Natural Polymers and Biopolymers – DNA and RNA.

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/
Types of Biological Molecules.

- **Water**
- **Proteins**
- **Lipids**
- **Nucleic acids**
- **Polysaccharides**
Natural Polymers and Biopolymers.

**Bio(Natural)-Polymers** are polymeric macromolecules produced by living organisms. **Bio-based polymers** are macromolecules synthetized by human starting from biological raw materials. **Synthetic polymers** are made from oil.

Since they are polymers, biopolymers contain monomeric units that are covalently bonded to form larger structures.

There are three main classes of biopolymers based on the differing monomeric units used and the structure of the biopolymer formed:

1. **polynucleotides**, which are long polymers composed of 13 or more nucleotide monomers;
2. **polypeptides**, which are short polymers of amino acids; and
3. **polysaccharides**, which are often linear bonded polymeric carbohydrate structures.

- Cellulose most abundant natural biopolymer
- Chitin next most abundant natural biopolymer
- DNA fundamental for reproduction
- Proteins essential for living cell control.

Examples of bio-polymers (natural)
Synthetic polymers are polymer macromolecules made from man and not existing in nature. When these synthetic products are made from natural building blocks, they are called Bio(derived)-Polymers. Bio(derived)-Polymers are much simpler and with a random molecular mass than natural polymers. This fact leads to a molecular mass distribution that is missing in bio(natural)-polymers.

All natural biopolymers of a type (say one specific protein) are all alike: they all contain the similar sequences and numbers of monomers and thus all have the same mass.

This phenomenon is called monodispersity in contrast to the polydispersity encountered in synthetic polymers.

As a result biopolymers have frequently a polydispersity index of 1.
Degradation of organic chemicals in the environment influences exposure and, hence, it is a key parameter for estimating the risk of long-term adverse effects on biota. Degradation rates, or half-lives, are preferably determined in simulation biodegradation tests conducted under conditions that are realistic for the particular environmental compartment (e.g. STP, surface water, sediment or soil). Simulation tests aim at mimicking actual environmental conditions such as redox potential, pH, T, P, microbial community, concentration of substance and occurrence/concentration of other substrates.

- aerobic biodegradability should be examined in a screening test for ready biodegradability
- If negative result in the previous test, biodegradation of the chemical may be examined in a simulation test to obtain data to be used for assessing the biodegradation rate (DT$_{50}$) in the environment or in a biological STP
- a screening test for inherent biodegradability may be conducted for describing the potential biodegradability under optimized aerobic conditions,
- In addition, potential biodegradability under anoxic conditions may be examined in a screening test for anaerobic biodegradability.
“Biodegradable” Macromolecules from Fossil and Bio Sources (Synthetic vs. Natural).

Raw materials

Fossil source

- PCL, PBAT, PBS

Bio(derived) polymers

Starch based materials, Cellofan, PLA Chitosan, PHA

Non-biodegradable

Many Cellulose derivatives

E.g. sugar based PE

Renewable source - biomass

- Bio(denominated) polymers

- Natural polymers

Properties

"Traditional" Plastics:

E.g. PE, PP, PS, PET, PA, PVC

Biodegradable

PCL = Poly(e-caprolacton)
PBAT = Poly(butylene adipate-co-terephtalate)
PBS = Polybutylen succinate
PE = Polyethylene
PP = Polypropylene
PS = Polystyrene
PET = Polyetylentereftalate
PA = Polyamide
PVC = Polyvinylchloride
PLA = Polylactic Acid
PHA = Polyhydrokyalkanoate
Protein, a linear sequence of amino acids codified by RNA through DNA, a linear sequence of nucleotides.
**EXPERIMENT**

**Question:** Can organic chemical compounds be generated under conditions similar to those that existed on primeval Earth?

**METHOD**

"Atmospheric" compartment

"Oceanic" compartment

Heat

Results

**Conclusion:** The chemical building blocks of life could have been generated in the probable atmosphere of early Earth.
Two Main Information Pathways in the Cell: Transcription and Translation.

PROTEIN SYNTHESIS

Step 1: Transcription
- DNA double helix
- RNA polymerase
- RNA nucleotides
- Messenger RNA leaves nucleus

Step 2: Translation
- Transfer RNA
- Amino acids
- Ribosomal RNA
- Anticodon
- Proteins
- Ribosome
- Polypeptide chain
- Transfer RNA with amino acid

Codon

Messenger RNA
Genetic Modified Organisms.
Bio-Degradable (Natural and Synthetic) Polymeric Materials.

**BIOBASED POLYMERS**

- Directly extracted from biomass
  - Polysaccharides
  - Proteins
  - Lipids
  - Animals
    - caseins
    - whey
    - collagen
    - silk
  - Plants
    - zein
    - soya
    - gluten
    - waxes
    - fats
    - oils
- Chemical treated biomass
  - Hydrolyzed
- Synthesized from bio-derived monomers
  - Polylactate
  - Other polyesters
- Produced from microorganisms
  - Bacterial compounds
  - Polyhydroxyalkanoates
  - cellulose
  - xanthan
  - curdlan
  - pullan

**Bio DERIVATIVES**

**NATURALS**

- Starch
  - potato
  - maize
  - wheat
  - rice
  - Other derivatives
- Cellulose
  - cotton
  - wood
  - other derivatives
- Gums
  - guar
  - locust bean
  - alginites
  - carragenanes
  - pectins
- Chitin/Chitosan
  - potato
  - cotton
  - gua
  - wood
  - other derivatives

Attilio Citterio
# Natural Bio-polymers from Renewable Sources – Classification by Structure

<table>
<thead>
<tr>
<th>Polysaccharides</th>
<th>Proteins</th>
<th>Polyesters</th>
<th>Lignin</th>
<th>Natural Rubber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Silk</td>
<td>DNA/RNA</td>
<td>poly-phenols</td>
<td>poly-isoprene</td>
</tr>
<tr>
<td>Starch</td>
<td>Wool</td>
<td>Polylactic Acid</td>
<td>PHA</td>
<td></td>
</tr>
<tr>
<td>Chitin/</td>
<td>Soy derivatives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitosan</td>
<td>Polyglutamic Acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alginate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biosynthetic</td>
<td>polypeptides</td>
<td></td>
</tr>
</tbody>
</table>
Flow of Glucose in *E. Coli.*

Each arrow = a specific reaction
Each arrow = a different enzyme (protein)
Biomass – Biobased Raw Materials.

Based on agricultural and forest products, and marine resources

Different routes to produce biopolymers

- Directly by extraction from natural occurring biopolymers in plants:
  - E.g. lipids, proteins, polysaccharides (e.g. starch)

- Chemical processes:
  - E.g. hydrolysis of biomass where bio-monomers is produced, which in turn is the building blocks in the biopolymer like polyesters and polylactate

- Polymers produced by organisms, polymerisation by microorganism:
  - E.g. bacterial cellulose and polyhydroxyalkanoates

**Renewable Resources**

- Direct conversion of a renewable source into a finished product
- Production of small molecules from renewable sources

**Bacterial Polyesters**

**Ethanol**
Monomeric building blocks of natural macromolecules

---

**building blocks of the cell**
- SUGARS
- FATTY ACIDS
- AMINO ACIDS
- NUCLEOTIDES

**larger units of the cell**
- POLYSACCHARIDES
- FATS/LIPIDS/MEMBRANES
- PROTEINS
- NUCLEIC ACIDS

----

...account for most of cell’s mass
Approximate Chemical Composition of a Bacterial Cell.

<table>
<thead>
<tr>
<th>TYPES</th>
<th>PERCENT OF TOTAL CELL WEIGHT</th>
<th>NUMBER OF EACH MOLECULE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Sugars and precursors</td>
<td>1</td>
<td>250</td>
</tr>
<tr>
<td>Amino acids and precursors</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>Nucleotides and precursors</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>Fatty acids and precursors</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Other small molecules</td>
<td>0.2</td>
<td>300</td>
</tr>
<tr>
<td>Macromolecules (proteins, nucleic acids, and polysaccharides)</td>
<td>26</td>
<td>&gt;6000</td>
</tr>
</tbody>
</table>
Approximate Chemical Composition of the *E. Coli* Bacterium and a Typical Mammalian Cell.

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PERCENT OF TOTAL CELL WEIGHT</th>
<th>E. Coli Bacterium</th>
<th>Mammalian Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous small metabolites</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Proteins</td>
<td>15</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>RNA</td>
<td>6</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>DNA</td>
<td>1</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Other lipids</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total cell volume</td>
<td>$2 \times 10^{-12}$ cm$^3$</td>
<td>$4 \times 10^{-9}$ cm$^3$</td>
<td></td>
</tr>
<tr>
<td>Relative cell volume</td>
<td>1</td>
<td>2000</td>
<td>2000</td>
</tr>
</tbody>
</table>
If there is a natural process to make it, there is also a process to degrade it.

→ **Natural Balance of nature**

Inherent Biodegradability

- Building block
- Polymer
- Depolymerase
- Enzymes
Benefits of Use of Biopolymers.

- Environmental compatibility
- Use does not pose environmental burden
- Renewable resources
  - Superior over petroleum-based (finite) resources
  - Boost for agricultural industry
- Potential biocompatibility
- Tailoring of structure by genetic manipulation

Mol. weight, stereochemistry sequence, chemical reactivity

May interfere with biodegradability/biocompatibility
Can have higher costs than synthetic polymers
Limits in the Use of Biopolymers.

- Premature degradation
- Unfavorable economic evaluation
- High production costs
- Medical/pharmaceutical use
- Environmental consequences
- Soil fertility
- Use of water to grow crops
Function of Bio(Natural) Polymers.

Example: polysaccharides

- Cell wall structure: structural function
- Extra-skeleton: structural function
- Starch granules: storage function
- Heparin: regulatory function
- Emulsan: emulsifying function
### Protein Functions.

<table>
<thead>
<tr>
<th>Vital activity</th>
<th>Example of proteins</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrition</td>
<td>Digestive enzymes</td>
<td>• Catalyzes the hydrolysis of proteins to polypeptides</td>
</tr>
<tr>
<td></td>
<td>e.g. trypsin,</td>
<td>• Catalyzes the hydrolysis of starch to maltose</td>
</tr>
<tr>
<td></td>
<td>amylase</td>
<td>• Catalyzes the hydrolysis of fats to fatty acids and glycerol</td>
</tr>
<tr>
<td></td>
<td>lipase</td>
<td></td>
</tr>
</tbody>
</table>
### Protein Functions (2).

<table>
<thead>
<tr>
<th>Vital activity</th>
<th>Example of proteins</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support and movement</td>
<td>Actin and myosin</td>
<td>• Responsible for muscle contraction</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>• Gives strength with flexibility in tendons and cartilage</td>
</tr>
<tr>
<td>Sensitivity and coordination</td>
<td>Hormones (e.g. insulin)</td>
<td>• Controls blood sugar level</td>
</tr>
<tr>
<td>Respiration and transport</td>
<td>Haemoglobin</td>
<td>• Responsible for the transport of $O_2/CO_2$ throughout body</td>
</tr>
<tr>
<td>Immune response</td>
<td>Antibodies</td>
<td>• Essential to the defense of body (e.g. against bacterial)</td>
</tr>
<tr>
<td>Growth</td>
<td>Hormones (e.g. tyrosine)</td>
<td>• Controls growth and metabolism</td>
</tr>
</tbody>
</table>
Sources for Biopolymers – Organisms.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Animals</th>
<th>Fungi</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly-saccharides</td>
<td>Proteins</td>
<td>Poly-saccharides (pullulan, chitin)</td>
<td>Poly-saccharides (dextran)</td>
</tr>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
<td>PHA</td>
</tr>
<tr>
<td>Lignin</td>
<td></td>
<td>chitin (glycogen)</td>
<td>PLA</td>
</tr>
<tr>
<td>Nat. rubber</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DNA and RNA.

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”
https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/
DNA: A Complex Polyester of Phosphoric Acid.

RNA and DNA a phosphate ester

Future: “Lab on the chip”

No material application yet!
Information Storage, Retrieval & Use

Nucleic Acids

Repeat Units

Nucleotides

Information Content

nitrogenous bases
  purine
  pyrimidine

Scaffolding

ribose + phosphate
Nitrogenous Bases: a Definition.

- **Aromatic rings** incorporating one or more atoms of nitrogen
- **Essentially flat**, or nearly so
- Nitrogen imparts a _weakly basic character_ to the ring
- **Pyridine** is a simple, _non-biochemical_ example:

\[
\text{Pyridine} \quad + \quad \text{H}^+ \quad \overset{\text{Pyridinium Ion}}{\rightleftharpoons} \quad \text{Pyridinium Ion}
\]
The Pyrimidine Bases.

- All are built on the pyrimidine platform
- Ring is numbered to assign the lowest possible numbers to the two nitrogens
- Connection to the ribose sugar is via a glycosidic bond from position 1
- All have an oxygen bonded to position 2
  (i.e. all are 2-oxo- substituted pyrimidines)
- **Position 4** will bear an oxo or amino group
- **Position 5** is methyl-substituted in one case
Pyrimidine Bases (2).

- **Cytosine**: 2-oxy-4-amino pyrimidine
  - Used in both DNA & RNA

- **Thymine**: 2,4-dioxy-5-methyl pyrimidine
  - (DNA only)

- **Uracil**: 2,4-dioxy pyrimidine
  - (RNA only)

(Root underlined)
• All are built on the purine platform
• Ring is numbered to assign the lowest possible numbers to the four nitrogens
• Connection to the ribose sugar is via a glycosidic bond from position 9
• 6-membered ring will be oxo or amino-substituted at positions 2 or 6
The Purine Bases (2).

Adenine
6-aminopurine

Guanine
2-amino-6-oxypurine

Found in both DNA and RNA (Root underlined)
Bases Absorb in the UV-Region.

Aromatic, $\lambda_{\text{max}} \sim 260$ nm

useful for:

- quantifying nucleic acids
- assessing purity
- monitoring structural changes (e.g. melting of double-stranded DNA)
Conformational Flexibility in the Bases.

Keto-enol tautomerism, e.g. of uracil or thymine

keto-form (strongly preferred)

enol-form

Importance: altered base pairing preference of the two tautomers
Keto-Enol Tautomerism in Guanine.

Keto form (strongly favoured)

Enol form

\[
\text{N} \quad \text{N} \quad \text{NH}_2
\]

\[
\text{N} \quad \text{N} \quad \text{NH}_2
\]
Other Bases (not found in nucleic acids).

Caffeine

Uric acid: final breakdown product in purine catabolism excreted in urine.
Riboses are components of the scaffolding for nucleic acids
The difference: 2'-OH vs. 2'-H
This difference influences:

- the secondary structure of RNA & DNA
- the stability of RNA and DNA
Nucleosides = Base + Pentose.

Base is linked via a glycosidic bond

Named by adding:
- -idine to the root name of a pyrimidine
- -osine to the root name of a purine

Sugars make nucleosides more water-soluble than the free bases they bear

β-N$_1$-glycosidic bonds in pyrimidine ribonucleosides

β-N$_9$-glycosidic bonds in purine ribonucleosides
Favored Conformations of Nucleosides.

How to avoid steric clash between the base and pentose rings?

Purine nucleosides
both syn & anti are OK

Pyrimidine nucleosides
anti favored
Nucleoside Functions.

- **Precursors of nucleotides**
- **Adenosine**: can also act as a hormone (hormone: blood-borne cellular stimulant)

Hormone (e.g. adenosine) is produced by one cell, is secreted into the bloodstream, binds to receptors on another cell surface & initiates changes in that target cell.
Adenosine is a white crystalline powder. It is soluble in water and practically insoluble in alcohol.

- Induces vasodilatation
- Induces smooth muscle contraction
- Release of neurotransmitters
- Induces sleepiness (countered by caffeine)
Nucleotides = Nucleoside + Phosphate.

Nucleotides are polyprotic acids (e.g. Adenosine 5’-monophosphate AMP)

\[
\begin{align*}
R-O-PO_3H_2 \leftrightharpoons & \; RO-PO_3H^- + H^+ \\
K_{a1} \\
R-O-PO_3H^- \leftrightharpoons & \; RO-PO_3^{2-} + H^+ \\
K_{a2}
\end{align*}
\]
Nucleotide Shorthand.

If the phosphate was at the 3’ position instead = Ap

= pA
if diphosphate ppA
if triphosphate pppA

If the phosphate was at the 3’ position instead = Ap
Adenosine TriPhosphate (ATP, pppA).

ATP is an high-energy molecule (coenzyme) used as an energy carrier in the cells of all known organisms; the process in which energy is moved.

It is present in the cytoplasm and nucleoplasm of every cell, and essentially all the physiological mechanisms that require energy for operation obtain it directly from the stored ATP. In animal systems, the ATP is synthesized in the tiny energy factories called mitochondria by a process called glycolysis.
Functions of Nucleotides.

- Precursors to the polynucleotides DNA & RNA
- Carriers of energy via phosphoryl group transfer
  
  \[
  \text{e.g. } \text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{Pi} + \text{energy}
  \]

- bases serve as recognition units
  - ATP is central to energy metabolism
  - GTP drives protein synthesis
  - CTP drives lipid synthesis
  - UTP drives carbohydrate metabolism

- Cyclic nucleotides are signal molecules and regulators of cellular metabolism and reproduction
A Cyclic Nucleotide: Adenosine 3’,5’-Monophosphate (cAMP).

(cyclic AMP or cAMP)

\[ \text{ATP} \rightarrow \text{cAMP} + \text{PPi} \]

Function: an important “second messenger” in intracellular signaling

Phosphodiester 6-membered ring
Linking Nucleotides by 5’-3’ Phosphodiester Bonds to Dinucleotides.

\[ \text{NpNpNpN} + \text{pppN} \rightarrow \text{NpNpNpNpN} + 2\text{Pi} \]
Nucleic Acids: Linear Polymers of Nucleotides.

- Polymers linked 5’ to 3’ by phosphodiester bridges
- Sequence is **always** read 5' to 3'
- In terms of genetic information, this corresponds to "N to C" in proteins
- Phosphodiester is weakly acidic: dissociated at neutral pH : anionic

The sample shown here is a DNA molecule with the sequence 5’-GACA-3’. The arrow gives the direction of the chain.
Sequence Shorthand.

or simply

5’pTGCAT-3’

or

pTGCAT
Classes of Nucleic Acids.

DNA - one type, one purpose: genetic material
- possesses primary & secondary structure (helices), but no tertiary structure

RNA - 3 types, 3 purposes
- primary, secondary & tertiary structures all occur
- ribosomal r-RNA - the basis of structure and function of ribosomes
- messenger m-RNA - carries the message
- transfer t-RNA - carries the amino acids
Two interwound chains, stabilized by hydrogen bonds between chains & stacking of the bases

"Base pairs" arise from H bonds:
- A on one strand pairs with T on the other
- G pairs with C

Erwin Chargaff had the base content data, but didn't understand its implications

Rosalind Franklin's X-ray fiber diffraction data provided crucial structural constraints

Francis Crick | James Watson | Linus Pauling |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>correctly interpreted the clues</td>
<td></td>
<td>blew it (for once)</td>
</tr>
</tbody>
</table>
Molecular Recognition Between Nucleic Bases.

PARING

DNA backbone

5'-boundary

Thymine

280 pm

Adenine

1110 pm

Cytosine

293 pm

Guanine

1080 pm

Attilio Citterio
DNA.

Vision through the axis

Lateral vision where is Evidenced the double chain (Double stranded DNA)

CGCGTTGACGACTGCGAGAATC

1. Original double helix
2. Strands separate
3. Complementary bases align opposite templates
4. Enzymes link sugar-phosphate elements of aligned nucleotides into a continuous new strand
Two Important Mechanisms.

Transcription:
- DNA
- Genetic Information
- messenger RNA

Traduction:
- messenger RNA
- chemical reactions, metabolism
- Proteins

A gene = a fragment of DNA which codes for a protein
# The Standard Genetic Code

<table>
<thead>
<tr>
<th>U</th>
<th>C</th>
<th>A</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU</td>
<td>Phe</td>
<td>UCU</td>
<td>Ser</td>
</tr>
<tr>
<td>UUC</td>
<td>Phe</td>
<td>UCC</td>
<td>Ser</td>
</tr>
<tr>
<td>UUA</td>
<td>Leu</td>
<td>UCA</td>
<td>Ser</td>
</tr>
<tr>
<td>UUG</td>
<td>Leu</td>
<td>UCG</td>
<td>Ser</td>
</tr>
<tr>
<td>CUU</td>
<td>Leu</td>
<td>CCU</td>
<td>Pro</td>
</tr>
<tr>
<td>CUC</td>
<td>Leu</td>
<td>CCC</td>
<td>Pro</td>
</tr>
<tr>
<td>CUA</td>
<td>Leu</td>
<td>CCA</td>
<td>Pro</td>
</tr>
<tr>
<td>CUG</td>
<td>Leu</td>
<td>CGA</td>
<td>Gln</td>
</tr>
<tr>
<td>AUG</td>
<td>Met</td>
<td>ACU</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACC</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACA</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACG</td>
<td>Thr</td>
</tr>
<tr>
<td>GUU</td>
<td>Val</td>
<td>GCU</td>
<td>Ala</td>
</tr>
<tr>
<td>GUC</td>
<td>Val</td>
<td>GCC</td>
<td>Ala</td>
</tr>
<tr>
<td>GUA</td>
<td>Val</td>
<td>GCA</td>
<td>Ala</td>
</tr>
<tr>
<td>GUG</td>
<td>Val</td>
<td>GCG</td>
<td>Ala</td>
</tr>
</tbody>
</table>

- **Translation Start Codon**: AUG
- **Translation Stop Codons**: UAA, UAG

- **Hydrophobic Amino Acids**: Val, Leu, Ile, Phe
- **Hydrophilic Non-Charged Amino Acids**: Ser, Thr, Alanine
- **Negatively Charged Amino Acids**: Asp, Glu
- **Positively Charged Amino Acids**: Lys, Arg
- **Cysteine**: Cys

---

Attilio Citterio

Politecnico di Milano
Auto Assembling and Molecular Recognition by DNA Molecule.

Assembling

Recognition

Duplication

Double alpha helix

Support for genetic information
PCR: Polymerase Chain Reaction.

30-40 cycles of 3 steps:

Step 1: denaturation
1 minute 94°C

Step 2: annealing
45 seconds 54°C
Forward and reverse primers!!

Step 3: extension
2 minutes 72°C
only dNTP’s
PCR Amplification.

Wanted gene

1st cycle

2nd cycle

3rd cycle

4th cycle

template DNA

$2^2 = 4$ copies

$2^3 = 8$ copies

$2^6 = 32$ copies

$2^{36} = 68$ billion copies

(Andy Vierstracte 1999)

Exponential amplification
Genome Dimension in Number of Base Pairs.

- **BACTERIA**: mycoplasma, *E. coli*
- **FUNGI**: yeast
- **PLANTS**: *Drosophila*
- **INSECTS**: shark
- **MOLLUSKS**: frog
- **CARTILAGINOUS FISH**: newt
- **BONY FISH**: human
- **AMPHIBIANS**: MAMMALS

Number of nucleotide pairs per haploid genome:
- *Attilio Citterio*
Super-coils of DNA.

Human

$3.3 \times 10^9$ pairs of bases

1 base each 3.4 Angstrom

Length = 1 meter !!!!
All inside the cell (~1 $\mu$m)
Alternative Packing of DNA Molecule.

Double helix

5 cm

$10^6$ Nucleotides $\Rightarrow v \sim 10^6 \text{ nm}^3$

DNA = $10^9 \ldots 10^{10}$ nucleotides

Chromatin "pearl necklace"

1 cm

Extended part of a chromosome

100 $\mu$m

Condensed part of a chromosome

700 $\mu$m

Chromatin fiber

30 nm

1 mm

Metaphasic chromosome

1.5 $\mu$m

5 $\mu$m
Histones and Chromatin.

Chromatin is a mass of genetic material composed of DNA and proteins that condense to form chromosomes during eukaryotic cell division. Chromatin is located in the nucleus of our cells. The primary function of chromatin is to compress the DNA into a compact unit that will be less voluminous and can fit within the nucleus. Chromatin consists of complexes of small proteins known as histones and DNA. Histones help to organize DNA into structures called nucleosomes by providing a base on which the DNA can be wrapped around. A nucleosome consists of a DNA sequence of about 150 base pairs that is wrapped around a set of eight histones called an octamer. The nucleosome is further folded to produce a chromatin fiber. Chromatin fibers are coiled and condensed to form chromosomes.

Autoassembling in Polymers: Flexible Macromolecules.

- Random coil
  - Free rotation
  - Solution
    - Fusion
  - Polymeric crystals
    - polyethylene
- Polymeric crystals
- Proteins
  - DNA, hemoglobin
- Rigid proteins
  - dentine, chitin

Primary auto-assembling

Secondary

Tertiary

Quaternary

Polymer auto-assembling
In all prokaryotic and eukaryotic organisms, three main classes of RNA molecules exist:

1) Messenger RNA (m RNA)
2) Transfer RNA (t RNA)
3) Ribosomatal RNA (r RNA)

The other are:

- small nuclear RNA (SnRNA),
- micro RNA (mi RNA)
- small interfering RNA (Si RNA)
- heterogeneous nuclear RNA (hnRNA).
RNA Functions.

Storage/transfer of genetic information

• Genomes
  • many viruses have RNA genomes
    single-stranded (ss-RNA)
    [e.g., retroviruses (HIV)]
    double-stranded (ds-RNA)

• Transfer of genetic information
  • m-RNA = "coding RNA" - encodes proteins

Structural

• e.g., r-RNA, which is a major structural component of ribosomes
  • BUT - its role is *not* just structural, also:
RNA Functions (2).

Catalytic

RNA in the ribosome has *peptidyltransferase* activity
- Enzymatic activity responsible for peptide bond formation between amino acids in growing peptide chain
- Also, many small RNAs are enzymes *ribozymes*'

Regulatory

*Recently discovered important new roles for RNAs*

*In normal cells:*
- in "defense" - esp. in plants
- in normal development

*As tools:*
- for gene therapy or to modify gene expression
# RNA Types & Functions

<table>
<thead>
<tr>
<th>Types of RNAs</th>
<th>Primary Function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-RNA - messenger</td>
<td>translation (protein synthesis), regulatory</td>
</tr>
<tr>
<td>r-RNA - ribosomal</td>
<td>translation (protein synthesis), &lt;catalytic&gt;</td>
</tr>
<tr>
<td>t-RNA - transfer</td>
<td>translation (protein synthesis)</td>
</tr>
<tr>
<td>hn-RNA - heterogeneous nuclear</td>
<td>precursors &amp; intermediates of mature mRNAs &amp; other RNAs</td>
</tr>
<tr>
<td>sc-RNA - small cytoplasmic</td>
<td>signal recognition particle (SRP), tRNA processing, &lt;catalytic&gt;</td>
</tr>
<tr>
<td>sn-RNA - small nuclear snoRNA - small nucleolar</td>
<td>mRNA processing, poly A addition, rRNA processing/maturation/methylation, &lt;catalytic&gt;</td>
</tr>
<tr>
<td>regulatory RNAs (si-RNA, mi-RNA, etc.)</td>
<td>regulation of transcription and translation, other?</td>
</tr>
</tbody>
</table>
RNA Structure.

The major bases found in DNA and RNA:

**DNA**
- Adenine
- Cytosine
- Guanine
- Thymine

**RNA**
- Adenine
- Cytosine
- Guanine
- Uracil (U)

**Thymine-Adenine Base Pair**
- 1110 pm
- 280 pm

**Uracil-Adenine Base Pair**
- 1110 pm
- 280 pm
Other Nucleosides in RNA.

Nucleoside bases found in RNA

- Adenine (A)
- Guanine (G)
- Cytosine (C)
- Uracil (U)

Examples of modified bases found in tRNA

- 1-methyladenine ($m^1A$)
- 7-methylguanine ($m^7G$)
- 3-methylcytosine ($m^3C$)
- Pseudouracil ($Ψ$)

Dihydouridine 1-methylguanosine 2-thiocytidine 5-methylcytidine Ribothymine
Single stranded bases within a stem are called a bulge of bulge loop if the single stranded bases are on only one side of the stem.

If single stranded bases interrupt both sides of a stem, they are called an internal (interior) loop.
RNA Tertiary Structure.

- red = guanine
- blue = cytosine
- orange = uracil
- gray = adenine

2 Dimensional

3 Dimensional

Simplified
Secondary structures of ciliate, vertebrate, and yeast (Saccharomyces) telomerase RNAs.

Structural elements in green represent conserved regions that bind to TERT. The structures that are present in some but not all species within a group are shown by dashed lines. The structures that define template region (red) and the template boundary (blue) are indicated.
Polysaccharides.

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”
See the related file: E4_15 polysaccharides.pdf
Protein Based Biopolymers.

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”
See the related file: E5_15 proteins.pdf
Biodegradable Plastics - Polylactic Acid and Poly(beta-alkanoates).

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/
Biodegradable Plastics.

Three main categories:

- **Chemically synthesised polymers**: PGA, PLA, polyvinyl alcohol, poly(ethylene oxide), poly(ε-caprolactone) ⇒ do not match all the properties of plastics
- **Starch**-based biodegradable plastics: blends of starch and plastic ⇒ only partially degradable
- **Polyhydroxyalkanoates** ⇒ similar properties and completely biodegradable

Main disadvantage: PRICES

- Synthetic plastics < 1 €/Kg
- Polylactic acid 3.00 - 4.00 €/Kg
- Starch compounds 2.00 - 4.00 €/Kg
- Polyhydroxyalkanoates > 5.00 €/Kg
Polylactic acid (PLA) is not a new polymer, it is known from 1932. Producing low molecular weight PLA is an easy process, however make high MW PLA is a more complex task.

Cargill-Dow has developed a new process which involves a selective depolymerization of low molecular weight PLA to a cyclic intermediate (lactide), which is purified by distillation.

The catalytic opening of lactide ring allow to prepare controlled MW PLA in continuous.

http://www.cargilldow.com/home.asp
Lactic acid is an optically active molecule (the central carbon is asymmetric) and exists in two enantiomeric forms (L and D lactic). Polymers with high L levels (natural) can be used to produce crystalline products, whereas the higher D materials (> 15%) are amorphous.
Polylactic acid (PLA) for Plastics Production.

Corn → Starch → Unrefined Dextrose → Fermentation → Lactic acid

Polymer Grades
- Fiber
- Film
- Thermoforming
- Bottle
- Woven
- Non-woven
- Etc.

Film → Apparel → Monomer Production → Lactide

PLA → Polymer Modification → PLA

PLA materials have mechanical properties between polystyrene and PET.
Coating with Polylactic Acid.

From a cooperation between Mitsui Chemicals Inc. and Cargill-Dow, LLC, SANYO in 2003 has realized the first optical disk in bio-plastic (polylactic acid).

Corn was used as raw material to obtain polylactic acid with appropriate optical and structural properties.

A mean of 85 maize seeds needs to make a disk and a maize-cob to make 10 disks. Word production of maize is about 600 millions tons, less than 0.1% is needed to make 10 billions of disks (the present word demand).
Biodegradability of PHB and PLA Products.

• Produced naturally by soil bacteria, the PHB are degraded upon subsequent exposure to soil, compost, or marine sediment.
• Despite their biodegradability the PHB still have good resistance to water and moisture vapor, and are stable under normal storage conditions and during use.
• PHB is used as nutrient only when phosphates, nitrogen, salts, water and heat allow the microorganisms to grow.
• These conditions are present in the compost and, in part, in soils, but not in the conditions of typical uses of articles formed by injection or extrusion. Therefore these materials are stable to use for years.
• PLA is hydrolyzed auto catalytically in humid environments at temperatures higher then the glassy transition (55°C). The lactic acid subunit is produced, which is used by microorganisms as food.
Biodegradability of PHB and PLA Products (2).

• The biodegradation rate of materials depends on the thickness and on environment temperature: in soil (mean temperature 8-15°C) objects biodegrade quite slowly (years), in not cured composts (with wide temperature excursions) faster and in professional composts (50-65°C) in weeks.

• Generally artifacts in PHB biodegrade at a rate similar to that of wood.

• PLA products degrade well only in professional composting structures (with temperature control).
Polyhydroxyalkanoates (PHA) – Characteristics.

- Linear polyesters;
- Thermoplastics;
- 100% resistance to water;
- Molecular weight: $2 \times 10^5 - 5 \times 10^6$;
- Biodegradable;
- Biocompatible;

Two types of PHA:

- scl-PHA $\Rightarrow$ if $R= H, CH_3, C_2H_5, C_3H_7$
  - $R = CH_3 \Rightarrow$ PHB poly-3-hydroxybutyrate
  - $R = C_2H_5 \Rightarrow$ PHV poly-3-hydroxyvalerate
- mcl-PHA $\Rightarrow$ if $R= (CH_2)_3CH_3$ to $(CH_2)_8CH_3$

- scl-PHA bear similar characteristics to polypropylene and mcl-PHA are similar to low density polyethylene
Microbial Polyesters.

3-PHB: 
\[-\text{O-CH-CH}_2-\text{C(O)-}]_n-\text{CH}_3\]

PHA (functionalized): 
\[-\text{O-CH-CH}_2-\text{C(O)-}]_n-\text{CH}_2\text{R}\]

4-PHB: 
\[-\text{O-CH}_2-\text{CH}_2-\text{CH}_2-\text{C(O)-}]_n-\]

R-configuration
- High crystallinity, 50-80 %
- Orthorhombic unit cell
- Thermoplastic, $T_m = 175^\circ$C
- Processable from melt
- Moldable
- Biodegradable

$M_w = 10^5 - 10^6$ Dalton

Okamura, Marchessault in: Conformation of Biopolymers 1967
# Physical Properties: Comparison PHA – PP.

<table>
<thead>
<tr>
<th>Property</th>
<th>PHB</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_m$ [°C]</td>
<td>175</td>
<td>176</td>
</tr>
<tr>
<td>Crystallinity [%]</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>$M_W$ [Dalton]</td>
<td>$5 \times 10^5$</td>
<td>$2 \times 10^5$</td>
</tr>
<tr>
<td>$T_g$ [°C]</td>
<td>4</td>
<td>-10</td>
</tr>
<tr>
<td>Density [g/cm$^3$]</td>
<td>1.250</td>
<td>0.905</td>
</tr>
<tr>
<td>Tensile strength [MPa]</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Extension at break [%]</td>
<td>6</td>
<td>400</td>
</tr>
<tr>
<td>UV resistance</td>
<td>good</td>
<td>poor</td>
</tr>
<tr>
<td>Solvent resistance</td>
<td>poor</td>
<td>good</td>
</tr>
<tr>
<td>Source</td>
<td>Sugar, Molasses</td>
<td>Petroleum</td>
</tr>
<tr>
<td>Cost [$/lb]</td>
<td>3.50</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Synthesis of PHB.

**Synthesis (microbial):**
- **Sugar**
- Microbial fermentation
  - Aqueous medium, 30 °C
  - Optically pure 100% R-configuration
- **Recovery**

**Synthesis (chemical):**
- **Stannous octanoate, Organic solvent, 120 °C, p**
- Racemic mixture
- **Purification**

**Reaction Formula:**
\[-\text{O} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{C(O)} \cdot \text{CH}_3 \]
PHB Accumulating Microorganisms.

- Alcaligenes
- Azotobacter
- Bacillus
- Rhodospirillum

(also archaebacterium)

Granules can make up to 90% of dry weight of biomass

Example of short-length PHB produced in activated sludge

Alcaligenes eutrophus now Ralstonia eutropha

Lee et al., 1996
The biosynthetic pathway of PHB and P(HB-HV) in Alcaligenes eutrophus.
**Non-limiting conditions:**

Carbon source (glucose) → **glycolysis** → Pyruvate → **Citric acid cycle** → Biomass + Energy

- Cell Proliferation, No polymer accumulation

**Limiting conditions:**

Carbon source (glucose) → **glycolysis** → Pyruvate → Acetyl-CoA → \( \beta \)-ketothiolase → Acetoacetyl-CoA

- Acetoacetyl-CoA reductase
- \( \beta \)-hydroxybutyryl-CoA → **PHB synthase** → PHB
In general:

Bacteria can be divided in two different groups:

- Bacteria that produce short-chain PHAs, $x = 0,1$
- Bacteria that produce long-chain PHAs, $x > 3$

Special cases:

**R. rubrum**
- $R = C_3H_7$ ($x = 2$)
- $R = C_4H_9$ ($x = 3$)

**B. thuringiensis**
- $R = CH_3$ ($x = 0$)
- $R = C_6H_{13}$ ($x = 5$)

**PHA Synthesis.**

*Alcaligenes eutrophus* grown on gluconate

<table>
<thead>
<tr>
<th>Depleted Nutrient</th>
<th>P(3HB) accumulation (g/g of protein / hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>0.10</td>
</tr>
<tr>
<td>Ammonium</td>
<td>0.40</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0.49</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.21</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.27</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.23</td>
</tr>
<tr>
<td>Iron</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Isolation of PHAs.

<table>
<thead>
<tr>
<th>Solvent Extraction</th>
<th>Alkaline Hypochlorite Treatment</th>
<th>Enzyme Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quick, efficient,</td>
<td>Efficient</td>
<td>Rather elaborate</td>
</tr>
<tr>
<td>High yields</td>
<td>Potential decrease</td>
<td>procedure</td>
</tr>
<tr>
<td>Granules disintegrated</td>
<td>in molec. weight</td>
<td>Granules intact</td>
</tr>
<tr>
<td></td>
<td>Granules intact</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic solvents</td>
<td>Caustic compounds</td>
<td>Lysozyme</td>
</tr>
</tbody>
</table>

Purification of the polymer is key for applications.
Polyhydroxyalkanoates (PHA’s).

- Sunlight → crops → Sugar solution
- Plastic Products → PHA
- Biodegradation to CO$_2$ and H$_2$O
Polyhydroxyalkanoates (PHA) – LCA.

• PHAs grown in corn stover studied
  ▪ Collect grain
  ▪ Harvest polymer

• Process involves:
  ▪ Fertilisers and pesticides (?)
  ▪ Harvesting and drying corn stover
  ▪ Extracting PHA
  ▪ Recycle solvent
  ▪ Purifying the plastic
  ▪ Blending the plastic to make resin

• Use of wheat straw as source of renewable energy

The findings:

• 1 kg of PHA requires 300% more energy than to make 1 kg of PE
  ▪ 2.65 kg of fossil fuel for PHA
  ▪ 2.29 kg for PHA from microbial fermentation
  ▪ 2.2 kg fossil fuel for PE (50% of which ends up in product)

• By burning wheat straw to power process results in Greenhouse gas saving

• However, it may be better environmentally to simply use renewable energy in a fossil fuel based process...

T. U. Gerngross, Nature Biotechnology, 17, (1999), 541 - 544
Oxo-Biodegradable: Poly(vinylalcohol) (PVA).

Poly(vinylalcohol) (PVA) is a synthetic organic polymer made from repetitive units of vinyl alcohol monomer. Cannot be prepared from this monomer but can be indirectly obtained by alkaline hydrolysis of polyvinyl acetate (PVAc).

\[
\text{CH}_2\text{-CH}_x\text{-CH}_2\text{-CH}_{1-x}\text{OH} \quad \text{OCOCH}_3
\]

Properties

Physical properties of commercial PVA depend on 5 factors;

- molecular weight
- hydrolysis degree (the number of non converted acetate units 1-x)
- degree and type of chain breaking
- cross linking degree (between the polymer chains)
- type, form and concentration of various additives

Changing these factors, properties as strength, brittleness, barrier characteristics to gases and water solubility can be controlled.
Biodegradability of Poly(vinylalcohol).

- PVA can be degraded using thermal, mechanical, photochemical, ultraviolet, biological and chemical processes. As concern biodegradation, a number of micro-organisms, at least 20 different genera of bacteria and a number of moulds and yeasts have known to degrade PVA. These organisms can occur in both artificial environments, such as anaerobic digesters, activated sewage sludge and composts, and natural environments such as aquatic systems and soil.

- The micro-organisms use PVA as a food source by producing a variety of enzymes that are able to react with it. The ultimate end products from this process are the naturally occurring substances carbon dioxide, water and biomass. Unusually, the degradation of polyvinyl alcohol takes place at random points along the entire length of the polymer chain at once. This is believed to be the reason for its relatively fast degradation. Other synthetic polymers, when biodegraded, are usually attacked gradually from both chain ends.
PVA Biodegradation in Liquid Culture.

Inoculum: Paper Mill Sewage Sludge

Mineralization (%)

Incubation time (days)
PVA Biodegradation in Liquid Culture.

Inoculum: Acclimated Microorganisms

Mineralization (%) vs. Incubation time (days)
Lignin, Natural Rubber, and Natural Fibers.

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”
Lignin: amorphous cross-linked polymer with high molecular weight.

- **S** = Syringyl
- **G** = Guaiacyl
- **SP** = Sinapyl **p**-hydroxybenzoate der.
- **β-O-4** = **β**-aryl ether
- **β-5** = phenylcoumaran
- **β-β** = resinol
- **4-O-5** = biphenylether
- Cynnamyl alcohol end group
- Phenolic end group

Monomers of lignin:
- Annual plant
- Resinous
- Foliage
Lignin of Soft Wood.

Formula Weight: 4477
Exact Mass: 4474.1354995(1)
Formula: C_{275}H_{308}O_{54}
Composition: C 73.77%  H 6.93%  O 19.30%
Cork (40% of Suberine).

Structure of suberine

Low density

Hydrophobicity and plasticity
Natural Rubber.

- Natural rubber consists of 1,4-cis-polyisoprene, extracted from the rubber tree (Hevea brasiliensis).
- It is produced in the tree by the biocatalyst hydroxynitrilelyase (2-hydroxyisobutyronitrile acetone-lyase).
- Synthetic rubber accounts for 75% of rubber usage. However, natural rubber has advantages of elasticity, resilience and thermal properties.
- Natural rubber is easily broken down in the environment, however vulcanization (treatment with sulfur) renders it resistant to biodegradation.
Attilio Citterio

ECOLOGY & ECOLOGY IN Bio-Isoprene SYNTHESIS and NR RECOVERY.

Alternative Sources

Hevea brasiliensis

Polyisoprene

Bio-isoprene

Polymer.
Natural Fibres.

- TEXTILE FIBER
  - Natural
    - Cotton
    - Wool
    - Silk
    - Jute
      - Mulberry
      - Tasar
      - Muga
      - Eri
  - Man made
Natural fibres are classified according to their origin:

1. Vegetable or cellulose
2. Animals or protein
3. Minerals

*Natural fibers (hemp, flax, china-reed, etc.) as reinforcing materials*

**Economics**
- Glass Fibers (~ $ 2/kg)
- Natural Fibers (~ $ .44 - $ .55/kg)

**Weight reduction**
- Glass Fibers 2.5-2.8 g/cm³
- Natural Fibers 1.2-1.5 g/cm³
Protein Based Natural Fibres: Silk and Wood.

**Silk**: fibre produced by insects (Bonbyx Mori) to protect larva

**Wool**: hair, produced by several animals for cold protection, composed of the protein keratin.
Cellulose Based Natural Fibres: Cotton and Flax.
Composite definition

- A composite is a material comprised of two or more physically distinct materials with at least one material providing reinforcing properties on strength and modulus (typically CaCO₃, SiO₂, Carbon black, Clay).

Natural Composites

- Bone
- Wood
- Bamboo: Natures fiber glass due to pronounced fibrillar structure which is very apparent when fractured.
- Muscle and other tissue

Engineering Composites

- Reinforced concrete beams
- Thermoset composites: Thermoset resins (polyurethanes, polyesters, epoxies)
  - Glass fibers, Carbon fibers, Synthetic fibers, metalfibers, or ceramic fibers
- Thermoplastic composites (polypropylene, nylon, polyester, TPU, polyimide)
  - Glass fibers, Carbon fibers, Synthetic fibers, metalfibers, ceramic fibers but also organic: kevlar, natural fibers (cellulose, hemp, flax, etc.)