Biomolecular Analysis

Archeological Chemistry Seminar

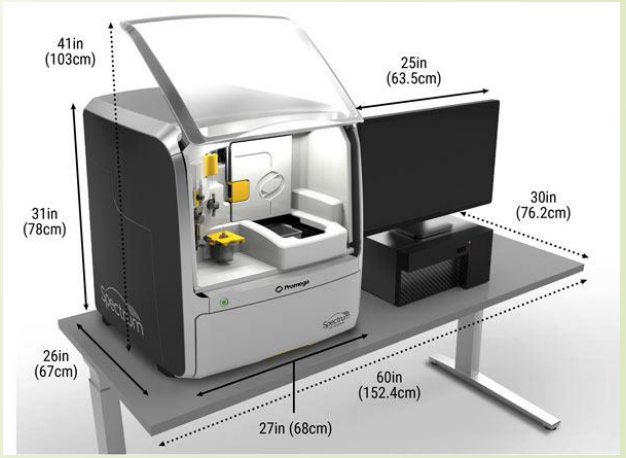
2023 AAS/ARAS Training Program

What is biomolecular analysis?

- Study of ancient molecules related to understand human biological and cultural evolution
  - Lipids (fats)
  - Carbohydrates
  - Proteins and amino acids
  - DNA
- Typical topics studied through biomolecular analysis
  - Organic residues on pottery/stone
  - Organic residues in soil (activity areas)
  - Amino acid analysis for food identification and dating
  - Genetic studies of plant and animal domestication
  - DNA relationships between groups/individuals

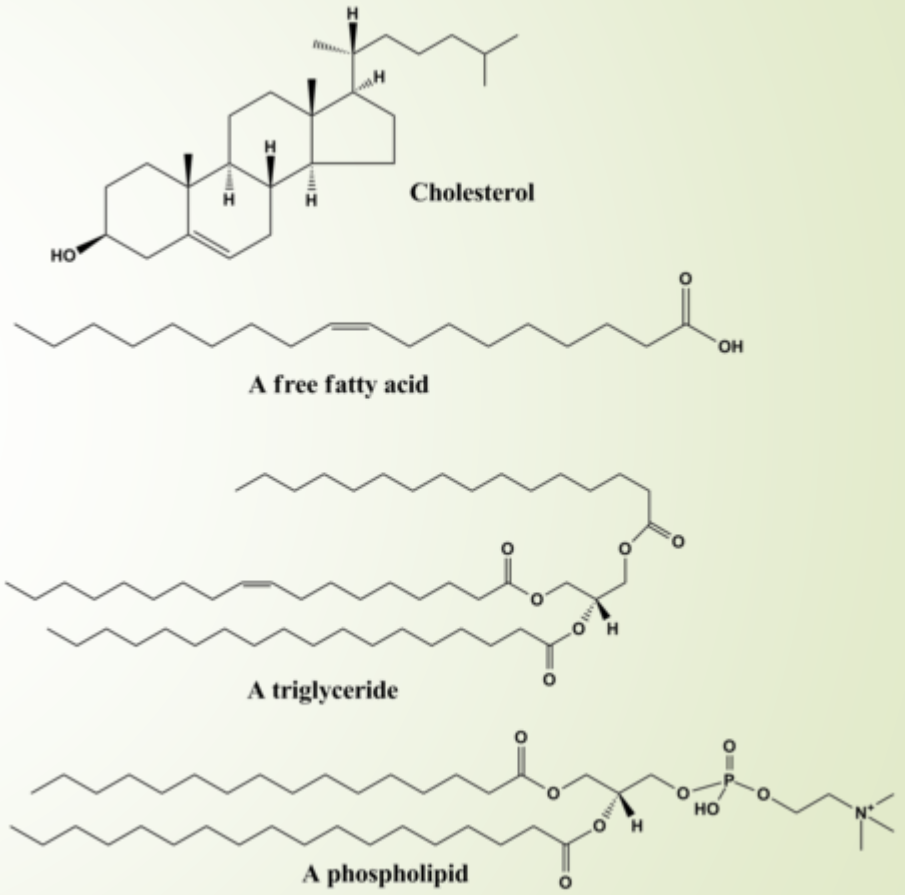
Techniques used in biomolecular analysis

- Lipids and Carbohydrates
  - Gas Chromatography-Mass Spectrometry (GCMS)
  - Liquid Chromatography-LCMS
- Proteins
  - Immunoassay
  - Electrophoresis
  - Isoelectric focusing
- Nucleic Acids (DNA and RNA)
  - Polymerase Chain Reaction (PCR)
  - Capillary Electrophoresis (CE)
  - Genetic Analyzer



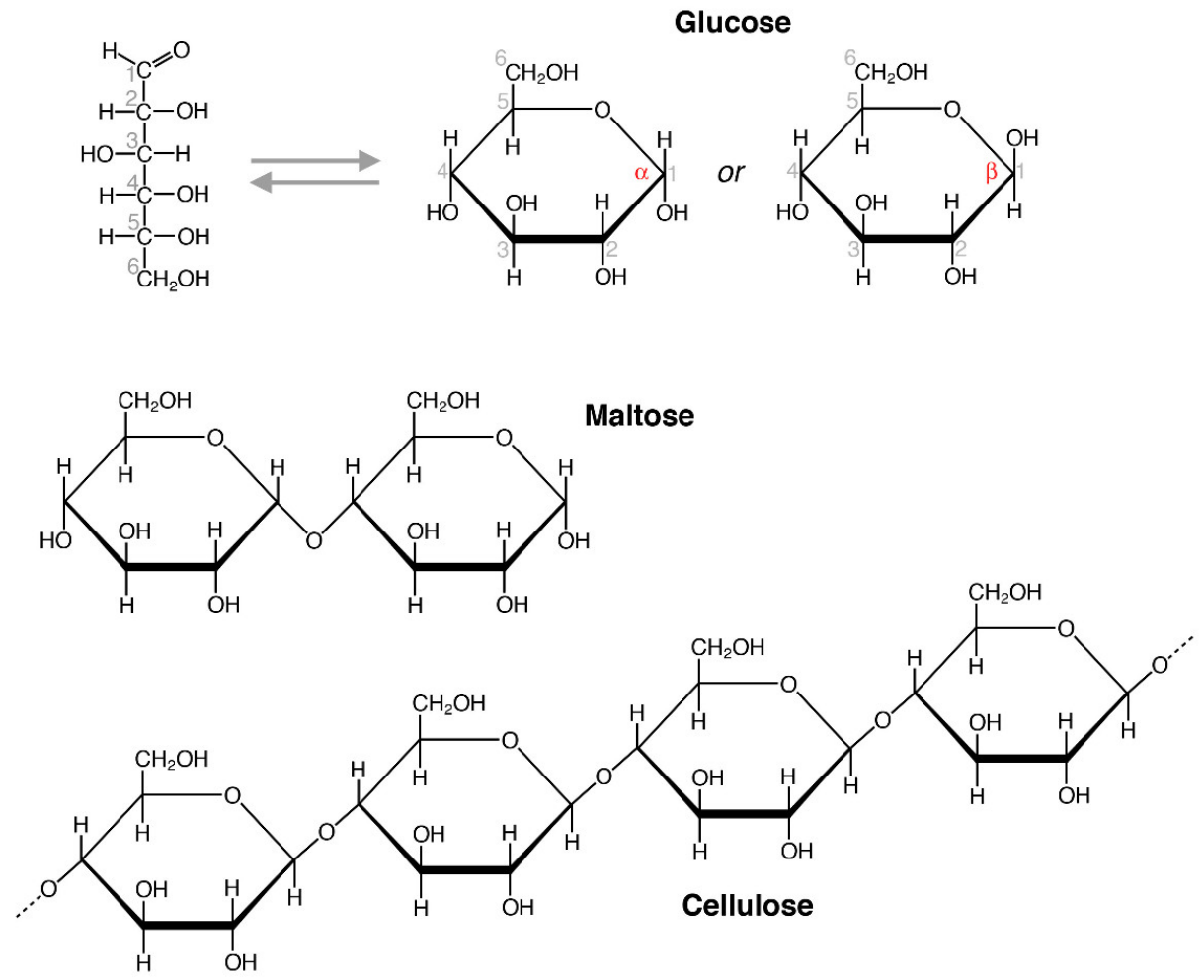
Lipids

- Lipids include:
  - Fats
  - Waxes
  - Sterols
  - Fat soluble vitamins
  - Glycerides
  - Phospholipids
- Mostly carbon-hydrogen bonds
- Mostly non-polar molecules
  - Do not dissolve in water

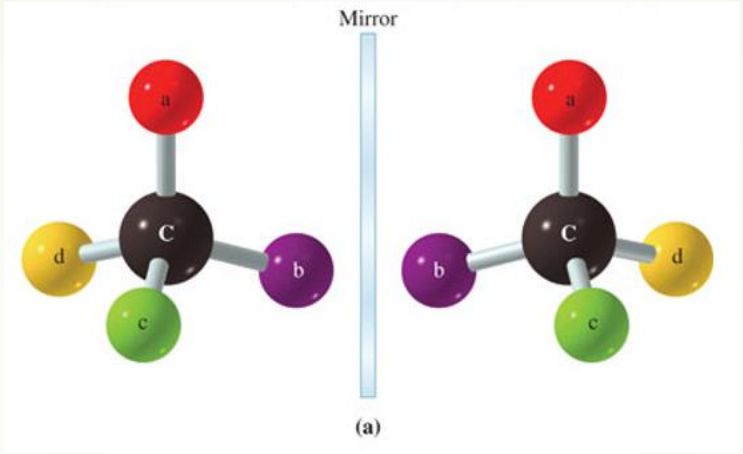


Carbohydrates

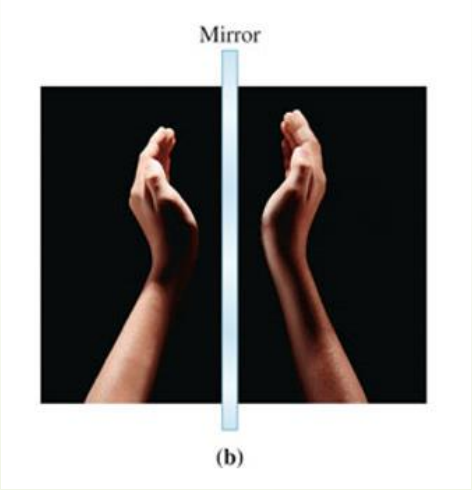
- Carbohydrates include:
  - Sugars
  - Saccharides
  - Starch
  - Cellulose
- Form ring structures
  - Linked by oxygen bonds
  - Can be left handed (L) or right handed (D)
  - Organisms use right handed sugars
- Biochemical functions:
  - Provide energy
  - Structural stability
  - Part of DNA/RNA structure



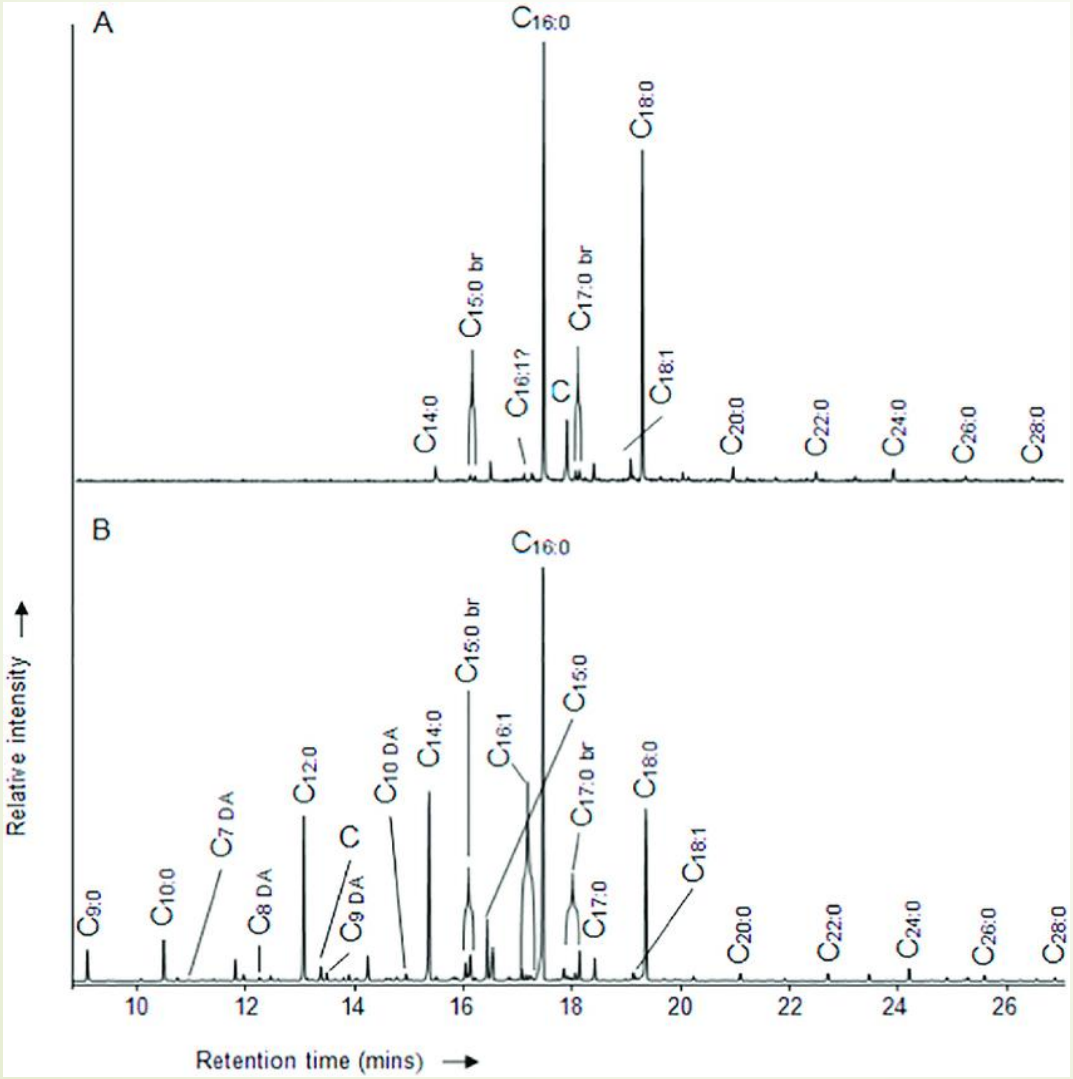
Enantiomers



Nonsuperimposable mirror images: Enantiomers



Organic Residue Analysis



Partial GC-MS chromatograms with the major fatty acid components (ion m/z 74) detected in two pottery samples from Scania, S. Sweden (LB16 (A) and SHM11 (B)). The fatty acid distributions are most likely indicative of a degraded animal fat (A) and a plant oil (B) component.

Fatty Acid Characteristics of Selected Mammals

Parameter	Mouse	Rat	Guinea pig	Dog	Pig	Cow	Horse	$p <$ value[‡]
n[†]	6	8	6	4	7	7	5	
16:0	22.5 ± 1.7	23.9 ± 1.4	15.5 ± 2.4	14.4 ± 0.7	17.9 ± 2.0	10.4 ± 1.3	8.9 ± 0.7	.0001
16:1*n*-7	2.5 ± 0.8	3.6 ± 0.8	1.2 ± 0.2	2.5 ± 0.4	1.2 ± 0.2	0.8 ± 0.1	2.8 ± 0.9	.0001
18:0	15.5 ± 1.8	11.8 ± 1.4	26.8 ± 0.4	19.1 ± 0.8	11.8 ± 1.6	13.8 ± 2.8	14.1 ± 0.9	.0001
18:1*n*-9	18.3 ± 1.7	18.9 ± 2.4	11.9 ± 0.7	15.5 ± 1.4	32.6 ± 4.7	26.2 ± 2.2	18.4 ± 2.1	.0001
18:2*n*-6	14.6 ± 1.4	18.4 ± 1.8	33.5 ± 2.7	25.1 ± 1.2	22.1 ± 2.2	32.9 ± 1.7	53.1 ± 3.1	.0001
18:3*n*-3	0.3 ± 0.03	0.2 ± 0.1	0.9 ± 0.4	0.4 ± 0.3	2.6 ± 0.5	0.1 ± 0.01	0.1 ± 0.1	.0001
20:3*n*-6	2.4 ± 0.7	0.2 ± 0.1	0.5 ± 0.08	1.5 ± 0.3	0.9 ± 0.2	2.4 ± 0.5	0.3 ± 0.1	.0001
20:4*n*-6	13.1 ± 1.1	14.1 ± 1.2	8.1 ± 0.9	20.6 ± 0.9	10.2 ± 1.1	12.3 ± 1.4	1.8 ± 0.5	.0001
22:6*n*-3	10.6 ± 0.7	8.6 ± 0.8	1.5 ± 0.2	0.7 ± 0.3	0.5 ± 0.3	0.7 ± 0.2	0.3 ± 0.1	.0001

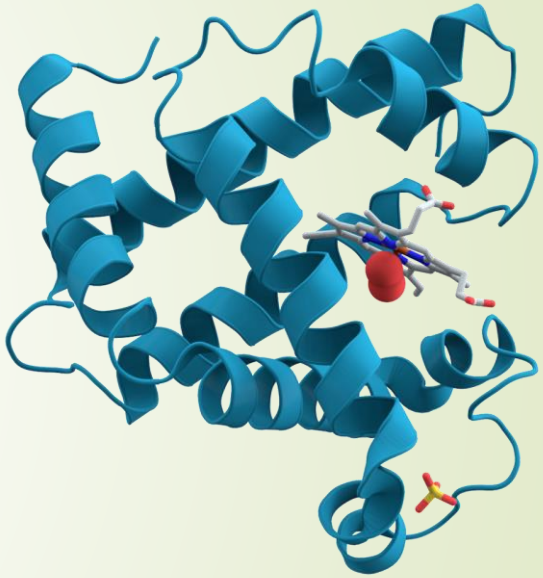
*Values are means ± SD.

[†]n = number of different animals in each species.

[‡]The significant differences among all species for a particular fatty acid are indicated, through a one-way analysis of variance.

Proteins

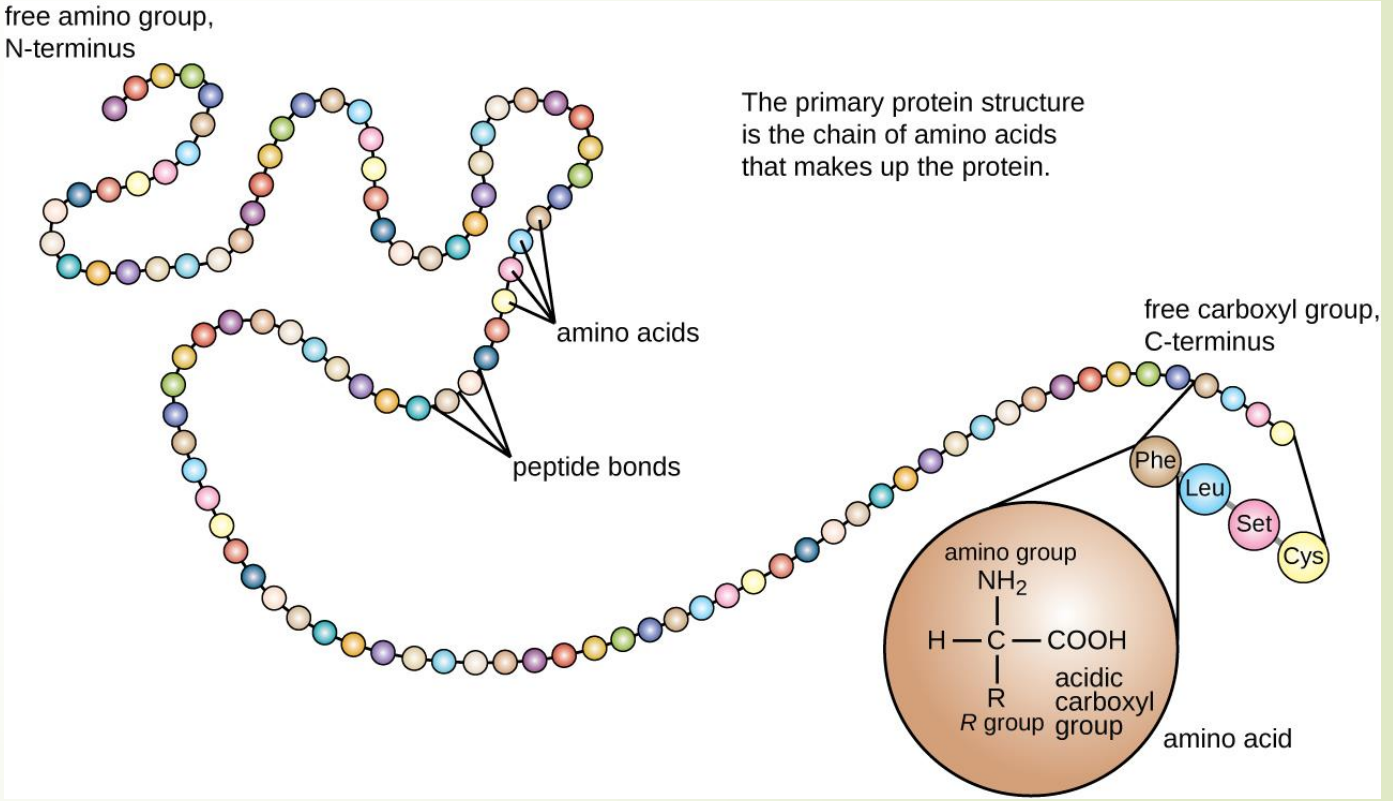
- Are large macromolecules
  - Comprised of amino acids
  - Form end-to-end (i.e. chain structure)
  - From 30-27,000 amino acids
  - Shorter chains called polypeptides
  - Individual amino acids are peptides
- Biochemical functions:
  - Catalyzing metabolic reactions
  - Assist in DNA replication
  - Provide structural stability
  - Assist in molecule transport



Myoglobin
(153 amino acids)

Amino Acids

- Organisms generally contain 20 different types of amino acids
  - Some organisms have a few more
- Linked together they create a chain structure that can bend around in distinctive shapes
  - There can be interactions between amino acids in different parts of the chain
  - Protein function depends on the overall shape of the protein
  - "Denaturing" causes the chain to unravel by using acids, bases, or heat
    - Cooking partially denatures proteins

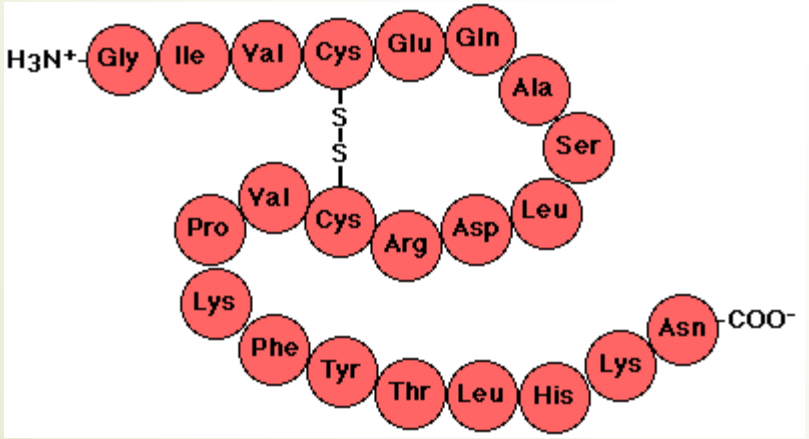


Amino Acids – Names and Abbreviations

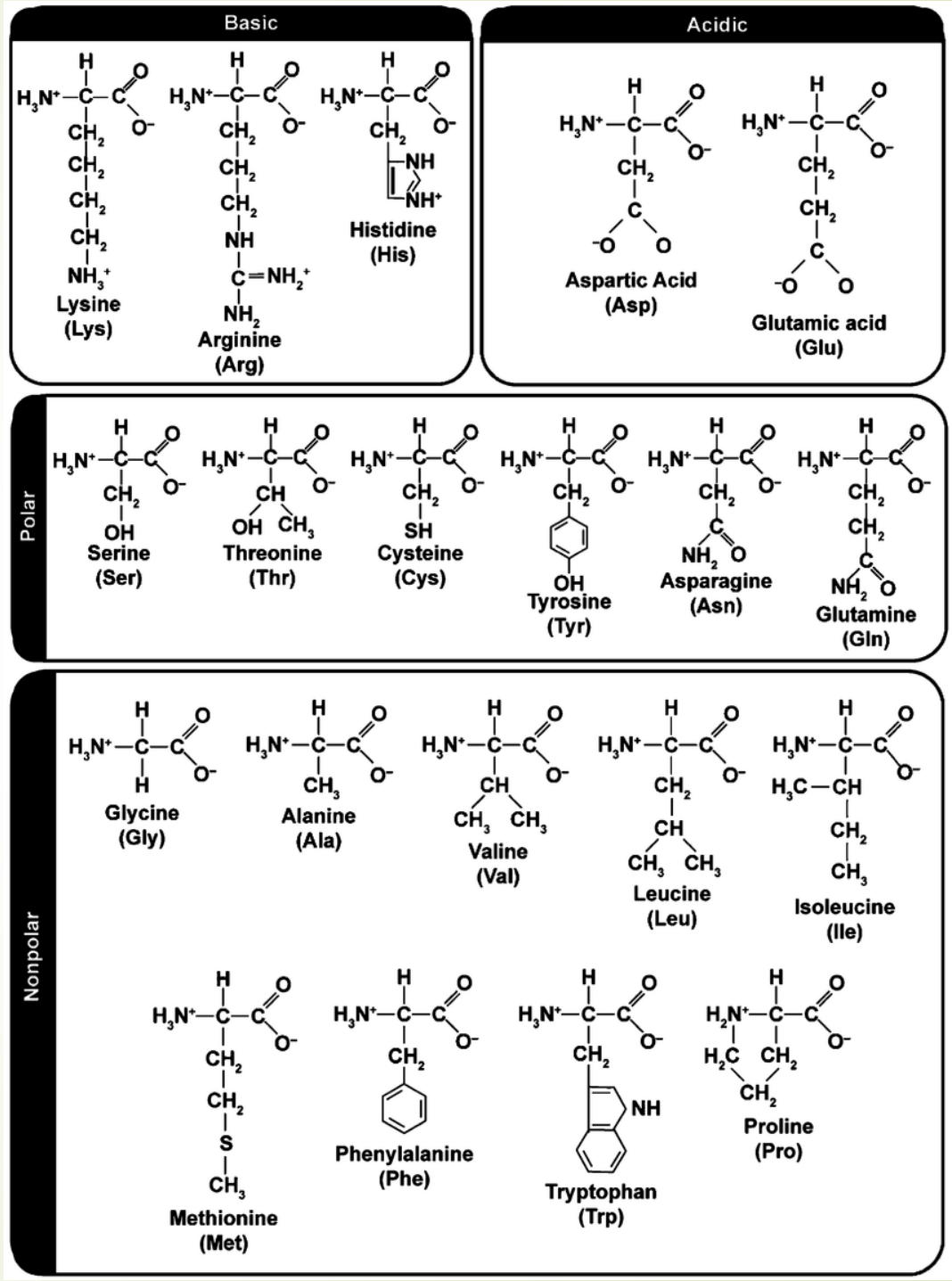
Amino acids in living organisms are all left handed (L) structures

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	ala	A
Arginine	arg	R
Asparagine	asn	N
Aspartate	asp	D
Cysteine	cys	C
Glutamate	glu	E
Glutamine	gln	Q
Glycine	gly	G
Histidine	his	H
Isoleucine	ile	I
Leucine	leu	L
Lysine	lys	K
Methionine	met	M
Phenylalanine	phe	F
Proline	pro	P
Serine	ser	S
Threonine	thr	T
Tryptophan	trp	W
Tyrosine	tyr	Y
Valine	val	V

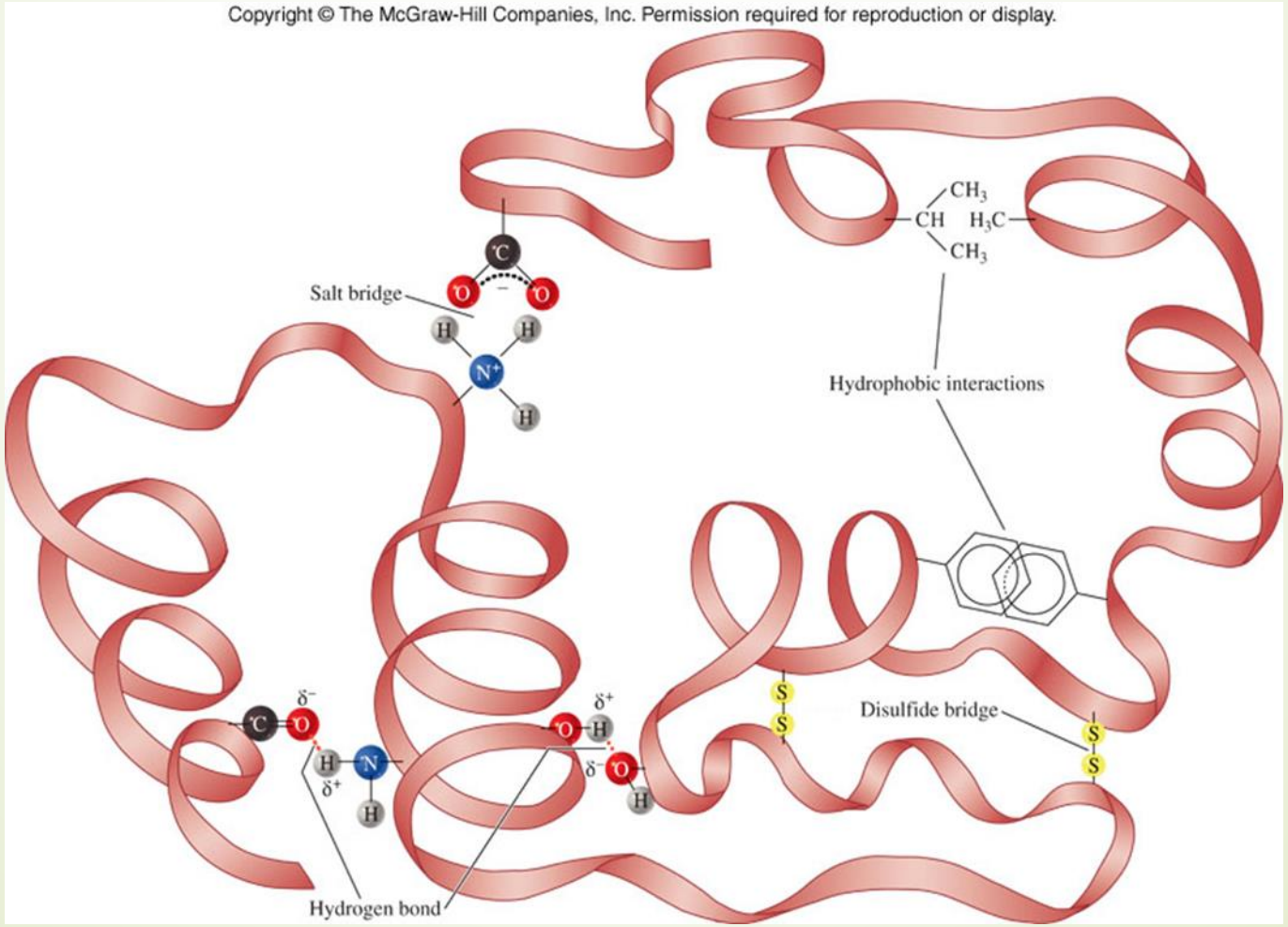
Amino Acid - Structure



Cross-linking dictates structure



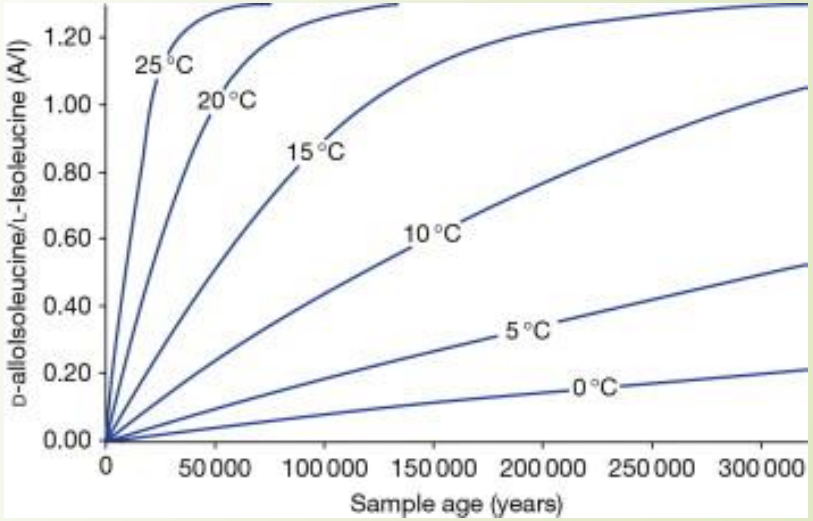
More Cross-linking Interactions



Amino Acid Racemization Dating

- Technique developed in the 1960s using fossil shell
  - Later extended to bone
- Uses slow conversion rate of left handed (L) amino acids into right handed (D)
  - Eventually, amino acids handedness would be 50:50 between L and D
  - Normally, it is difficult to analyze handedness (they must be separated first)
  - One amino acid is easily separated

    L-isoleucine → D-alloisoleucine
  - Conversion rate is temperature dependent, higher temps = faster conversion
  - Researchers studied L-isoleucine between 100°C to 150°C
    - Extrapolated conversion rate to deep sea temps 2-4°C
  - Other researchers have applied it to bone (e.g. Arizona)
- Still considered controversial



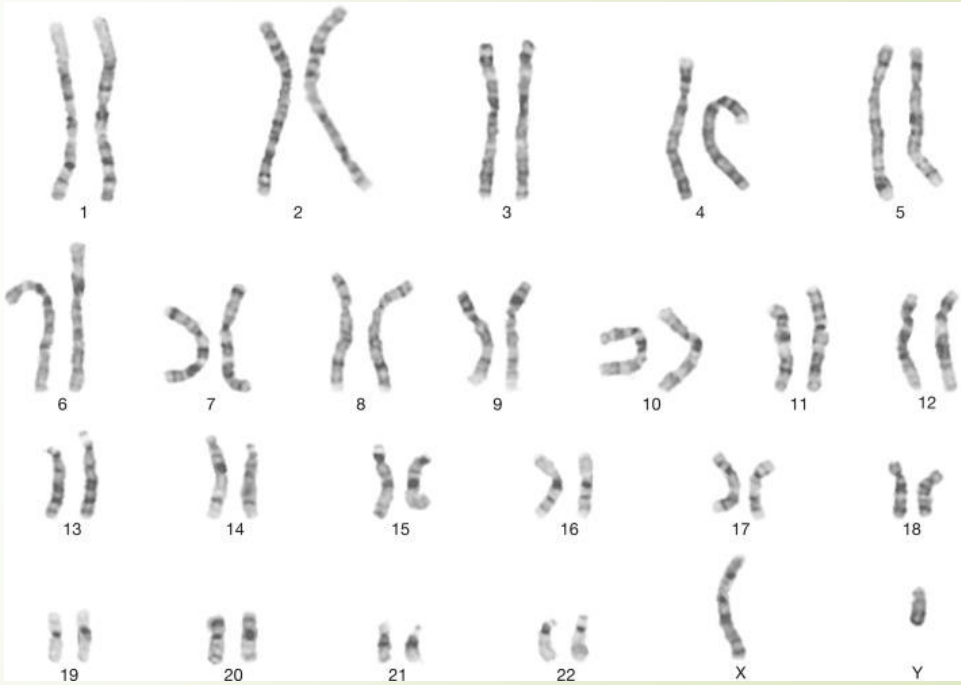
Deoxyribonucleic Acid (DNA)

- Archaeogenetics –
  - The study of ancient DNA using various molecular genetics methods and DNA resources
  - Ancient population group migrations
  - Domestication events
  - Plant and animal evolution

- FYI, the oldest DNA ever sequenced came from?
  - 2 million year old mammoth found in Greenland

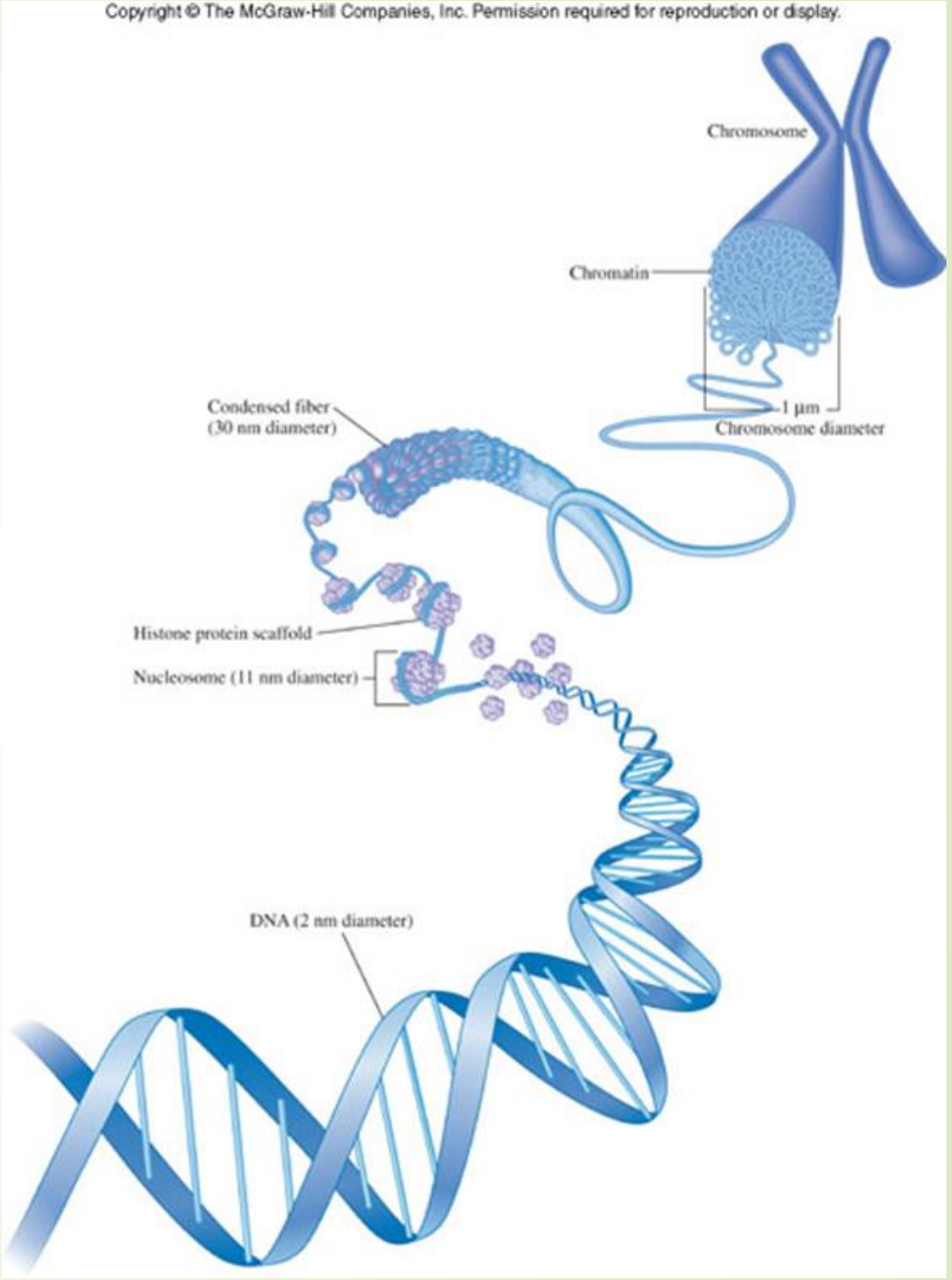
DNA Facts

- All organisms on Earth share the same four DNA nucleotides
  - Physical differences in organisms are the result of their nucleotide sequence
- In humans, the nucleotide sequence is about 3.2 billion nucleotides long
  - Divided into 46 long chains, called chromosomes
  - Chromosomes are paired, thus 23 pairs of chromosomes
  - 23 chromosomes are derived from the mother, 23 chromosomes are derived from the father
  - Genes are shorter parts of the chromosomes

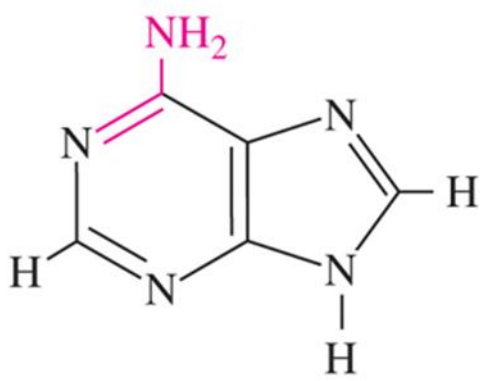


Chromosome Structure

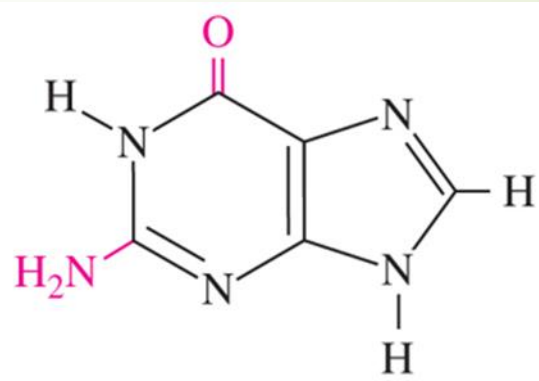
- Chromosomes are made of a tight bundle of DNA strands
  - The strands are paired together in a double helix structure
  - The strands are held together by chemical bonds between the nucleotide bases



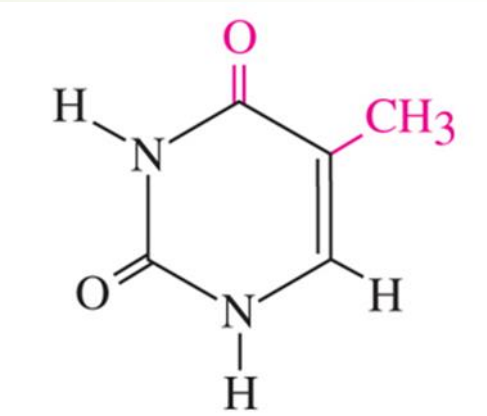
DNA Nucleotide Bases



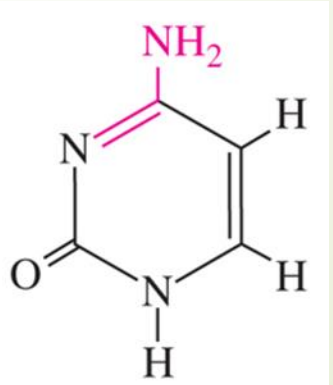
Adenine (A)



Guanine (G)



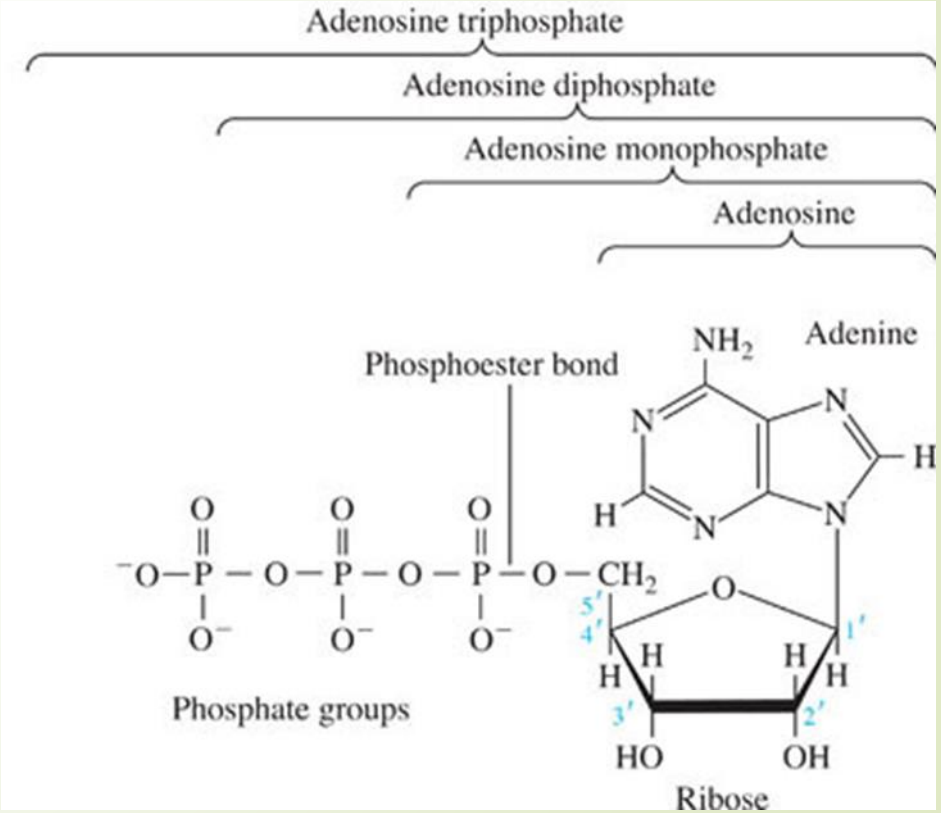
Thymine (T) (in DNA)

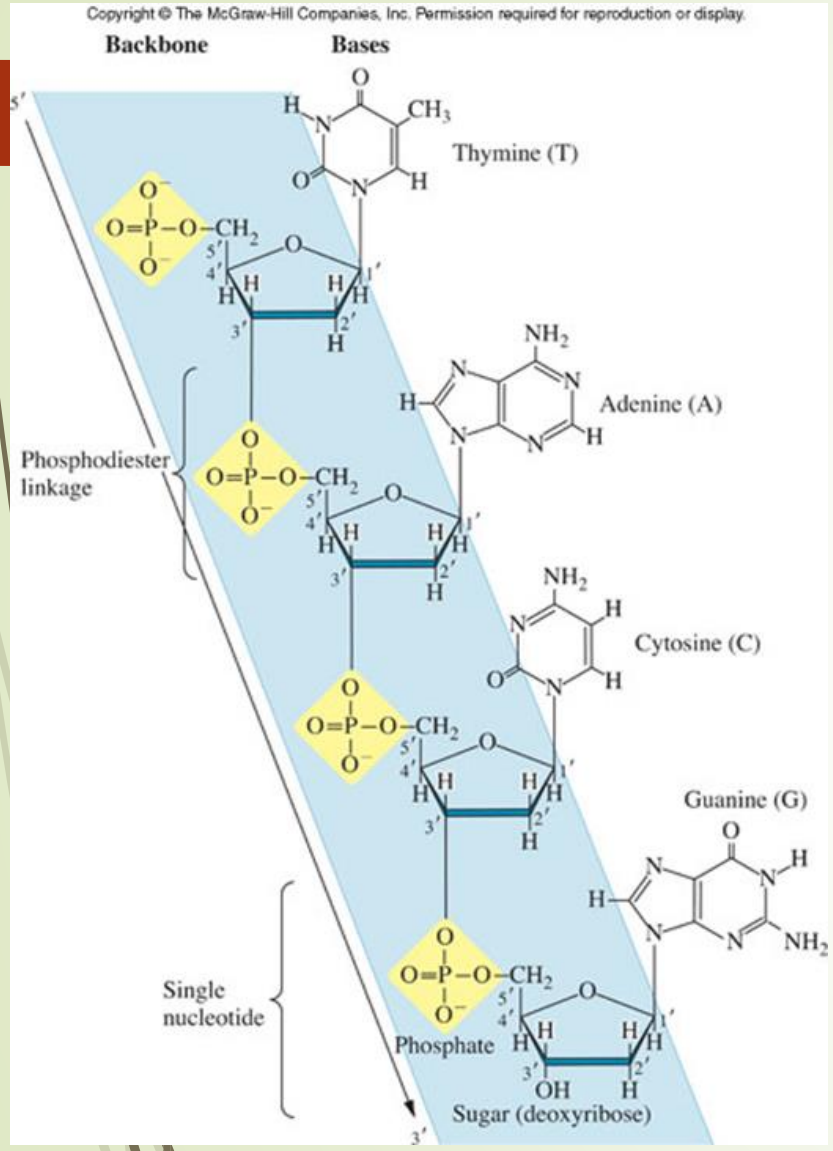


Cytosine (C)

A Single Nucleotide

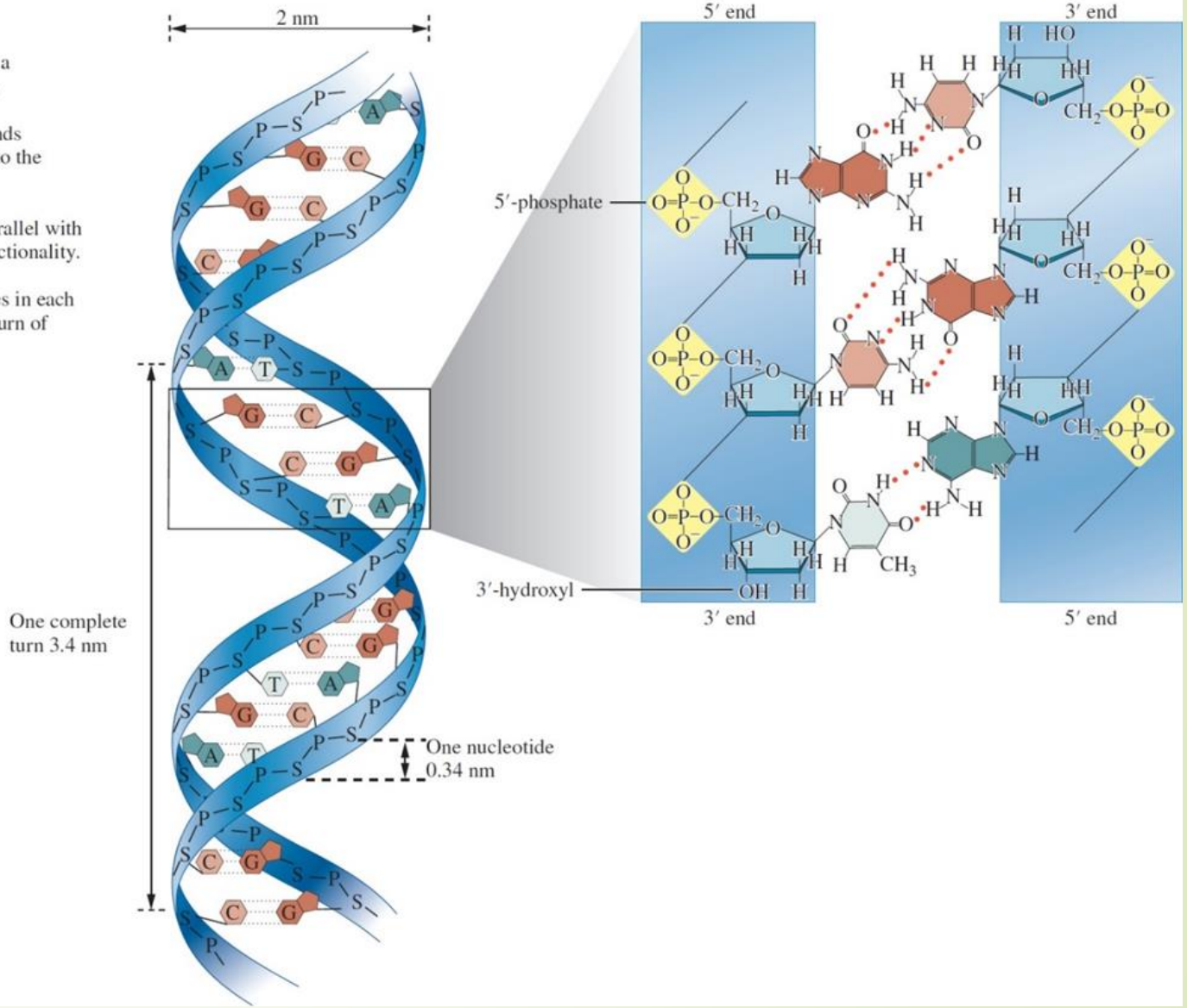
- A single nucleotide is made of three parts:
  - Base (in this case adenine)
  - Sugar (ribose)
  - Phosphate groups





Key Features

- Two strands of DNA form a right-handed double helix.
- The bases in opposite strands hydrogen bond according to the AT/GC rule.
- The two strands are antiparallel with regard to their 5′ to 3′ directionality.
- There are ~10.0 nucleotides in each strand per complete 360° turn of the helix.

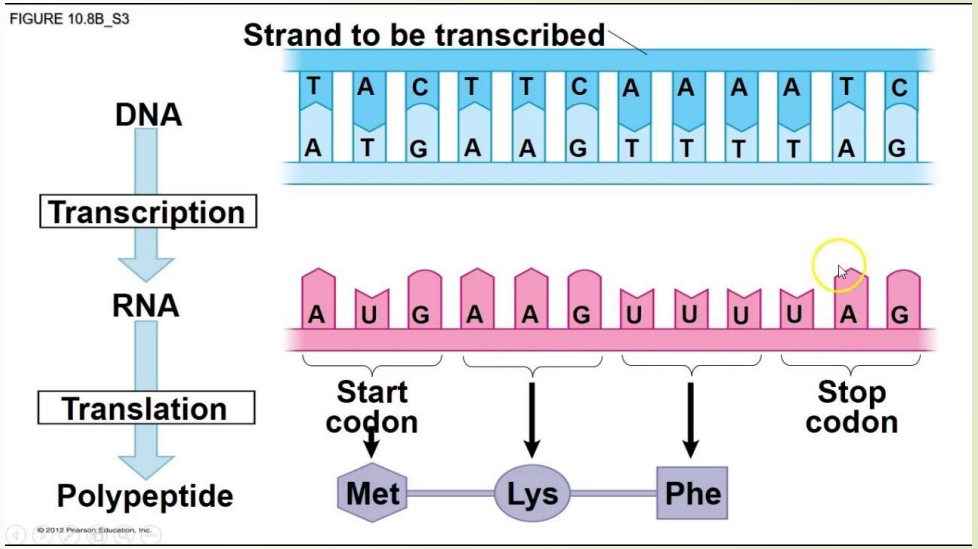


Information Flow in Biological Systems

- Central Dogma – inside cells there is a one-way street to the flow of information

  DNA → RNA → Proteins

- Transcription – the process by which a single strand of DNA serves as a template for the synthesis of an RNA molecule
  - Essentially it is making a copy
- Translation – converts from the sequence of nitrogenous bases to another sequence of amino acids
  - Essentially translating one language into another
  - Protein synthesis



DNA Replication

- DNA of parents is replicated and handed down to offspring
- The replication process is not perfect
  - Occasionally, a different nucleotide replaces the intended nucleotide
- This new DNA sequence is handed down through many generations before another change occurs
- This allows certain traits, common to a particular group or tribe, to be followed in the DNA over time
  - Reveals when mobility when those unusual DNA sequences show up in unusual places

Sequencing DNA

- Ancient DNA degrades over time
  - Often fragments into smaller pieces
  - Often difficult to put the pieces back in the right places
- There are many different techniques for sequencing
  - Full genome – Sequencing by synthesis (uses DNA Polymerase)
  - Short sequences – high throughput methods

DNA Sequencing Changed the Game
The World's Oldest DNA
Looking for Londoners

Image References

page #	
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