Expanding the genetic code

Only 20 amino acids are used in the biosynthesis of proteins

- no fundamental reason other amino acids cannot be used in proteins

Chemical synthesis (with or without chemical ligation) can be used introduce a range of functional groups

- chemical synthesis is often not economical and limited to short peptides
- need to fold the protein following synthesis and purification

Some may be introduced biosynthetically by adjusting the growth condition

- e.g. inducing protein expression in the presence of selenomethionine would incorporate selMet in place of Met

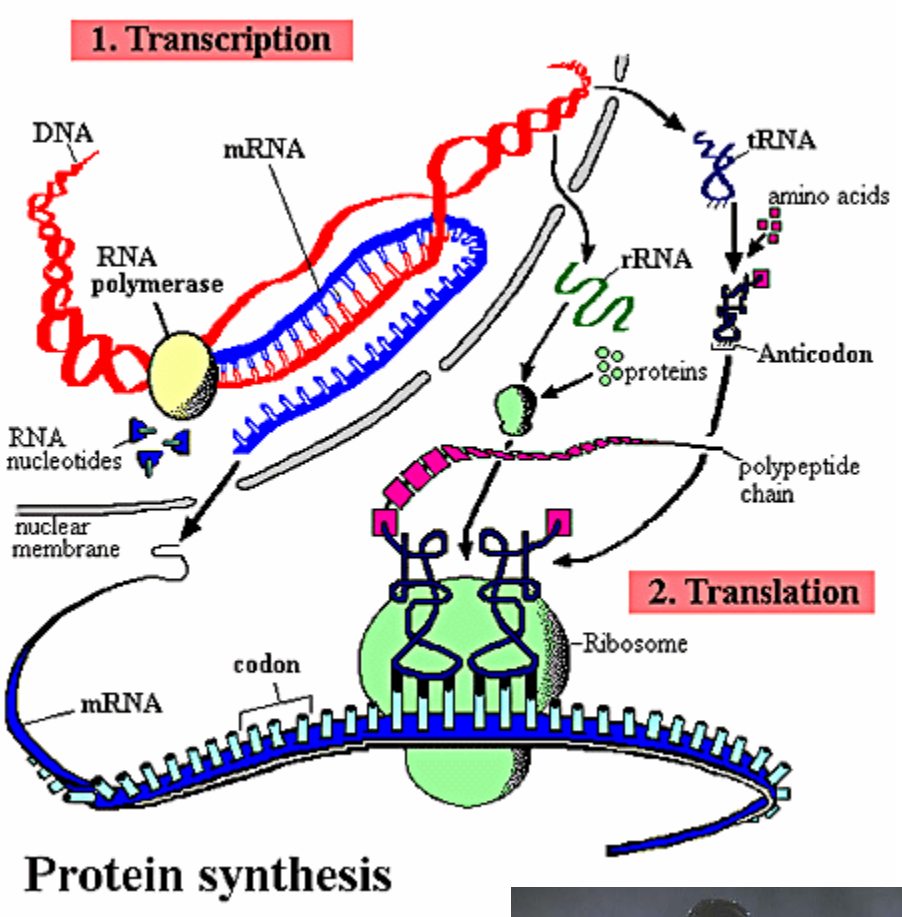
“Non-natural” amino acids may be introduced to expand the chemical properties available in native enzymes

- acidity, nucleophilicity, H-bonding potential
- may also be used to selectively modify protein for biophysical, chemical, structural studies

Analog	Target AARS	Whole cells	Purified proteins	Applications
Azetidine-2-carboxylic acid	ProRS	(144)		
3,4-Dehydroproline	ProRS	60% (145)		
Perthiaproline	ProRS		(146)	Drug carrier
Canavanine	ArgRS	(147)		Measure of stress resistance
Ethionine	MetRS	(148)	(149)	
Norleucine	MetRS	38% (150)		Increased enzyme activity (26)
	LeuRST252Y		(151)	
	$IleRS_{Ala}$*			
Selenomethionine	MetRS	100% (18)	(19)	Crystallography
Aminohexanoic acid	MetRS		(149)	
Telluromethionine				Crystallography
Homoallylglycine	MetRS		(24)	Alkene functionality
Homopropargylglycine	LeuRST252Y[a]		(151)	Staudinger ligation (134)

Hendrickson et al, ARB 73, 147 (2004)

Biosynthetic incorporation of nonnatural amino acids



Protein synthesis

tRNA converts genetic information in the form of RNA sequence into the amino acid sequence in protein

Aminoacyl synthetase (E) activates amino acids and loads them onto tRNA

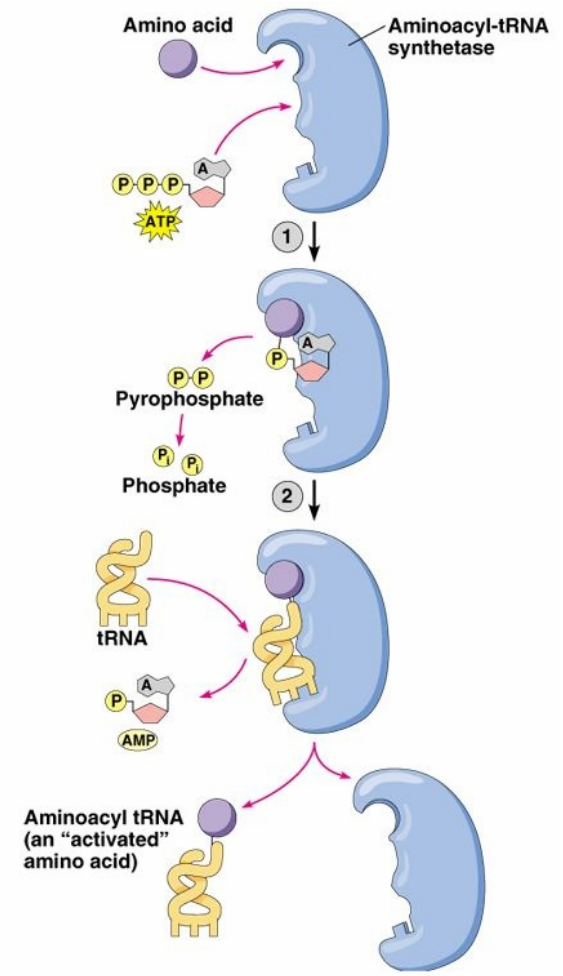
E + ATP + AA ➔ E(AA-AMP) + PPi

E(AA-AMP) + tRNA ➔ AA-tRNA + AMP + E

E : alanyl-tRNA synthetase, cysteinyl-tRNA synthetase, …

tRNA : $tRNA^{ala}$, $tRNA^{cys}$, …

Loading of tRNA



Amino acyl tRNA synthetase (aaRS) ensures correct amino acids get loaded on each tRNA

- there is a certain amount of promiscuity
- differentiating similar amino acids is chemically difficult—e.g. Val and Ile differ by a single methylene
- isoleucyl-tRNA synthetase (IleRS) may load val onto tRNAile
- there is an editing mechanism

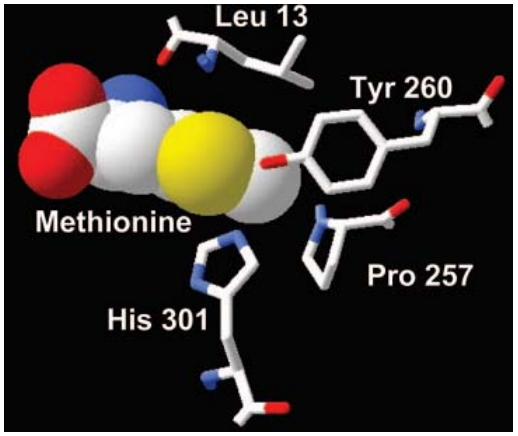
Nonnatural amino acids may be introduced into proteins by engineering novel aaRS/tRNA pairs

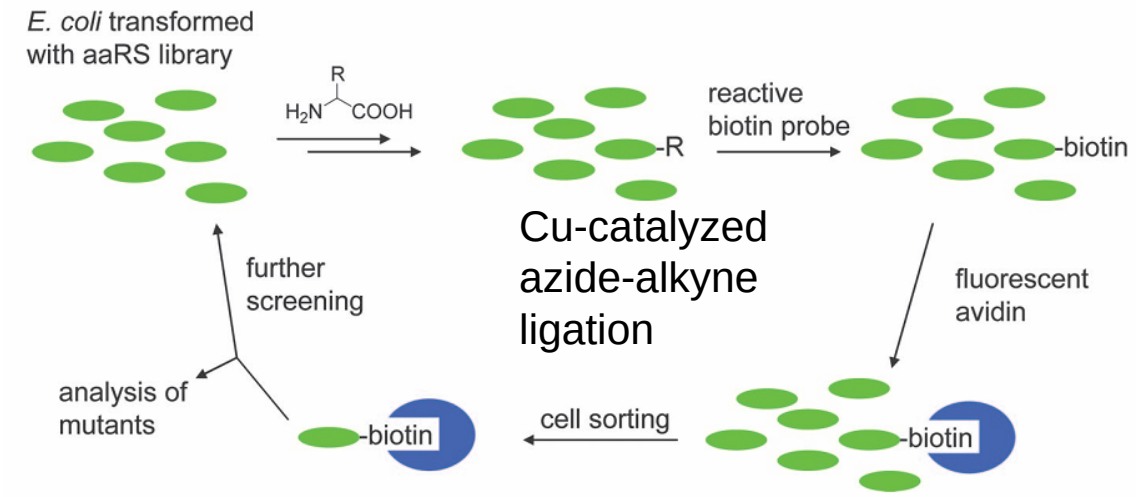
The aaRS/tRNA pair needs to be "orthogonal" to the existing sets of aaRS/tRNA to ensure nonnatural amino acids are introduced selectively at predetermined positions only

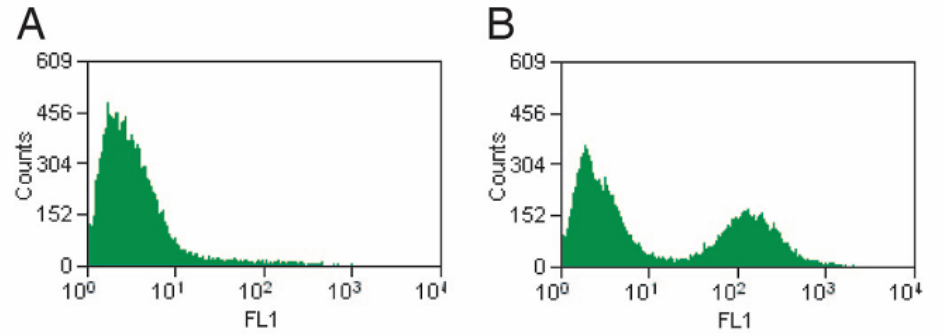
Engineering novel aaRS

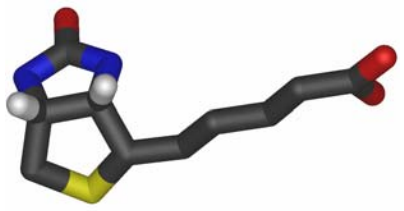
High resolution E coli MetRS structures are available with and without bound methionine

Use cell-based directed evolution to engineer aaRS to bind new amino acid









biotin

Link et al, PNAS 103, 10180 (2006)

Engineering tRNA

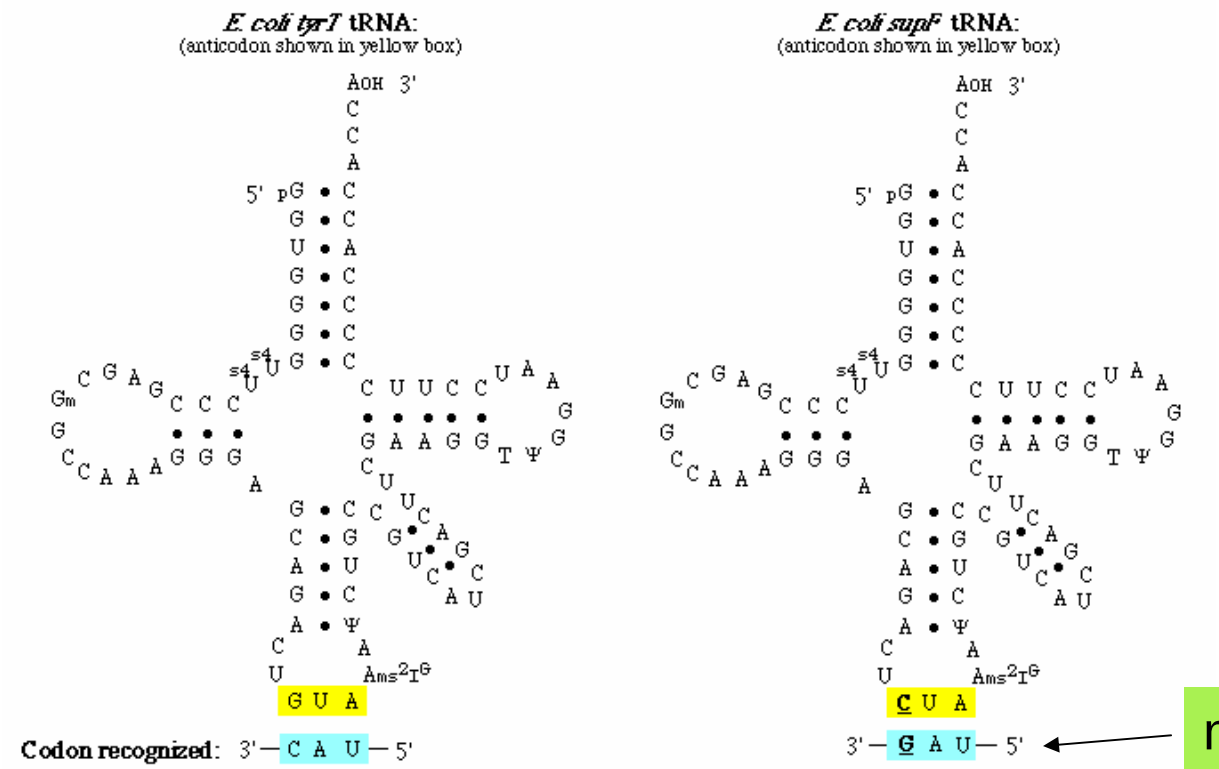
Novel tRNA would recognize a codon not used in nature

termination codon (TGA, TAG, TAA), four base codon

Suppressor tRNA ignores the termination codon in an mRNA and instead adds an amino acid

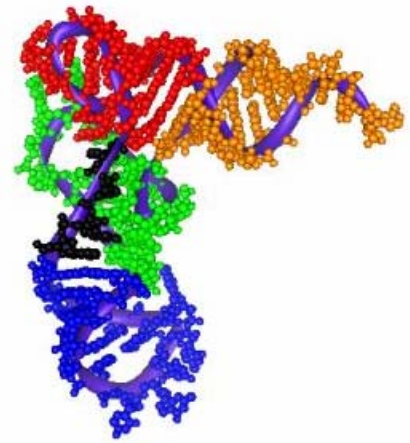
tyrT

GGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACTGTAAATCTGCCGTCATCGACTTCGAAGGTTCGAATCCTTCCCCCACCACCA
CCACCCCAAGGGCTCGCCGGTTTCCCTCGTCTGACATTTAGACGGCAGTAGCTGAAGCTTCCAAGCTTAGGAAGGGGGTGGTGGT

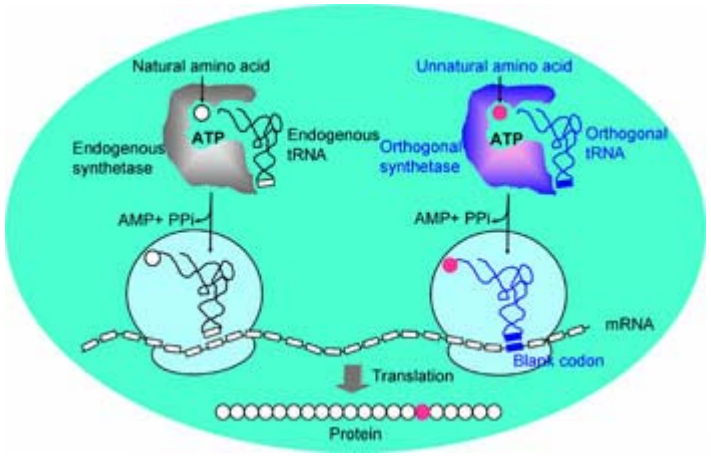


mRNA

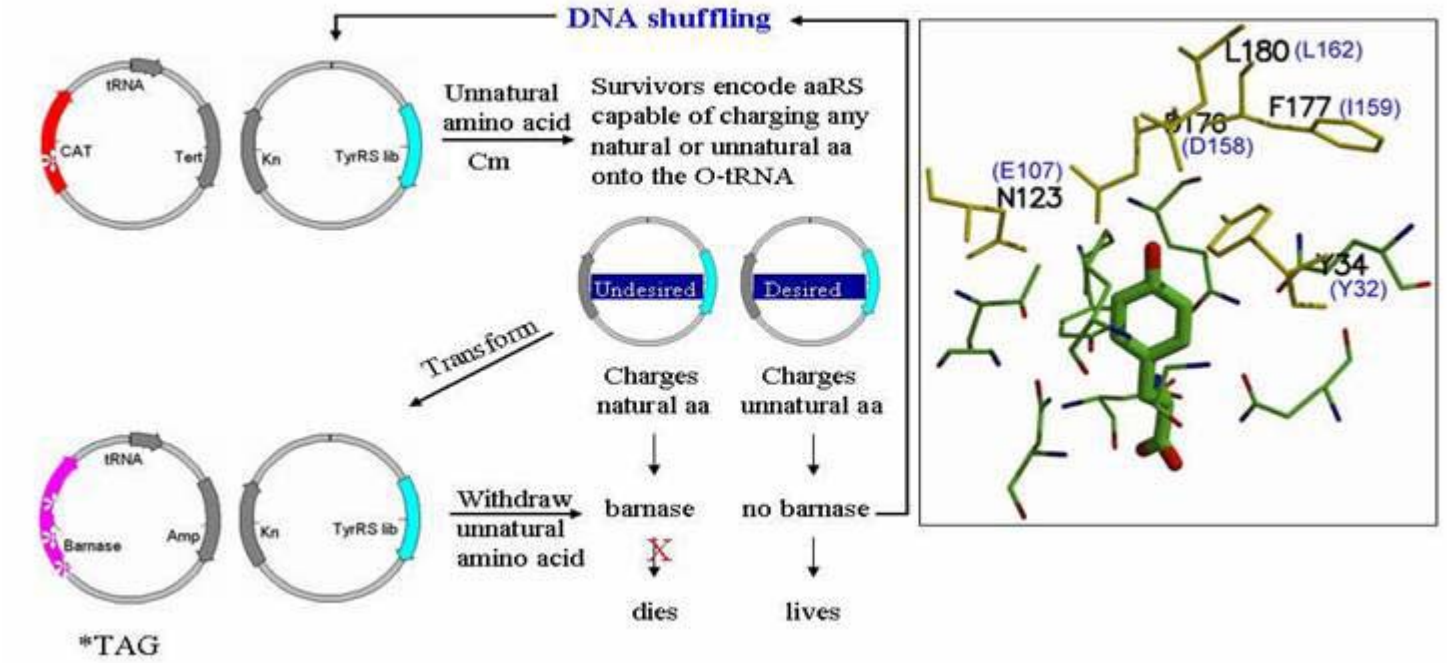
TAG: amber
TAA: ochre
TGA: opal



Designing orthogonality



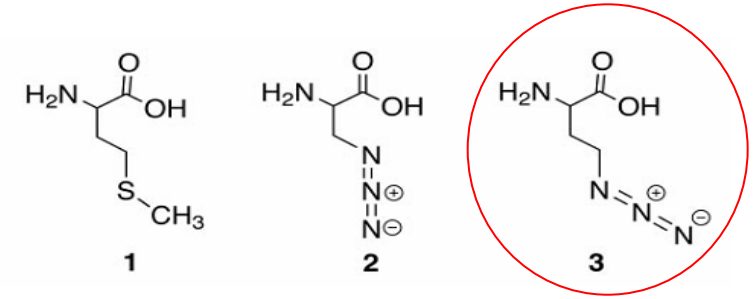
1. Orthogonal synthetase must load orthogonal tRNA
2. Endogenous synthetase must not load orthogonal tRNA
3. Orthogonal synthetase must not load endogenous tRNA



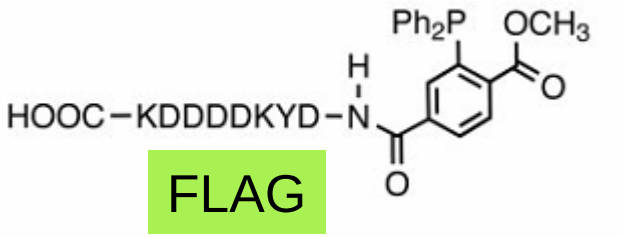
Putting it together

Nonnatural amino acids with chemically useful groups may be introduced

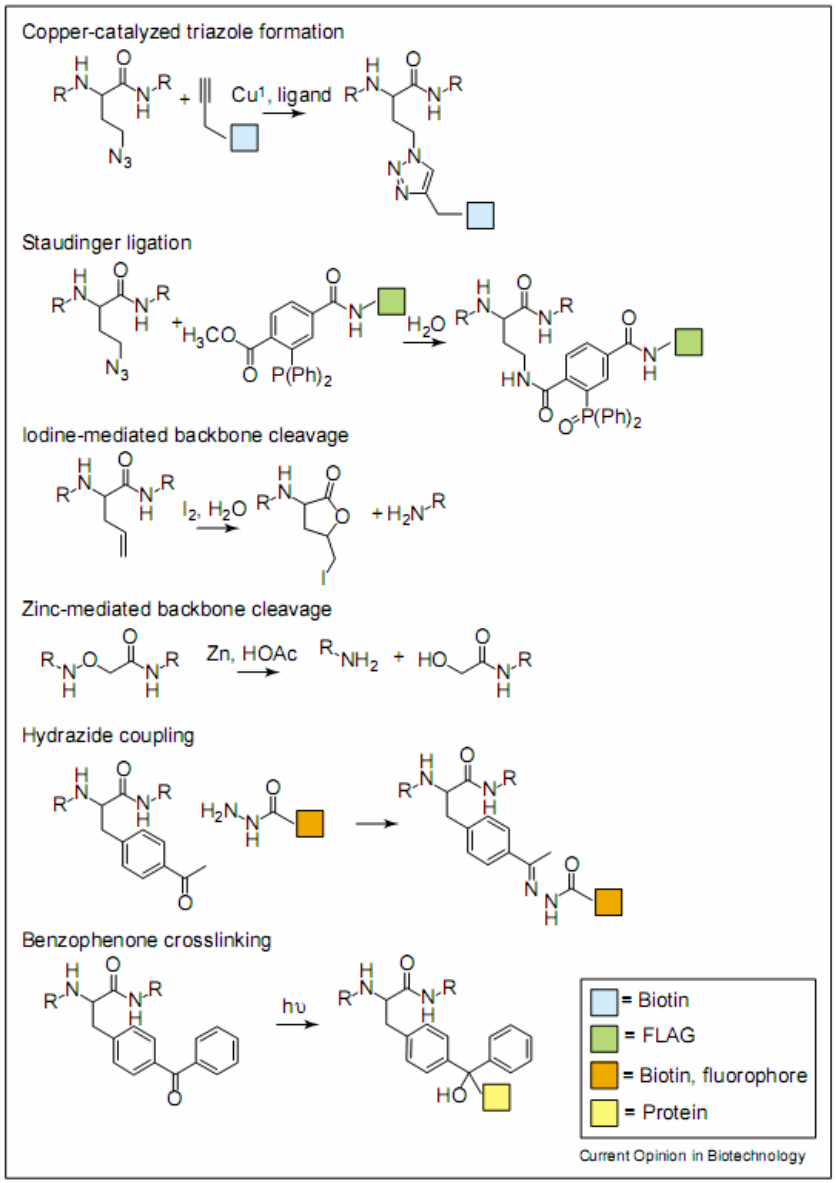
Proteins may be further modified chemically to modulate protein-protein recognition or to selectively label the protein



potential substrates for metRS



Kiick, et al, PNAS 99, 19 (2002)



Link et al, CO in Biotech 14, 603 (2003)