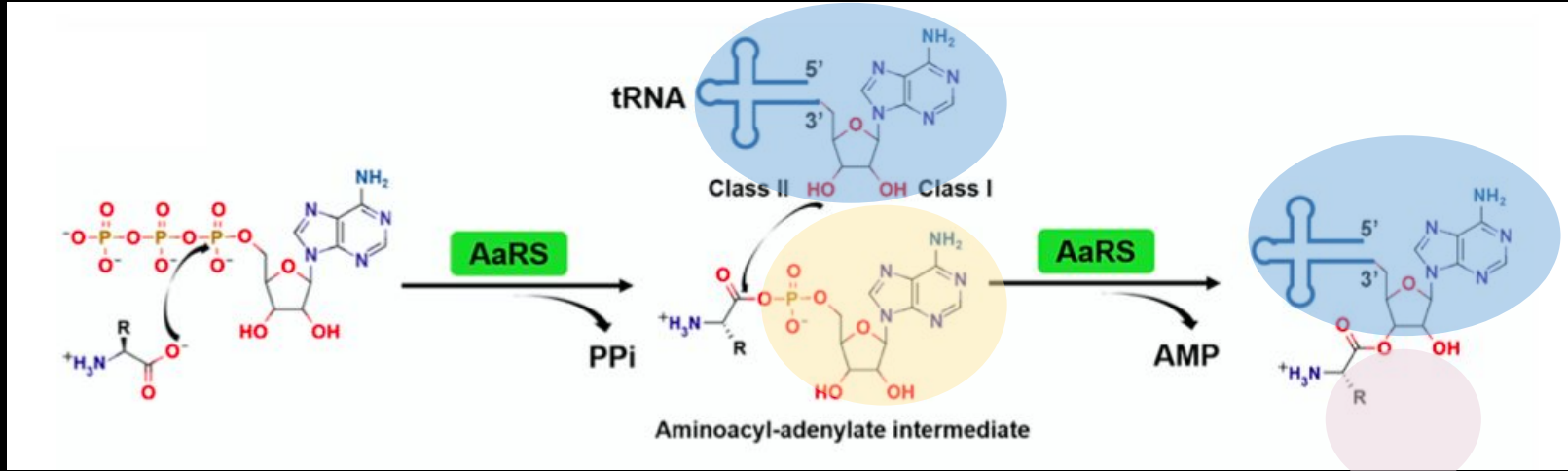


Rajendran V, Kalita P, Shukla H, Kumar A, Tripathi T. Aminoacyl-tRNA synthetases: Structure, function, and drug discovery. *Int J Biol Macromol.* 2018 May;111:400-414.

Second step: The specific tRNA for that AARS binds to the complex and forms a covalent bond with the aa by releasing AMP. An amino acidenzyme-tRNA complex is formed.

Third step: The charged tRNA is detached from the AARS.

Aminoacylation process



Pang, L., S.D. Weeks, and A. Van Aerschot, Aminoacyl-tRNA Synthetases as Valuable Targets for Antimicrobial Drug Discovery. *Int J Mol Sci*, 2021. 22(4)

Amino acid

ATP

Amino acid

ATP

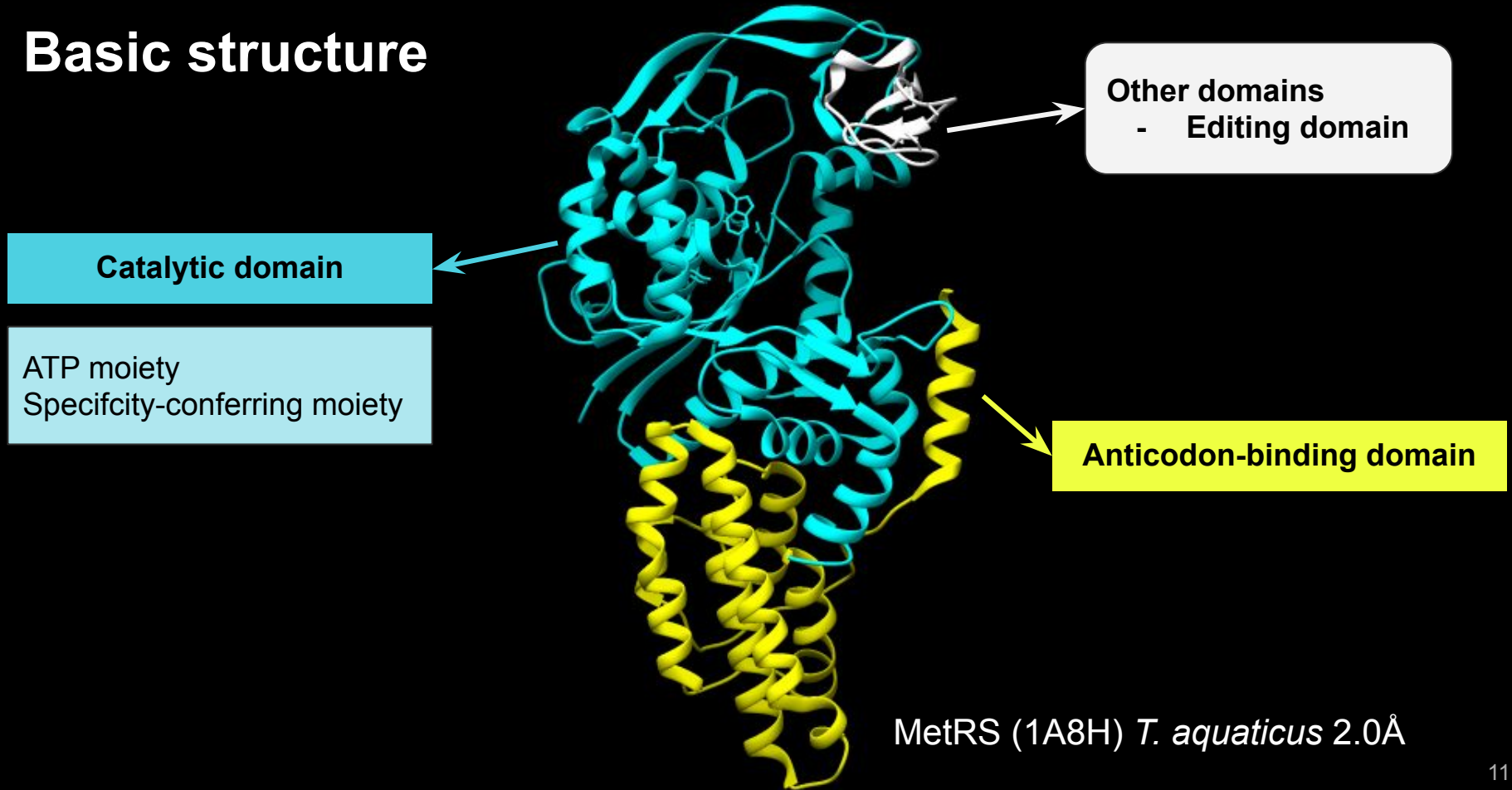
tRNA

1. Amino acid activation: adenylation

2. Transfer of aminoacyl adenylate

3. charged tRNA

Basic structure



AARS structural classification



Class I



Class II

SHARED MOTIFS	HIGH and KMSKS	1 P and 2 R
CATALYTIC DOMAIN	Rossmann fold	Seven beta-strands
APPROACH TO tRNA	Minor groove	Major groove
TRANSFER AMINO ACID TO	3'-OH	2'OH
BIND ATP IN	Extended configuration	Bent configuration
ARE FOUND FORMING	Monomers with few exceptions that are dimeric (e.g., MetRS, TyrRS, and TrpRS)	Dimers and tetramers

AARS SUBCLASSES

Class I

Ia: IleRS, LeuRS, MetRS, and ValRS

Ib: CysRS, GluRS, and GlnRS

Ic: TyrRS and TrpRS

Id: ArgRS

Ie: **LysRS**

Class II

IIa: GlyRS, HisRS, ProRS, SerRS, and ThrRS

IIb: AspRS, AsnRS, and **LysRS**

IIc: AlaRS, PheRS, GlyRS and PyIRS

SCOP Classification

	Class I Aa-tRNA synthetases	Class II Aa-tRNA synthetases
C D	<p>Class: Alpha and beta proteins</p> <p>Fold: Rossmann fold</p> <p>Superfamily: Nucleotidyl transferase</p> <p>Family: Class I aminoacyl-tRNA synthetases</p>	<p>Class: Alpha and beta proteins</p> <p>Fold: Class II aaRS/BPL domain-like</p> <p>Superfamily: Class II aaRS and biotin synthetases</p> <p>Family: Class II aminoacyl-tRNA synthetase (aaRS)-like</p>
A B D	<p>Class: All alpha proteins</p> <p>Fold/Superfamily/Family: Anticodon-binding domain of a subclass of class I aminoacyl-tRNA synthetases</p>	<p>Class: All beta proteins</p> <p>Fold: OB-fold</p> <p>Superfamily: Nucleic acid-binding proteins</p> <p>Family: Anticodon-binding domain</p>

Catalytic domain - Aa tRNA class I

Catalytic domain

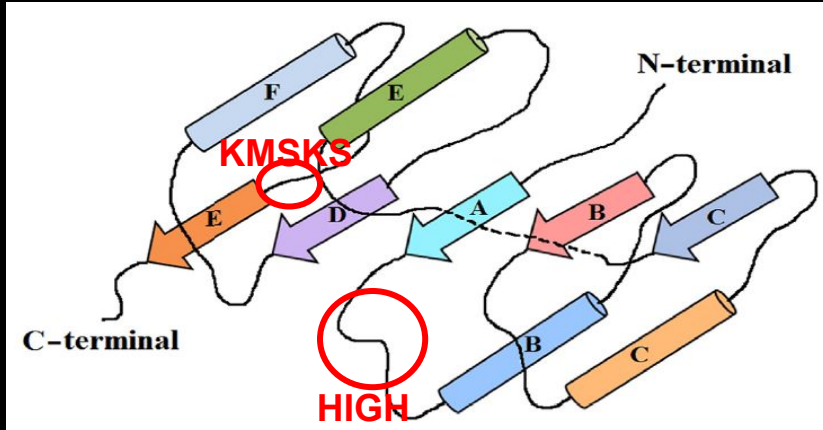


Rossmann Fold

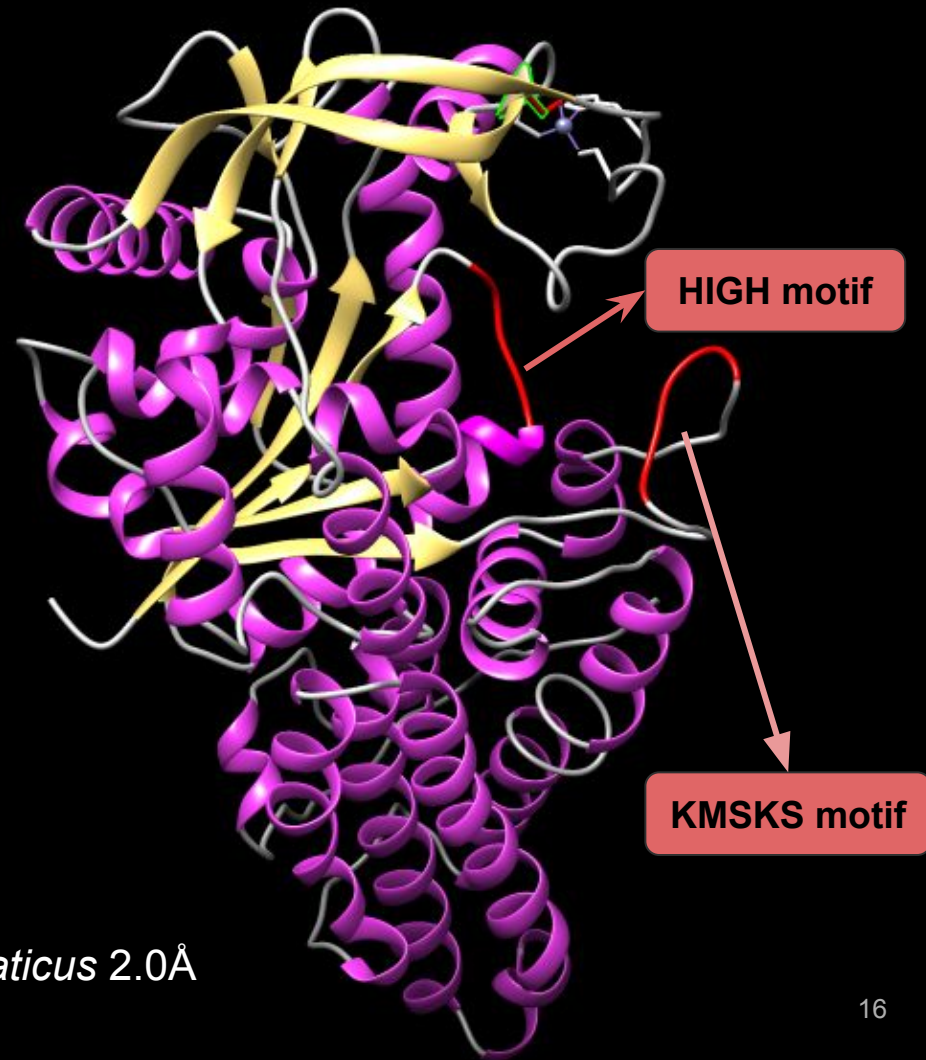
- **HIGH motif** → loop joining first β -strand to subsequent α -helix
- **KMSKS motif** → loop immediately following the 5th β -strand .

MetRS (1A8H) *T. aquaticus* 2.0Å

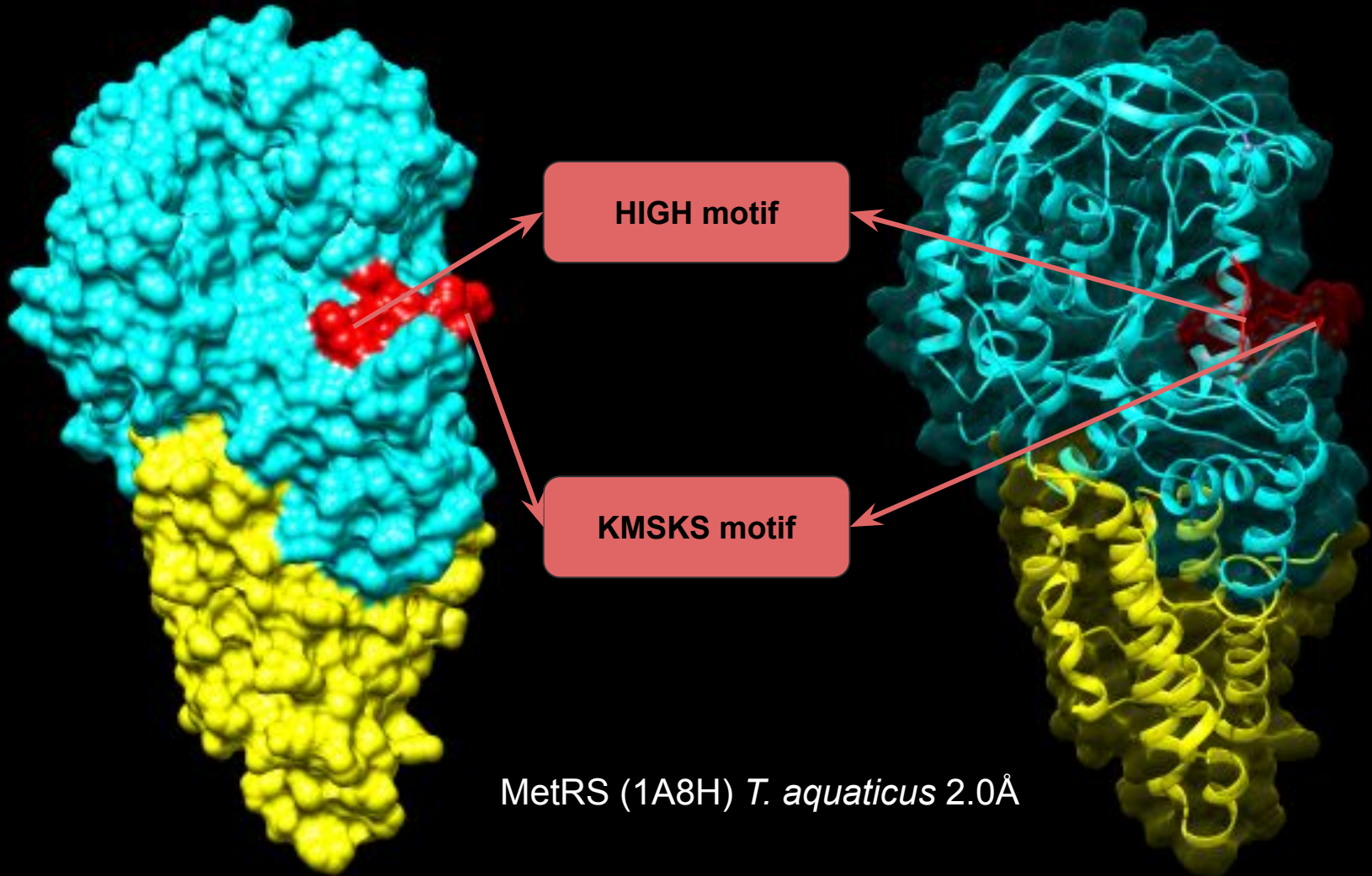
Rossmann fold - Class I



Rajendran V, Kalita P, Shukla H, Kumar A, Tripathi T. Aminoacyl-tRNA synthetases: Structure, function, and drug discovery. *Int J Biol Macromol.* 2018 May;111:400-414.



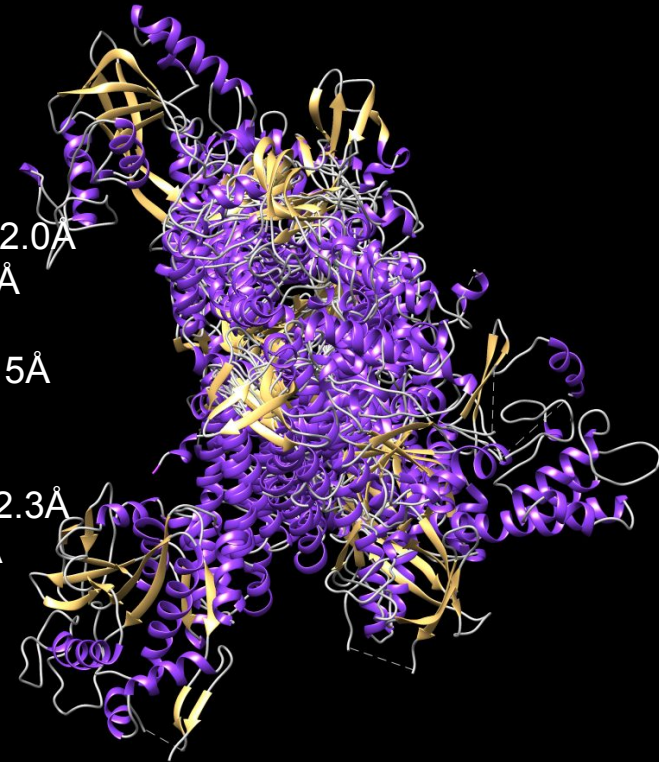
MetRS (1A8H) *T. aquaticus* 2.0Å



Catalytic domain Superimposition - Class I



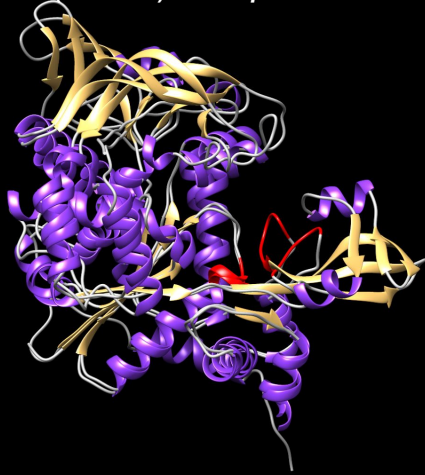
LeuRS (1H3N) *T. thermophilus* 2.0Å
MetRS (1A8H) *T. aquaticus* 2.0Å
CysRS (1U0B) *E. Coli* 2.3Å
GluRS (4H3S) *S. cerevisiae* 2.15Å
TyrRS (4OUD) *E. Coli* 2.65Å
TrpRS (5VOI) *E. Coli* 1.9Å
ArgRS (1IQ0) *T. Thermophilus* 2.3Å
LysRS (1IRX) *P. horikoshii* 2.6Å



Class Ia

LeuRS (1H3N) *T. thermophilus* 2.0Å

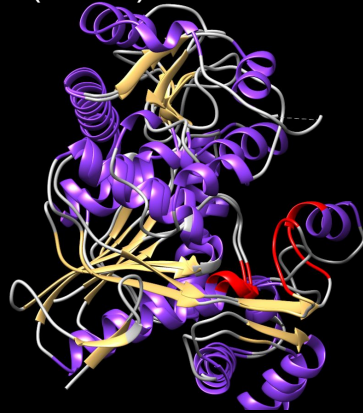
MetRS (1A8H) *T. aquaticus* 2.0Å



Class Ib

CysRS (1U0B) *E. Coli* 2.3Å

GluRS (4H3S) *S. cerevisiae* 2.15Å



Class Ic

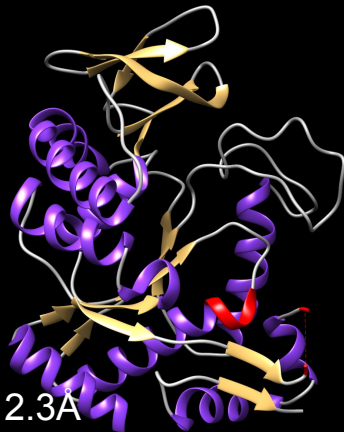
TyrRS (4OUD) *E. Coli* 2.65Å

TrpRS (5VOI) *E. Coli* 1.9Å



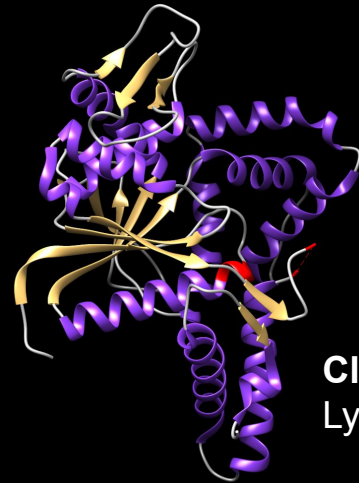
Class Id

ArgRS (1IQ0) *T. Thermophilus* 2.3Å



Class Ie

LysRS (1IRX) *P. horikoshii* 2.6Å



Catalytic domain - Aa tRNA class II

Catalytic domain

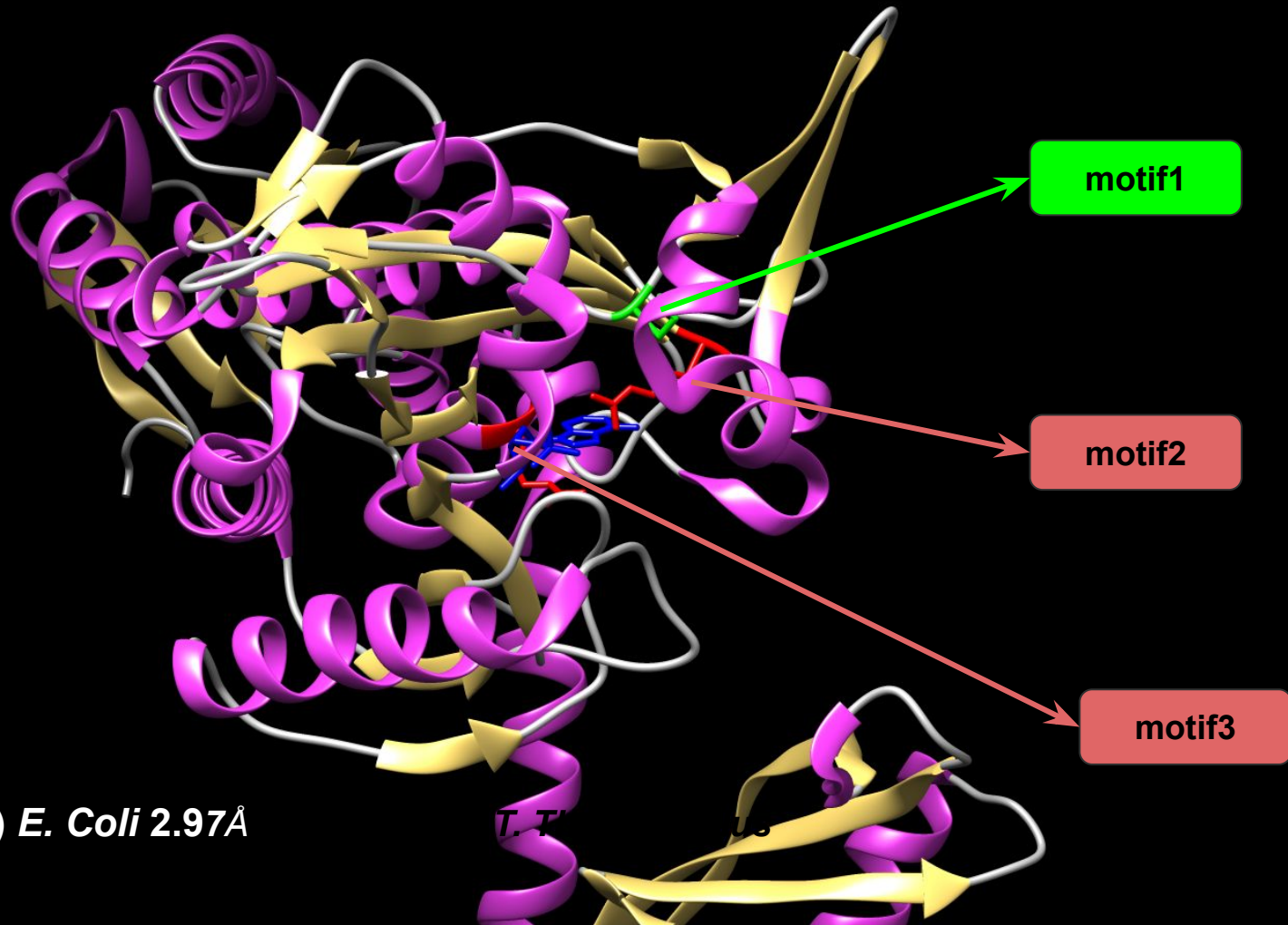


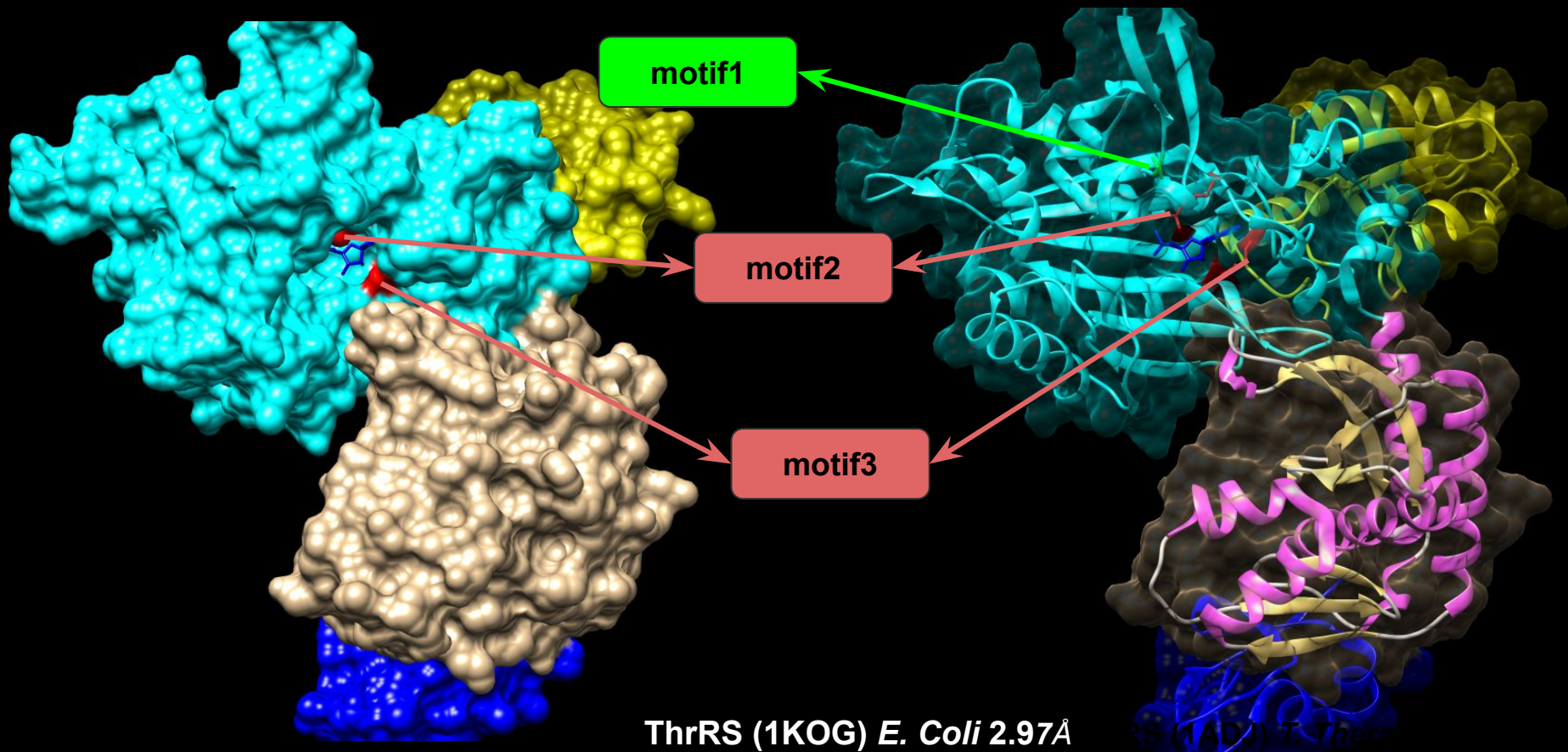
Anti-parallel β -sheets

- **Motif 1** → Pro residue followed, by a distorted β -strand, important for homo-dimerization
- **Motif 2** and **motif 3** → conserved Arg residue involved in ATP binding

HisRS (1ADJ) *T. Thermophilus* 2.7Å

ThrRS (1KOG) *E. Coli* 2.97Å





Catalytic domain Superimposition - Class II

SerRS (1SET) *T. thermophilus* 2.5Å

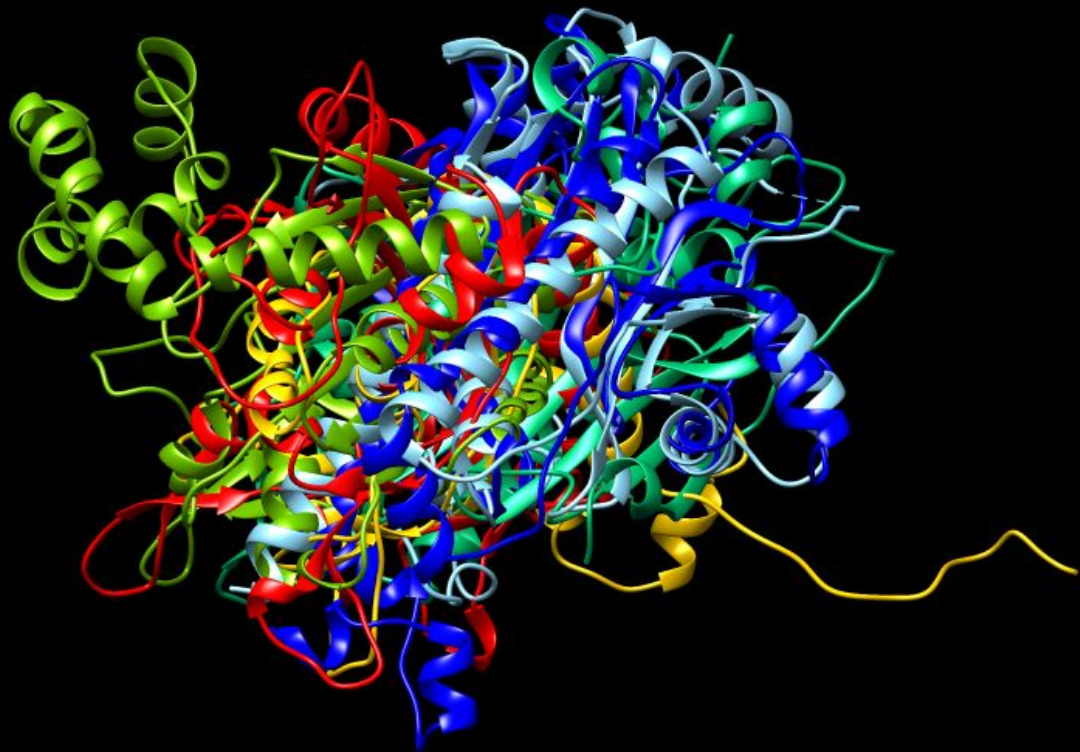
HisRS (1H4V) *T. thermophilus* 2.4Å

AspRS (1EOV) *S. cerevisiae* 2.3Å

AsnRS (1X55) *T. thermophilus* 2.3Å AB

PheRS (1B7Y) *T. aquaticus* 2.5Å

GlyRS (1ATI) *T. thermophilus* 2.75Å



Class IIa

SerRS (1SET) *T. thermophilus* 2.5Å

HisRS (1H4V) *T. thermophilus* 2.4Å



Class IIb

AspRS (1EOV) *S. cerevisiae* 2.3Å

AsnRS (1X55) *T. thermophilus* 2.3Å



Class IIc

PheRS (1B7Y) *T. aquaticus* 2.5Å

GlyRS (1ATI) *T. thermophilus* 2.75Å



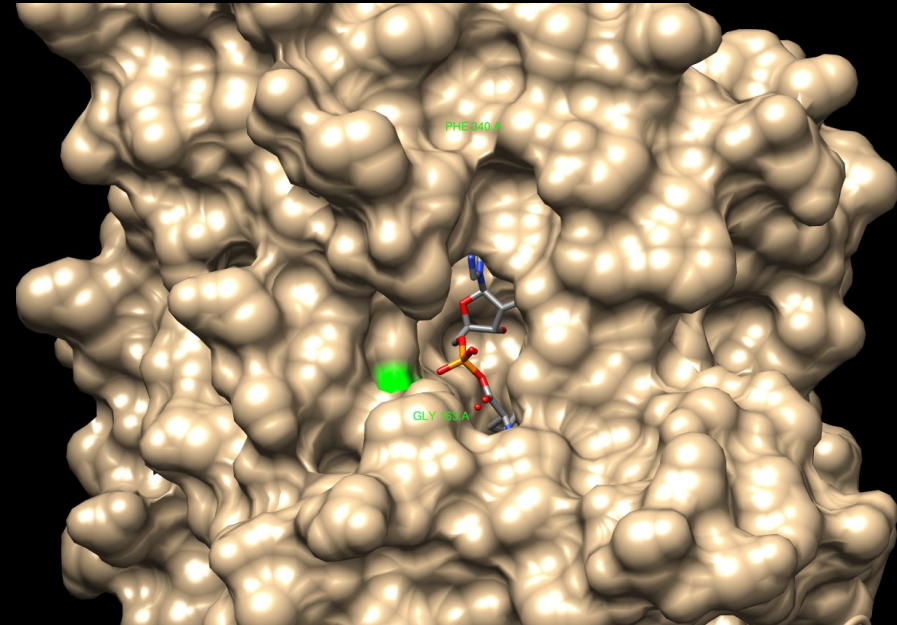
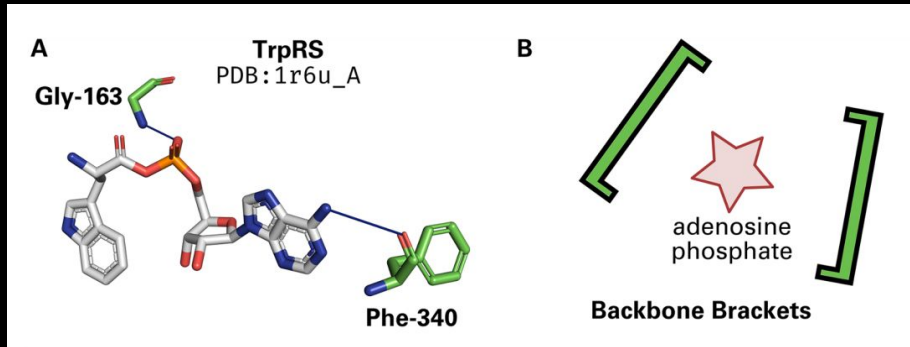
Insight into tRNA synthetase function and interactions

Interaction with:

- 1) **ATP → ATP binding moiety in CD**
- 2) **Aa → Aa binding moiety in CD**
- 3) **tRNA → critical residues in CD and ABD**

1) ATP binding moiety - Class I: Backbone Brackets

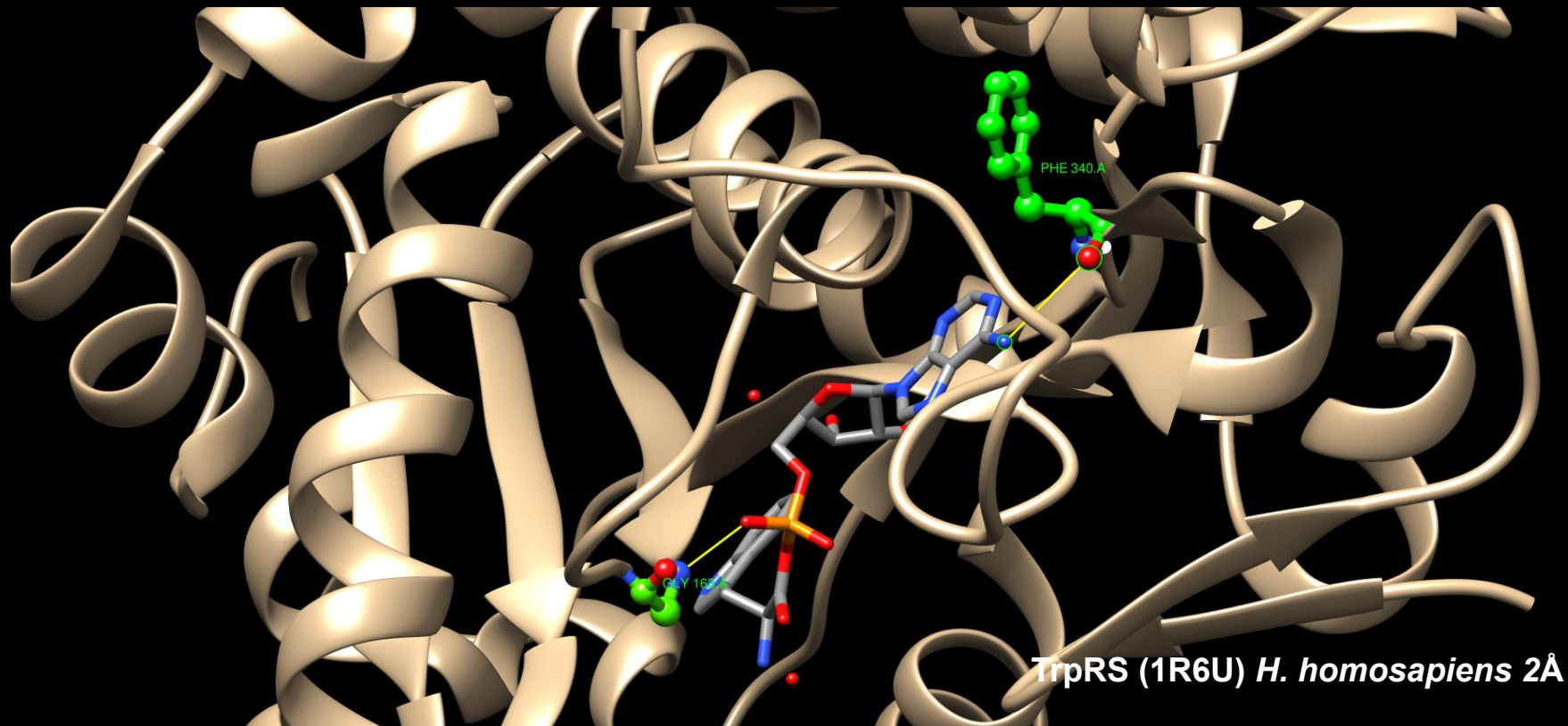
Highly conserved **hydrogen bonds** between the peptide bond nitrogen or oxygen atom and the adenosine phosphate part of the ligand.



TrpRS (1R6U) *H. homospiciens* 2Å

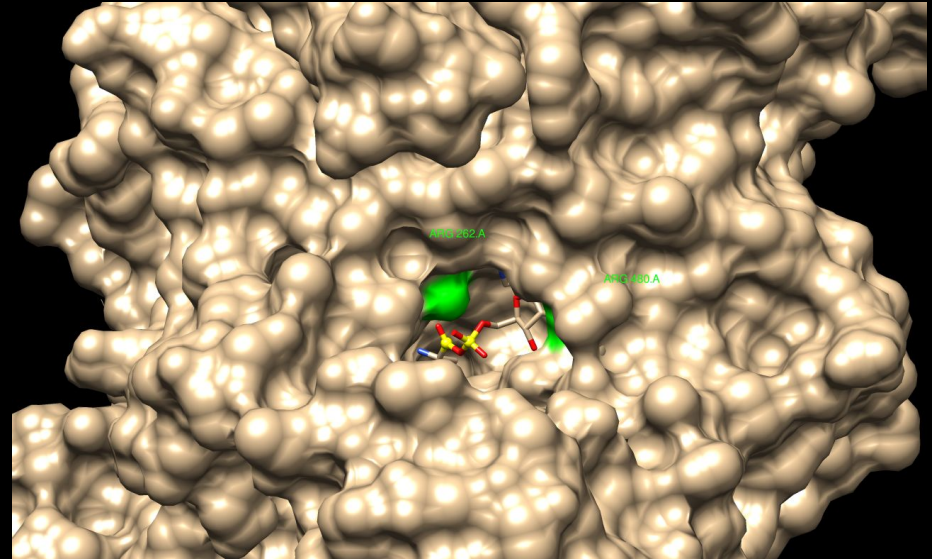
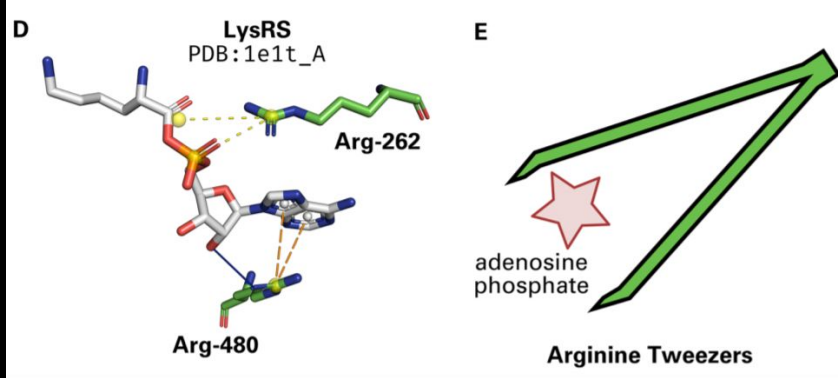
Kaiser F, Bittrich S, Salentin S, Leberecht C, Haupt J et al. Backbone Brackets and Arginine Tweezers delineate Class I and Class II aminoacyl tRNA synthetases. *PLoS Comput Biol.* 2018; 14(4): e1006101.

1) ATP binding moiety - Class I: Backbone Brackets



1) ATP binding moiety - Class II: Arginine Tweezers

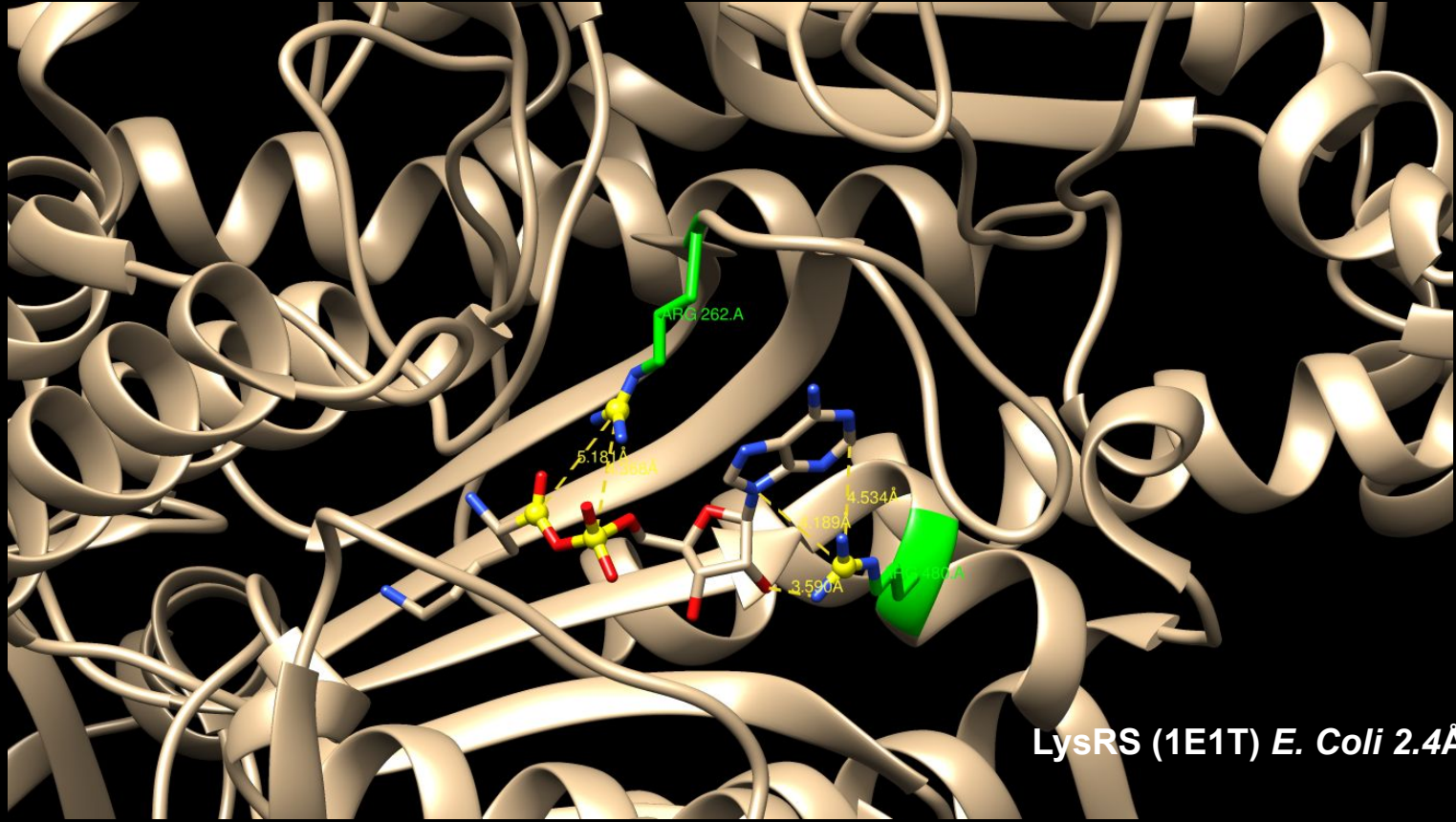
Two arginines with conserved salt bridges and side chain orientations in Class II Aa t-RNA synthetases. Salt bridges as well as π -cation interactions are established



LysRS (1E1T) *E. Coli* 2.4Å

Kaiser F, Bittrich S, Salentin S, Leberecht C, Haupt J et al. Backbone Brackets and Arginine Tweezers delineate Class I and Class II aminoacyl tRNA synthetases. *PLoS Comput Biol.* 2018; 14(4): e1006101.

1) ATP binding moiety - Class II: Arginine Tweezers



LysRS (1E1T) *E. Coli* 2.4Å

2) Amino acid recognition

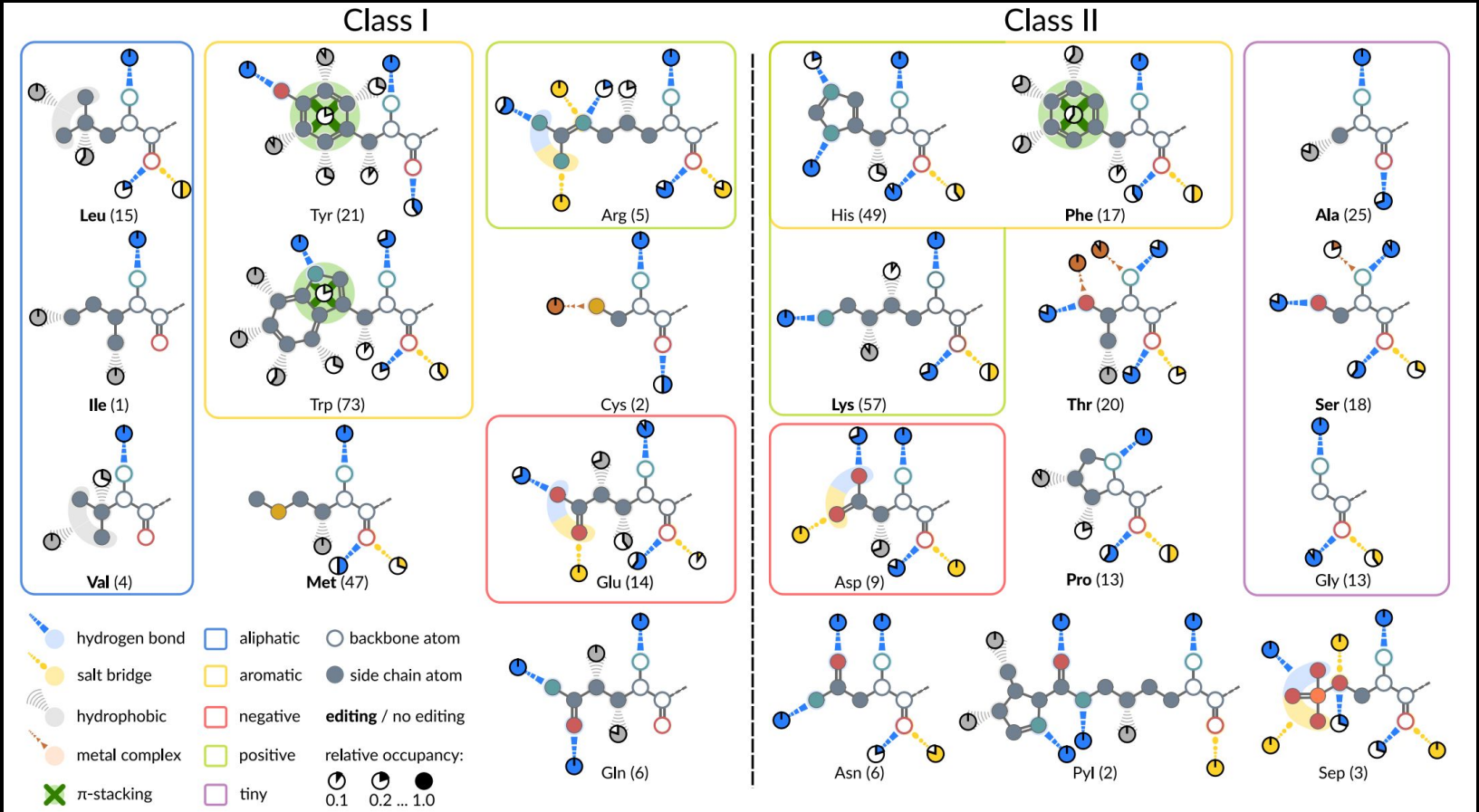
Double sieve mechanism: pre-transfer editing and post-transfer editing.

Affinity of an amino acid for the active site relays on:

- Size
- Charge
- Use of metal ions that bind to specific chemical groups

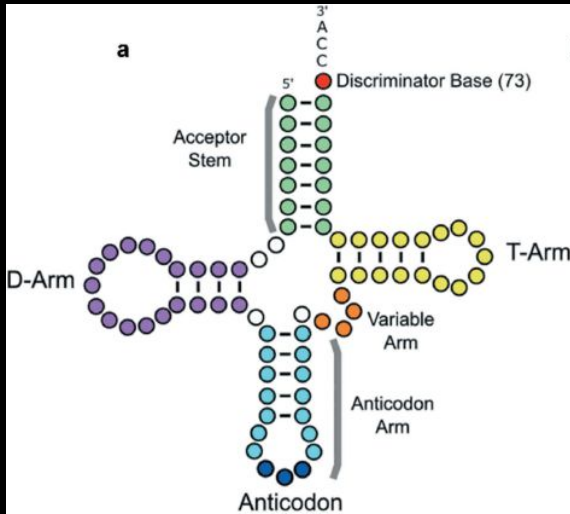


HisRS (1KMM) *E. Coli* 2.6Å



tRNA structure

2'/3' OH
Aa attachment



Acceptor stem

Variable loop

T loop

D loop

Anticodon stem

Anticodon loop
Codon recognition

Image extracted from: Berg M, Giguere D, Dron J, Lant J, Genereaux J, Liao C et al. Targeted sequencing reveals expanded genetic diversity of human transfer RNAs. RNA Biology. 2019;16(11):1574-1585.

tRNA anticodon loop recognition

	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	UAA	STOP	UGA	STOP	A
	UUG	Leu	UCG	Ser	UAG	STOP	UGG	Try	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G
A	AUU	Iso	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC	Iso	ACC	Thr	AAC	Asn	AGC	Ser	C
	AUA	Iso	ACA	Thr	AAA	Lys	AGA	Arg	A
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg	G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G

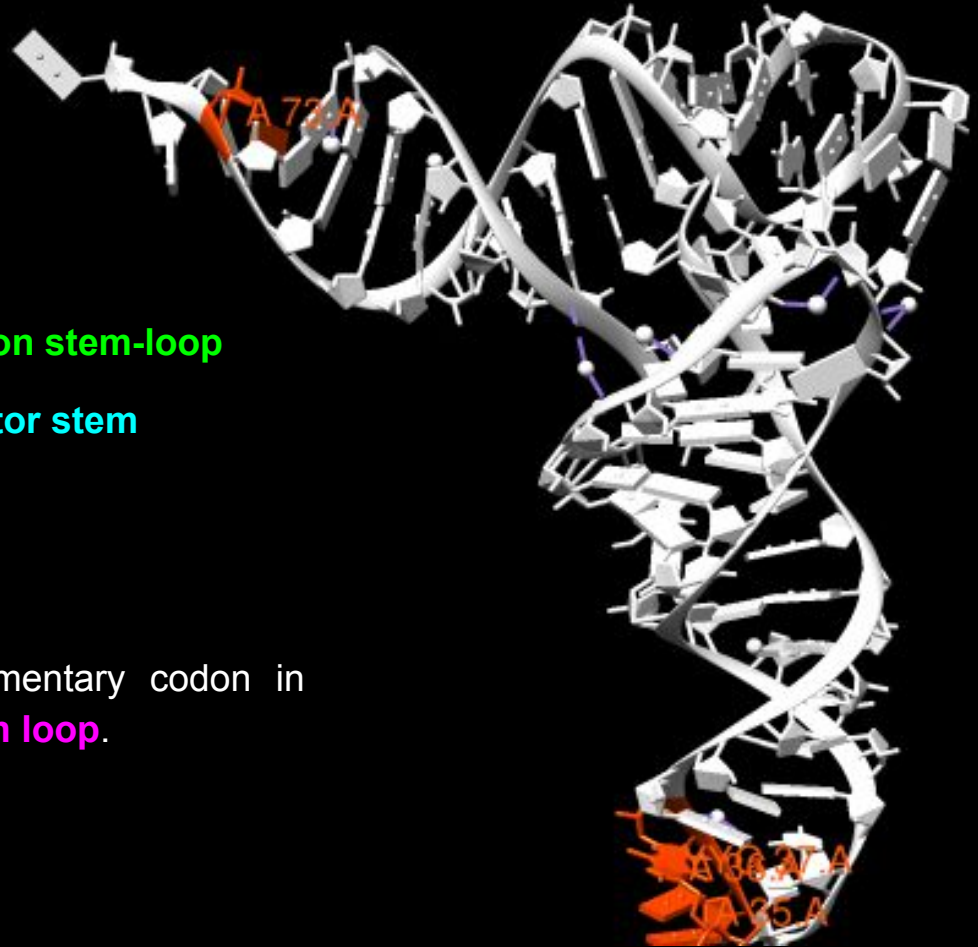
Extracted from <https://www.innovabiologia.com/>

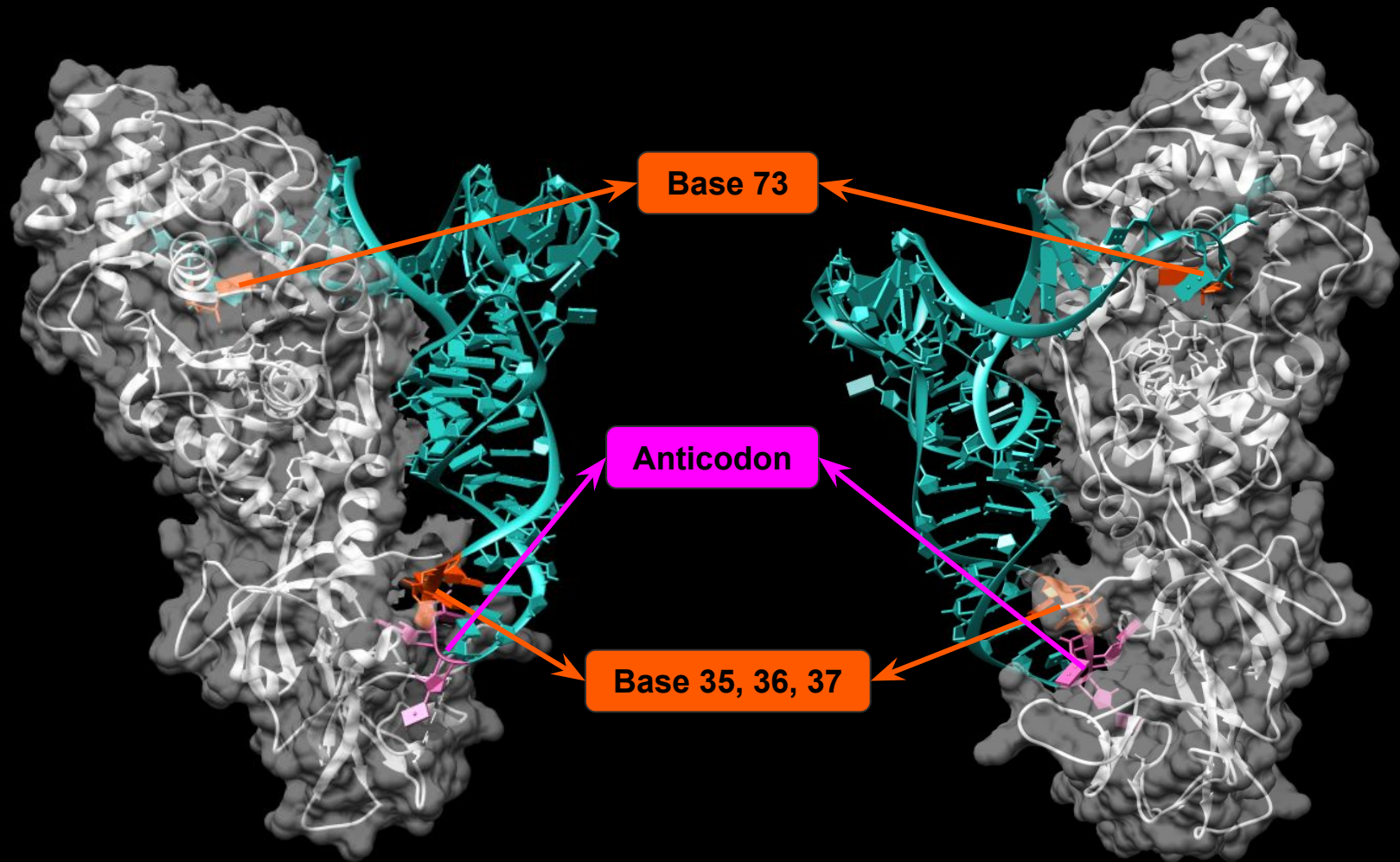
3) tRNA recognition

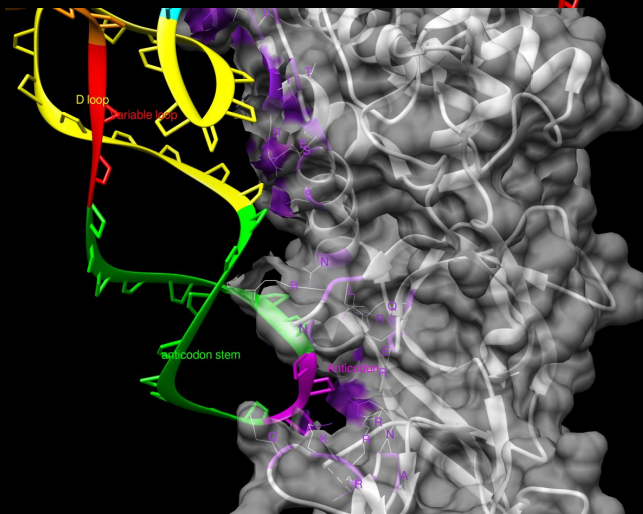
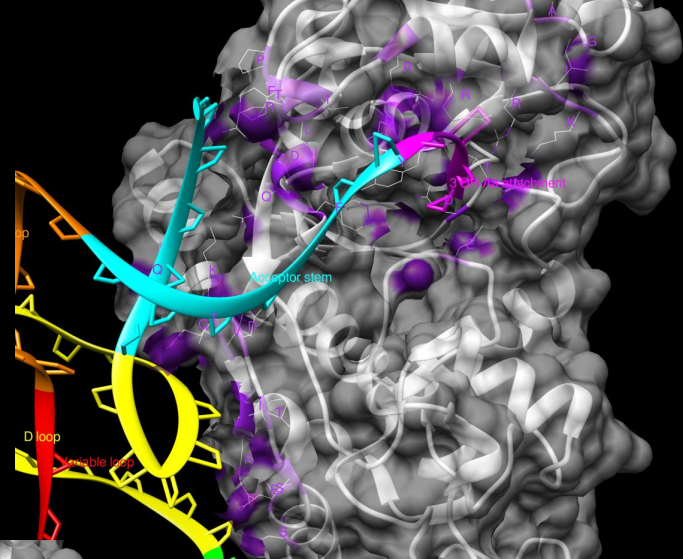
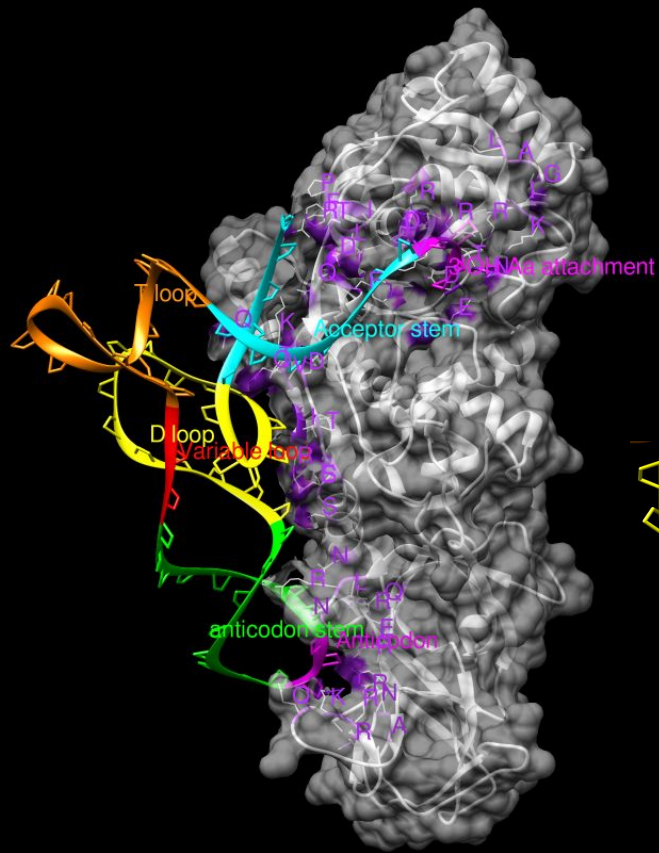
Aided by identity determinants:

- **Bases 35, 36 and 37** of the **anticodon stem-loop**
- Discriminatory **base 73** in the **acceptor stem**
- **Anticodon**

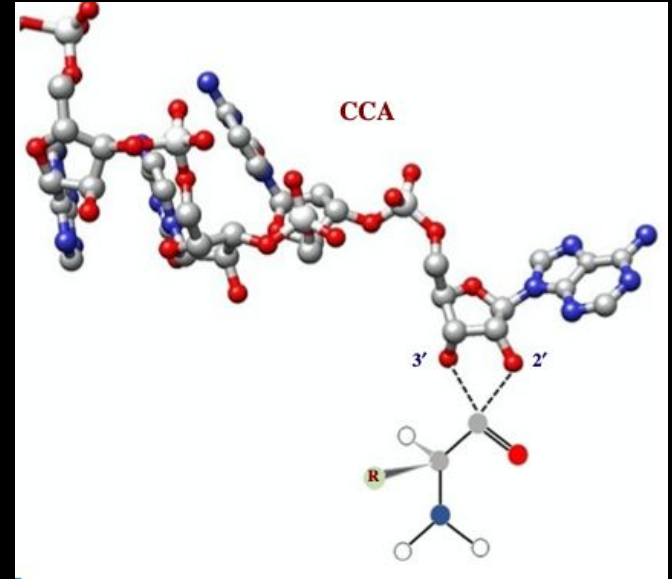
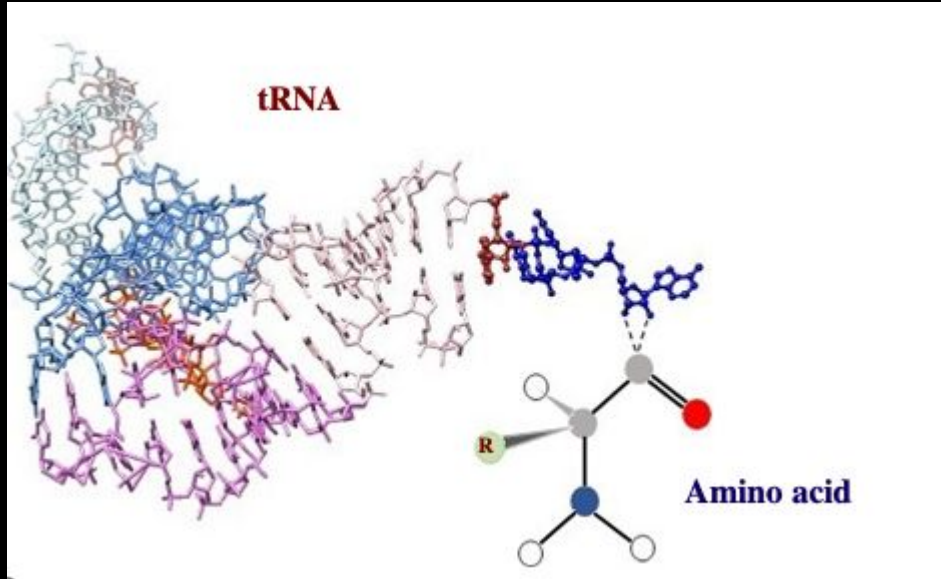
When charged, tRNA bind to complementary codon in mRNA in ribosomes through the **anticodon loop**.







Aa-AMP transfer to tRNA



This two images were extracted from: Pal S. Translation. Fundamentals of Molecular Structural Biology. 2020;;:311-346.

Aa-tRNA synthetases as a drug target for antibiotics

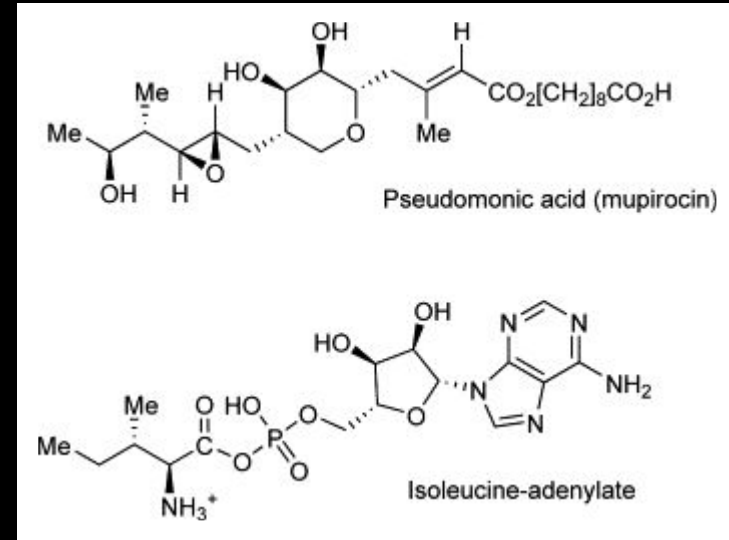
Mupirocin

Produced naturally by *Pseudomonas fluorescens*

Mechanism of action:

Inhibiting the activity of bacterial isoleucyl-tRNA synthetase.

Treatment of **skin infections** caused by *S. aureus* and *S. pyogenes*



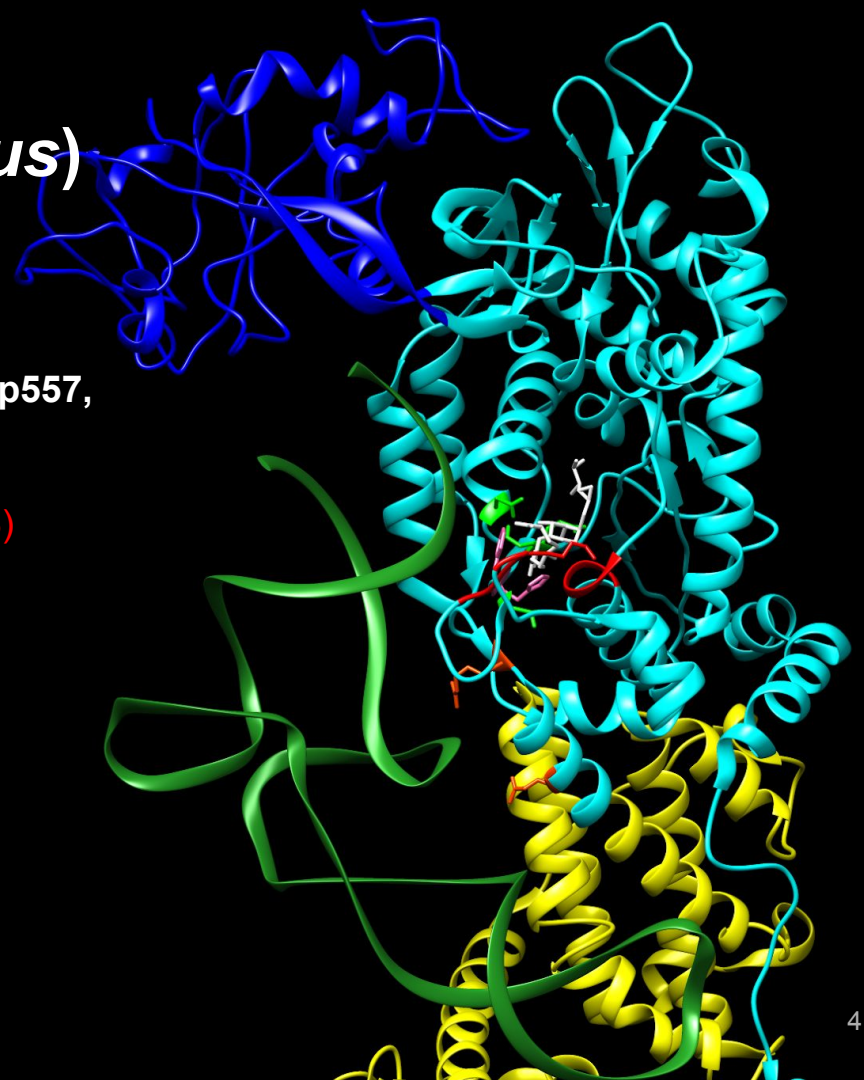
Patrick A. Frantom, John S. Blanchard, in *Comprehensive Natural Products II*, 2010

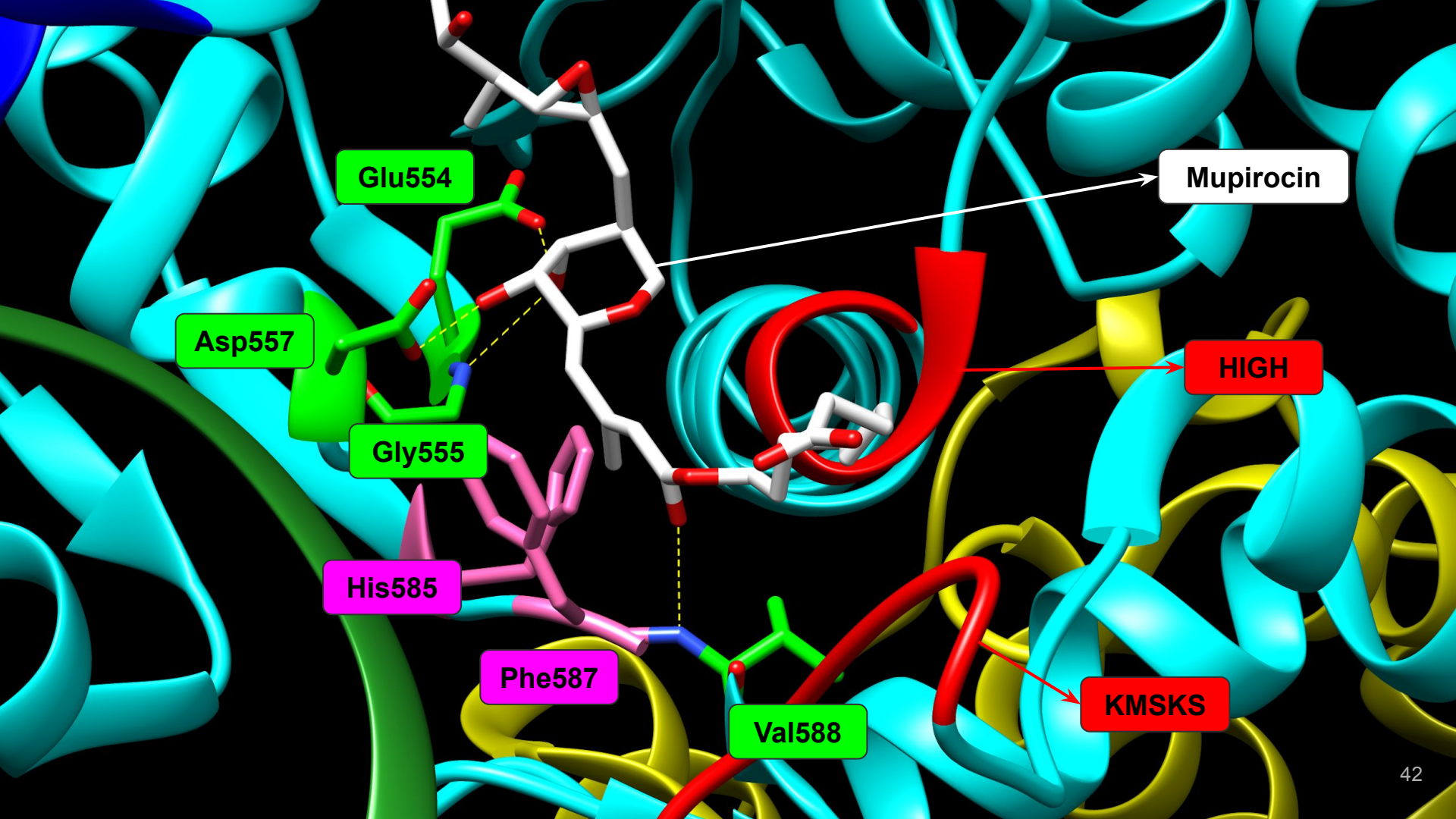


Why binding *S. aureus* Ile-tRNA synthetase and not Human?

Ile-tRNA synthetase (*S. aureus*)

- **Catalytic domain 1-200, 395-644**
 - **Mupirocin recognition** Glu554, Gly555, Asp557, His585, Phe587 and Val588
 - **KMSKS motif 594-599** (ATP recognition 598)
 - **HIGH motif 64-67**
 - **tRNA recognition 632 and 640**
- **Anticodon-binding domain 201-394**
- **Editing domain 201-394**





Model building

1. Obtain human IleRS sequence from UniProt



2. Building a PSSM against SwissProt



3. Align against PDB with the PSSM



4. Select the templates (1FFY, 1JZS, 1QU2, 1QU3)



5. Run rough STAMP to obtain structural alignment

6. Convert to Clustal



7. HMM of templates using hmmbuild



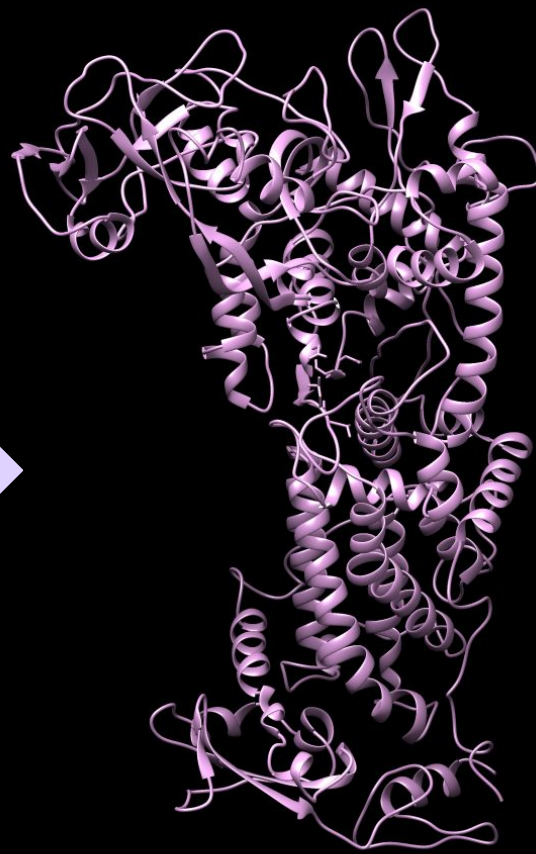
8. hmmalign with HMM and sequences



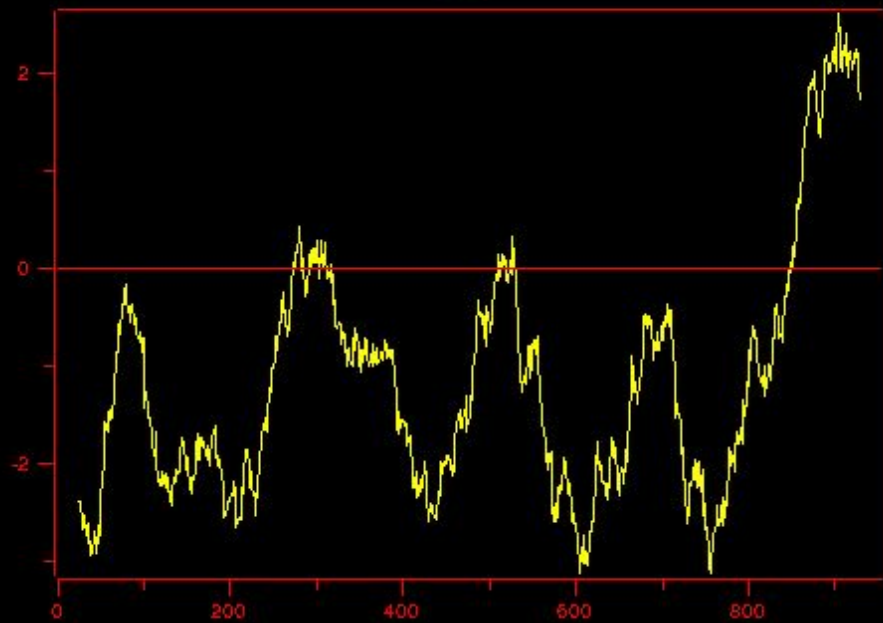
9. Alignment based on templates structure



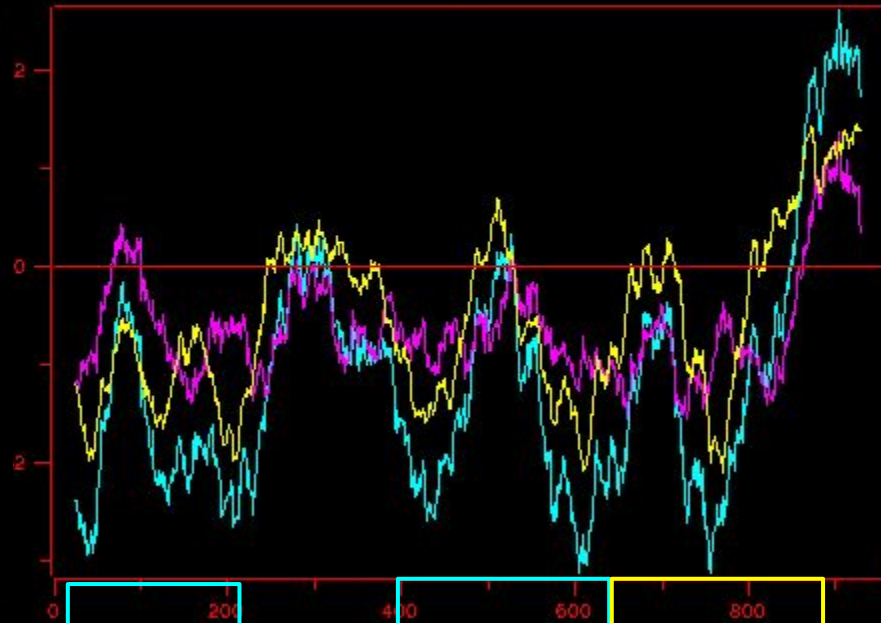
10. Run modeler



obj1



obj1

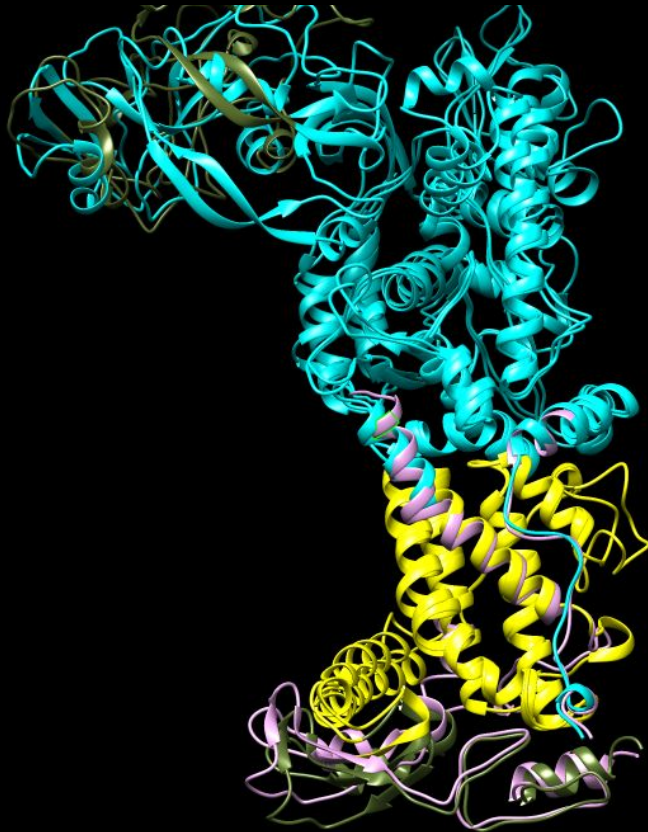


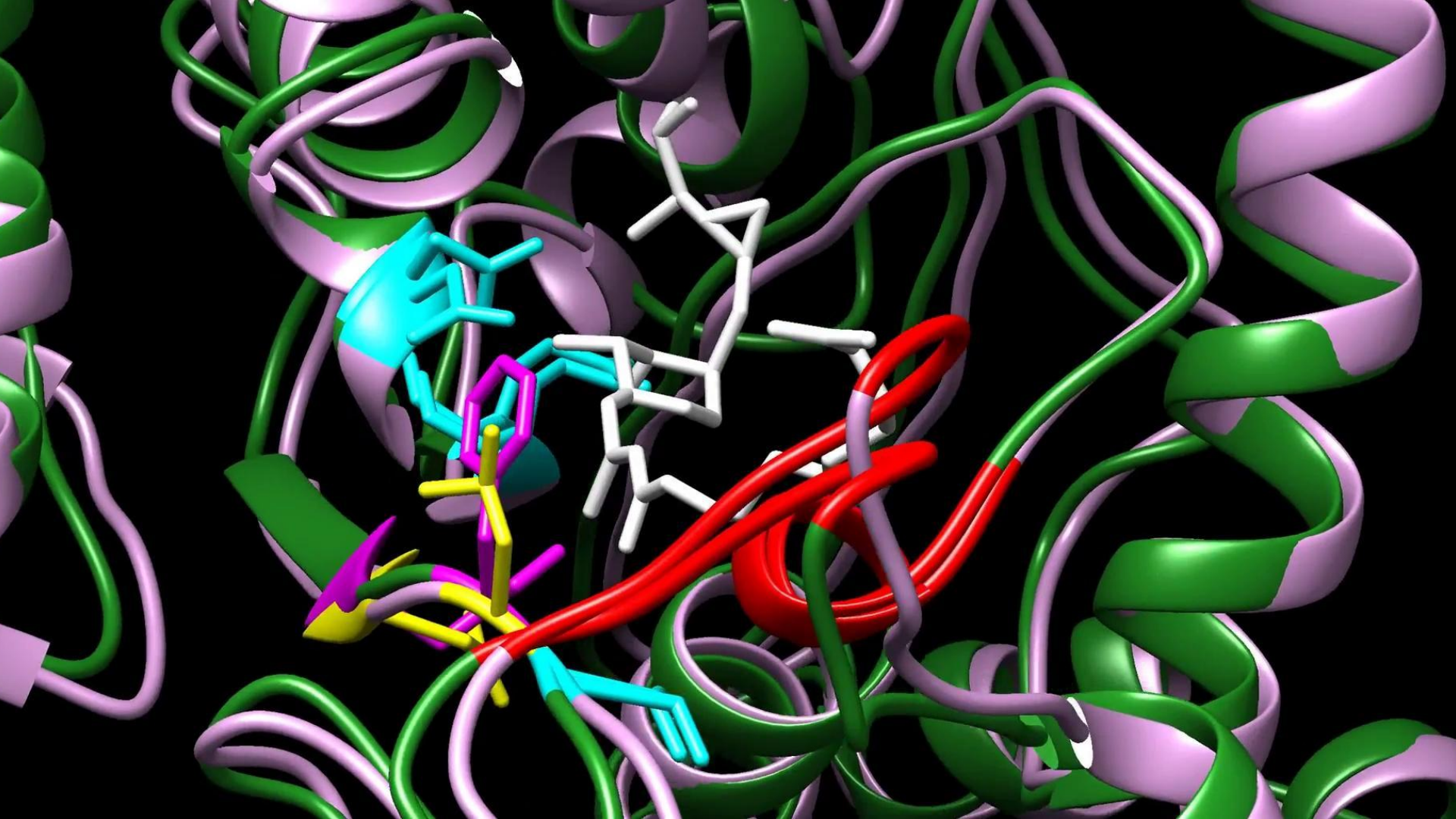
surf

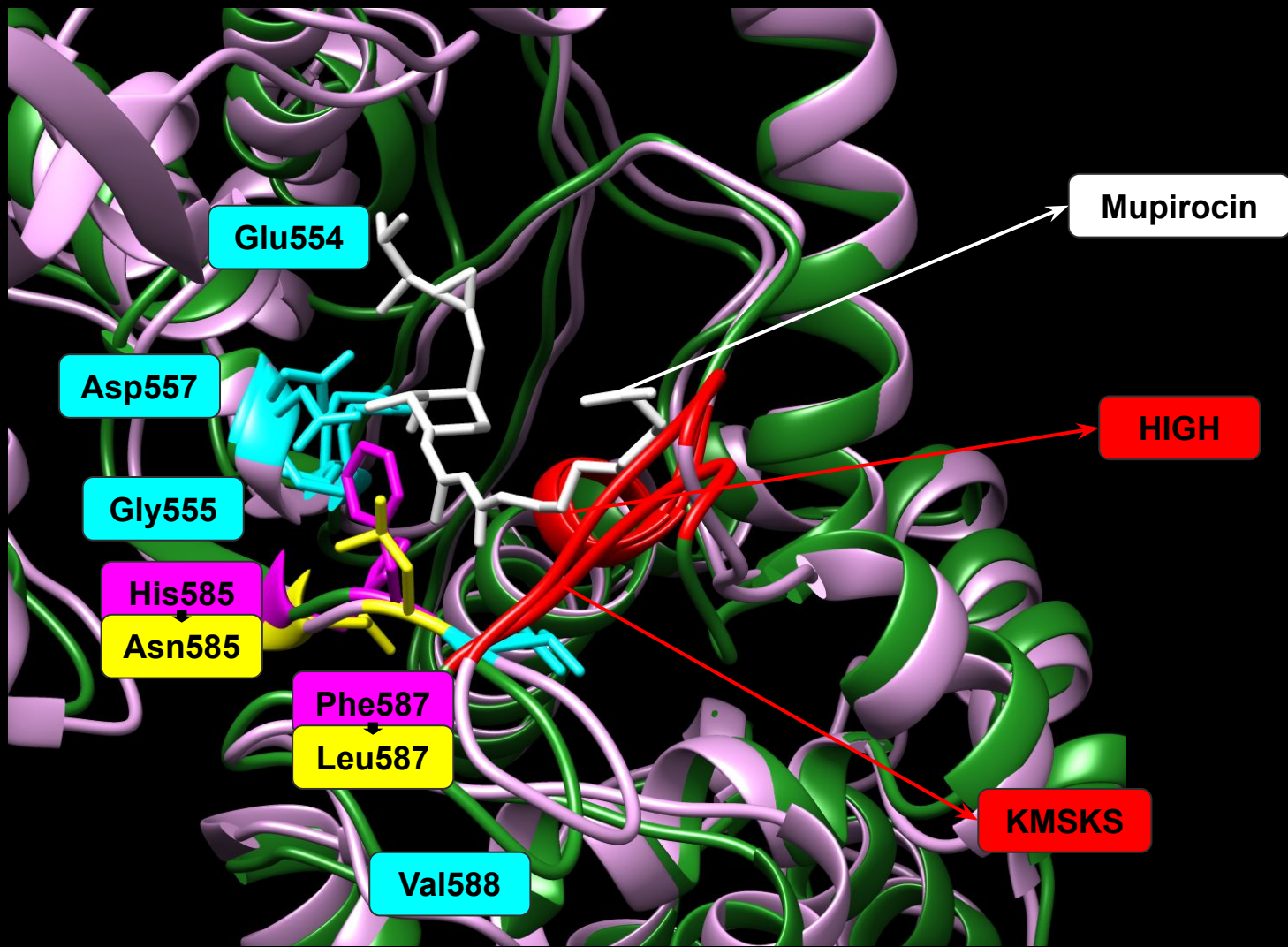
combined

pair

Ile-tRNA synthetase from **human** and *S. aureus*








```

1 1FFY_2 100.0% 100.0% 1 [ . . . . . : . . . . . HIGH . . . . . 1 . . . . . 120
-----MDYEKTLMPKTD FPMRG--GLPNKEPQIQEKWDAEDQYHKALEKNKGNETFLHDGPPYANGNLHMGHALNKILKDFIVRYKTMQGFYAPYVPGWDTHGLPIEQALTKKG---
2 humanile 98.6% 18.1% ACWPAGLSRHARPLSNKMLQQVPENINFPAAEEKILEFWTEFNCFQECLKQSKHKPKKFTFYDGPFFATGLPHYGHILAGTIKDIVTRYAHQSGFHVDRRFGWDC HGLPVEYIDKTLGIR

1 1FFY_2 100.0% 100.0% 121 : . . . . . : . . . . . 2 . . . . . 240
--VDRKMKSTAEFREKCKEFALAEQIELQKDFRRLGVRGDFNDPYITLKPEYEAQAIRIFGEMADKGLIYKGGKPVYVSPSSSESLA--EAEIEYHDKRSASIVYAFNVKDDKGVVDADA
2 humanile 98.6% 18.1% GPEDVAKMGITEYNNQCRAIVMRYSAEWKSTVSRLLGRWIDFDNDYKTLYPQFMESVWVFKQLYDKGLVYRGVVKMVPFSTACNTPLSNFESHQNYKDVQDPSVVFVTFPLEEDE----TV

1 1FFY_2 100.0% 100.0% 241 : . . . . . 3 . . . . . : . . . . . 360
KFIINTTTPWTIPSNVAITVHPPELKYGYQN--VNGEKYIIAEALSDAVA EALDWDKASIKLEKEYTGKLEWVVAQHPFLDRES---LVINGDHVTTDAGTGCVHTAPGHGEDDYIVGGQ
2 humanile 98.6% 18.1% SLVAWTTTPTWTLPSNLAVCVNPEMYYVVIKIDVARGRLLILMEARLSALYKLESDYEILERFPGAYLKGKKYRPLFDYFLKCKENGAFVTLVDNVYKEEETGVVHQAPYF GAEDYRVCMD

1 1FFY_2 100.0% 100.0% 361 . . . . . 4 . . . . . : . . . . . 480
YELP-----VISPIDDKGVFT EEGQFEGMFYDKANKAVTDLLTEKGALLKLDIFITHSYPHDWRTKKPVIFRATPQWFASISKVRQDILDAIENTNFKVNWG-KTRIYNMVRDRGEWVTS
2 humanile 98.6% 18.1% FNIIRKDSL PVPVDASGCFTEVTDFAGQYVKDADKSIIRTLKEQGRLLVATTFTHSYPCWRS DTPLIYKAVPSWVFRVENMVDQLLRNNDLCVWPELVREKRFGNWLDKARDWITS

1 1FFY_2 100.0% 100.0% 481 . . . . . 5 . . . . . : . . . . . 600
RQRVWGVPLPVFYAENGEIIMTKETVNHVADLFAEHGSNIWFEREAKDLLPEGFTHPGSPNGTFTKETDIMDVWFDSGSSHrgVLETRP-----ELSF PADMYL EGS DQYRGWFNSSIT
2 humanile 98.6% 18.1% RNRVYGTPIPLWVSDDFEEVVCIGSVAEEL EELSGAKISDLHRESVDHLTIPS----RCGKGLSHRISVEFDCWFESGMPYAQVHYPFENKREFEDAFADPADI EGD QTRGWYFTLLV

1 1FFY_2 100.0% 100.0% 601 . . . . . KMSKS . . . . . : . . . . . 7 . . . . . 720
TSVATRGVSPYKFLLSHGFLVMDGEGK KMSKSLGNVIVPDQVVKQKGAD IARLWVSSDYLL--ADVRISDEILKQTSDDYRK-----IRNTLRFMLGNINDFNPDTSDIPESELLEVA
2 humanile 98.6% 18.1% LATALFGQPPFKNVIVNGLV LASDGQ KMSKR KKNYPDPVSI IQY GADALRLYLINSPVVAENLRKFEKEGVRDVLKDVLLPWYNAYRFLIQVNLRLQKEEEIEFLY NENTVRES-PNIT

1 1FFY_2 100.0% 100.0% 721 . . . . . Mupirocin binding differences . . . . . : . . . . . 8 . . . . . 840
DRYLLNRLREFTASTINNYENFDYLNIIYQEVQNF INVELSNFYLDYGKDILYIEQRDSHIRRSMQT VLYQILVDMTKLLAPILVHTAEVWS-----HTPHVK
2 humanile 98.6% 18.1% DRWILSFMQSLIGFFETE MAAYRLTYVPRLVKFDVILTN-WYVRMNRRLKGENGMEDCVMALET-LFSVLLSCLRLMAPYTPFLT ELMYQNLKVLIDPVSVDQDQD TLSIHVLMPLRVR

1 1FFY_2 100.0% 100.0% 841 . . . . . 9 . . . . . : . . . . . 960
EESVH-----LADMPKVVEVDQALLDK-----WRTFMNLRDDVNRALETARNEKVIGKSL EAKVTIASNDK-----FNASEFLT SFDALHQLFIVSVQVVKLDDQATAYEHG
2 humanile 98.6% 18.1% EELIDKKTESAVSQMSVIELGRVIRDRKTIPIKYPLKEIVVIHQDPEALKDIKSLKEYIIEELNVRKVTLSTDKNKYGIRLRAEPDHMVLGKRLKGAFKAVMTSIKQLSSELEQFQKT

1 1FFY_2 100.0% 100.0% 961 . . . . . 0 . . . . . : . . . . . 1080
DIVIEH---ADGKERCWNYSEDLG-----AVDELTHLCPRCQVVKSLV-----AVDELTHLCPRCQVVKSLV-----AVDELTHLCPRCQVVKSLV-----
2 humanile 98.6% 18.1% GTIVVEGHELHDEDIRLMYTFDQATGGTAQFEAHSDAQALVLLDVTDPDQSMVDEGMAREVINRIQKLRKKCNLVPTEITVYKAKSEGTYLNSVIESHTEFIFTTIKAPLKPYPVSPSD

1 1FFY_2 100.0% 100.0% 1081 . . . . . 1 . . . . . : . . . . . 2 1200
-----
2 humanile 98.6% 18.1% KVLIEKQTQLKGSLEIITL TRGSSLPGACAYVNLNICANGSEQGVLLLENPKGDNRLDLLKLSVVTISIFGVKNT ELAVFHDETEIQNQTDLLSLSGKTL CVTAGSAPSLINSSSTLL

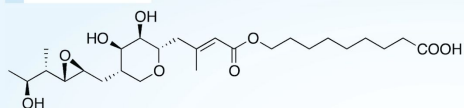
1 1FFY_2 100.0% 100.0% 1201 . . . . . : . . . . . ] 1292
-----
2 humanile 98.6% 18.1% CQYINLQLLNAK PQECLMGTVGTLLENPLGQNGLTHQGLLYEAAKVFGLRSRKLKLFNETQQTQEITEDIPVKTLNMKTYVYVSVLPTTADF

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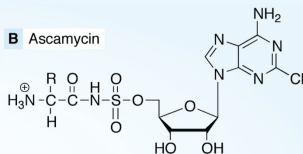
Actual landscape

Natural products

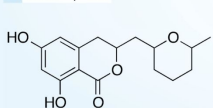
A Pseudomonamic acid



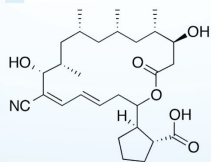
B Ascarnycin



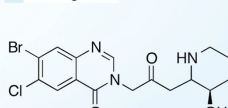
C Cladosporin



D Borrelidin

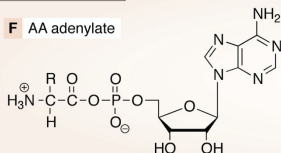


E Halofuginone

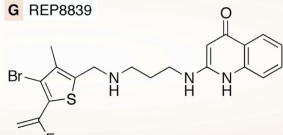


Synthetic inhibitors

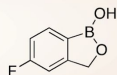
F AA adenylate



G REP8839

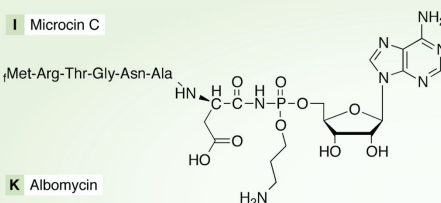


H AN2690

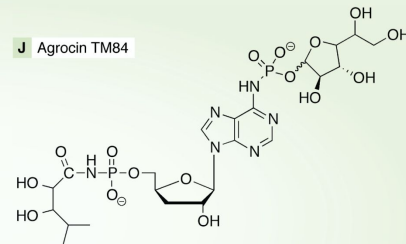


"Trojan horse" inhibitors

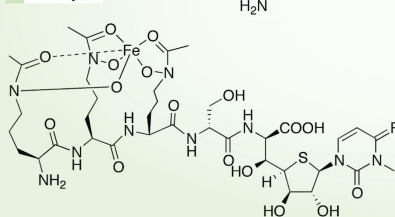
I Microcin C



J Agrocin TM84



K Albomycin



Adapted from Francklyn CS, Mullen P. Progress and challenges in aminoacyl-tRNA synthetase-based therapeutics. J Biol Chem. 2019 Apr 5;294(14):5365-5385.

Take home message

- 1. Aminoacyl tRNA synthetases have a crucial role in translation process and therefore they're essential for life.**
- 2. Divergence between eukaryotes and prokaryotes makes them a promising drug target.**
- 3. There is a lot of work to be done in this field**

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PEM questions

- How many different aminoacyl tRNA synthetases are normally in the cell?
 - 20
 - 40
 - 35
 - 43
 - All the answers are incorrect
- About aminoacyl tRNA catalytic domains fold?
 - They vary according to the class
 - They vary according to the subclass
 - Are only formed by Rossmann folds
 - Are only formed by beta-sheets
 - Are only formed by alpha helices
- Aminoacyl tRNA synthetases can be found forming...
 - monomers
 - dimers
 - tetramers
 - always monomers
 - a, b and c are correct
- Aminoacyl tRNA synthetases interact with...
 - ATP, tRNA, amino acids
 - tRNA, amino acids, ribosomes
 - ATP, aminoacids, ribosomes
 - ATP, tRNA, ribosomes
 - ATP, codon and ribosome
- Aminoacyl tRNA synthetases interact with ATP by
 - Arginine Tweezers (Class II) and
 - Backbone brackets (Class I)
 - a and b are correct
 - They don't interact with ATP
 - None of the answers is correct

6. The HIGH motif is found in...
 - a. Class II Aminoacyl tRNA synthetases
 - b. All type Aminoacyl tRNA synthetases
 - c. Only in LeuRS, IleRS and MetRS
 - d. Class I Aminoacyl tRNA synthetases
 - e. The HIGH motif is not conserved in Aminoacyl tRNA synthetases.
7. Class I Aminoacyl tRNA synthetases and Class II Aminoacyl tRNA synthetases have respectively...
 - a. 7 and 5 subclasses
 - b. 4 and 7 subclasses
 - c. 1 and 3 subclasses
 - d. 5 and 3 subclasses
 - e. They don't have subclasses
8. Mupirocin is an antibiotic that blocks:
 - a. All Class I Aminoacyl tRNA synthetases
 - b. All Class II Aminoacyl tRNA synthetases
 - c. IletRNA synthetase
 - d. His tRNA synthetases
 - e. It's not functional
9. Regarding mupirocin...
 - a. There has already been reported some strains of mupirocin resistant *S. aureus*
 - b. It is used against infections of *S. aureus* and *S. pyogenes*
 - c. It blocks IletRNA synthetases active site
 - d. It's already in the market
 - e. All the answers are correct
10. About Aminoacyl tRNA synthetases...
 - a. They are not good drug targets
 - b. They are really good targets
 - c. They don't play an essential role in cells
 - d. There's no investigations currently studying this field
 - e. All of the answers are incorrect