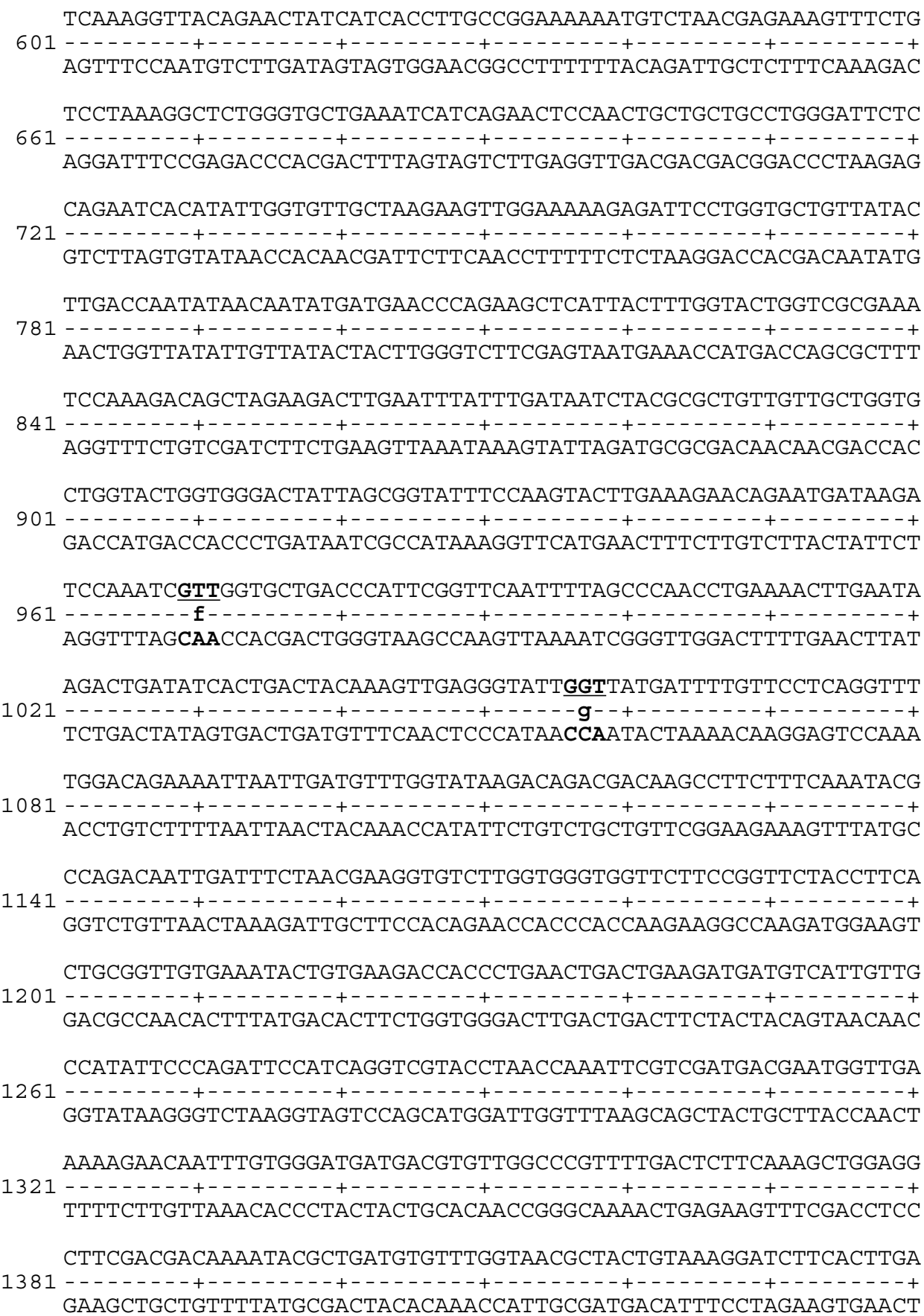
Recitation Section 8
March 2-3, 2005

Molecular Biology—Transcription and Translation

Today we return to take another look at the yeast cystathionine beta synthase (CBS) protein. Below is the sequence of the yeast CBS gene and surrounding DNA. We will use this sequence for parts A and B of today's section.

The sequences of both strands of the DNA duplex are shown: the top strand reads 5' to 3' left to right (1 to 2040); the bottom, complimentary, strand reads 5' to 3' right to left (2040 to 1).

```
5′-  CAACTTCACCCAAGTAAGGATAATCAGCTCTGTCGTGACTGATAAATGCTATATCCGGCA
   1 ---------+---------+---------+---------+---------+---------+
3′-  GTTGAAGTGGGTTCATTCCTATTAGTCGAGACAGCACTGACTATTTACGATATAGGCCGT

     TATGCAGTCCACACGGCATTACCGTTTCACTAATTTATTGCCATCTTCCTCCACAGTTTT
  61 ---------+---------+---------+---------+---------+---------+
     ATACGTCAGGTGTGCCGTAATGGCAAAGTGATTAAATAACGGTAGAAGGAGGTGTCAAAA

     GCACCGAAAGGAAAAAAAGAAACCAACACCGAAAATTTTTTTCTCCTAAAGGTTAAAGTA
 121 ---------+---------+---------+---------+---------+---------+
     CGTGGCTTTCCTTTTTTTCTTTGGTTGTGGCTTTTAAAAAAAGAGGATTTCCAATTTCAT

     AACGCAAGGCACTTCACCAGGCTTGTATATATAAATGTCGTGATGCTTCTATGCCAAAGT
 181 ---------+---------+---------+---------+---------+---------+
     TTGCGTTCCGTGAAGTGGTCCGAACATATATATTTACAGCACTACGAACATACGGTTTCA

     AAAAGGCAACACTTGAAGATTTCGTTGTAGGCCACTTGCTCAAAGGACATCTAGATAAAT
 241 ---------+---------+---------+---------+---------+---------+
     TTTTCCGTTGTGAACTTCTAAAGCAACATCCGGTGAACGAGTTTCCTGTAGATCTATTTA

     ACGACGTAAGAATAAAAATGACTAAATCTGAGCAGCAAGCCGATTCAAGACATAACGTTA
 301 ---------+---------+--------c+--------b+---------+---a-----+
     TGCTGCATTCTTATTTTTACTGATTTAGACTCGTCGTTCGGCTAAGTTCTGTATTGCAAT

     TCGACTTAGTTGGTAACACCCCATTGATCGCACTGAAAAAATTGCCTAAGGCTTTGGGTA
 361 ---------+----e----+--d------+---------+---------+---------+
     AGCTCAATCAACCATTGTGGGGTAACTAGCGTGACTTTTTTAACGGATTCCGAAACCCAT

     TCAAACCACAAATTTATGCTAAGCTGGAACTATACAATCCAGGTGGTTCCATCAAAGACA
 421 ---------+---------+---------+---------+---------+---------+
     AGTTTGGTGTTTAAATACGATTCGACCTTGATATGTTAGGTCCACCAAGGTAGTTTCTGT

     GAATTGCCAAGTCTATGGTGGAAGAAGCTGAAGCTTCCGGTAGAATTCATCCTTCCAGAT
 481 ---------+---------+---------+---------+---------+---------+
     CTTAACGGTTCAGATACCACCTTCTTCGACTTCGAAGGCCATCTTAAGTAGGAAGGTCTA

     CTACTCTGATCGAACCTACTTCTGGTAACACCGGTATCGGTCTAGCTTTAATCGGCGCCA
 541 ---------+---------+---------+---------+---------+---------+
     GATGAGACTAGCTTGGATGAAGACCATTGTGGCCATAGCCAGATCGAAATTAGCCGCGGT
```


TCAAAGGTTACAGAACTATCATCACCTTGCCGGAAAAAATGTCTAACGAGAAAGTTTCTG
601
AGTTTCCAATGTCTTGATAGTAGTGGAACGGCCTTTTTTACAGATTGCTCTTTCAAAGAC
TCCTAAAGGCTCTGGGTGCTGAAATCATCAGAACTCCAACTGCTGCTGCCTGGGATTCTC
661
AGGATTTCCGAGACCCACGACTTTAGTAGTCTTGAGGTTGACGACGACGGACCCTAAGAG
CAGAATCACATATTGGTGTTGCTAAGAAGTTGGAAAAAGAGATTCCTGGTGCTGTTATAC
721
GTCTTAGTGTATAACCACAACGATTCTTCAACCTTTTTCTCTAAGGACCACGACAATATG
TTGACCAATATAACAATATGATGAACCCAGAAGCTCATTACTTTGGTACTGGTCGCGAAA
781
AACTGGTTATATTGTTATACTACTTGGGTCTTCGAGTAATGAAACCATGACCAGCGCTTT
TCCAAAGACAGCTAGAAGACTTGAATTTATTTGATAATCTACGCGCTGTTGTTGCTGGTG
841
AGGTTTCTGTCGATCTTCTGAAGTTAAATAAAGTATTAGATGCGCGACAACAACGACCAC
CTGGTACTGGTGGGACTATTAGCGGTATTTCCAAGTACTTGAAAGAACAGAATGATAAGA
901
GACCATGACCACCCTGATAATCGCCATAAAGGTTCATGAACTTTCTTGTCTTACTATTCT
TCCAAATC**GTT**GGTGCTGACCCATTCGGTTCAATTTTAGCCCAACCTGAAAACTTGAATA
961
f
AGGTTTAG**CAA**CCACGACTGGGTAAGCCAAGTTAAAATCGGGTTGGACTTTTGAACTTAT
AGACTGATATCACTGACTACAAAGTTGAGGGTATT**GGT**TATGATTTTGTTCCTCAGGTTT
1021
g
TCTGACTATAGTGACTGATGTTTCAACTCCCATAA**CCA**ATACTAAAACAAGGAGTCCAAA
TGGACAGAAAATTAATTGATGTTTGGTATAAGACAGACGACAAGCCTTCTTTCAAATACG
1081
ACCTGTCTTTTAATTAACTACAAACCATATTCTGTCTGCTGTTCGGAAGAAAGTTTATGC
CCAGACAATTGATTTCTAACGAAGGTGTCTTGGTGGGTGGTTCTTCCGGTTCTACCTTCA
1141
GGTCTGTTAACTAAAGATTGCTTCCACAGAACCACCCACCAAGAAGGCCAAGATGGAAGT
CTGCGGTTGTGAAATACTGTGAAGACCACCCTGAACTGACTGAAGATGATGTCATTGTTG
1201
GACGCCAACACTTTATGACACTTCTGGTGGGACTTGACTGACTTCTACTACAGTAACAAC
CCATATTCCCAGATTCCATCAGGTCGTACCTAACCAAATTCGTCGATGACGAATGGTTGA
1261
GGTATAAGGGTCTAAGGTAGTCCAGCATGGATTGGTTTAAGCAGCTACTGCTTACCAACT
AAAAGAACAATTTGTGGGATGATGACGTGTTGGCCCGTTTTGACTCTTCAAAGCTGGAGG
1321
TTTTCTTGTTAAACACCCTACTACTGCACAACCGGGCAAAACTGAGAAGTTTCGACCTCC
CTTCGACGACAAAATACGCTGATGTGTTTGGTAACGCTACTGTAAAGGATCTTCACTTGA
1381
GAAGCTGCTGTTTTATGCGACTACACAAACCATTGCGATGACATTTCCTAGAAGTGAACT

```
     AACCGGTTGTTTCCGTTAAGGAAACCGCTAAGGTCACTGATGTTATCAAGATATTAAAAG
1441 ---------+---------+---------+---------+---------+---------+
     TTGGCCAAGAAAGGCAATTCCTTTGGCGATTCCAGTGACAACAATAGTTCTATAATTTTC

     ACAATGGCTTTGACCAATTGCCTGTGTTGACTGAAGACGGCAAGTTGTCTGGTTTAGTTA
1501 ---------+---------+---------+---------+---------+---------+
     TGTTACCGAAACTGGTTAACGGACACAACTGACTTCTGCCGTTCAACAGACCAAATCAAT

     CTCTCTCTGAGCTTCTAAGAAAACTATCAATCAATAATTCAAACAACGACAACACTATAA
1561 ---------+---------+---------+---------+---------+---------+
     GAGAGAGACTCGAAGATTCTTTTGATAGTTAGTTATTAAGTTTGTTGCTGTTGTGATATT

     AGGGTAAATACTTGGACTTCAAGAAATTAAACAATTTCAATGATGTTTCCTCTTACAACG
1621 ---------+---------+---------+---------+---------+---------+
     TCCCATTTATGAACCTGAAGTTCTTTAATTTGTTAAAGTTACTACAAAGGAGAATGTTGC

     AAAATAAATCCGGTAAGAAGAAGTTTATTAAATTCGATGAAAACTCAAAGCTATCTGACT
1681 ---------+---------+---------+---------+---------+---------+
     TTTTATTTAGGCCATTCTTCTTCAAATAATTTAAGCTACTTTTGAGTTTCGATAGACTGA

     TGAATCGTTTCTTTGAAAAAAACTCATCTGCCGTTATCACTGATGGCTTGAAACCAATCC
1741 ---------+---------+---------+---------+---------+---------+
     ACTTAGCAAAGAAACTTTTTTTGAGTAGACGGCAATAGTGACTACCGAACTTTGGTTAGG

     ATATCGTTACTAAGATGGATTTACTGAGCTACTTAGCATAAATAAGAACCCACGCTTCAA
1801 ---------+---------+---------+---------+---------+---------+
     TATAGCAATGATTCTACCTAAATGACTCGATGAATCGTATTTATTCTTGGGTGCGAAGTT

     ATAAAAGCAAACATAGAAGCAAAATCCGTCATTCCTTTCCTATTCAATTGCACCGTTCTC
1861 ---------+---------+---------+---------+---------+---------+
     TATTTTCGTTTGTATCTTCGTTTTAGGCAGTAAGGAAAGGATAAGTTAACGTGGCAAGAG

     TTTATATAACTACTTAATTAAATAGCGCCTATACGAAGCAGCATTGTTCTATTATTTTTA
1921 ---------+---------+---------+---------+---------+---------+
     AAATATATTGATGAATTAATTTATCGCGGATATGCTTCGTCGTAACAAGATAATAAAAAT

     CAAATTCCTTATCATGCATGCATCACATCAGTGTTTGAATCTGTTAACTTTTCACTTTAT
1981 ---------+---------+---------+---------+---------+---------+
     GTTTAAGGAATAGTACGTACGTAGTGTAGTCACAAACTTAGACAATTGAAAAGTGAAATA
```

A. Transcription and Translation—Practice

RNA Polymerase complex binds to the two TATA-type elements underlined and bolded above. Once bound, RNA polymerase starts making mRNA somewhere between 40 and 120 base pairs downstream of the last TATA element.

1. If transcription starts 40 base pairs downstream of the last TATA element, at base pair #253, write the sequence of the first 10 nucleotides of the resulting mRNA. Label 5’ and 3’ ends.

2. If transcription starts 90 base pairs downstream of the last TATA element, at base pair #303, write the sequence of the first 10 nucleotides of the resulting mRNA. Label 5’ and 3’ ends.

3. In each case, what are the first eight amino acids of the resulting protein? In this case, does the transcription start site influence the sequence of the resulting protein?

4. Does translation terminate at the TAA at the underlined position 353 (**a**)? Why or why not?

5. How would your answer to 3 change if the C/G base pair at position 339 (**b**, bold) was deleted? What effect, if any, do you expect this mutation to have on the resulting CBS protein?

6. How would your answer to 3 change if an A/T base pair was added between 328 & 329 (**c**, bold)? What effect, if any, do you expect this mutation to have on the resulting CBS protein?

7. How would your answer to 3 change if the A/T base pair at position 383 (**d**, bold) were changed to a G/C? What effect, if any, do you expect this mutation to have on the resulting CBS protein?

8. Give a single base change (substitution, deletion, or addition of a single base and it's partner on the other strand) that would cause termination of the polypeptide chain at TAA codon 374 (**e**, underlined).

9. Give an example of a nonsense mutation (codon --> stop codon).

10. Give an example of missense mutation (codon --> codon for another amino acid).

11. Give an example of silent mutation (codon ---> codon for the same amino acid).

12. A mutation in what codon(s) always results in a change in the primary sequence of the resulting protein?

13. The actual stop codon for CBS is at bases 1839-1841 (underlined). What would the effect of the A1841C mutation be on the CBS protein?

B. Transcription and Translation—Functional effects

Recall that yeast that lack CBS protein can not synthesize the amino acid cysteine. Mutant versions of the human CBS protein can lead to a very serious disease called homocystinuria. Some symptoms include mental retardation, early strokes and heart disease.

Recall also that in Section 3 we encountered a crystallographic model of a form of CBS protein. Consider the model again. This model is of a truncated form of the human CBS protein. Yeast and human proteins are very similar (72%), and some sections are identical (38%).

I. Mutation 1-- Treatable

1. Take a look at bases 969-971 (**f**, bold).
    a. What amino acid is encoded?

    b. What kind of amino acid is it?

    c. Where in a protein might you expect to find it?

In the human CBS protein, this amino acid corresponds to Ile278.

2. What type of amino acid is Ile? Would you expect to find it in the same environment as the amino acid in 1 or not? Explain.

Choose “Isoleucine 278” on the crystallographic model exercise.

3. What type of secondary structure element is it located in?

4. What type of amino acids would you expect to surround it?

Choose “Isoleucine 278 and Neighbors” on the crystallographic model exercise.

5. What amino acids is Ile278 in contact with?

6. What type of amino acids are those?

A human mutation leading to a serious but somewhat treatable case of homocystinurea is caused by the equivalent of G969A and T970C mutations in yeast.

7. What amino acid would be created in yeast with those substitutions?

8. Is a mutation causing this amino acid substitution more likely in humans or in yeast? Why?

9. What would be the effect of this mutation be on the tertiary structure of CBS? Why?

The only known treatment for homocystinurea is to supplement patient's diet with large quantities of vitamin B_6. B_6 is the co-factor for human CBS.

10. In general, why would adding more co-factor to a deficient enzyme help restore some of the function?

11. How do you suppose does the treatment overcome the deficiency in the mutant CBS?

II. Mutation 2-- Untreatable

12. Take a look at bases 1056-1058 (**g**, bold).
    a. What amino acid is encoded?

    b. What kind of amino acid is it? Does it have any special properties?

    c. Where in a protein might you expect to find it?

In the human CBS protein, this amino acid corresponds to Gly307. Choose "Glycine 307" on the crystallographic model exercise.

13. Does it belong to any secondary structure element?

14. What type of amino acids would you expect to surround it?

Choose "Glycine 307 and Neighbors" on the crystallographic model exercise.

15. What amino acids is Gly307 in contact with?

16. What type of amino acids are those?

A human mutation leading to a severe and untreatable case of homocystinurea is caused by the equivalent of G1056A mutation in yeast.

17. What amino acid would be created in yeast with that substitution?

18. Is a mutation causing this amino acid substitution more likely in humans or in yeast? Why?

19. What would be the effect of this mutation be on the tertiary structure of CBS? Why?

This mutation in humans does not respond to the vitamin B_6 treatment.

20. What are the possible reasons that one mutation would respond to treatment and another would not?

C. Transcription and Translation—Conclusions

1. Where is all the information needed to properly execute transcription and translation recorded?

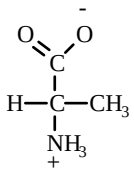
2. Is all this information accessible to the same machinery?

3. Is the stop codon in position 1839 where transcription of the CBS gene stops? Why or why not?

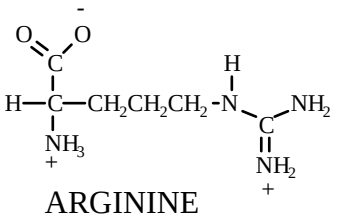
The Genetic Code

	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 **trp**	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 **ile**	ACU **thr**	AAU **asn**	AGU **ser**	U
	AUC **ile**	ACC **thr**	AAC **asn**	AGC **ser**	C
	AUA **ile**	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

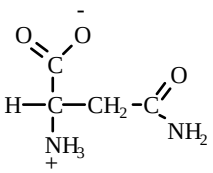
STRUCTURES OF AMINO ACIDS
at pH 7.0



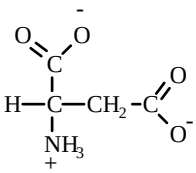
ALANINE
(ala)



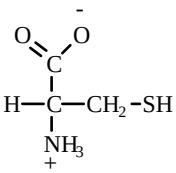
ARGININE
(arg)



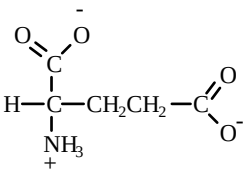
ASPARAGINE
(asN)



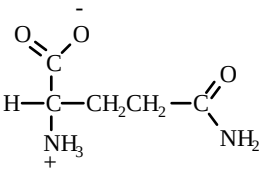
ASPARTIC ACID
(asp)



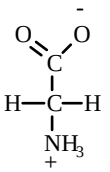
CYSTEINE
(cys)



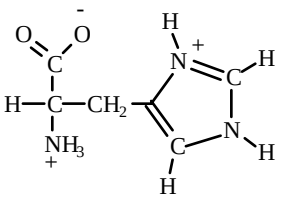
GLUTAMIC ACID
(glu)



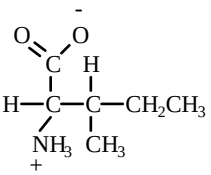
GLUTAMINE
(glN)



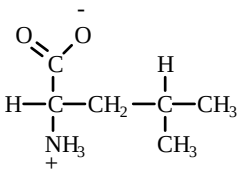
GLYCINE
(gly)



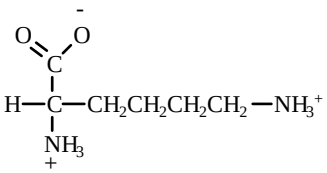
HISTIDINE
(his)



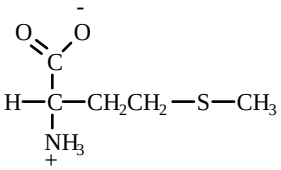
ISOLEUCINE
(ile)



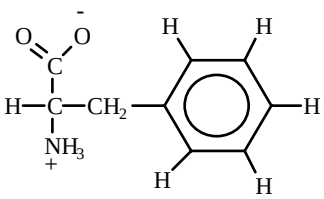
LEUCINE
(leu)



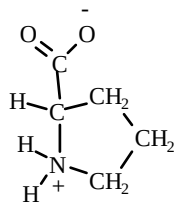
LYSINE
(lys)



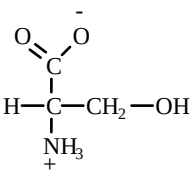
METHIONINE
(met)



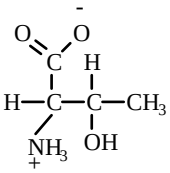
PHENYLALANINE
(phe)



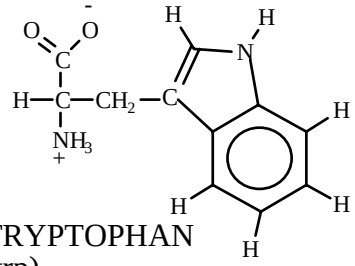
PROLINE
(pro)



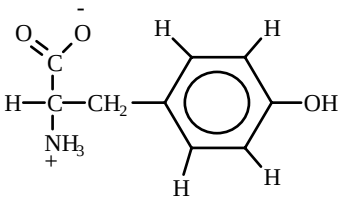
SERINE
(ser)



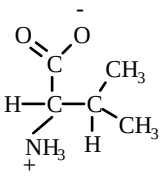
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)