Chapter 3
The Families of Biological Molecules

Diversity is essential to the sustainability of living systems. This is true for species in ecosystems as it is for molecules in cells, tissues, and organisms. Yet, the same way different species are linked by common ancestors and may be grouped in taxonomic classes according to common characteristics they share, molecules may be grouped in classes and classified according to common chemical and physical characteristics. One of such characteristics is solubility in water (in other words: how polar atoms are distributed in the 3D structure of molecules). One class of biological molecules, the lipids, includes only low water solubility (“hydrophobic”) molecules, this being the characteristic that defines this class. Other classes include molecules that are mostly moderately or highly soluble in water and can be recognized for the dominant presence of specific chemical groups: OH in saccharides (also referred to as “carbohydrates”) and a combination of amino and carboxyl groups in amino acids. Lipids, saccharides, and amino acids may combine with molecules of its own class to form either polymers (molecules formed by successively covalently attaching smaller molecules), such as polysaccharides and proteins, or supramolecular assemblies (organized arrangements of molecules that are in contact but are not covalently attached), such as the lipid bilayer of cell membranes. It is common to find molecules and supramolecular assemblies that combine elements from different classes, such as nucleotides, which contain saccharides. Proteins are extremely versatile in this regard because proteins’ interactions with saccharides, lipids, and nucleic acids (nucleotide polymers) are ubiquitous in virtually all cells. Figure 3.1 depicts the basic principles that support the organization of biological molecules in different classes.
3.1 Lipids and the Organization of Their Supramolecular Assemblies

Lipids are highly hydrophobic molecules that nonetheless may have polar chemical groups in their composition. If part of the 3D structure of the molecule is very polar and the other is non-polar, the molecule is referred to as amphiphilic, which stresses its dual nature: while the polar part will tend to interact with water and other polar molecules, the other will tend to minimize its interaction with water and other polar molecules. Nevertheless, it is important to bear in mind that lipids are molecules in which hydrophobicity dominates, even if they are amphiphilic. This is a qualitative definition with no clear boundaries in terms of molecular structure, which is nonetheless a useful working definition because hydrophobicity grants lipids the ability to organize in supramolecular assemblies that are very distinctive from polar molecules. Take lipid bilayers as example: they are extensively organized supramolecular assemblies that are very stable and yet do not involve covalent bonds between...
lipid molecules. Lipids spontaneously self-associate in aqueous environments, and amphiphilic lipids in particular may self-associate in a very organized way. This results from the so-called hydrophobic effect, although the most appropriate term would be “entropic effect.”

The entropic effect results from the second law of thermodynamics, which in one of its possible statements implies that all physical and chemical events tend to evolve in a way so that total entropy (“disorder”) increases. Consider Fig. 3.2: strongly amphiphilic molecules of generic cylindrical or rectangular cuboid shape will spontaneously form a bilayer to minimize the contact of non-polar regions with water. The driving force for this event may be counterintuitive at first glance: the bilayer is the arrangement that corresponds to the most disordered system. This may seem absurd because we tend to focus our attention in the solute (the lipids, in this case) and forget the solvent (water); yet, the gain in entropy refers to both. The lipids become ordered relative to each other, but the contact of hydrophobic groups with water molecules imposes restrictions to the orientational freedom of water, which is very costly in terms of entropy. When two lipid molecules associate, less water molecules are forced to order and, although the lipid molecules become more ordered relative to each other, the whole molecular systems (water included) becomes more disordered, in agreement with the entropic formulation of the second law of thermodynamics. Thus, this is named the entropic effect (occasionally imprecisely referred to as “hydrophobic effect”). The same principle applies when 3, 4, 5, … \( n \) molecules are considered forming large assemblies of lipids, such as lipid bilayers (see Sect. 3.1.1).

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**Fig. 3.2** Simplified explanation of the entropic effect. Most membrane lipids have two aliphatic (hydrocarbon) chains and a polar “head.” Polar heads usually contain phosphate (phospholipids) and other polar groups (left panel). Non-polar regions of lipids tend to associate with each other because fewer molecules of water get exposed to the aliphatic chains (right panel). Exposed aliphatic chains force molecules to orient their oxygen atom away from the hydrocarbon constituents, i.e., they force a certain degree of order to the solvent.
Broadly speaking, diacyl lipids (i.e., lipids containing two acyl—aliphatic—chains) with polar “heads,” such as most of the lipids found in cell membranes, have a generic shape with the characteristics depicted in Fig. 3.2, and the entropic effect compels these molecules to an ordered and parallel self-association. This is the physical process that sustains the stability of cell membranes as we shall discuss in Sect. 3.1.1. Now we will look closer into the chemical structure of diacyl lipids: although these lipids tend to assemble as bilayers, the differences in their chemical nature determine which chemical processes they are able to participate in (e.g., cell signaling) and their tendency to associate with membrane proteins and/or form specific domains in the membrane. Most triacyl lipids occurring in nature, such as triacylglycerols (often referred to as triglycerides), lack a bulk polar “head” group and therefore lack the propensity to form ordered supramolecular structures such as membranes (Fig. 3.3). Instead, they self-associate disorderly, forming aggregates, such as the lipid droplets found in cells where these lipids are synthesized (hepatocytes) or stored (adipocytes). Figure 3.3 shows histological images of the adipose tissue in which the presence of lipid aggregates occupying almost all cellular volume is detected.

Fig. 3.3 Lipids storage in the adipose tissue cells (adipocytes). (a) Triacylglycerols lack bulk polar “head” groups, which makes them not prone to self-assemble into bilayers. (b) Triacylglycerols aggregate in disordered agglomerated structures that can be stored adipocytes. In Panel (b), the lipids were extracted from the histological preparations of adipocytes, leaving white empty areas; arrows point nucleus. (Figure reprinted with the permission of Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina, Universidade de Lisboa, FMUL)
Fatty acids can be considered the basic unit common to most lipids in human cells. They consist of a linear unbranched aliphatic chain (“tail”) with a carboxyl group (Fig. 3.4a). The aliphatic chain can be saturated (i.e., having all the carbon–carbon bonds as simple bonds, without double or triple bonds) or unsaturated. Most fatty acids in humans have an even number of carbons, ranging from 10 to 28. Fatty acids are important molecules in the energetic metabolism of the organism, and they can be found either in “free” form (unattached to other molecules) or attached to other molecular structures through ester bonds (Fig. 3.4d, e). Free fatty acids differ from each other in the number and position of double bonds they have and in the length (in practice, the number of carbons) of the chains. The diversity attainable by changing these characteristics is virtually infinite, but, in practice, not all combinations are detected in nature (Table 3.1 displays examples of the most frequent fatty acids found in the human cells). Naturally, the nomenclature of fatty acids highlights their characteristic differences in terms of number and position of double bonds and chain length (Fig. 3.4b). The more recent nomenclature systems are very descriptive of these characteristics, but although older, traditional nomenclature systems are widely used. The recent nomenclature identifies the carbon atoms in a chain by number, starting consecutively from the carbon of the carboxyl (see Fig. 3.4b). Double bonds, if existent, are identified by the number of the carbon of lowest order forming the bond. The $\omega$, or $n$, nomenclature numbers the carbons in reverse order (see Fig. 3.4b). Another nomenclature, still popular among many biochemists, identifies carbons by Greek letters in alphabetical order ($\alpha$, $\beta$, $\gamma$, $\delta$, etc.) starting from the carbon adjacent to the carboxyl group (see Fig. 3.4b). A process of metabolic degradation of lipids is titled “$\beta$-oxidation” because of the importance of carbon $\beta$ in the reactions of this metabolic pathway (see Sect. 7.4.4).

Glycerolipids such as diacylglycerols and triacylglycerols result from the chemical conjugation of a glycerol molecule with fatty acids through esterification (Fig. 3.4c). Yet, glycerolipids may also conjugate other groups besides fatty acids. Usually, a phosphate group is used to bridge glycerol to a polar group, forming glycerol phospholipids (Fig. 3.4c). The phosphate binds both the glycerol and the polar group through phosphodiester bonds, which are ubiquitous in biochemical processes for their importance in molecular structure and energetics. Glycerophospholipids are named according to the fatty acids attached to glycerol and the chemical nature of the polar group (Fig. 3.4e).

Sphingolipids constitute another class of lipids present in many human cells, being responsible for rigid domains in the membranes. Figure 3.5 shows that although these lipids are similar in structure to glycerophospholipids, they are chemically different. These differences are responsible for the formation of rigid bilayers when conjugated to phospholipids and cholesterol.

Sterols, of which cholesterol in an example (Fig. 3.6), are extremely hydrophobic alcohols. These molecules are characterized by a flat rigid system of carbon rings, cyclopentaphenanthrene, and have some remarkable properties:
Fig. 3.4  The chemical structure of lipids. (a) Pictoric representation of free fatty acids, highlighting the polar carboxylic group in blue and the non-polar aliphatic chain in light blue; double bonds impose constraints in the conformation of the chain. (b) Illustrative examples of the application of different nomenclature systems; recent systems (numbering carbons from the carboxylic group) are more precise and descriptive, but older systems (Greek lettering and ω-numbering from the non-carboxylic ending carbon) are still widely used. Trivial (non-systematic) names (Table 3.1) were never abandoned for their "user-friendly" nature. (c) Fatty acids form ester bonds with other molecular structures to form more complex lipids such as triacylglycerols (detailed example in Panel d), glycerophospholipids, or sphingolipids. Fatty acid residues are present in all. In sphingomyelins, the polar group attached to phosphate is either choline or ethanolamine (Panel e). The example of specific triacylglycerol in (d) is 1-palmitoyl-2,3-dioleoyl-glycerol. (e) Phosphatidylglycerol lipids form different classes depending on the chemical nature of the "polar head" (fatty acid chains in this example are illustrative).
Table 3.1 Nomenclature of the most common fatty acids found in humans, both saturated (only simple carbon–carbon bonds) and unsaturated

| Number of C (chain length) | Saturated | Unsaturated | Number of double bonds | Nomenclature system |
|----------------------------|-----------|-------------|------------------------|--------------------|
| 14                         | Myristic  | Palmitoleic | 1                      | 16:1 Δ9            |
| 16                         | Palmitic  | Palmitoleic | 1                      | 16:1 Δ9            |
| 18                         | Stearic   | Oleic       | 1                      | 18:1 Δ9            |
|                            | Linoleic  | 2           | 18:2 Δ9,12              | 18:2 Δ9,12         |
|                            | α-Linoleic| 3           | 18:3 Δ9,12,15           | 18:3 Δ9,12,15      |
|                            | γ-Linoleic| 3           | 18:3 Δ6,9,12            | 18:3 Δ6,9,12       |
| 20                         | Arachidic | Arachidonic | 4                      | 18:4 Δ5,8,11,14    |
| 24                         | Lignoceric|             |                        | 18:4 Δ5,8,11,14    |

There are trivial names and descriptive abbreviations. Trivial names usually refer to the neutral (protonated) form of the acid, but one should bear in mind that at most physiological pHs (including plasma pH, 7.2–7.4), the acidic groups are unprotonated (pH above 4–5). Abbreviated nomenclatures highlight the number of carbon atoms in the aliphatic chain and number and position of unsaturated carbon–carbon bonds (Δ). Carbon numbering varies depending on adopting modern or older (ω, n) systems. Older systems are still widely used in some fields such as nutrition and occasionally used in biochemistry.

Fig. 3.5 Molecular structure of sphingolipids. As with phosphatidylglycerol lipids, sphingolipids form different classes depending on the chemical nature of the “polar head” (the fatty acid chain in this example is illustrative). Glu, glucose; Gal, galactose; GalNAc, N-acetyl-galactosamine; NeuAc, N-acetylmuramidate.
1. Only one small polar group present (OH in cholesterol; C=O in the equivalent ketones)
2. A flat molecule formed of four almost coplanar rings
3. When placed in lipid bilayers, it acts as a fluidity regulator (not too rigid to compromise dynamics and not too fluid to compromise barrier integrity)

**Fig. 3.6** Chemical architecture of steroids. (a) Cyclopentaphenanthrene is the basic structure of (b) steroids, in which R is a generic radical group. The numbering system of carbons is highlighted. Cyclopentaphenanthrene is rigid, planar, and hydrophobic. (c) Sterols are a sub-family of steroids having an alcohol group in carbon 3, which can be found in the lipid membranes of animals, fungi, and plants but not bacteria. They are the targets of some anti-infectious drugs in humans (this issue is addressed in Fig. 3.9)

Cholesterol is a vital molecule to humans (Box 3.1). In addition to its properties in membranes, it takes part in other biochemical processes such as the synthesis of some hormones. Nevertheless, the seriousness of problems associated with its ingestion in excessive amounts in unhealthy diets, associated with its very low solubility in aqueous media, turns this hero molecule into a bad reputation killer.

It is curious to note that although cholesterol is essential to many species in nature, including humans (Fig. 3.6c), its synthesis is relatively recent in evolutionary terms as it uses molecular oxygen, which is only present in atmosphere since the advent of photosynthesis.
Box 3.1 Cholesterol: A Hero with Bad Reputation

High concentration (“level”) of cholesterol circulating in the blood is a major risk factor for cardiovascular diseases, which affect a significant fraction of the whole population in many countries. The campaigns to prevent cardiovascular disease are often centered in reducing dietary cholesterol, which gives the impression that cholesterol is some kind of poison or toxin that should be banned. The persistent unhealthy doses of cholesterol in diet are indeed harmful, but this must not create the illusion that cholesterol is by itself malicious to cells. Virtually all molecules in excessive doses are harmful and cholesterol is not an exception. Moreover, the human body is so dependent on cholesterol that it synthesizes its own cholesterol and has homeostatic mechanisms to regulate its production in connection with several metabolic processes.

The way the human body works at biochemical and physiological levels is crucially dependent on cholesterol. This extremely hydrophobic molecule intervenes in three major processes in humans: (a) it contributes to the balance of physical-chemical properties of lipid membranes dynamics in cells; this includes the plasma membrane and the intracellular membranes; (b) it is a precursor for the bile acid synthesis; bile acids are important to absorb the lipids existing in food after ingestion; and (c) it is also a part of the synthesis of vitamin D and hormones as important as estrogen in women and testosterone in men. Because of its hydrophobicity, the solubility in aqueous media, such as blood, is very low. There are specialized structures, lipoproteins, which incorporate cholesterol and form stable emulsions in aqueous medium (see Sect. 3.1.2). When cholesterol is not contained in lipoproteins and/or when these structures deteriorate due to oxidation, cholesterol depots may be formed in the endothelial tissue of the blood vessels. These depots are typical of atherosclerosis. In the most extreme cases, the blood vessels may be interrupted and neighboring tissues are not irrigated. Lack of nutrients and oxygen (ischemia) may cause severe lesions in tissues. Vulnerable atherosclerotic plaques may also detach and clog vessels. Any of these conditions is called infarction. Myocardial infarction, commonly known as a heart attack, is one example. Cerebral infarction is another example, commonly known as stroke.

A demonstration of how cholesterol is indispensable for the human body is the Smith–Lemli–Opitz syndrome. This is a rare disease characterized by failure to thrive; mental retardation; visual problems; physical defects in hands, feet, and/or internal organs; increased susceptibility to infection; and digestive problems, among others. Smith–Lemli–Opitz syndrome is a genetic disease caused by a defect in cholesterol synthesis, namely, deficiency of the enzyme 3β-hydroxysterol-Δ7-reductase, the final enzyme in the sterol synthetic pathway that converts 7-dehydrocholesterol (7DHC) to cholesterol (the complete pathway of cholesterol synthesis is described in Box 8.8 in Chap. 8). This results in low plasma cholesterol levels and elevated levels of cholesterol precursors, including 7DHC.
3.1.1 The Structure of Biological Membranes

The concept illustrated in Fig. 3.2 can be extended to the association of many lipid molecules. The entropic effect will cause the molecules to self-associate orderly, ultimately forming bilayers of lipids with a hydrophobic core (Fig. 3.7). If these bilayers are extensive enough for the overall structure to bend, then the bilayer curves allow the sealing of the hydrophobic borders. All hydrophobic aliphatic chains became protected from water because the only areas in direct contact with the surrounding aqueous environment are the external and internal (“luminal”) surfaces formed by polar headgroups of the lipids. This is depicted in Fig. 3.7.

Lipid vesicles form spontaneously, being the simplest models of biological membranes. Amazingly, there is not a single cell known in nature that does not have their membranes formed with lipid bilayers to some extent. Diversity arises from the different kinds of lipids used and from their combination with other molecules, but the structural arrangement of the membrane itself is configured by lipid bilayers.

Some cells (e.g., bacteria, fungi, and plants) may also have a cell wall in addition to the cellular membrane, which serves for structural and/or protection purposes.

In fact, lipid membranes are relatively malleable and fragile. Yet, such malleability and apparent fragility are very important characteristics from a dynamic point of view: membrane division and fusion, for instance, are favored as these processes do not imply covalent bonds to be broken or formed among lipids and lipid bilayers are flexible. Membrane fusion is advantageous in many biological circumstances (Fig. 3.8). In biotechnology, mainly in pharma and cosmetics, lipid vesicles are valuable tools due to their properties (Box 3.2).
The dynamic structure of lipid bilayers. (a) Lipid membrane fusion occurs in nature associated with a plethora of cellular events. Some of these events such as endocytosis or budding of vesicles from the Golgi complex require inducing a curvature in membranes so that vesicles are formed. (b) This induction is accomplished by the action of specialized proteins that are curved and adhere to the surface of lipid bilayers (b, top), stabilize spontaneous curvatures (b, middle), or insert into one leaflet of the bilayer only, thus forcing the membrane to bend (b, bottom). (c, d) Membrane fusion implies that two different bilayers come together, which is due to the action of proteins that insert the two bilayers and enroll conformational changes that lead to the contact of the lipid bilayers. First, a common bilayer is formed from the mix of the two membranes (hemifusion state), and then total fusion merges the two entities that were initially enclosed in their own membranes. Panel (e) shows the example of membrane fusion during neurotransmission, namely, a neurotransmitter being released at a synapse. The proteins responsible for fusion are named soluble NSF attachment receptors, SNAREs (represented by green, pink, and blue filaments). Panel (d) shows the details of hemifusion and fusion of an envelope virus such as influenza virus, HIV, SARS-CoV-2, or dengue virus with the target cell. In this case, viral proteins at the surface of the virus insert the membrane of the target cell and undergo conformational changes ending in fusion and consequent release of viral contents in the cytosol.
Box 3.2 Lipid Vesicles in Pharma and Cosmetics

For many decades, lipids were considered relatively inert biologically, with functions of storage of energy in the adipose tissue or constitution of a matrix for cell membranes. Thus, in general, there was little interest in research to discover the properties, structures, biosynthetic pathways, biological utilization, and other functions of lipids. In the present, the situation is completely opposite. Lipids are regarded as important biological molecules that in addition to being energy stores and membrane components, participate in the regulation of many biochemical processes in cells and endocrine physiological regulation in the human body. Moreover, lipids are now important tools in pharma and cosmetic industries because they can be used in formulations that distribute and deliver drugs or other biologically active molecules in the human body. In most of these formulations, the lipids self-assemble in bilayers that form extensive vesicular systems, liposomes (see below figure), able to encapsulate molecules having desirable functions. It is a very versatile system as hydrophobic molecules may be accumulated in the lipid areas and hydrophilic molecules may remain solubilized in the aqueous spaces inside the vesicles.

In cosmetic applications, the liposomes may be part of formulations to be applied topically in the skin. The lipids help in diffusing the whole formulation through the outer layers of the skin. Moreover, the simple fact that lipids and water are forming an emulsion will help the formulation hydrating the desired areas of the skin.

Liposome systems used in pharma and cosmetic industries
Box 3.2 (continued)

Liposomal encapsulation can substantially improve the action of a drug, such as the decreased toxicity observed with amphotericin B (see Fig. 3.9). Conventional amphotericin B has been generally considered the drug of choice for many types of systemic fungal infections. These infections are a major threat to those whose immune systems are compromised, such as patients undergoing chemotherapy for cancer, bone marrow transplant recipients, and AIDS patients. However, amphotericin B is very toxic, thus limiting its utility. For these patients, who have a high rate of morbidity and mortality, there is a dosage form distinct from conventional amphotericin B, which consists of amphotericin B complexed with two phospholipids in approximately a 1:1 drug-to-lipid molar ratio: 1,2-Dimyristoyl-glycero-3-phosphocholine (DMPC) and 1,2-Dimyristoyl-glycero-3-phosphorylglycerol (DMPG), present in a 7:3 molar ratio. Doxorubicin is another example. Liposomal doxorubicin is designed to target to tumor cells and spare healthy tissue, maintaining efficacy while reducing toxicity. Conventional doxorubicin, drug commonly used to treat cancer, is limited by its potential for causing a variety of severe side effects.

Researchers are developing innovative liposomes with refined drug delivery properties to be part of future medicines. Some have their surface modified with proteins and other selected polymers to target meaningful cells. Synthetic phospholipids are suitable for specific applications in liposome targeting and gene therapy. Gene therapy is based on the efficient delivery of genes to their intended targets. Researchers have successfully put DNA into liposomes and have achieved fusion of these liposomes to cells. Scientists have also succeeded in protecting these liposomes from degradation and are able to modulate their circulation time.

Because lipid bilayers are the basic structure of cell membranes, several drugs directed to bacteria and fungi target the organization of lipid bilayers of these pathogens. Several antimicrobial peptides, for instance, are cationic so they bind and disrupt the lipid membranes of bacteria, which are highly anionic. Polyene antibiotics such as nystatin B and amphotericin B bind specifically to ergosterol, causing the selective membrane permeability and lysis of fungi (Fig. 3.9) because ergosterol only exist in the membrane of fungi.
Fig. 3.9 Examples of membrane-targeting drugs. Some drugs such as (a) amphotericin B, a fungicide used in the treatment of infections with Candida sp. among others, or (b) filipin, a fungicide also toxic to human cells and therefore not used in therapies, and (c) cationic amphipathic peptides with antibacterial properties target cell membranes. (a, right) Amphotericin B binds to ergosterol forming ordered complexes in which the hydrophobic sides of the polyene rings face the lipids and the polar sides face each other forming a hydrophilic pore. Filipin is not so selective to ergosterol when compared to cholesterol as amphotericin B; therefore, it is more toxic to human cells. Panel (b) (right) shows atomic force microscopy images of pores created by filipin in cholesterol-containing bilayers (arrows). (b, center) Large structures in the surface of the lipid bilayer are filipin aggregates. Panel (c) (left) also shows atomic force microscopy images but of an individual bacterium (E. coli). Exposure of the bacterium to the cationic amphipathic peptide BP100 caused the collapse of the bacterial membrane. The bacterial membrane is anionic, thus attracting electrostatically the peptides, which then aggregate on the lipid bilayer causing perturbation and increasing permeability. (c, bottom) This perturbation may be caused by formation of pores or by unspecific destruction of the lipid organization (c, right shows the action of rBPI23, a peptide derived from bactericidal/permeability-increasing protein potentially useful against meningitis). (Figures in Panel b (right) are reprinted with permission from Santos et al., Biophys J. 75:1869–1873, 1998. Figures in Panel c (left) are reprinted with permission from Alves et al., J. Biol. Chem. 285:27536–27544, 2010)
While lipid vesicles depicted in Fig. 3.7 are formed only by lipids (a single pure lipid or a mixture of lipids), biological membranes are often composed of lipids, proteins, and saccharides. How these components organize in the membrane has been the subject of intensive scientific research over the years (Fig. 3.10). Nowadays, a biological membrane of a human cell is regarded as a lipid bilayer having a heterogeneous distribution of lipids both in each layer and among layers. This heterogeneity leads to the formation of specific domains of lipids having defined functions. Rigid platforms, for instance, may serve to anchor proteins on the membrane (Fig. 3.11). The cell membrane is directly connected to the cytoskeleton through an array of proteins, the so-called cytoskeleton anchors. The outer surface of cell membranes may have lipids and proteins that are glycosylated (i.e., covalently attached to saccharides) contributing to a rich chemical diversity on the surface of cells (Fig. 3.10).

Fig. 3.10 The structure and organization of cell membranes. (a) Historical evolution of the concept of cell membranes (according to Ole Moritsen; figure reprinted with permission from Biol. Skr. Vid. Selsk. 49:7–12, 1998). The lipid bilayer was described after the discovery that proteins interact with lipids. The concept that there are integral proteins embedded in the lipid bilayer forming a dynamic structure came with the fluid mosaic model by Singer and Nicolson, in 1972, which is still accepted as the basic framework of membranes. Nevertheless, from then on, the organization of cell membranes has been continuously unraveled. Lipids are now known to self-associate in lateral domains of different composition, and some membrane proteins are bound (“anchored”) to the cytoskeleton (models proposed by Israelachvili and Sackmann). (b) The modern view on cell membranes, in which lipid colors represent heterogeneous lipid compositions. In the outer surface of cell membranes, glycosylated (i.e., saccharide-containing, black) lipids and proteins are present with different functionalities (see Sect. 3.2). Cytoskeleton and cytoskeleton-binding proteins are represented in brown.
Heterogeneities in lipid membranes. Although phospholipids are frequently depicted in an oversimplified form with ordered stretched aliphatic chains, as in Fig. 3.2, in reality most molecules in lipid bilayers in physiological conditions in cells have very flexible and dynamic acyl chains. Panel (a) shows molecular dynamics simulation of lipids with one single lipid in green to highlight the bent conformation (courtesy of Dr. Claudio Soares, ITQB-UNL, Portugal). Lipids with longer and saturated chains adopt stiffer and linear conformations as they interact more tightly with each other. Mixing stiff and fluid lipids results in partial segregation of the lipids. The atomic force microscopy image of a mixture of a fluid unsaturated lipid (palmitoyl-oleoyl-phosphatidylcholine, POPC—50% molar) with a saturated rigid lipid (dipalmitoylphosphatidylcholine, DPPC—50% molar) is shown in Panel (b) (upper left); segregated areas are clear and the height profile along the line seen in the top image (bottom graph) shows that the segregated areas are higher, therefore corresponding to more rigid areas of the membrane. (b, right) Epifluorescence images of lipid bilayers having one of the lipids tagged with a fluorescent dye confirm segregation of both lipids (figures reprinted with permission from Franquelim et al., J. Am. Chem. Soc. 130:6215–6223, 2008; and Franquelim et al., Biochim. Biophys. Acta. 1828:1777–1785, 2013). (c) These more rigid areas of the membrane are more adapted to anchor proteins and membrane receptors. Ceramides and cholesterol, for instance, further enhance these characteristics. Proteins covalently attached to lipids are typically found in these rigid platforms.
The lipid composition of cell membranes varies a lot from species to species, from organelle to organelle in the same cell, and from the inner leaflet to the outer leaflet in the same membrane (Fig. 3.12). This variety of compositions grants the necessary diversity to membranes so that they are specific for certain functions, in spite of the common feature to all membranes of all cells: in the end, they are all constructions based on lipid bilayers.

Because lipid bilayers are hydrophobic barriers, hydrophilic molecules, such as glucose, cannot freely transverse them, which poses challenges to cells. To overcome such challenges, the cells have channels and transporters in their membranes. These are proteins specific for certain molecules or ions that facilitate or enable the translocation of such molecules or ions across the membranes. This subject will be revisited in Sect. 5.3.1, after careful consideration of protein structure.

### 3.1.2 The Structure of Lipoproteins

Lipoproteins are organized assemblies of lipids and proteins covering a wide range of sizes and densities. They circulate in the blood and are responsible for the transport of lipids among different tissues (Fig. 3.13). These assemblies have a lipidic core formed mainly by triacylglycerols and cholesterol esters, surrounded by a monolayer of phospholipids and cholesterol. The global arrangement is largely determined by the entropic effect as the monolayer of phospholipids minimizes the contact between apolar components of the core and water molecules in blood (Box 3.3).
Fig. 3.13  Lipoprotein structure, size, and density. Lipoproteins consist of a monolayer of phospholipids (lilac) and cholesterol covering a lipid droplet of triacylglycerols and cholesterol esters (represented by the orange and blue circles, respectively). There are also proteins at the surface (represented in magenta), the apolipoproteins, which are specific of each class of lipoproteins and serve for cell recognition, i.e., interact with specific receptors in cells. Lipoproteins are grouped according to their densities and sizes, although the most common nomenclature refers to density (HDL high-density lipoprotein, LDL low-density lipoprotein, VLDL very-low-density lipoprotein). Chylomicra are the structures formed with dietary lipids in the enterocyte (intestines) and released in plasma.

Box 3.3 Lipoproteins: The Burden of Lipid Transport
Lipids have extremely low solubility in aqueous media. Therefore, as a consequence of the entropic effect (see Sect. 3.1), when placed in aqueous medium, they tend to self-associate. Most phospholipids, having a hydrophilic “head” and two hydrophobic acyl chains, tend to pack side-by-side and form bilayers. Cholesterol is not prone to form very organized supramolecular assemblies itself but is able to insert in the lipid bilayers and contribute to its stability. Triacylglycerols (“triglycerides”) and esters of cholesterol (cholesteryl esters; see next figure) do not have the amphipathic properties and structural requirements to form lipid bilayers. Instead, triacylglycerols and cholesteryl esters amalgamate in an aggregate having no polar surface. These aggregates tend to be spherical, the geometry that minimizes the surface area exposed to the solvent. The lipid aggregates tend to grow until free lipids are nearly absent, unless a phospholipid monolayer covers the surface of these aggregates, forming an entropically favorable interface. The phospholipid monolayers stabilize the lipid aggregates and an emulsion is formed. Emulsion means the lipids are heterogeneously distributed in microscopic scale because the lipids are clustered in aggregates, but homogeneously distributed in macroscopic scale since the aggregates are evenly disseminated in the solvent.

(continued)
Example of a cholesteryl ester: cholesteryl nonanolate. The molecule is composed of a cholesterol moiety and a fatty acid moiety (nonanoic acid in this case).

In the human body, very large lipid aggregates are found in the cells of the adipose tissue (adipocytes), occupying almost all cytoplasmatic space. It is a storage place (Fig. 3.3 in the main text). Smaller aggregates are found emulsified in blood, in association with specific proteins. These smaller aggregates are dragged by blood and serve as lipid transporters. In both cases, the aggregates are covered by monolayers of phospholipids having the polar groups exposed to aqueous environment and the acyl “tails” in contact with the lipids. The ensemble formed by the emulsified lipid aggregate covered with a phospholipid monolayer and associated with specific proteins is named lipoprotein (see Fig. 3.14 in the main text). The proteins themselves are named apolipoproteins. The lipoprotein, as a whole, is the lipid carrier entity; apolipoproteins’ main function is binding to specific receptors so that lipids are delivered to target cells only.

Lipoproteins vary among them in the proportion of triacylglycerols, cholesteryl esters, and proteins, which directly impacts in their compactness and density. Early studies on the properties of lipoproteins achieved separation of several classes of lipoproteins based on their different densities, so the density-based nomenclature was naturally adopted, from high-density lipoproteins (HDL) to very-low-density lipoproteins (VLDL) and chylomicra (see below table). There is a concomitant change in volume, but it is not given importance regarding lipoprotein classification. It is also worth highlighting that apolipoproteins are also divided in classes (A, B, C, etc.), and HDL are the only lipoproteins not bearing apolipoprotein B, which is a distinctive feature.

Properties of plasma lipoproteins

| Plasma lipoproteins          | Density (g ml⁻¹) | Diameter (nm) | Apolipoprotein | Physiological role             |
|------------------------------|------------------|---------------|----------------|-------------------------------|
| Chylomicron                  | <0.95            | 75–1200       | B48, C, E      | Dietary fat transport         |
| Very-low-density lipoprotein | 0.95–1.006       | 30–80         | B100, C, E     | Endogenous fat transport      |
| Intermediate-density lipoprotein | 1.006–1.019     | 15–35         | B100, E        | LDL precursor                 |
| Low-density lipoprotein      | 1.019–1.063      | 18–25         | B100           | Cholesterol transport         |
| High-density lipoprotein     | 1.063–1.21       | 7.5–20        | A              | Reverse cholesterol transport |

(continued)
Box 3.3 (continued)

During digestion, the lipids are partially degraded and emulsified in the intestinal lumen by bile acids, molecules similar to cholesterol but having several polar groups. Lipids and other nutrients are uptaken by the intestinal cells, enterocytes (see next figure). Chylomicra are formed in these cells and released in the blood. 80% to 90% of the lipids in chylomicra are triacylglycerols, which account for their low density. The remaining cargo is free cholesterol (1–3%), cholesteryl esters (3–6%), and phospholipids (7–9%). Chylomicra circulate in the blood, where degradation of their triacylglycerols into free fatty acids occurs. Part of these fatty acids is delivered to adipose tissue and peripheral tissues. The chylomicra remnants bind to liver cells that have specific receptors that recognize their proteins. The excess of nutrient uptaken after digestion is converted to lipids in the liver, where they form VLDLs in a process similar to the assembly of chylomicra in the intestine. VLDLs are released from the liver cells. In the bloodstream, they are depleted of free fatty acids meanwhile formed by the hydrolysis of triacylglycerols, resulting in the intermediary-density lipoproteins (IDL) and low-density lipoproteins (LDL), which transfer lipids to peripheral tissues having LDL receptors. Liver cells having LDL receptors also bind LDL. In contrast, HDLs transport cholesterol from peripheral tissues to the liver, where there are cells having specific receptors for HDL. The cholesterol is then used to synthesize bile acids. HDLs are the only lipoproteins that dispose of cholesterol. This characteristic renders the name “good cholesterol” to HDL-associated cholesterol in public health campaigns for lay audiences. This name makes no sense on biochemical grounds but helps to spread the message that in cardiovascular risk evaluation, it is important to differentiate between cholesterol that is being removed and cholesterol that is being incorporated. Interestingly, from the biochemical point of view, cholesterol to be disposed is associated with lipoproteins having no apolipoprotein B, and cholesterol to be incorporated is associated with apolipoprotein B-containing lipoproteins.

Origin and fates of plasma lipoproteins

(continued)
Box 3.3 (continued)

LDLs are degraded inside the cells after being uptaken by endocytosis. The LDL receptor is segregated in the endocytic vesicle, which then divides in two: one empty vesicle having the receptors in the membrane returns to the surface of the cell, and the other vesicle has the proteins and lipids of the lipoproteins and joins the lysosome. LDL-derived cholesterol may then either be used in cell membranes, or to synthesize steroid hormones or bile acids, or simply be stored as cholesteryl esters. The exact destination of cholesterol in the cell depends on the type of cell and its metabolic state. Dietary cholesterol suppresses the synthesis of cholesterol by the body, and high free cholesterol levels inhibit the synthesis of LDL receptors. Cellular uptake is thus inhibited in the presence of excess cholesterol and the level of LDL in the blood increases. Moreover, the LDLs take more time to be uptaken and circulate in blood for longer periods. This increases the chances of having the LDL exposed to oxidative agents such as NO, hydrogen peroxide, or the superoxide ion. Oxidized LDLs are then removed from circulation by macrophages, but the macrophages get their properties severely altered after incorporating oxidized LDL, becoming the so-called foam cells. Foam cells accumulate in the walls of endothelia, releasing growth factors and cytokines that stimulate the migration of smooth muscle cells that proliferate in the site of accumulation of foam cells and form collagen matrices. This consists in the deposition of atherosclerotic plaques, which pose severe cardiovascular risk.

Interestingly, the proteins responsible for triacylglycerols conversion into fatty acids in the heart (the enzymes named “heart lipoprotein lipases”) have much higher affinity for triacylglycerols than the corresponding proteins in the adipose tissue. The affinity parameter, $1/K_M$, which will be addressed in Sect. 4.2.1, is about tenfold higher in the heart. During starvation, the levels of plasmatic triacylglycerol drop, but delivery of fatty acids from triacylglycerols is kept in the heart even when suppressed to the adipose tissue.

Different classes of lipoproteins differ in density (due to differences in the relative amounts of proteins, phospholipids, cholesterol, triacylglycerols, and cholesteryl esters in their composition), size, and specific proteins associated (see Table in Box 3.3). Nevertheless, these classes are named after the differences in density only, which relates to the most practical property that can be used for their separation in different fractions (see Fig. 3.13). Lipoproteins formed in the intestine with dietary lipids are known as chylomicra, and the remaining classes range from very-low-density lipoproteins (VLDLs) to high-density lipoproteins (HDLs). Intermediary (IDLs)- and low-density lipoproteins (LDLs) are in between.

The different classes of lipoproteins have different functions and different target tissues for their action (see Box 3.3). Target recognition depends on the specificity of the proteins present on the lipoprotein surface (referred to as apolipoproteins to highlight that only the proteic part is being addressed) for well-defined receptors.
3.2 Saccharides and Their Polymers and Derivatives

Saccharides, at variance with lipids, are extremely polar, therefore hydrophilic, molecules. They are linear aldehydes or ketones with hydroxyl groups bound to the carbons that do not form the carbonyls (C=O). Many of these molecules have only C, H, and O in their composition and fit the formula \((\text{CH}_2\text{O})_n\). This spurious characteristic consecrated the designation “carbon hydrate,” which is still widely used to identify saccharides despite its total inadequacy in chemical terms: not all saccharides obey to \((\text{CH}_2\text{O})_n\), and this does not reflect a hydration of carbon, only a specific molar proportion between C, H, and O atoms. Referring to saccharides as “sugars” is equally inadequate and misleading. “Sugar” is related to a property, sweetness, which not all saccharides possess and extends to molecules other than saccharides, such as peptide sweeteners (see Box 3.4). Saccharides or oses are therefore the preferred nomenclatures for biochemists although “carbon hydrates” and “sugars” are also commonly used.

Saccharides are the most abundant biomolecules and owe this ubiquity to their reactivity and structural plasticity, which enable a great variety of functionalities, including energetic storage, cell communication, and cell protection against mechanical aggressions and dehydration. In order to understand such structural plasticity and the functionalities arising therefrom, one has to start with the basic chemistry and reactivity of saccharides. Although this is a wide and complex world in the realm of biochemistry, we will devote ourselves to the understanding of the most important saccharides in human biochemistry only. One will stick to the basics of this fascinating world for the sake of clarity and focus on processes that are foundational for other medical disciplines such as histology and physiology.

Box 3.4 Sweeteners and Sugar Substitutes
The problem of popularization of high caloric diets stimulated the search for sugar substitutes. Sucrose, the most commonly used sugar in cooking, is a natural sweet molecule from which a certain amount of energy can be used by the human body after metabolization. However, there are molecules known as high-intensity sweeteners that have many-fold the sweetening power of sucrose. Saccharin, for instance, is approximately 300-fold sweeter than sucrose when equal quantities are compared. Aspartame and acesulfame K are approximately 200-fold sweeter than sucrose. For sucralose, the ratio raises to an impressive 600-fold. A specific chemical modification in aspartame, advantame, grants an impressive 20,000-fold increase in sweetness relative to sucrose. Therefore, much less mass of sweetener is needed to achieve the sweetness of a food or beverage. Even though the “caloric content” of a unit mass of the molecule may be equivalent to sucrose in some cases, the total amount used is several orders of magnitude less and the total calories in the diet drops drastically.
The chemical structure of most popular sweeteners, including sucrose and the artificial ones, is very different (see next figure). Sucrose is a disaccharide composed of the residues of the monosaccharides glucose and fructose. Sucralose is prepared from sucrose via the substitution of three hydroxyl groups for chlorides. Saccharin and acesulfame K have much different structures. Aspartame is the methyl ester of the dipeptide l-aspartyl-l-phenylalanine.

The molecular structure of sweeteners must be such that they bind to a specific receptor molecule at the surface of the tongue. The receptor is coupled to a G-protein (see Sect. 5.4), which dissociates when the sweetener binds to the receptor. This dissociation leads to the activation of an enzyme that triggers a sequence of events resulting in signals that are transmitted to and interpreted by the brain. The sweetness perception depends on fine details of the interaction between the sweetener and its receptor. The importance of fine details in molecular shape to sweetness is illustrated by the case of aspartame, as its stereo isomer, l-aspartyl-d-phenylalanine methyl ester, has a bitter, not a sweet, taste.

There has been a long and continuous controversy on the impact of artificial sweeteners on health, which has driven a lot of research about the possible toxicity of their metabolic products. Saccharin has been very controversial and banned in some countries. In the body, aspartame is broken down into/absorbed as products that include aspartate, phenylalanine, and methanol, which is toxic. Phenylalanine is toxic to individuals with phenylketonuria, a genetic disease wherein individuals cannot process phenylalanine. Products containing aspartame must therefore be labeled for phenylalanine. Regardless of the controversies and limitations in their use, artificial sweeteners have an important role in the improvement of the quality of life of diabetics, who are limited in the consumption of sucrose and other saccharides.
The simplest conceivable saccharides have three-carbon chains, i.e., they are trioses (“tri” for three carbons, “ose” for saccharide). Depending on the position of the carbonyl group, C=O, which may be terminal (aldehyde) or not (ketone), the saccharide is an aldose or a ketose. In the specific case of trioses, only glyceraldehyde and dihydroxyketone are possible (Fig. 3.14). But even in these cases, two kinds of common chemical reactions in nature are possible: esterification and reduction (Fig. 3.15).

The chemical structure of glyceraldehyde deserves close attention as its central carbon (carbon number 2; C2) has four different substituents (i.e., it is bound to four different atoms or groups of atoms), being referred to as a chiral carbon or chiral center. Imagine the permutation of the H and OH substituents, for instance. A different molecule results from this switch (Fig. 3.16).

Fig. 3.14 Important glycerol-related structures. Glycerol is related to an aldehyde; glyceraldehyde, which has a ketone isomer; and dihydroxyketone, which in turn is related to acetone, also named dimethyl ketone, or propanone. The aldehyde and ketone groups of glyceraldehyde and dihydroxyketone, respectively, are highlighted in the chemical structures by a shadowed box.

Fig. 3.15 Examples of very frequent reactions involving saccharides. Glycericaldehyde may be reduced to glycerol upon chemical reduction of the C=O group in carbon 1 (carbons are numbered starting with the one from the carbonyl group, similarly to fatty acids, in which carbons are numbered starting in the carboxyl group). Phosphoric acid (HPO₄²⁻) may react with carbon 3, for instance, to form an ester, glyceraldehyde-3-phosphate.
Although being isomers, both molecules cannot be overlapped because the orientation in space of the H and OH substituents is different. The difference is clear if the molecule is represented in a three-dimensional (3D) perspective. A closer look reveals that both molecules are mirror images of each other, i.e., they are enantiomers. To distinguish between both enantiomers of glyceraldehyde, one is named “L,” and the other is named “D.” These labels were arbitrarily assigned by Emil Fischer but are used to name saccharides and amino acids by extrapolation from glyceraldehyde (Fig. 3.16): D stands for right (dextro in Latin) and refers to the structure having the OH group in the chiral carbon to the right when it is projected toward the observer; L stands for left (levo in Latin) and refers to the structure having the OH group in the chiral carbon to the left when it is projected toward the observer. The chemical structure of glyceraldehyde deserves close attention as its central carbon (C2) has four different substituents (i.e., it is bound to four different atoms or groups of atoms), being referred to as a chiral carbon or chiral center. Imagine the permutation of the H and OH substituents, for instance: a different molecule results therefrom (Fig. 3.16).

In chemistry research, there are two other naming conventions for enantiomers independent from each other: the R- and S-system, which is based on a classification of the substituent group based on the atomic number of atoms bound to the central atom (chiral center), or the + and − system, based on optical activity, i.e., on direction of rotation of incident plane-polarized light. Symbols + and − are sometimes replaced by d- (dextrorotatory) and l- (levorotatory), but d- and l- are easy to confuse with D- and L- and prone to misunderstanding. Both systems are more robust than Fischer’s D and L because they are not dependent on the comparison with glyceraldehyde. Chemists tend to use R/S or +/−, but biochemists are still “attached” to the D/L system for a simple reason: chiral diversity among biological saccharides and amino acids is very restricted. By far the most abundant saccharides in human biochemistry are D. Interestingly L is the preferred form in amino acids. Natural evolution favored one form specifically probably because it is sim-
pler to have only one form as the building block for saccharide polymers (polysaccharides) and amino acid polymers (proteins). A small protein with 100 amino acid residues that could be D or L would have 2100 different possible isomeric structures. Because only L-amino acids are used, only one isomeric form is allowed. Why specifically L-amino acids and D-saccharides and not the other forms? It is not clear; probably it originated from ancient primordial random processes that later propagated and converged by evolution into the specific enantiomers found in nature nowadays.

When longer carbon chains are considered, more complex saccharides are possible depending on the:
1. Length of carbon chains
2. Position of the carbonyl group in the carbon chain
3. Number and location of chiral centers

Although many different saccharides can be found in the human body, pentoses and hexoses are the most frequent in metabolic processes, so we will now focus on these molecules, namely, ribose and glucose (Fig. 3.17).

![Fig. 3.17 D- and L-isomers of ribose (pentose) and glucose (hexose). D-forms are the more relevant forms in nature](image)

Aldoses such as glyceraldehyde, ribose, and glucose react with water, forming a hydrate. This happens at the C=O group because the oxygen atom attracts the electrons leaving the C deficient in electrons, therefore prone to interact with the electrons of water oxygen (Fig. 3.18). The reaction is reversible, so aqueous solution of aldoses contains mixtures of their aldehyde and hydrate forms. Nevertheless, pentoses and hexoses may react intramolecularly in a way that is similar to hydration. Because the carbon chain is able to bend and is dynamic (similarly to saturated aliphatic chains in lipids), the carbonyl group may contact alcohol groups (OH—hydroxyl) in the same molecule and react with it. The result is the formation of a cyclic molecule by the conversion of the carbonyl group in a hemiacetal group (Fig. 3.18). The cyclization is reversible, and in cells, the cyclic forms of ribose and glucose coexist with the linear forms, although the cyclic forms are dominant. Upon cyclization, two enantiomers are formed because the hydroxyl group in C1 may be
linked to any of the two sides of the plane of the ring: in the \( \alpha \)-anomer, the OH group in C1 is in the opposite side of the plane of the ring relative to the terminal carbon, C6 (\(-\text{CH}_2\text{OH}\)), and in the \( \beta \)-anomer, they are both at the same side. Glucose adopts a “chair” conformation at variance with the strict planar ring of ribose (Fig. 3.18), but \( \alpha \)- and \( \beta \)-anomers exist the same way. The existence of the enantiomers has drastic implications in the polymerization of hexoses.

**Fig. 3.18** Hydration of saccharides. (a) Hydration of glyceraldehyde forms a hydrate. This reaction is reversible, so glyceraldehyde coexists with its hydrate. The carbon originally present as a carbonyl group is the only carbon with two bonds to oxygen in the hydrate. A similar reaction may occur intramolecularly in (b) pentoses and (c) hexoses. Panel (b) shows in detail the reaction of C=O (C1, in CHO) with the OH group in C4, analogous to hydration. A cyclic pentose is thus formed. As with hydration, the reaction is reversible and both forms coexist, although the cyclic form is more abundant. (c) The cyclic form of the hexose glucose is not planar as ribose is, as the molecular hexagon is flexible and adopts other conformations, such as the “chair” conformation. (d) Cyclization results in the formation of two anomers because the OH group formed at C1 may be placed on two different sides of the newly formed molecular ring: in the \( \alpha \)-anomer, the OH group in C1 is in the opposite side of the plane of the ring relative to the terminal carbon, C6 (\(-\text{CH}_2\text{OH}\)), and in the \( \beta \)-anomer, they are both at the same side. Both the “chair” (c) and the more simplistic planar (d) representation of hexoses are used in this book.
3.2.1 From Monomers to Polymers: Polysaccharides

Saccharides such as hexoses and pentoses may react with each other forming chains that may reach considerable size. Molecules built from the association of smaller molecules of a kind are generally named polymers, and polymers made of unit saccharides such as ribose or glucose are named polysaccharides. The units forming polysaccharides are referred to as monosaccharides. A covalent association of two monosaccharides is a disaccharide. Association of “few” monomers forms “oligosaccharides”; the size boundary between oligosaccharides and polysaccharides is not well defined.

Two monosaccharides may associate by dehydration. Take the example of two glucose molecules forming a maltose molecule (a disaccharide) by dehydration (Fig. 3.19). C4 in one molecule and C1 in the other become covalently attached by an acetal linkage, also named O-glycosidic bond. Water resulting therefrom is formed with the oxygen previously attached to C1. The reverse process is the hydrolysis of maltose into two glucose monomers, which although thermodynamically favorable, is a very slow reaction. When degrading enzymes are not present, the process is meaningless in practice.

![Fig. 3.19](image_url) Formation of disaccharides. (a) Two glucose molecules may associate by dehydration. When C1 in α-glucose (α anomer) reacts with C4 in another glucose, maltose is formed. Maltose is thus a disaccharide formed by linking two glucose molecules through an acetal or O-glycosidic bond. This bond is named “α-(1,4)” to stress that C1 in the α-anomer binds to C4 in the other molecule. (b) Sucrose and lactose are other examples of disaccharides. Both are formed from the conjugation of different constituent monosaccharides: glucose and fructose in the case of sucrose and galactose and glucose in the case of lactose. Sucrose involves an α-(1,2) bond, whereas lactose involves a β-(1,4) linkage.
The stereochemistry (i.e., the spatial orientation of the chemical groups in the molecule) is very important because the covalent linkage of two molecules imposes restrictions on the way molecules can move in space. Depending on whether monomers are α- or β-anomers, different degrees of restriction arise. The flexibility of the conjugate is very much dependent on the enantiomers because the interaction between molecular groups in the disaccharide is very different (Fig. 3.20a). This effect is amplified in large polymers; polysaccharides may have a wide range of flexibilities, from extremely stiff and straight to coiled and deformable depending on the enantiomers used. Cellulose, for instance, is a glucose polymer formed with β-(1,4) bonds that is extremely mechanically resistant, whereas amylose is an example of a flexible polymer formed by a α-(1,4) backbone (Fig. 3.20b, c). Cellulose properties determined its evolutionary selection toward structural functions in plants, forming cell walls, which impacts in the macroscopic properties of wood, for instance. Amylose is a component of starch, a molecule that curls forming helices and is stored in plants for use in the energetic metabolism. Starch is the most common polysaccharide in human diet. Cellulose and amylose are striking examples of how apparently small details may actually determine profound differences in molecular properties and structures and therefore also in function.

Carbon 6 is also available for reaction, so linear polymers formed of C1–C4 chains may branch when C1–C6 bonds are also formed (Fig. 3.20d). Amylose turns into amylopectin when α-(1,6) links are formed. Glycogen is the human storage polysaccharide and is very similar to plant amylopectin (Fig. 3.20e). They differ only in the frequency of branching and average size of α-(1,4) segments. The advantage of having glycogen as energy storage relative to a linear (unbranched) polysaccharide relates to the fact that glycogen is enzymatically degraded by saccharide hydrolysis of the terminal units. A branched molecule has several termini which can all be degraded at the same time, making glucose readily available at high rate.

Depending on their chemical composition and stereochemistry, polysaccharides found in nature have one of three functions: (1) structural/mechanical protection, (2) energetic storage, and (3) water-binding (protection against dehydration). Several examples are in Table 3.2. Hyaluronic acid, a polysaccharide with sulfate groups (SO₄²⁻ has similar properties to PO₄³⁻), forms an extracellular mesh with collagen in the connective tissue, forming a flexible but resistant hydrated histological structure (Fig. 3.21).

3.2.2 Molecular Conjugates of Mono- and Oligosaccharides

We have seen in previous sections that saccharide monomers offer diverse possibilities of reaction, and so they are molecules that form many conjugates in nature. The most important derivatives are phosphate esters. Phosphoric acid is able to form up to three ester bonds (Fig. 3.22), although the triesters are not commonly found in nature. Yet, diesters are important and enable saccharide phosphates to form polymers (e.g., nucleic acids) or bridge saccharides with other organic molecules.
Conformational restrictions in saccharide polymers. (a) Two monosaccharides such as \( \text{D-glucose} \) forming a disaccharide have very different restrictions to articulate and move depending on whether the glycosidic bond is \( \alpha-(1,4) \) or \( \beta-(1,4) \). (b) When several monomers bind to form a polymer, successive \( \alpha-(1,4) \) or \( \beta-(1,4) \) bonds confer distinct properties to the polymer: \( \alpha-(1,4) \) bonds enable bending between monomers, which results in curled polymers such as (c) amylose, and the stiffer \( \beta-(1,4) \) links between monomer favor linear straight polymers, such as cellulose. Therefore, cellulose is found in structural elements of plants, while amylose is used by plants as energy storage. Humans also use a poly-\( \alpha-(1,4) \) saccharide as energy storage. (d) Periodic \( \alpha-(1,6) \) branching further enables a globular organization of this polysaccharide. The final result is a regularly branched polymer of \( \text{D-glucose} \) named (e) glycogen. Glycogen synthesis is initiated by a protein and elongation requires several enzymes (see Sect. 8.2).
Table 3.2 Examples of the function of polysaccharides found in nature: structural (Str), energy storage (Sto), and water-binding hydration (Wat)

| Polysaccharide | Monosaccharide 1 | Monosaccharide 2 | Bond | Branching | Location | Main function |
|----------------|------------------|------------------|------|-----------|----------|--------------|
| **Bacteria**   |                  |                  |      |           |          |              |
| Peptidoglycan  | d-GlcNAC         | d-MurNAC         | β-(1,4) | α-(1,6)  | Bacterial wall Capsule | Str            |
| Dextran        | d-Glc            | –                | α-(1,3) | –         |          | Wat³         |
| **Animals**    |                  |                  |      |           |          |              |
| Chitin         | d-GlcNAC         | –                | β-(1,4) | α-(1,6)  | Insects, crabs | Str            |
| Glycogen       | d-Glc            | –                | α-(1,4) | α-(1,6)  | Liver, muscles | Sto            |
| Hyaluronic acid| d-GlcUA          | d-GlcUA          | β-(1,4) | –         | Connective tissue | Str, Wat        |

³Capsular materials like dextrans may be overproduced when bacteria are fed with saccharides to become reserves for subsequent metabolism

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**Fig. 3.21** The extracellular matrix has hyaluronic acid in its composition. (a) Histological preparations of cock crest highlighting the hyaluronic acid matrix (*left*, conventional electron microscopy; *center*, platinum–carbon replica; *right*, preserved blue-dyed hyaluronic and extracellular heavily glycosylated proteins, the “proteoglycan matrix”) (figures reprinted with the permission of Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina, Universidade de Lisboa, FMUL). (b) Schematic representation of the molecular organization of extracellular matrix, which is composed of gel-forming saccharides attached to a backbone of hyaluronic acid that is intertwined among collagen fibrils. Polysaccharides form gels due to the high density of H bonds. These gels confer structure and mechanical protection to cells and retain water, which prevents desiccation of the tissues.
Phosphate groups forming esters are anionic in aqueous environment in the most common biological pH ranges. This means that neutral molecules, such as glucose, become charged when esterified with a phosphate. The consequence is an increase in solubility in water and a decrease in the ability to cross lipid bilayers, for instance. This is deemed important as glucose metabolism starts by forming glucose phosphate (Fig. 3.22a).

Phosphate ester hydrolysis is a spontaneous but very slow process, which makes it under enzymatic control in cells. In addition, many chemical processes occurring in cells, such as condensation of polymers with formation of water, are unfavorable processes (there is “excess” water in most cell environments); enzymes speed the reaction but do not shift the equilibrium toward condensation. The use of phosphate derivatives of the monomers in the process of condensation facilitates the reaction as phosphates are so-called good leaving groups: they alter the reactivity of transient chemical species in the course of the mechanism of reaction.

ATP (adenosine triphosphate, Fig. 3.22b) is among the biological molecules that are saccharide derivatives and involves a phosphate ester. A phosphate diester bond bridging two other phosphates is another interesting characteristic of this molecule. The energy balance involved in the hydrolysis of phosphate–phosphate bonds makes this molecule pivotal in energetic metabolism. Divalent cations such as Mg2+ are usually associated with ATP and other molecules having diphosphate groups. This reduces electrostatic repulsion between the oxygen atom of water and the negative charge of phosphate groups, facilitating the hydrolysis of phosphate derivatives.

Probably not so famous as ATP, but equally important in biochemistry, is coenzyme A (Fig. 3.23c). This is a relatively small but complex molecule. Amazingly, coenzyme A has phosphate and saccharide groups but owes its reactivity to a terminal thiol (–SH) group. This thiol group may bind an acetyl residue through a thioester bond, but may also bind a fatty acid, forming acyl-CoA, which is involved in lipid metabolism.

Nicotinamide adenine dinucleotide (NAD+) is another interesting case of saccharide derivative that also contains phosphates. NAD+ intervenes in redox reactions as it may accept and donate electrons, changing from NAD+ to NADH + H+ or vice versa. One extra phosphate group turns NAD+ into NADP+, which has similar redox properties but can only bind to specific enzymes that usually do not bind NAD+. This implies that there are specific metabolic roles for NADP+, distinct from NAD+. The phosphates are involved in enzyme recognition but not in the redox activity itself (Fig. 3.22d). The same happens with flavin adenine dinucleotide (FAD+ and FADH2; Fig. 3.22e).

Nucleotides themselves deserve closer attention because they polymerize to form the so-called nucleic acids. They will be left for further discussion in the next section. To finalize, it should be stressed that many therapeutic drugs are also saccharide derivatives, such as digoxin (Fig. 3.22f), used in the treatment of heart conditions. Azidothymidine (AZT) is another example. It is an analog of thymidine that may inhibit the action of reverse transcriptase of HIV. It was the first drug used in the treatment of AIDS. Cellular enzymes convert AZT into the effective 5-triphosphate form (Fig. 3.22g). Once bound to reverse transcriptase, the azide group, N3, is responsible for chemical inhibition. Inspired by the success of AZT (Fig. 3.23), many nucleosides are now under development to create new inhibitors of HIV reverse transcriptase to fight AIDS.
3.2 Saccharides and Their Polymers and Derivatives

Fig. 3.22 The importance and ubiquity of phosphates. (a) Phosphates form esters or diesters bridging two organic molecules. Phosphate confers an anionic charge to the newly formed chemical entity because the ionization of the phosphate group occurs at pH > 2, increasing its solubility in aqueous medium and decreasing its ability to translocate lipid membranes. This is the case for glucose-6-phosphate, which is “trapped” in the cytosol of cells, where it will be processed in different metabolic pathways. (b) Adenosine triphosphate, ATP, and (c) coenzyme A, CoA, are important biological molecules with a saccharide residue bound to a phosphate group. ATP also contains a phosphodieste-ter bond, very important for its reactivity in cells. CoA has a couple of phosphate groups bound to each other, but its reactivity in cells is dictated by the sulfhydryl group, also named thiol (-SH).
Fig. 3.22 (continued) Nicotinamide adenine dinucleotide (NAD\(^+\)) is another important molecule with saccharide residues bound to phosphates. (d) NAD\(^+\) may be reduced to NADH. Redox reactions of NAD\(^+\)/NADH take place in a specific cyclic residue of the molecule, involving a nitrogen atom (right). (d) NAD\(^+\)/NADH phosphate (NADP\(^+\)/NADPH) also enter in redox reactions in human metabolism. NADH and NADPH cannot be distinguished by their reducing properties because the phosphate group that distinguishes them is not related to the nitrogen atom that grants the redox properties. Yet enzymes use specifically NADH or NADPH and so there is no redundancy between these molecules. (e) FAD\(^+\) and FADH\(_2\) are molecules similar to NAD\(^+\) and NADH in the adenine nucleotide and their role in metabolic redox reactions. (f) Digoxin and digitoxin are examples of drugs with monosaccharides in their structure; more specifically, three residues are specifically combined as part of a unique structure. (g) Another example of drug that is a saccharide derivative is azidothymidine (AZT), which is converted to a triphosphate in cells and is able to insert in the active center of the reverse transcriptase of HIV because it is similar to the natural substrate. However, the natural substrate does not have the N\(_3\) group. The presence of this group blocks the conversion of the viral RNA into DNA.
3.2.3 Molecular Conjugates of Oligosaccharides

It is worth stressing that combination of saccharide monomers may generate a big
diversity of products when compared to amino acids, for instance (Fig. 3.24). Two
glucoses, for instance, can bind via six carbons in each monomer, thus being able to
form 36 different molecules. Considering the anomers, the diversity increases. It is
not surprising that oligosaccharides are present in the surface of cells as receptors of
unique structure (see an example in Box 3.5), while amino acids form polymers
(proteins) having domains with few restricted and well-defined structures. Moreover,
monosaccharides or oligosaccharides are frequently formed in nature attached to
proteins.

**Box 3.5 The ABO Blood Groups**

There are different blood groups according to different immunogenic mole-
cules present in erythrocytes. The most important classification of blood
groups is based on three antigens, A, B, and O, that form four groups: A, B,
O, and AB—the ABO blood groups. The ABO blood group antigens are oli-
gosaccharide chains attached to proteins and lipids located in the outer sur-
face of erythrocytes. One single residue of a small oligosaccharide determines
whether the antigen is A, B, or O (see below figure).
ABO group antigens: Fuc represents the monosaccharide fucose; Gal, galactose; GalNAc, \( N\)-acetylgalactosamine; and GlcNAc, \( N\)-acetylglucosamine

The immune system of an individual produces antibodies against the ABO antigens not present in his own erythrocytes. Individuals in A group will produce antibodies against B and vice versa. Type O, the most common, does not contain the last residue, which is the antigen, in its structure (in fact the original nomenclature was 0—zero—but became the letter O). So, in blood group O will produce both anti-A and anti-B. Individuals in blood group AB are rare and, naturally, have no anti-A and no anti-B antibodies. This has tremendous implications in blood transfusions as a patient cannot receive erythrocytes against which he/she has antibodies. AB individuals can, in principle, receive blood from any donor; O individuals can donate blood to any individual; A and B individuals can only donate and receive blood to/from individuals belonging to the same blood group.

It is believed that ABO antibody production is stimulated when the immune system contacts in foods or in microorganisms with the saccharide antigens that are absent in the erythrocytes. The functions of the ABO blood group antigens are not known. Individuals who lack the A and B antigens are healthy, suggesting that any function the antigens have is not important, at least not in modern times.

Hemolytic disease of the newborn (HDN) is a serious medical problem that occurs almost exclusively in infants of blood group A or B who are born to group O mothers. This is because the anti-A and anti-B formed in group O individuals tend to be of the IgG type, which can cross the placenta. HDN tends to be relatively mild mainly because fetal erythrocytes do not express adult levels of A and B antigens. However, the precise severity of HDN cannot be predicted.
Fig. 3.24  Saccharides combine into very diverse structures. (a) A combination of two amino acids generates one single dimer, but there are several ways that two monosaccharides can combine to form a disaccharide. Saccharides are better suited to form highly specific structures at the surface of (b) cell membranes or (c) proteins. (b) Saccharide tags are covalently bound to lipids, usually rigid lipids such as ceramide for a better anchoring to the membrane. Glycolipids (i.e., associations of saccharides and lipids) determine blood groups, for instance (see Box 3.5). (c) The same principle applies to oligosaccharides attached to proteins, i.e., glycoproteins. The side chain of the amino acid asparagine may react with a saccharide by dehydration forming an N-glycosidic bond (analogous to an O-glycosidic bond but involving N instead of O). Likewise, the side chain of the amino acid serine may react with a saccharide forming an O-glycosidic bond. An oligomeric sequence of saccharides attached to an hypothetical protein IgG are shown in (c) (bottom) as an example.
3.2.4 Polymers of Saccharide Conjugates: Nucleic Acids

Nucleotides that compose deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are formed by 2-deoxyribose or ribose, respectively, linked to a heterocyclic base, a purine (adenine, guanine) or a pyrimidine (cytosine and uracil or thymine), and a phosphate group attached to carbon 5 of the ribose residue. To avoid ambiguity with numbering of carbons of the heterocyclic base, the carbon numbers of the ribose and deoxyribose are identified with a prime: phosphate ester linkage occurs at C5\(^\prime\) (Fig. 3.25). The physical and chemical characteristics of the heterocyclic bases are extremely important as they are determinant for the way nucleotide polymers (nucleic acids) organize. The bases are planar, cyclic, aromatic molecules with N and O atoms able to participate in hydrogen bonding in the plane of the ring. The bases are low polarity groups poorly solvated, so both faces of the plane of the base rings will be fairly hydrophobic and thus subject to significant entropic effects.

![Fig. 3.25](image_url) Conjugation of building blocks in nucleic acids. (a) Nucleotides are formed with a heterocyclic base, purine (adenine or guanine) or pyrimidine (cytosine, uracil, or thymine), a ribose or 2-deoxyribose, and a phosphate. The phosphate group forms a phosphodiester bond in C5 and the heterocyclic base binds to C1. (b) The nucleotide deoxythymidine phosphate is shown as example. (c) A dimer of nucleotides may be formed by dehydration, which creates a phosphodiester linkage between the monomers via C5\(^\prime\) and C3\(^\prime\). In RNA X=OH, and in DNA X=H.
There are four different possible nucleotides in RNA and DNA. RNA is formed by adenosine-5′-monophosphate (AMP), guanosine-5′-monophosphate (GMP), cytidine-5′-monophosphate (CMP), and uridine-5′-monophosphate (UMP). DNA is formed by deoxyribose, which is denoted by a prefix: dAMP, dGMP, dCMP, and dTMP. dTMP stands for thymine-5′-monophosphate using deoxyribose; DNA does not contain dUMP.

Because nucleotides are phosphate monoesters, they can form additional phosphoester links to other alcohols, such as the OH groups in other nucleotides. In other words, they can polymerize by dehydration reactions. Nucleic acids are formed by phosphodiester bonds between C5′ of one nucleotide and C3′ of another nucleotide (Fig. 3.25c). The result is a linear polymer having the heterocyclic bases and the phosphate groups in opposing sides, the phosphate groups being anionic (Fig. 3.26a). Some simplified representations of nucleic acids pinpoint this characteristic (e.g., Fig. 3.26b), which remains elusive when the nucleic acid is simply represented by a sequence of letters identifying the nucleotides (T, thymine; C, cytosine; G, guanine; A, adenine; U, uracil) (Fig. 3.26c, d). By convention, nucleic acid sequence is written from the C5′ to the C3′ endings, 5′ → 3′.

**Fig. 3.26** Natural polymers of nucleotides—nucleic acids. (a) Deoxyribonucleic acid (DNA) has 2-deoxyribose residues and uses thymine but not uracil. Ribonucleic acid (RNA) has ribose residues and uses uracil but not thymine. Both polymers are formed by C3′–C5′ phosphodiester bonds. (b–d) For the sake of simplicity, the chemical structure of the monomers is usually omitted, and other forms of presenting the nucleotide residues sequence are preferred. The simplest and more common form represents the nucleotides by a one-letter code (the first letter of the base name: T, A, C, or G). Which ending is the free, C5′ or C3′, is not explicitly mentioned, but it is established by convention that the sequences are presented in the sense 5′ to 3′.
Fig. 3.27 The detailed structure of nucleic acids. (a) Heterocyclic bases are flat. At the edges of heterocyclic bases, in the plane of the rings, hydrogen bonding may occur. Purines and pyrimidines fit each other, as in pairs T–A and C–G, which is known as Watson–Crick base pairing. Because bases are so flat, relatively hydrophobic on both sides, and undergo base pairing, nucleic acids may bind complementary sequences of nucleotides in the same polymer or from a different polymer. The entropic effect will cause this arrangement to twist around its long axis forming a double helix in which the polar parts of the molecule, phosphate and pentose residues, are exposed to the aqueous medium shielding the relatively hydrophobic bases. In the center of this helix, the bases stack parallel to each other and are slightly rotated relatively to each other. (b) This kind of organization can be found even in some domains of the transfer RNAs (PDB 2TRA). The OH groups present in C2′ groups of RNA (asterisk in c, right) but not DNA (c, left) have structural implications in the conformation of nucleic acids as these groups contribute to shield the core of the double strands from aqueous environment. (c) DNA forms a more stable and regular double helix because it lacks the OH group in C2′. (Panel c was reproduced from Goodsell, The Machinery of Life, 2009)
Unlike polysaccharides, nucleic acids are amphiphilic molecules (Fig. 3.26), so the entropic effect will be a significant driving force for folding in aqueous environment. The heterocyclic bases will tend to nucleate to minimize the contact with water molecules. The crystal structure of transfer RNA (tRNA) shows that the bases stack parallel to each other, which is favored by their strictly planar structure. In addition, the nucleic acid tends to twist along its major axis forming a helix that exposes the phosphates to the aqueous medium and has the base stacks in its core. In addition, most of the helical regions in tRNA consist of two sequences of the RNA chain running in opposite directions with bases in opposite sequences contacting each other close enough and with the adequate stereochemical arrangement to establish hydrogen bonding between them. This adequate arrangement only occurs if purines pair with pyrimidines, as in pairs A–U and G–C (Fig. 3.27). This is known as Watson–Crick base pairing. Hydrogen bonding occurs at the edges of heterocyclic bases, in the plane of the rings.

In regions in which the two opposing antiparallel sequences of tRNA have a considerable array of complementary base pairs, both RNA sequences fold into a helical structure to keep the parallel stacked base pairs in the core surrounded by the pentose and phosphate ester backbone. This is the double-helix structure frequently associated with DNA but equally present in RNA. Ribosomal RNA structure is similar to that of tRNA (Fig. 3.27). It is also worth stressing that DNA polymers may have complementary RNA polymers, which may associate and fold into helices. However, the alcohol group, OH, at C2′ makes the structure of RNA less compact due to its volume and polarity.

The structure of RNA is not only less compact, but it is also less chemically stable. The OH group at C2′ is close to the phosphate diester bond with which it can react to hydrolyze RNA (Fig. 3.28). Because RNA molecules have transient functionalities and are not stored for very long periods in the cells, this limitation of RNA is not a problem. DNA is less prone to hydrolysis because it lacks the OH group in C2′, being the molecule that natural evolution selected to store genetic information for longer periods.

Fig. 3.28  The chemical stability of nucleic acids. Intramolecular hydrolysis of the phosphodiester bonds of RNA caused by a base (top). The absence of a hydroxyl group at C2′ increases the hydrolytic stability of DNA relative to RNA (bottom shaded structures)
Some drugs target DNA taking advantage from the parallel stacking of heterocyclic bases. Notably, most of these molecules are composed of hydrophobic planar heterocyclic groups able to intercalate the base pairs of DNA (Fig. 3.29). Some of these molecules are used in oncology because they prevent cell multiplication.

**Fig. 3.29** Examples of nucleic acids-targeted drugs. (a) Actinomycin D is an antibiotic with anticancer activity. It binds DNA because it has a flat polycyclic and relatively hydrophobic group able to intercalate the stacked bases of DNA, preventing RNA synthesis. (b) Adriamycin is also an anticancer drug that operates with the same mechanism of action: intercalation of a polycyclic flat hydrophobic group between the base pairs of DNA, preventing cell proliferation. (b) Mitomycin C has a different mechanism of action: it is a DNA cross-linker by covalently linking two guanines. The direct contact with these nucleotide residues is possible because this drug is a polycyclic flat and relatively hydrophobic molecule.
3.3 Amino Acids and Their Polymers: Peptides and Proteins

Chemically speaking, amino acids are molecules that simultaneously have carboxyl (-COOH) and amine (–NH₂) groups. Biochemists focus on α-amino acids, in which these groups are bound to the same terminal carbon (so-called α-carbon in older organic chemistry nomenclatures), because naturally occurring proteins are polymers of α-amino acids. These amino acids have the structure depicted in Fig. 3.30. Besides the amino and carboxylic acid groups, the α-carbon (also named central carbon) attaches to a hydrogen atom and another group, so-called lateral chain and represented by R. In nature, R is one of the 20 possible groups with few exceptions that are usually derivatives of these groups.

Another interesting peculiarity of naturally occurring amino acids besides being α-amino acids is that they are almost exclusively L-enantiomers as the α-carbons are chiral centers. The other enantiomer is named D. The L and D nomenclature for the stereochemistry of the amino acids was established by Emil Fisher in analogy with glyceraldehyde, which also has a single chiral center with two possible enantiomers (see Sect. 3.2). Another nomenclature, more complex and following modern rules, exists to describe the stereochemistry of amino acids, but the predominance of L-amino acids and the simplicity of the L vs. D system resulted in the long-term longevity and universality of this system.

One simple empirical rule to distinguish L- from D-enantiomers is to adopt the perspective of the chemical structure of the amino acid along the H-αC axis (Fig. 3.31). The groups COOH, NH₂, and H appear projected as the vertices of a triangle. You can now recognize “CORN” written clockwise, in L-enantiomers, or counterclockwise, in D-enantiomers. This is the CORN rule of thumb.

The chemical nature of the lateral chain, R, is determinant for biochemical processes in which amino acids participate and for the structure that proteins adopt when such amino acids are present. Broadly speaking, amino acids can be grouped in four different categories based on polarity and acidic/basic nature of R (Fig. 3.32): acidic, basic, neutral polar, and neutral non-polar. Other classification systems are based on the chemical nature of R: hydrocarbons, carboxylic acids, amides (–CONH₂), acyclic nitrogen-containing, hydroxyl, sulfur-containing, and nitrogen heterocycles. Figure 3.32 includes grouping of the amino acids according to polar-
Fig. 3.31  The \(l\) vs. \(d\) nomenclature revealed by the CORN rule of thumb. When \(R = H\) (this happens in glycine, the simplest amino acid), chirality does not exist as two equal substituents (\(H\), in this case) are attached to the central carbon. The example of \(l\)- and \(d\)-alanine is presented, highlighting they are mirror images.

Chirality and charge and shows the chemical nature of the side chains. Table 3.3 clarifies the relationship between the names of amino acids in extent and the three-letter and one-letter code abbreviated nomenclature. It also summarizes the most relevant properties of amino acids.

![Periodic Chart of Amino Acids](image)

Fig. 3.32  Periodic chart-like arrangement of the natural amino acids. (Figure reprinted with the permission of Bachem, Bubendorf, Switzerland)
Table 3.3  Natural amino acid nomenclature (three-letter and one-letter code) and main properties

| Nomenclature rationale for 1-letter code | Amino acid | 3-letter code | 1-letter code | Main properties |
|-----------------------------------------|------------|---------------|---------------|----------------|
| First letter is unique                  | Cysteine   | Cys           | C             | Thiol side chain susceptible to oxidization to form disulfides. |
|                                        | Histidine  | His           | H             | Essential amino acid with imidazole side chain. The imidazole side chain has a $pK_a$ of approximately 6.0, which implies that relatively small shifts in most frequent physiologically relevant pH values will change its average charge, which in turn may impact significantly on protein structure. |
|                                        | Isoleucine | Ile           | I             | Essential amino acid isomer of leucine. Chiral side chain. |
|                                        | Methionine | Met           | M             | Side chain possesses a S-methyl thioether, which may be a source of sulphur for cartilage healing. It has been suggested that Met is able to strengthen the structure of hair and nails because its side chains may cross react. |
|                                        | Serine     | Ser           | S             | Residues of Ser are found in some phospholipids (besides proteins). |
|                                        | Valine     | Val           | V             | Essential amino acid. Like Leu and Ile, Val is a branched-chain amino acid. |
| First letter not unique. Most frequent amino acids have priority. | Alanine    | Ala           | A             | D-Ala occurs in bacterial cell walls and in some peptide antibiotics. Side chain is very small (methyl group). |
|                                        | Glycine    | Gly           | G             | Side chain consists in H, making Gly the only achiral and the smallest possible amino acid. |
|                                        | Leucine    | Leu           | L             | Essential branched-chain amino acid. |
| First letter not unique and less frequent: letter with phonetic similarity or side chain chemical nature | Proline | Pro | P |
|---|---|---|---|
| Arginine ArgR | The guanidium group in the side chain is positively charged at physiological pH ranges therefore prone to binding negatively charged groups. This group has also the ability to form multiple H-bonds. |
| Asparagine AsnN | Its side chain is curiously an amide (like in peptide bonds). Owes its name to asparagus because it was first detected in asparagus juice. |
| Aspartate AspD | Essential amino acid with a benzyl side chain, which makes it fluorescent and neutral. |
| Glutamate GluE | In addition to its role in proteins and amino acid metabolism, in neurosciences Glu is a very relevant neurotransmitter. |
| Glutamine GlnQ | Its side-chain is curiously an amide (like in peptide bonds) formed by replacing the side-chain hydroxyl of Glu with an amine functional group. |
| Phenylalanine PheF | Essential amino acid with a benzyl side chain, which makes it fluorescent and neutral in proteins. |
| Threonine ThrT | Essential amino acid. Chiral side chain. The hydroxyl group in the side chain is prone to glycosylation and phosphorylation. |
| Tyrosine     | Tyr | Y   |
|--------------|-----|-----|
| Tyr has a phenol group in the side chain, which makes it fluorescent. More importantly, the phenol group functions as a receiver of phosphate mediated by protein kinases (so-called tyrosine kinases) resulting in alterations on the activity of the target protein. |

| Tryptophan (side chain with double ring) | Trp | W   |
|-----------------------------------------|-----|-----|
| Essential amino acid having a fluorescent indole functional group in the side chain. The indole group is bulky and hydrophobic, so Trp is commonly found in lipid-contacting domains of proteins, such transmembranar regions of membrane proteins or fusion domains of viral proteins. |

| Lysine | Lys | K   |
|--------|-----|-----|
| Essential amino acid. Like in Arg, Lys side chain participates in hydrogen bonding and is cationic at physiological pH range, therefore prone to binding negatively charged groups. |

| Unknown (unknown amino acid) | -- | X   |
|-------------------------------|----|-----|
| Undefined amino acids in peptides or proteins structure are generically represented by X. |
It should be kept in mind that the ionization states of amino acids vary with pH, so depending on pH, amino acids may have different global charges. The example of His is presented in Fig. 3.33. His is peculiar as the side chain changes ionization (pK_{a} \sim 6) not far from the range of plasmatic and cytoplasmatic pHs. The intermediate value of the neutrality range (from pH 6 to 9.2), the so-called isoelectric point, pI, is 7.6, within the range of plasmatic and cytoplasmatic pH range, which happens only for His.

**Fig. 3.33** Schematic variation of the global charge of His with pH. The molecule has three ionizable groups, one acidic and two basic. Therefore, allowed global charges range from −1 to +2. However, in most common physiological pHs, the global charge is nearly nil. The pK_{a} corresponding to the three deprotonations with increasing pH are 1.8, 6.0 and 9.2

### 3.3.1 From Monomers to Polymers: Peptides and Proteins

Amine and carboxylic groups may react by dehydration, forming amide bonds (Fig. 3.34a). Amide bonds connecting several amino acids form a peptide. Many amino acids connected through amide bonds form a protein. There is no precise limit to separate the number of amino acid monomers in peptides and proteins although 30 is usually taken as a reference value.

Among biochemists, amide bonds forming peptides or proteins are generally referred to as peptide bonds. Because peptide bonds are very planar (C=O, C–N, and N–H bonds are coplanar) due to electron distribution limitations imposed by specific molecular orbitals, and have the R groups in close vicinity, the chain of peptide bonds forms a polymer backbone that is not freely flexible. It articulates with spatial constraints, which means that the polymeric chain tends to adopt fixed angles between its amide groups; these angles are the ones that allow accommodating the side chains of the amino acids and adapting the orientation of the amide
bonds to each other (Fig. 3.34b). As shown in Fig. 3.32, there is a wide diversity of side chains in charge, polarity, and size. All these parameters influence the way a protein folds to cope with the electrostatics, hydrogen bonding, entropic effects (hydrophobicity), and occupation of 3D space. In the end, altogether these factors determine that amino acid polymers have two different preferred kinds of conformations: α-helices and β-sheets. Many other folds exist but are not as common because these two are the ones that better suit stabilization of amino acid sequences.

**Fig. 3.34**  The conformational organization of proteins. (a) Dehydration reactions among amino acids lead to polymerization through amide (also known as “peptide”) bonds. (b) The amide bonds CO, CN, and NH bonds are coplanar because of the electronic distribution among the connected OCN set of atoms. This implies that when the polymeric chain folds, flexibility is limited, and specific arrangements tend to be adopted, which include (c) α-helices and (d) β-sheets. Whether a certain sequence of amino acids adopts the conformation of α-helix, β-sheet, or any other, depends largely on the amino acids involved, their order, and environmental factors such as solvent polarity, pH, and temperature. (c, d) Both α-helices and β-sheets are conformations that enable the occurrence of frequent intramolecular hydrogen bonding and externalize the location of side chains. Proteins may be formed almost exclusively of α-helices, such as (c, bottom right) myoglobin, or (d, bottom) β-sheets, such as porin, or be a mixture of both. (Images of porins is a courtesy of Dr. Claudio Soares, ITQB-UNL, Portugal)
In a α-helix, the peptide bond sequence (i.e., the peptide or protein “backbone”) adopts a helical structure projecting the side chains, R, to the exterior of the helix. It is a very stable structure because there are almost no constraints to spatially accommodate R and because the vast array of C=O and N–H groups in the backbone interacts strongly through frequent hydrogen bonds. Many proteins, such as myoglobin (Fig. 3.34c), are composed of several helical segments in their amino acid sequence. To facilitate protein representation and reading, helical segments are usually represented as a helical ribbon or a cylinder. This highlights the conformation of the segments, although it overlooks what specific amino acids are involved.

β-sheets are extended conformations that turn in specific points resulting in several linear amino acid residue sequences antiparallel to each other. Like in helices, this enables frequent hydrogen bonding in the protein backbone and projection on the side chain groups to the exterior of this compact arrangement. A certain degree of bending is allowed, and big extensions of β-sheets are usually associated with very stable proteic structures, such as membrane pores. β-sheet representation is usually done with straight ribbons (Fig. 3.34d).

The complete protein structure is described in three or four levels. The primary level is simply the sequence of amino acids that compose the protein, conventionally counted from the free amine terminal to the free carboxyl terminal. This elucidates the chemical nature of the protein but tells us little about what are the domains engaging α-helices, β-sheets, or none, which form the secondary-level structures. These secondary-level structures tend to interact with each other toward mutual stabilization by means of electrostatic forces, hydrogen bonding, and entropic effect contributions (Fig. 3.35). The tertiary level arises therefrom: α-helices, β-sheets, and other local arrangements that organize in space to form the protein structure.

**Fig. 3.35** Intramolecular forces that contribute to stabilize protein structures. Secondary-level structures such as helices interact intramolecularly or intermolecularly through electrostatic forces (1), hydrogen bonding (2) or entropic effect, which determines exposure of polar groups such as –COO− and –NH3+ to aqueous solvent and association of hydrophobic groups with minimal exposure to the aqueous environment (3). Two Cys residues in contact may react through the thiol groups (–SH) in the side chains forming disulfide bonds (S–S) that strongly contribute to the structure of proteins (see as an example the structure of insulin in Fig. 3.36)
itself. Occasionally, there are different parts of the global geometry of the protein that form fairly independent and separable parts, frequently having specific dynamics and specific functions. These are known as domains. An upper level exists for proteins that associate with other proteins, equal or not, to form organized protein assemblies: the quaternary-level structure. The different levels for protein structure are illustrated in Fig. 3.36, using as example insulin, whose structure was discovered by Dorothy Hodgkin, who also discovered the structure of cholesterol (see Fig. 2.10).

![Fig. 3.36](image)

**Fig. 3.36** The hormone insulin, from primary- to quaternary-level structure. (a) Amino acid (three-letter code) sequence, the primary-level structure. (Note that insulin is formed by two different chains covalently connected to each other.) (b) Segments engaging helical secondary-level structure are represented as helical ribbons. (c) The protein folds into a tertiary-level structure that is stabilized by disulfide bonds (yellow in the protein structure). Disulfide bonds are the result of oxidation of two thiol (−SH) groups to form a S–S bond (e). It is common that Cys residues react this way in proteins. (d) Six insulin monomers associate forming a homohexamer, the quaternary-level structure. The quaternary-level structure is stabilized by the presence of two zinc ions (central sphere) and due to contacts between hydrophobic surfaces of monomers (entropic effect). Insulin is stored in the pancreatic beta cells and secreted into the bloodstream in the form of aggregates of these compact hexamers. Upon dilution in the blood, insulin dissociates and the active form is believed to be the monomer.
Hydrogen bonding is frequently the strongest non-covalent factor in keeping the tertiary and quaternary levels of the structure of proteins. Enolase is a good example. Although there are no covalent bonds between both proteins in the dimer, hydrogen bonds are frequent (Fig. 3.37). Altogether, the sum of all hydrogen bonds creates a strong network of adhesion forces in the contact surface of the proteins. Hydrogen bonds are directional; they occur in a well-defined direction between chemical groups at a definite distance; this further contributes to maintain the structure of proteins. The extreme contribution of hydrogen bonding to polymer structure may not be intuitive, but one should bear in mind that Kevlar, an extremely resistant material used in protective items such as bulletproof vests, owes its properties in part to hydrogen bonding (Fig. 3.37).

![Fig. 3.37](image)

**Fig. 3.37** Examples of hydrogen bonding contribution to polymer structure. (a) Enzyme enolase (PDB 1IYX) is a dimer in which both subunits are attached by a dense array of hydrogen bonds in the contact surface between them. (b) The structure of an amide polymer (such as proteins) commercially known as Kevlar. It involves a dense network of hydrogen bonds, which confers high resistance, and Kevlar is used in protective materials such as helmets and bulletproof vests. Comparing the molecular-level details of Kevlar and β-sheets in proteins, there is a parallelism between the resistance of Kevlar and the extreme stability of aggregates formed by the juxtaposition of β-sheets in amyloid plaques (see Box 3.6).
Kevlar was named after its inventor, the chemist Stephanie Louise Kwolek, who had planned to attend a medical school but started a temporary job in chemistry and finally quit a medical career. The historic parallelism between artificial polymeric materials and biological molecules dates back to 1920, when Hermann Staudinger proposed that rubber and other polymeric molecules such as starch, cellulose, and proteins are long chains of short repeating molecular units linked by covalent bonds, a disruptive concept at that time. Staudinger used the term macromolecule (“makromoleküle”) for the first time, a term now very popular among biochemists. Paul Flory, a chemist pioneer of the studies of three-dimensional organization of polymers and its relation to dynamics, also worked for the rubber industry during certain periods of his career. His work opened the field to structure–function relationships in macromolecular biochemistry. Flory was awarded the Nobel Prize in Chemistry in 1974 “for his fundamental achievements, both theoretical and experimental, in the physical chemistry of macromolecules.” Thinking of natural protein fabrics, such as silk and spider webs, and artificial fabrics made of nylon and other polymers helps us realize that in the molecular world, the boundaries between nature and human artifacts are very faint.

Hermann Staudinger (1881–1965), Paul Flory (1910–1985), and Stephanie Kwolek (1923–2014)

As mentioned before, there are also covalent contributions to the tertiary level of structure of proteins, namely, disulfide bonds (or “bridges”) and attachment of metal or other non-proteic groups to more than one amino acid residue. Disulfide bonds are formed by oxidation of two contacting Cys thiol (-SH) groups originating from an S–S bond between the Cys residues (cystine). Cell cytosol is a relatively strong reducing environment, and the contribution of disulfide bonds in cytosolic proteins is limited. However, in other circumstances, disulfide bridges form and are strong stabilizers of protein structure at the tertiary level. Insulin, a proteic hormone, is an example (see Fig. 3.36).

Metals can bind multiple ligands and covalently link different amino acid residues in a protein, therefore also contributing to stabilize a tertiary-level structure. Frequently, metals bind to the thiol group of Cys. In the electron transfer chain proteins, several metallic complexes are present, which in addition to chemical functions also contribute to the stability of the proteins (Fig. 3.38; see also Sect. 6.2.2).
Fig. 3.38 Contribution of metals to the structure of proteins. (a) Metal complexes, such as iron–sulfur centers, are common among the proteins of the electron transfer chain. Iron complexes with sulfur atoms but also with the thiol group of the side chain of Cys residues, resulting in stabilization of the structure of the proteins where they insert. The nitrogen atoms in the side chains of His are also prone to metallic complexation. Panel (b) shows a detail of metallic complexation in Complex IV (PDB 1OCC) of the electron transfer system (see also Sect. 6.2.3). An iron ion (red sphere) complexes simultaneously the N atoms of two His side chains (blue), stabilizing the tertiary-level structure of the protein. It also binds to a non-protein molecule, the heme a (red organic structure), which is also associated with the Complex IV.

When one refers to quaternary-level structure, one usually refers to proteins that associate with high specificity and well-defined function, such as hemoglobin, for instance. This does not include pathological cases in which aggregation of proteins leads to loss of function and increase in toxicity. Extensive tertiary-level alterations are observed when amyloid fibers form upon aggregation of proteins, or when prions trigger conformational changes of native proteins (see Box 3.6), for instance. These are referred to as protein folding diseases as folding is the expression used to comprise secondary- and tertiary-level structure altogether.

Box 3.6 Amyloids and Prions: When Misfolding Turns into Disease

The relationship between the structure and function of proteins has been one of the main focuses of modern biochemistry for decades. Mutated proteins may have important changes in their structure and may thus be defective in their function, which is not surprising. However, the knowledge that proteins without mutations can fold in diverse forms, some of them pathogenic, is recent. Protein folding is the key to important diseases such as Alzheimer’s, in which massive stacks of β-sheet-folded proteins accumulate in the brain. These stacks form plaques of insoluble protein in the extracellular tissue, which cannot be broken down by enzymes. When these plaques were found for the first time, they were described as related to saccharides and named amyloids. Although the chemical nature of the plaques is now known not to be related to saccharides, the name “amyloid” is still used, and the group of diseases is known as amyloidoses.

Amyloid plaques grow with an ordered structure forming long filaments (fibrils). There are about 20 different proteins that can act as the building blocks of these fibrils, each of which is associated with a different disease. In
so-called systemic amyloidoses, the precursors of these plaques are transported through the bloodstream from their point of origin to their point of deposition. Localized amyloidoses are of greater clinical significance, as they mainly affect the central nervous system, the extracellular matrix of which is particularly susceptible to damage.

Transmissible spongiform encephalopathies (TSEs), which include mad cow disease (bovine spongiform encephalopathy, BSE) and Creutzfeldt–Jakob disease (CJD) in humans, are forms of amyloidoses in which the diseased brain degenerates to a porous sponge-like structure. These diseases appear when human protein called prions misfold (see below figure). The human prion (named PrPc) is a component of the membrane of healthy nerve cells that may misfold in a particular way. Amazingly, the misfolded prion may induce misfolding in a neighboring prion if contact among both molecules occurs. This has the appearance of an infection-like process in which the misfolded molecule “infects” the “healthy” molecule. “Infectious” prions can be transmitted in the diet, triggering a domino effect in healthy prions.

In Alzheimer’s disease, β-amyloid plaques are formed by cleavage of the amyloid–precursor protein (APP) by two different enzymatic activities, which release peptide fragments that are 40 or 42 amino acids long. When these peptides fold into β-sheets and aggregate, fibrils are formed, surrounding neurons and causing damage. This does not happen when the same peptides fold differently. It is only in β-sheets that hydrophobic amino acids are exposed, and they rapidly bind to hydrophobic groups on other peptides due to the entropic effect. The β-sheet structure, being highly ordered, is prone to regular stacking, ultimately leading to fibrils (see below figure).
3.3.2 Structure and Function in Proteins

Proteins can adopt many different structures at the tertiary level, from extended rods to compact globules. Extended proteins may associate in fibers, and globular proteins may have flexible domains able to bind other molecules. It is clear that proteins with extended conformation, like keratin, collagen, or silk fibroin, are good to maintain the structure of tissues or biomaterials, whereas globular proteins are good to intervene in dynamical processes, which justifies why enzymes are globular proteins. While this is generally true, it should also be acknowledged that the frontier between both is not always clear. For instance, actin is a globular protein that binds other actin molecules to form quaternary-level fibers in the cytoskeleton and muscle contractile system. Actin is an example of a globular protein having structural functionality (Fig. 3.39; see also Sect. 10.1.1). Another interesting molecule that challenges the classical dichotomy of structural/extended vs. functional/globular proteins is lung elastin. When relaxed, it is a globular protein but it stretches to encompass lung expansion. Elastin molecules are interconnected covalently by the side chain of four Lys residues: cross-linked desmosin bonds (Fig. 3.39). This way, the continuous alteration between the globular and extended conformation of elastin confers to the lung the ability to expand and contract without histological lesions. To finalize, one should stress the fact that there are also proteins that have both extended and globular domains. This is the case of myosin, another central protein in muscle contraction, in which an extended domain forms oligomeric fibers (Fig. 3.39; see also Sect. 10.1.1).

Fig. 3.39 (continued) form very resistant biomaterials such as spider silk. It is important to realize that collagen has a Gly residue at every third position of its amino acid sequence. Gly is a very small amino acid because the side chain is replaced by H. This creates a line along the helix surface where two other alike helices may dock in close contact. Lys side chains are exposed in collagen and form cross-links that strength the collagen fibers. The chemical process of this cross-linking reaction between the endings of the Lys side chains depends on vitamin C (ascorbic acid). (d) Lys residues are also involved in cross-links of elastin, a connective tissue protein of unusual elasticity. The cross-links involve four Lys residues forming a desmosin arrangement. (e) Actin is a globular protein that self-associates forming fibers important in muscle contraction. (f) Myosin, on the other hand, is a combination of an extended domain with a globular domain (“head”) having catalytic activity. The extended domain associates with other proteins forming fiber-like oligomers responsible for muscle contraction (see Sect. 10.1.2).
Fig. 3.39  Typical protein structures and protein–protein interactions. Proteins such as (a) keratin and (b) collagen (PDB 1BKV) adopt string-like conformations (very extended helices) that associate to form fibers that stabilize structures like hair or nails (keratin) or the connective tissue (collagen). (c) Silk fibroin forms very extended $\beta$-sheets that associate antiparallel to each other to
Besides tight parallel packing in collagen fibers, proteins bind covalently to other proteins in different fibrils of collagen through side chains of Lys residues (Fig. 3.39). When this strong covalent meshing of collagen is disrupted, the properties of the connective tissue are very much affected causing diseases (Box 3.7). Likewise, mutations of the Gly residues impact dramatically on collagen structure and connective tissue function. This has a particular effect on bones since early age. In addition, bones lose resistance and break easily. This disease is known as osteogenesis imperfecta, Lobstein syndrome, or, more commonly, “brittle bone disease.”

**Box 3.7 Scurvy: An Example of a Pathology Directly Associated with Protein Structure**

Scurvy is a pathology characterized by fatigue, anemia, gingivitis (gum disease), and skin hemorrhages caused by diets with a prolonged deficiency of ascorbic acid (vitamin C). It was a frequent disease in sailors on long voyages in the pioneering intercontinental discoveries of the fifteenth century. Many men died until it was discovered that scurvy could be cured and prevented by consuming citrus, such as oranges, lemons, and limes. The Portuguese sailor Vasco da Gama was the first European to lead a fleet that reached India by sea, linking Europe and Asia, connecting the Atlantic and the Indic. The drama of scurvy in his first trip to India (1497–1499) is eloquently described in an epic poem by Luís de Camões, in *The Lusiads* (1572):

> And 'twas that sickness of a sore disgust,<br>the worst I ever witness'd, came and stole<br>the lives of many; and far alien dust<br>buried for aye their bones in saddest dole.<br>Who but eye-witness e'er my words could trust?<br>of such disform and dreadful manner swole<br>the mouth and gums, that grew proud flesh in foysen<br>til gangrene seemed all the blood to poysen:<br>“Gangrene that carried foul and fulsome taint,<br>spreading infection through the neighbouring air:<br>No cunning Leach aboard our navy went,<br>much less a subtle Chirurgeon was there;<br>but some whose knowledge of the craft was faint<br>strove as they could the poisoned part to pare,<br>as though 'twere dead; and here they did aright; —<br>all were Death's victims who had caught the blight.

“The Lusiads” (Canto V, 81 and 82) version reproduced here was translated to English in 1880 by Richard Burton

Nearly two thirds of the sailors of the entire fleet of Vasco da Gama died during the trip, although documents from that time clearly show that it was known empirically by Portuguese sailors that a diet based on fruits and other unprocessed foods was a treatment for scurvy. A manuscript from a pilot in

(continued)
the fleet of Pedro Álvares Cabral, discoverer of Brazil in 1500, says that a diet of fresh foods, including sheep, chicken, ducks, lemons, and oranges, was used to heal scurvy.

It was only in the eighteenth century that the Scottish doctor James Lind related scurvy to diets poor in citrus on a reasonably scientific way. Ascorbic acid was discovered by the biochemist Albert Szent-Györgyi (born Hungarian, later US citizen), who was awarded the Nobel Prize in Medicine or Physiology in 1937 (Szent-Györgyi also performed important studies on muscle contraction; see Box 10.1 in Chap. 10). Ascorbic acid is part of several biochemical pathways, the synthesis of collagen being one of them. Specifically, it is mandatory in protein hydroxylation, which is a posttranslational modification in which a hydroxyl group (–OH) is added to a protein residue. Collagen is naturally hydroxylated in healthy individuals. Ascorbic acid is also mandatory in the biosynthesis of carnitine. Impaired synthesis of carnitine and collagen account for the common symptoms of scurvy.

The primary defects behind rotten or loose teeth, rigid tendons, or cartilage fragility observed in scurvy reside in the connective tissue. Without ascorbic acid, collagen is not hydroxylated and a nonfibrous, defective incomplete collagen is formed instead of fibrous collagen. The enzyme prolyl 4-hydroxylase, for instance, hydroxylates a Pro residue using an iron atom that is oxidized in the process. Ascorbic acid is needed to reduce the iron and make the enzyme active again (see below figure).

Ascorbic acid oxidation in the reaction catalyzed by prolyl 4-hydroxylase

Lysyl hydroxylases are also operative. Hydroxylation of Pro and Lys residues, both exposed in the triple helix of collagen, favors intermolecular adhesion interactions by hydrogen bonding and further chemical modifications, which are strengthened with age (this is one of the reasons why meat from young animals is more tender than from older animals).
Carnitine is involved in the transport of fatty acids into the mitochondria, where they are oxidized (see Sect. 7.4.3). Ascorbic acid is used by two different enzymes in the carnitine biosynthesis. Without ascorbic acid, production of carnitine declines and fatty acids cannot be used as energy source. This leads to fatigue, which is one of the symptoms of scurvy. Curiously, fatigue appears first than other symptoms. This may be explained by the fact that the enzymes in carnitine biosynthesis require higher concentrations of ascorbic acid to function (they have “lower affinity” for ascorbic acid) when compared to hydroxylases.

Other pathologies, such as the Ehlers–Danlos syndrome (EDS) and osteogenesis imperfecta (OI), are genetic pathologies associated with deficiencies on protein–protein interactions in collagen. A fraction of the Lys residues in collagen react with each other forming covalent cross-links in collagen fibers. In some forms of EDS, this crosslinking is impaired, rendering the skin less firm and less resistant, hyperelastic. Collagen contributes to the mechanical strength of skin, joints, muscles, ligaments, blood vessels, and visceral organs. In OI, replacement of Gly residues in the collagen amino acid residue sequence destroys the capacity of the protein to assemble in perfect triple helices because all other residues are bulkier than Gly. This leads to an extremely severe condition that is characterized by alterations in the physical properties of collagen and perturbations in the biochemical processes involving collagen homeostasis. The relationship between the collagen fibrils and hydroxyapatite crystals when bones are formed is altered, causing brittleness. For this reason, OI is also known as “brittle bone disease.”

One remarkable property of many globular proteins is the ability to both bind other molecules and change conformation upon binding. Taking adenylate cyclase as example, binding of molecules such as ATP or ADP causes a very mobile domain of the protein to change position (red domain in Fig. 3.40). This often leads to dynamic distortions of the ligand molecules because of the contact of amino acid residues. The results may be such that covalent bond is formed or broken, thus chemically transforming the ligand molecule into a product. In practice, the protein action is to increase the rate of transformation of the ligand in the product. If only the ligand, not the protein, is chemically transformed in this process, this can be seen as an enzymatic catalysis, i.e., increase of chemical reaction velocity caused by proteins, the enzymes. The ligand (reactant) is named substrate in these cases.
As discussed in the previous section, the tertiary-level structure of a protein is determined and maintained by arrays of spots in which attractive or repulsive forces between groups of atoms exist. This is a relatively delicate balance. When a significant number of such “force spots” is altered, the configuration of the protein adapts a different tertiary-level structure, which corresponds to the new balance of forces. Likewise, in cases in which a quaternary-level structure exists, the changes in the conformation of one protein monomer at the surface of contact with other monomer may impose alterations in the “force spots” (hydrogen bonds, electrostatic repulsion and attraction, entropic factors, etc.) so that the second monomer changes conformation to adapt. In practice, this means that conformational changes in one protein may be transmitted to a neighboring protein that is in contact with it. In other words, tertiary-level structural changes may be transmitted and amplified to other proteins throughout the quaternary-level structure. Hemoglobin is a good example. It is formed by two subunits, $\alpha$ and $\beta$, forming a dimer that associates with other dimer—a tetramer that is in fact a dimer of dimers. Dimers are numbered 1 and 2; therefore, hemoglobin is a tetramer of four subunits $\beta_1$, $\alpha_1$, $\beta_2$, and $\alpha_2$ (Fig. 3.41). $\alpha$–$\beta$ attractive forces are stronger than 1–2 attractive forces, but both are sufficient to transmit to neighboring monomers conformational changes. This affects the affinity of the hemoglobin monomers to oxygen. Each hemoglobin monomer is covalently associated with a non-proteic group, i.e., a prosthetic group, of the porphyrin family (Box 3.8). In this case, the porphyrin binds in its center an iron ion, forming a heme. The iron ion complexes with the heme through four bonds in the plane of the porphyrin and to a His residue side chain orthogonally to the heme plane. Other orthogonal bond, opposite to His, is established with small molecules having electron
donor atoms, such as O₂ or CO. When an O₂ molecule binds to the iron in the heme, the position of the iron slightly shifts, which in turn affects the position of the His residue. When the His residue is pulled, the whole structure of the protein changes slightly. This change in conformation induces a change in conformation of the neighboring monomers. As a consequence, the neighboring monomers acquire increased affinity to bind an oxygen molecule. So, binding of O₂ to a monomer increases the chances that a second O₂ molecule binds to another monomer in the hemoglobin tetramer relative to a monomer in a tetramer without bound O₂ molecules. This is called positive cooperativity: several entities influencing each other making more likely a certain event to occur.

Fig. 3.41 Structure-function relationships in hemoglobin. (a) Hemoglobin is a tetramer, each subunit containing a heme group (red). Human deoxyhemoglobin (left; PDB 2HHB) and oxyhemoglobin (right; PDB 1GZX) show subtle but important changes in conformation. (b, left) Binding of molecular oxygen to the heme group (PDB 1HHO) causes a (b, right) shift in the orientation of the heme relative to the His residue relative to the heme in deoxyhemoglobin (PDB 4HHB). This slight distortion in the position of the heme leads to a variation in the conformation of the protein, which propagates to neighboring monomers in the tetramer. (c) The neighboring monomers acquire higher affinity for O₂.
Box 3.8 The Importance of Heme Groups in Proteins
Many natural proteins are associated with prosthetic groups of similar chemical nature called porphyrins. Porphyrins are macrocyclic compounds related to porphin (see below figure). Hemoglobins, for instance, bind porphyrin groups, such as heme B (see below figure). Hemes are porphyrins that bind iron ions in the center. The remarkable capacity to bind metallic ions of charge +2 or +3 in the center of the ring may explain the success of these molecules during natural selection and subsequent ubiquity of porphyrins in nature. The porphyrin macrocycle has 26 delocalized ($\pi$) electrons in total, being classified as aromatic from a chemical point of view. This system of delocalized electrons extends to the nitrogen atoms and is available to bind the cationic metals. It is also responsible for the intense absorption bands in the visible region of electromagnetic radiation. This is the reason why compounds with porphyrins, such as hemoglobin and chlorophyll, are deeply colored. Heme-containing proteins, hemoproteins, and other metal-containing proteins, metallo-proteins, due to their unique electronic properties, are adequate for the transient binding of diatomic gases that occurs during their transportation in blood and electron transfer (i.e., electron donation and reception to and from other compounds). It is possible that hemoproteins evolved from ancient proteic forms, whose function was electron transfer in sulfur-based photosynthesis in the ancestors of cyanobacteria before molecular oxygen existed in atmosphere.

Chemical structure of porphyrins. Porphyrins, such as heme B (bottom), are macrocyclic compounds related to porphin (top)
In addition to the peculiar intrinsic properties of hemes and other porphyrin–metal associations, the interaction between the porphyrin and the amino acid residues of the site where it inserts in proteins is of extreme importance. Variations in the shape, volume, and chemical composition of the binding site, in the mode of heme binding and in the number and nature of heme–protein interactions, result in significantly different heme environments in proteins having different biological roles. The outcome is a fine-tuning of the heme properties. Take the 3D structure of the hemoglobin amino acids’ residues sequences as example. The position of a His residue is such that its protonation interferes with oxygen release from the iron ion. Acidification of the medium causes the protonation of His, which in turn facilitates the release of oxygen. This is known as Bohr effect and is not a simple curiosity: in the pulmonary vasculature, the pH is higher than in the peripheral tissues because the pH is affected by the local abundance of CO2; therefore, the Bohr effect helps in increasing the efficacy of binding oxygen in lungs and releasing in peripheral tissues.

The porphyrin groups are equally important to stabilize protein structure and resistance to proteolysis, although these properties are frequently overlooked. Even in cases in which the porphyrins are not covalently bound to the protein, the interplay between amino acid residues and the non-proteic groups is very specific.

Hemoglobin monomers interact cooperatively to bind up to four oxygen molecules. Myoglobin, a protein abundant in muscles, also binds O2, but this protein occurs as a monomer, in contrast to hemoglobin. Comparing hemoglobin to myoglobin makes the effect of cooperativity clear. The fraction of myoglobin binding O2 relative to total myoglobin increases nearly linearly up to saturation. To be more precise, the variation is hyperbolic. In contrast, hemoglobin binds O2 critically at a triggering narrow concentration range, in which it reaches saturation. Hemoglobin changes from highly unsaturated to almost saturated in a narrow O2 partial pressure interval. Interestingly, the narrow interval of transition to near saturation corresponds to the partial pressure of oxygen found in peripheral tissues (Fig. 3.42), away from lung alveoli. Therefore, cooperativity among the hemoglobin monomers enables that hemoglobin saturates with oxygen in the lungs and delivers its cargo in peripheral tissues. Myoglobin would not be adequate for this function as it is almost saturated in both situations. Myoglobin is fit for oxygen storage in muscle cells. Release of oxygen occurs when the consumption in mitochondria is such that the cell is almost depleted in oxygen (Fig. 3.42).
Fig. 3.42 Bridging oxygen-binding biochemistry to physiology. (a) Myoglobin is a monomeric oxygen-binding protein. Like a hemoglobin monomer, it also binds oxygen through a heme group (red organic structure). (b) The absence of cooperativity in myoglobin when compared to hemoglobin implies distinct binding capacities at different oxygen partial pressures, pO₂. Hemoglobin has an abrupt transition from low to high binding when pO₂ changes from values typical from peripheral tissues to values typical of lungs. (c) As the erythrocytes pass adjacent to alveoli, O₂ and CO₂ diffuse freely across arterial and lung cells driven by partial pressure gradients.
It is worth stressing that hemoglobin transports most, but not all, oxygen used in tissues. Oxygen, like carbon dioxide, is a small, hydrophilic molecule, which easily dissolves in aqueous media. Although being hydrophilic, it is very small and diffuses freely in tissues. This is the reason why cells do not need oxygen transporters or channels. The same happens with CO₂, but it has lower affinity for hemoglobin. In addition, CO₂ is converted to HCO₃⁻ that equilibrates with H₂CO₃ (see Sect. 2.1.1). Therefore, plasmatic CO₂ transport is not dependent on a specific protein.

Although the direct binding of CO₂ to hemoglobin is not significant, hemoglobin is very important in the chemistry and physiology of CO₂ in the human body as the protein itself is a weak base or weak acid depending on pH. In the peripheral tissues, in which CO₂ is present at higher partial pressure, CO₂ diffuses to plasma and therefore in erythrocytes. Carbonic anhydrase then converts CO₂ to H₂CO₃ that acidifies the medium. Acid pH leads to the protonation of hemoglobin, which has lower affinity for O₂. Near the lung alveoli, plasmatic CO₂ diffuses to the alveoli due to the gradient in the partial pressure of CO₂ (Fig. 3.42). This drop in plasmatic partial pressure of CO₂ causes carbonic anhydrase to convert H₂CO₃ in CO₂, therefore shifting the equilibrium \( \text{HCO}_3^-/\text{H}_2\text{CO}_3 \) toward the consumption of \( \text{HCO}_3^- \) and \( \text{H}^+ \). The slight drop in pH causes the deprotonation of protonated hemoglobin, which has higher affinity for oxygen. Thus, there is a coupling between pH and the efficiency of oxygen capture, transport, and release (Fig. 3.43). The coupling of hemoglobin structure with pH is known as the “Bohr effect.”

![Bohr effect diagram](image-url)

**Fig. 3.43** The Bohr effect associated with hemoglobin. CO₂ levels in blood influence the transport of O₂ by hemoglobin through plasma pH because protonation/deprotonation of hemoglobin affects cooperativity in O₂ binding.
The same way oxygen binding, transport, and release by hemoglobin is affected by pH, it is also affected by binding of 2,3-bisphosphoglycerate (2,3-BPG) in the interface between monomers. 2,3-BPG binding to deoxyhemoglobin affects oxygen fixation in a way such that the binding curve of oxygen by hemoglobin is shifted toward higher partial pressures of oxygen (Fig. 3.44). This is far from being a simple curiosity: 2,3-BPG forms from 1,3-BPG, a metabolite of glycolysis (see Sect. 6.1.3). When glycolysis is highly active, 2,3-BPG is formed, and release of O$_2$ from hemoglobin is more effective, which is convenient for the cell as higher glycolytic activity implies, in principle, a higher demand of oxygen for the human cells. The fixation of oxygen in lungs remains unaffected.

**Fig. 3.44** Coupling glycolysis to oxygen transport. (a) 2,3-BPG (2,3-bisphosphoglycerate) forms from 1,3-bisphosphoglycerate, an intermediate metabolite of glycolysis; it is therefore a chemical signal of glycolytic activity. (b) 2,3-BPG binding to hemoglobin affects cooperativity in such a way that the O$_2$ binding curves are shifted in a way that O$_2$ release in peripheral tissues is facilitated but the O$_2$ capture in the lungs is not affected.

### 3.3.4 Enzymes

The previous section showed how dynamic the binding of proteins to other molecules may be. In the case of hemoglobin, oxygen binds to the iron of the heme group, but it is common that more complex molecules bind directly to side chains of certain amino acid residues. Specific sets of amino acid residues in a protein may precisely locate and orient in space and have the correct physical properties (charge, polarity, hydrogen donor/acceptor groups, etc.) to specifically bind molecules that establish attractive forces with them (Fig. 3.45). The electronic clouds of these molecules are distorted by the contact with the amino acid residues, which in turn adapt their tertiary-level structure to the presence of the molecules. This mutual adaptation between protein and bound molecules frequently weakens some chemical bounds of the molecules, which may be destroyed. Likewise, formation of other
bonds is possible. The result is that the molecule that bound to the protein is converted in a different molecule. If the resulting product unbinds from the protein and the protein returns to the same state as before binding the initial molecule, then the protein is an enzyme, i.e., a proteic natural catalyst, and the initial molecule that binds the enzyme to undergo a chemical reaction is said to be a substrate, as previously mentioned in Sect. 3.3.2.

Fig. 3.45 Determinants of substrate specificity in enzymes—an example. (a) Malate dehydrogenase (PDB 2DFD) is a homodimeric enzyme that catalyzes the oxidation of malate to oxaloacetate concomitantly to the reduction of NAD⁺ to NADH (highlighted in orange). (b) A specific set of amino acids of the enzyme has the right properties (charge, H-binding ability, etc.), the right location and the right orientation to simultaneously fix the malate molecule. This set of residues form the so-called active site (or active center) of the enzyme. NAD⁺ binds to other site, specific for it, in close vicinity to the active site and participates in the oxidation of malate facilitated by the action of the amino acids (bottom right structure)

Because the enzymes interact with the substrate and facilitate its conversion to products, they increase the velocity of chemical reactions enormously, typically, above 10⁷-fold. In some cases, the increase may be 10¹⁷-fold, which is a figure difficult to realize intuitively. Considering that a 2 × 10⁸-fold increase in the velocity of a relaxed walk (~1.5 ms⁻¹) would leave us traveling at the speed of light (~3 × 10⁸ ms⁻¹), this intuitive perception becomes more clear. 10¹⁷-fold is more than the difference between a relaxed walk and 100 million faster than the speed of light in vacuum. One will see in Chap. 4 that enzymes accelerate reactions but cannot turn impossible reactions in possible reactions. However, enzymes turn very slow reactions (so slow that in practice reactions are as if they would not be able to occur) into fast reactions. So, in practice it is almost like if an impossible reaction was transformed into a possible reaction by the intervention of an enzyme.

3.3.4.1 The Importance of Studying Enzymes

Enzymes are interesting molecules because the dynamics of their tertiary-level structure implies catalytic activity, which is amazing and shaped life as it exists today. Even viruses need enzymes to be effective. Because enzymes are so profi-
cient in speeding reactions, controlling the activity of enzymes is, in practice, controlling the course of chemical reactions in a cell. This is an essential piece to impose order in the chemistry of the cells as controlling the activity of enzymes assures that certain reactions only occur to significant extent when and where the enzyme is inside the cell. This prevents conflicting reactions in a regulated cell and enables that certain reactions can be coupled. Imagine substrate A and substrate B; now imagine that enzyme $E_A$ converts A in B and a second enzyme, $E_B$, converts B in C. The simultaneous presence of both enzymes in the same cell compartment has the practical consequence that A is converted in C. This coupling of reactions may reach considerable complexity, with many substrates, reactions, and enzymes being involved, sometimes with branched and cyclic reaction sequences (recall Figs. 1.3 and 1.4). Such sets of reactions are referred to generally as “metabolisms.” Regulation of metabolism is largely dependent on enzymes. The mechanisms of metabolic regulation are extremely important and will be addressed in Chap. 5. A regulated metabolism is a sine qua non condition for the state of organisms we call “health.”

Yet, the structure–activity relationship in enzymes and their significance in metabolic regulation are only part of the importance of studying enzymes. Enzymes can operate outside cells and be used in industrial processes in pharma, food, or detergent processing and manufacturing, for instance. More importantly, in biomedical sciences and clinical practice, they can be used as valuable diagnosis. When enzymes that were supposed to be confined in cellular compartments in specific tissues are found with increased levels in plasma, this is a sign of tissue lesions with rupture of cell membranes (and consequent leakage of enzymes to the plasma). The death of cells in tissues implies a constant flow of intracellular contents to the plasma, but in non-injured tissues, this occurs with a very limited extent. A severe lesion in the liver, heart, or other organ leads to unusually high increased levels in the plasma of enzymes that are specific of that organ. Prostatic-specific acid phosphatase (PSAP, Fig. 3.46), for instance, is an enzyme produced by the prostate and can be found in high amounts in the blood of men who have prostate cancer. A short number of other diseases cause moderate increased levels of PSAP, but only direct lesion of the prostate such as in tumors in this organ causes very increased levels of the protein in plasma. PSAP is then classified as a marker of prostate cancer.

Being highly irrigated (see Box 8.1), and particularly exposed to the action of drugs and other exogenous chemicals, and viruses, the liver is an organ that suffers frequent insults that lead to the presence of hepatic enzymes in plasma. Two transaminases, alanine transaminase (ALT) and aspartate transaminase (AST), are markers of lesions frequently assayed in blood samples when hepatitis, poisoning, or alcoholic liver disease is suspected. However, one should bear in mind that these enzymes are also present in other organs, albeit in small concentrations. A full diagnosis is composed of data that takes into consideration not only biochemical analysis but also the symptoms, the history, lifestyle, and other diagnostic results.
The enzymes lactate dehydrogenase (LDH) and creatine kinase (CK) are of particular interest because they are markers of heart muscle lesion. Although these enzymes also exist in muscles other than the heart, there are differences in the amino acid composition that enable detection of the heart variants. Enzymes with similar activity and extensive structure homology are known as isoenzymes. For instance, three CK isoenzymes have been discovered: CK-MM or CK3, found mostly in skeletal muscle; CK-BM or CK2, found mostly in myocardium; and CK-BB or CK1, which is concentrated in lungs and the brain. Because of this distribution of CK isoenzymes, a pulmonary embolism is associated with elevated levels of CK-BB. On the other hand, an acute myocardial infarction is associated with elevated levels of CK-MB, and injuries of the skeletal muscle cause elevated levels of CK-MM. LDH isoenzymes are tetramers, both in the heart and other muscles. These tetramers may disassemble and reassemble in the form of mixed heterogeneous tetramers because the structure of the monomers is very similar. In any case, the presence of dominant heart isoenzymes can be detected in plasma in case of myocardial infarction. The plasma enzyme changes in acute myocardial infarction are shown in Fig. 3.47. CK-MB isoenzyme peaks first, AST next, and LDH last.

There are also nonenzymatic markers that are used in the diagnosis of acute myocardial infarction: myoglobin and two cardiac troponins, troponin I (cTnI) and troponin T (cTnT). CK-MB and the heart LDH isoenzyme are the most important for their heart specificity. Cardiac troponins are also important as their serum levels are frequently elevated during the first hours of acute myocardial infarction, even at a time when CK and CK-MB activities are still within the reference range but are not as consecrated as the enzymatic markers.

Fig. 3.46 A marker of prostate cancer. (a) Human prostatic-specific acid phosphatase, PSAP (PDB 1cvi), and (b) aspartate aminotransferase, ASP (PDB 3hi0), are markers of prostate cancer and hepatitis, respectively. Pyridoxal 5-phosphate is a cofactor that is shown bound to ASP (yellow)
Fig. 3.47 Biomarkers for lesions of the myocardium, the heart’s muscle. (a) Myocardium infarc-

tion involves partial tissue death (necrosis) caused by a local deficit of oxygen supply, consequence

of an obstruction of the tissue’s blood flow. (b) Cardiac muscle enzymes, such as CK-MB, AST,

and LDH, appear in the blood after the infarct. The combined information of CK-MB and LDH

allow to estimate the time of the infarct, which in turn helps devising a therapeutic strategy
While the scientific and clinical discipline of studying enzymes for direct clinical diagnosis, clinical enzymology, is expanding and gaining importance, it is curious to mention that dead brain tissue does not release into the blood any significant amounts of enzymes. Despite the frequency of cerebral infarcts (strokes), no test for brain enzymes is currently available due to the blood–brain barrier (BBB, Fig. 3.48), the network of capillaries that irrigates the central nervous system. The cells of these capillaries are connected by tight junctions and adhesion molecules that severely restrict the diffusion of hydrophilic macromolecules into the cerebrospinal fluid. Small gas molecules, such as O₂ and CO₂, diffuse passively through the barrier, and some nutrients and hormones are actively transported with specific proteins (this will be revisited, for instance, in Box 9.3, which discuss the glucose transport through the BBB).

Fig. 3.48 The blood–brain barrier (BBB). (a) The network of brain capillaries that forms the BBB conserved through plastination, a technique used to conserve anatomical structures (image reprinted with permission of von Hagens Plastination, Germany; © www.vonHagens-Plastination.com). The network of very thin arteries that penetrate the brain forms a very reticulated mesh. (b) The capillaries are associated with a thick basement membrane and astrocytic endfeet (brown cells covering the endothelium). Passage of molecules across the endothelial cells of the BBB is highly selective. Enzymes released from nerve cells upon a stroke cannot reach the blood, which is highly detrimental for diagnosis. (Image by Ben Brahim Mohammed, reproduced from Wikimedia Commons under a CC-BY license)
3.3.4.2 The Nomenclature of Enzymes

Because an enzyme is very specific for the substrate or for a small family of closely related molecules of very similar structure, it is very unique. In the early days of metabolic studies, enzymes were named individually, one at each time, with no concern for general rules of nomenclature. With time, the diversity of names and multitude of criteria to identify newly discovered enzymes was such that the lack of a nomenclature that could be used worldwide was detrimental to the progress of enzymology (the scientific discipline devoted to study enzymes). The International Union of Pure and Applied Biochemistry (IUPAB), an international organization emerging from the joint efforts of many national societies of biochemists around the globe, appointed a working group to propose general rules that could be used to classify and identify enzymes. The result was a nomenclature based on the kind of reaction catalyzed by the enzyme consisting of:

1. A name based on the contraction of “substrate + suffix ase” (e.g., urea + ase = urease, an enzyme that catalyzes a reaction with urea). This type of nomenclature has some flexibility.
2. A rigid four-number code preceded by EC (for “Enzyme Commission”), which is unique for each enzyme (or sets of isoenzymes). The numbers refer to a family of enzymes and three successive sub-families (Box 3.9). EC 5.2.1.3, for instance, identifies an enzyme from family 5 (“isomerases”—catalyzes an isomerization reaction), first sub-family 2 (“cis–trans isomerization”), and the total code identifies specifically retinal isomerase, an enzyme involved in vision (see Sect. 2.2).

Part of the whole tree of enzyme nomenclatures is presented in Box 3.9.

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Box 3.9 Enzyme Classification and Nomenclature

IUBMB (International Union of Biochemistry and Molecular Biology) is the organization responsible for recommendations on the nomenclature and classification of enzymes. Enzyme classification and strict nomenclature rules allow the unambiguous identification of enzymes. A working group, named “Enzyme Commission,” was established in 1956 to propose a universal classification and nomenclature system. Nearly 659 enzymes were known by then and the chaos in enzyme naming was clear. Nowadays, more than 5500 enzymes are known, and it would be virtually impossible to communicate in enzymology if an official universal classification and nomenclature systems had not been established.

In 1961, the Enzyme Commission presented its report, in which enzymes are divided in six classes according to the reaction they catalyze. Classes and three levels of subclasses are numbered. The Enzyme Commission thus identified enzymes through a four-number code preceded by the letters EC to clearly identify that the numeric code corresponds to the classification set by the Enzyme Commission. The Enzyme Commission itself has been renamed but the classification system is still the same. The initials EC have remained although the commission they refer to has not.

(continued)
Box 3.9 (continued)

Besides the numeric EC code, a name is also used because names are more intuitive and immediate than numeric codes. The most commonly used name for the enzyme is preferred, provided that it is unambiguous, but there are alternative systematic names that attempt to describe unambiguously the catalysis. Systematic names consist of two parts. The first contains the name of the substrate or, in the case of a bimolecular reaction, of the two substrates separated by a colon. The second part, ending in -ase, indicates the nature of the reaction, e.g., oxidoreductase, oxygenase, transferase (with a prefix indicating the nature of the group transferred), hydrolase, lyase, racemase, epimerase, isomerase, mutase, and ligase.

In practice, the enzyme classification and nomenclature stems from the classification of enzyme-catalyzed reactions, not from protein structures. A single protein may have two or more EC numbers if it catalyzes two or more reactions. This is the case, for example, for two proteins in *Escherichia coli*, each of which catalyzes the reactions both of aspartate kinase and of homoserine dehydrogenase. It may also happen that two or more proteins with no detectable evidence of homology catalyze the same reaction. For example, various different proteins catalyze the superoxide dismutase reaction and share a single EC number, EC1.15.1.1. This latter case is relatively rare, but it is almost universal that proteins catalyzing the same reaction in different organisms, or sets of isoenzymes in one organism, are homologous, with easily recognizable similarities in sequence.

Take class EC 1 of enzymes, oxidoreductases, as example. This class contains the enzymes catalyzing oxidation reactions. Since the oxidation of one group must be accompanied by the reduction of another, they are grouped together as oxidoreductases. The systematic enzyme name is in the form “donor/acceptor oxidoreductase.” The substrate that is being oxidized is regarded as being the hydrogen donor. The name is commonly “donor dehydrogenase.” Although the term reductase is sometimes used as an alternative, it is important to remember that the recommended name does not define the equilibrium position of the reaction or the net direction of flux through the enzyme in vivo. The term “donor oxidase” is used only when O₂ is the acceptor.

**Enzyme classes:**
There are six classes of enzymes:

**EC 1—Oxidoreductases** catalyze reactions in which a substrate donates one or more electrons to an electron acceptor, becoming oxidized in the process.

**EC 2—Transferases** catalyze reactions in which a chemical group is transferred from a donor substrate to an acceptor substrate.

(continued)
EC 3—Hydrolases catalyze reactions in which a bond in a substrate is hydrolyzed to produce two fragments.

EC 4—Lyases catalyze non-hydrolytic reactions in which a chemical group is removed from a substrate leaving a double bond.

EC 5—Isomerases catalyze one-substrate one-product reactions that can be regarded as isomerization reactions.

EC 6—Ligases catalyze the joining together of two or more molecules coupled to hydrolysis of ATP or an analogous molecule. These enzymes are also sometimes called synthetases, a name that was already in use before creation of the original Enzyme Commission.

In reality, all of the enzymes in classes 1–3 satisfy the definition of transferases. However, as these three classes are all large compared with the other three groups, it is convenient to break them into three classes and to reserve the name transferase for enzymes that are not oxidoreductases or hydrolases.

**Enzyme subclasses:**
Each of the six classes is divided into subclasses on the basis of the salient differences between the enzymes in the class. In EC 1, for example, the subclasses define the type of substrate acted on:

EC 1.1: Acting on the CH–OH group of donors  
EC 1.2: Acting on the aldehyde or oxo group of donors  
EC 1.19: Acting on reduced flavodoxin as donor  
EC 1.97: Other oxidoreductases

This last subclass is numbered EC 1.97 because it is provisional. In due course, the enzymes it contains may be reclassified more appropriately. The original report had two subclasses EC 1.99 and EC 1.98 that were removed when sufficient information was available to place the enzymes they contained elsewhere.

Classes EC 3–5 are divided into subclasses on the basis of types of substrate, in much the same way as in EC 1. In EC 2, however, it was more useful to emphasize the nature of the transferred group. So, for example, we have:

EC 2.1: Transferring one-carbon groups  
EC 2.2: Transferring aldehyde or ketone residues  
EC 2.3: Acyltransferases  
EC 2.8: CoA-transferases

In EC 6, the division into subclasses is made on the basis of the type of product:

EC 6.1: Forming carbon–oxygen bonds  
EC 6.2: Forming carbon–sulfur bonds  
EC 6.3: Forming carbon–nitrogen bonds

(continued)
**EC 6.4:** Forming carbon–carbon bonds  
**EC 6.5:** Forming phosphoric ester bonds

**Enzyme sub-subclasses:**  
The subclasses are divided into sub-subclasses in much the same way as the way the subclasses themselves are defined. For example, **EC 1.16** (oxidoreductases oxidizing metal ions) contains two sub-subclasses:

**EC 1.16.1:** With NAD$^+$ or NADP$^+$ as acceptor  
**EC 1.16.2:** With oxygen as acceptor

As with the numbering of subclasses, 99 (or a smaller number if necessary) is used for sub-subclasses containing a miscellaneous group of enzymes. For example, subsection **EC 1.6** contains oxidoreductases acting on NADH or NADPH, and within this, there is **EC1.6.99** for miscellaneous acceptors.

There are also sub-sub-subclasses so that each enzyme is identified by four different numbers. The division of sub-subclasses into sub-sub-subclasses follows the same rationale as before. An exhaustive visit of the fourth level of classes is not justified here.

**Final note:** Text based on “Enzyme Classification and Nomenclature” by S Boyce and K Tipton (Encyclopedia of Life Sciences, 2001) and “Current IUBMB recommendations on enzyme nomenclature and kinetics” by A, Cornish-Bowden (Perspectives in Science, 2014, 1, 74–87)

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IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) (1985) Nomenclature and symbolism for amino acids and peptides, J Biol Chem 260:14–42  
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Challenging Case 3.1: Biochemistry in Dali’s (he)art

Source

This case is based on a biographic paper by Juan Esteves de Sagrera that describes the influence of science in general, biochemistry in particular, in the work of the surrealist artist Salvador Dali: “Dali científico. Las ciencias biomédicas en la obra del pintor ampurdanés” (“Dali scientist. The biomedical sciences in the work of the painter from Ampurdania”).¹

The emblematic Dali painting “Galatea de las esferas,” from 1952, highlighted in the paper in which this case is based. (Fundación Gala-Salvador Dalí, Figueres)

Case Description

Salvador Dali was not an ordinary man. Known for his eccentricity and geniality, the artist is famous for his prolific surrealist work in painting. His inspiration for the oneiric-like landscapes and rich details in his paintings is less known but worth exploring. Surprising as it might be at first glance, science was a major inspiration for Dali, mainly chemistry, biochemistry, and psychoanalysis. While psychoanalysis would obviously inspire him for the dimensions of human nature, chemistry and biochemistry provided a repository of hidden geometries inherent to the principles of matter. With the right doses of mysticism, the organization of matter was translated into the decoding of life. The atomic implications of quantum physics and, above all, the structure of DNA had a strong fascination over Dali. DNA was regarded by Dali as the key to life. The importance of DNA in Dali’s work is such that some scholars in arts name one of his creative periods as “the DNA period.”

Dali used to read a lot and forge ideas from his readings. His personal home library had more than 100 books annotated by him on physics, quantum mechanics, the origin and evolution of life, and mathematics. He also subscribed to scientific magazines. This passionate avidity for scientific information was kept throughout his life. In 1985, for instance, at the age of 81, Dali was an active spectator of a

¹ de Sagrera JE (2004) Dali científico Las ciencias biomédicas en la obra del pintor ampurdanés. Offarm 23:122–128.
scientific meeting on culture and science that took place in his hometown. He also read *A Brief History of Time* by Stephen Hawking.

In the 1940s, Dali was influenced by the atomic nature of matter and atomic nuclear energy. “La desmaterialización de la Nariz de Nerón” (1947) is an example of this influence (see next figure). In the following decade, the 1950s, Dali became fascinated by the quantum theory and praised Werner Heisenberg, the German scientist known for the “uncertainty principle” in quantum mechanics. The paintings frequently became corpuscular as if made of atomic units (e.g., “*Galatea de las esferas*,” 1952; see previous figure).

In the 1960s, the structure of DNA dominates. Interestingly, DNA first appears in Dali’s work in 1957, only 4 years after Watson and Crick published their note on the structure of DNA, many years before the importance of DNA structure was duly recognized and became an icon. In this painting, “*La paisaje de la mariposa*,” DNA coexists with butterflies, which also happens in other representations. Butterflies in Dali’s paintings are symbols of tranquility and peacefulness. In 1971, the advertising poster of the National Congress of Biochemistry (of Spain) was painted by Dali having the structure of DNA forming Jacob’s Stairway to Heaven.

In the last period of his highly creative life, Dali develops intense interest for virtual imaging and the sense of three dimensionality. In 1970, Dali used the Fresnel lens to create holograms. Dennis Gabor was awarded with the Nobel Prize in Physics for his work with lasers and development of the holographic method in
1971. Gabor’s work was his source of inspiration, which demonstrates how Dali was up to date with scientific novelties. The painter’s main interest however was stereoscopic painting, which is simpler and closer to his art. Stereoscopy is an elegant trick to create an optical illusion having two similar paintings and a pair of mirrors forming the arrangement depicted in the next figure. This technique consists of presenting two similar images to the left and right eye of a viewer in a way that the perception of integrated vision in the brain is three-dimensional, i.e., the sum of the two flat images in the brain creates the illusion of depth. Dali painted pairs of very similar paintings, eventually with different objects and colors in the background, which when presented to the vision of each eye individually through the reflection in mirrors resulted in a virtual 3D painting. This technique is still used in 3D viewing devices to be coupled to the screen of mobile phones. Amazingly, Dali learned about stereoscopy from the work of Gerrit Dou, in the seventeenth century. This Dutch painter probably used lenses and mirrors to create his stereoscopic paintings and was helped in this task by Van Leeuwenhoek, the seminal founder of modern optical microscopy.

The basic principle of stereoscopy. Two similar images are placed adjacent to two planar mirrors in a symmetrical W-like setup. The viewer observes the images having the common edge of the mirrors in between the eyes at close distance so that each eye receives an individual image. The overlay composition of both images takes place in the brain.

Two stereoscopic paintings of Dali have important representations of DNA: “El pie de Gala” (1975–1976) and “La estructura del ADN” (1975–1976) (shown in the next figure). The intention to bring the representation of DNA closer to the scientific standard representation of macromolecules with balls and sticks models is obvious. This path that deviates Dali from artistic standards toward the language of science is regarded by many as detrimental to his artistic creation. For them, the beginning of the end for Dali was his progressive metamorphosis from a genial painter to an academic artist and a dilettante “dandy man” of science.
Questions

1. Take into consideration the painting “La estructura del ADN” (The structure of DNA). Is there a correspondence between the structure represented and the atomic-resolution structure of DNA we know today?
2. Now take into consideration the painting “La estructura del ADN” as perceived in the reflections of the stereoscopic setup. What are the differences relative to the painting?
3. Search for the 1957 painting “Paisage de mariposas” (Landscape of butterflies) in the Internet and correlate the structure of DNA as depicted with the structure proposed 4 years before by Watson and Crick.
4. In a famous painting besides “El pie de Gala,” Dali also merged two of his main inspirations: the lifetime muse and wife, Gala, and DNA. “Galacidalacidesoxyribonucleicacid” (Museum S. Petersburg, Florida, USA) was the strange title given to this painting. Imagine Gala would refer to galactose and try to represent the structure of a hypothetical nucleic acid having a galactose residue replacing the ribose residue.

Biochemical Insights

1. No. The graphics are obviously similar but there is no relation to the atomic resolution structure of DNA.
2. Reflections in the mirror result in a symmetrical but not identical structure, as is the case with enantiomers.

3. There is impressive realism in the way the blue spheres correspond to the charged phosphate backbone, and the yellow spheres’ core correspond to the bases’ residue stacking. The polar vs. nearly non-polar nature of the blue vs. yellow regions is the primordial physical reason for the double-helix folding of DNA.

4. Relative to ribose, galactose would have an extra hydrophilic and reactive OH group (see below figure) to accommodate in the polymeric structure of a nucleic acid imposing steric constraints (see figure). This is probably one of the reasons why evolution favored pentoses relative to hexoses.

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**Final Discussion**

Dali used to say about himself that he was a better writer than painter. Nevertheless, his legacy in literature is largely overlooked. Dali also felt dazzled by geometric pattern in scientific disciplines other than biochemistry. The eyes of the flies and some viruses under the electronic microscope, for instance, were fascinating for him. Yet, strangely, his most obsessive natural structure besides DNA was the rhinoceros’ horn, which he associated with logarithmic function in mathematics. Strangely, he found a similarity in rhino horns and DNA as both have spiral forms. DNA and rhino horns were the key to the composition of all beings. A strange association for biochemists, to whom rhino horns are little more than keratin. Coincidence or not, rhino horns are also believed to have miraculous pharmacologic properties in some cultures.

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**Further Reading**

“Dali y la estrutura de doble hélice”. S. Grisolia (25/04/2003) ABC, hemerotace (www.abc.es/hemeroteca/historico-25-04-2003/abc/Sociedad).
Challenging Case 3.2: From Best with Proteins to Worst with DNA

Source

This case is based on the biography of Linus Pauling, “Linus Pauling. A Natureza da Ligação Química” (“Linus Pauling. The nature of the chemical bond.”), written by the Portuguese author Raquel Gonçalves-Maia, from the University of Lisbon. ²

(Left) Cover of the book Linus Pauling. A natureza da ligação química (reproduced with permission of Dr. Raquel Gonçalves-Maia, and Edições Colibri); (right) Oil portrait by Leon Tadrick with Linus Pauling and a model of the alpha-helix, 1951. (Image provided by the Ava Helen and Linus Pauling Papers, Oregon State University Libraries)

Case Description

Biochemistry owes a lot to Linus Pauling. Not only did Linus Pauling unravel the basics of the detailed structure of helical domains of proteins, but he also was a fierce defender of the importance of biochemistry to understand life. “All the activities of living beings are chemical in nature” he said once. In fact, through a chemical vision of the world, he worked across physics, biology, pharmacology, and medicine. He also engaged activism for social causes. His merits in science and his efforts for a better society were properly recognized with the Nobel Prize in Chemistry (1954) and the Nobel Prize for Peace (1963). However, no one is perfect and even the very best can and do fail. Over-confident in his chemical knowledge and intuition, which led him to the structure of the protein helix, Linus Pauling rushed to a grossly erroneous structure of DNA. He could have been the discoverer of the DNA double helix, yet he proposed an unreasonable triple helix of inconceivable organization.

² Gonçalves-Maia R (2017) Linus Pauling. A natureza da ligação química. Edições Colibri, Lisbon. ISBN 978-989-689-692-8.
3.5.2.1 The Protein Vision

In the 1930s, several research groups were committed to unlock the mysteries of the origins of life and how cells could transmit information from generation to generation, i.e., how could cells divide and still be functional. Proteins were everywhere, so ubiquitous that they were thought by most as the molecules responsible for information storage and transmission. Pauling expertise was chemistry, yet biology was an irresistible challenge.

Pauling’s *The Nature of The Chemical Bond* was the bestselling book in science in the twentieth century and undeniably the most influential book in the history of chemistry. The book was dedicated to Gilbert Lewis, who had proposed before that chemical bonds consisted of electron sharing. In a letter of August 25, 1938, Lewis reacted positively to the book: “I have returned from a short vacation for which the only books I took were a half a dozen detective stories and yours ‘Chemical bond’. *I found yours the most exciting of the lot.*” Pauling knew the previous work of Hermann Emil Fischer (1852–1919), who discovered that proteins are formed by amino acid residues connected through amide bonds (also known as “peptide bonds” among biochemists). Pauling believed that proteins were long polypeptide chains. Others believed proteins would be formed by amorphous conglomerates of small peptides. The seminal work of William Astbury in X-ray crystallography seemed to support the long polypeptide hypothesis, but the evidence was not totally conclusive. Serendipitously, Astbury worked with wool, hair, and other materials rich in keratin and collagen, i.e., materials of almost purely aligned helices. The genius of Pauling’s chemical input consisted in deducing the structure of polypeptides from the three-dimensional arrangement of the consecutive chemical bonds in the amino acid residue sequence. He realized that the planar arrangement of the amide bond and H bonds could be the master factors in stabilizing the overall structure. In 1936, Pauling published a general theory on the structure of proteins3 together with Alfred Ezra Mirsky, a visiting professor at his lab. This structure was born out of chemical reasoning and intuition. Hard evidence was yet to come. It took about a dozen years … with World War II in between.

Working with paper models, Pauling considered it reasonable to admit proteins would form helices having 3.7 (α-helix) or 5.1 (γ-helix) amino acid residues per turn. In 1950, Pauling and Robert Bohr Corey, an X-ray crystallographer, published a landmark paper titled “Two-hydrogen spiral configurations of the polypeptide chain”,4 followed by several other papers in the scientific periodical *The Proceedings of the National Academy of Sciences* of the USA,5 commonly known as PNAS. The helical structure of proteins was finally unveiled (see next figure).

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3 Mirsky AE, Pauling L (1936) On the structure of native, denatured, and coagulated proteins. Proc Natl Acad Sci U S A 22:439–447.
4 Corey RB, Pauling L (1950) Two-hydrogen spiral configurations of the polypeptide chain. *J Am Chem Soc* 72:5349–5349.
5 Pauling L et al (1951) The structure of proteins: two hydrogen-bonded helical configurations of the polypeptide chain. Proc Natl Acad Sci U S A 37:205–211.
Pauling and Corey also demonstrated that H-bonding was the key structural main determinant of another stable protein structure: β-sheets\(^6\) (see next figure).

Globally speaking, Linus Pauling united the physical chemistry of chemical bonding to structural biochemistry of complex biological molecules first and then united molecular structure to function in biology. In this sense, he was one of the founders of modern biochemistry. He was awarded with the Nobel Prize in Chemistry for this multidisciplinary contribution. For the first time in history, a Nobel Prize was awarded for multiple contributions during a lifetime, not for a single event discovery.

\(^6\)Pauling L, Corey RB (1951) Configurations of polypeptide chains with favored orientations around single bonds: two new plated sheets. Proc Natl Acad Sci U S A 37:729–740.
The DNA Vertigo

Linus Pauling was very close to proposing the correct structure of DNA, but he failed. Not only did he not propose the correct structure, but he actually proposed a wrong structure. A cascade of capital errors dragged him to a proposal that was not compatible with his chemical knowledge and reasoning at all:

Error #1—Pauling underestimated the importance and complexity of nucleic acid polymers. Probably the fascination with proteins, with about 20 different natural amino acid monomers, and conformational plasticity, convinced him that proteins rather than nucleic acids, with only four different monomers, were responsible for “heritage” in cell division. This was a matter of intense debate in the 1940s, and Pauling was on the wrong side of the barricade.

Error #2—Pauling also underestimated the time and effort needed to deduce DNA structure from the fragile experimental evidence available. Astbury had very low-resolution X-ray diffraction data from DNA. Other researchers were ahead in this front. Maurice Wilkins and Rosalind Franklin, under the guidance of John Randall, were studying DNA thoroughly and methodically. None had the prestige of Linus Pauling, but they were focused and determined.

Error #3—Pauling wanted to be the first to publish DNA structure and rushed to a beginner’s mistake. In the last day of 1952, Pauling and Corey submitted to PNAS a manuscript titled “A proposed structure of the nucleic acids” without solid evidence for their pitch. The structure proposed consisted of a triple helix (see next figure) with the phosphate groups forming a core densely packed by a network of hydrogen bonds. The bases pointed outward.

Perspective drawing of a portion of the nucleic acid structure as proposed by Pauling in the article cited in the footnote 7 (left), showing the phosphate tetrahedral near the axis of the molecule, the β-d-ribose rings connecting the tetrahedral into chains, and the attached purine and pyrimidine rings (represented as purine rings in the drawing). On the right, a plan of the proposed nucleic acid structure having several nucleotide residues is shown.

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7 Pauling L, Corey RB (1953) A proposed structure of the nucleic acids. Proc Natl Acad Sci U S A 39:84–97.
The double-helix structure was proposed few years later, based on solid experimental evidence and generally accepted. Likewise, the hypothesis of DNA being the molecule responsible for heritable legacy became increasingly demonstrated and accepted.

Questions

1. Other researchers, contemporary to Linus Pauling, tried to resolve the structure of protein helices assuming that the number of amino acid residues per turn would be an integer. Knowing the helix structure as proposed by Pauling, what makes this assumption unrealistic?

2. The helix with 3.6 residues per turn is named α-helix and the helix with 5.1 residues per turn is named γ-helix. π-helices have 4.4 residues per turn. There is another helix structure named 3_10-helix, in which H-bonding is established between an amino acid residue and the amino acid 3 residues earlier. What variations are expected among these types of helices in packaging density, angles between residues, and side chain projection?

3. Why were protein materials such as collagen and keratin adequate to be studied by X-ray crystallography?

4. What makes the Pauling’s DNA structure unconvincing?

Biochemical Insights

1. The amino acid residues in a α-helix tilt 100° relative to each other, making 3.6 residues (i.e., 360°) per turn (near the value of 3.7 predicted by Pauling). The N–H group of a peptide bond forms an H bond with the C=O group of the fourth residue before; in symbolic terms, this is represented by \( i + 4 \rightarrow i \). The alignment between amino acid residues is dictated by H-bonding, which occurs between C=O and N–H groups. A perfect integer in all types of helices would imply that C=O would align with C=O every N (integer) residues. In addition, in the α-helix, because the number of residues per turn deviates significantly from an integer, the side chains of the residues are also not aligned; therefore steric hindrance is minimized. This stabilizes the structure and contributes to explaining why α-helices are dominant in nature relative to other helices. It is worth highlighting that α-helices are dominant in nature over 3_10- and π-helices. The γ-helix proposed by Pauling remains conceptual: it was never found in nature.

2. The π-helix is \( i + 5 \rightarrow i \) with 4.4 residues per turn and the 3_10-helix is \( i + 3 \rightarrow i \) with 3.0 residues per turn. Less residues per turn imply increasing the angle between consecutive residues. Three residues correspond to \( 360°/3 = 120° \) tilt between residues. The packing density of the helix increases, and the side chains
of every three residues are aligned linearly with steric hindrance among them, as illustrated in the below figure, in comparison with an $\alpha$-helix.

Schematic representation of an $\alpha$- and $3_{10}$-helix

3. Collagen and keratin are ordered arrays of proteins almost exclusively composed of $\alpha$-helices. They are thus homogenous structures repeated in space. This property is prone to the formation of crystals, a sine qua non condition to obtain an X-ray diffraction pattern from which structure can be deduced.

4. Phosphate groups are deprotonated even at neutral pH, which makes H bond between phosphate groups unrealistic. Also, the proposed structure has highly hydrophilic groups in the core shielded by not so hydrophilic groups, the bases, exposed to the solvent, which is also unrealistic. In addition, there is steric hindrance between the bases.

**Final Discussion**

Linus Pauling made consistent work from the theoretical chemistry he rooted in quantum mechanics to the structure of biological macromolecules. His work highlights the importance of H-bonding to protein structure but extends much further than the unveiling of the $\alpha$-helix and $\beta$-sheet. He also worked on hemoglobin pathologies and antigen–antibody structural complementarity. In this sense, he can be considered one of the founding fathers of immunochemistry. His mistake with DNA is nothing compared to the greatness of new ideas he donated to biochemistry. His beginner’s mistake with DNA is an opportunity to reflect on how strongly we should rely on the power of evidence against mere intuition, the border between self-confidence and overconfidence, and how much science is modulated by competition of scientists for reputation.
Challenging Case 3.3: London Fatberg Analysis—Impact of Urban Obesity on the Metropolitan Vasculature

Source

This case is based on the documentary “Fatberg autopsy: secrets of the sewers” (Channel 4, UK, 2018), produced by BBC studios in conjunction with the UK water and wastewater services Thames Water8, 9, which exposed the contents of one huge blockage of congealed fat, a “fatberg,” trapping daily use chemicals and other human waste in their composition, discovered underneath the streets of London. The Whitechapel fatberg, for instance, would have risked raw sewage flooding onto the streets had it not been discovered during a routine inspection. In the event of a blockage, contents of the sewers could come back up through domestic pipes causing flooding; the threat to public health would be immense. Yet, the documentary reveals another face of fatbergs. The molecular contents in the chemical analyses (cleverly named “autopsy” in the documentary), being made up of materials people flush down the toilet or pour down the sink, such as cooking oil and medicines, reveal a lot about our society: rapidly changing cooking habits and obsession with body image, for instance. The documentary was produced by BBC Studios—The Science Unit; Executive Producer: Paul Overton; Series Producer: Rob Liddell; Director: Nick Clarke Powell. Another analysis followed, this time to a fatberg in Sidmouth (UK), by researchers of the team of Professor John Love, University of Exeter (UK).

Protagonists of the documentary “Fatberg autopsy: secrets of the sewers,” Rick Edwards and Carla Valentine. (Courtesy of Thames Water)

Case Description

The study in the documentary by Channel 4 analyzed the contents of one supersized fatberg discovered underneath the streets of South Bank in Central London. Fatbergs are part of a growing urban problem across the UK as the sewage infrastructure

8(2018) Fatberg Autopsy promises ‘unforgettable sight’. Thames Waters media. https://corporate.thameswater.co.uk/media/News-releases/Fatberg-Autopsy.
9(2018) Fatberg autopsy: secrets of the sewers. Thames Waters media. https://corporate.thameswater.co.uk/Media/News-releases/Fatberg-Autopsy-Secret-of-the-Sewers.
struggles to cope with the population’s changing habits. The South Bank mass is thought to be larger than the first large-scale fatberg discovered under Whitechapel, East London, which weighed the same as 11 double decker buses and stretched the length of two football/soccer pitches. “We and other water companies are facing a constant battle to keep the nation’s sewers free from fatbergs and other blockages,” Thames Water’s waste networks manager, Alex Saunders, said.\(^\text{10}\) Fatbergs are so big that water companies are forced to employ teams of “flushers” to remove them. The filming crew of Channel 4 registered the activity of a “flushers” team while removing the South Bank fatberg, chunk by chunk, and the setup of an improvised lab big enough and adequate to manipulate fatberg chunks but having all necessary equipment for biochemical analysis and film shooting. A disused pumping station at Abbey Mills, dubbed “the cathedral of sewage,” was the elected laboratory/studio venue (see next figure). The aim was to produce a standout documentary, uncover what was making fatbergs an ever-growing problem, and reveal what that specific fatberg could tell us about the dirty secrets of the people who contributed to it.

Thames Water fatberg fighters prepared for action (left) and the laboratory/studio setup at Abbey Mills, where fatberg material was crushed into small pieces and analyzed (center and right). In “Fatberg autopsy: secrets of the sewers,” Rick Edwards, the presenter, discusses with Carla Valentine, anatomical pathology technologist, the interpretation of the “autopsy”. (Images reproduced from the Evening Standard website (left)\(^\text{11}\) and Thames Waters media (center and right)\(^\text{8,9}\). (Courtesy of Thames Water)

Almost every aspect of the production involved contact with material that should never really leave a sewer—from filming underground with Thames Water’s team of “flushers” to setting up a makeshift studio with a crew of 78, who were all kitted out head to toe in hazmat suits. Filming professionals were told by Thames Water that due to gases building up behind fatbergs, every bit of kit needed to be “intrinsically safe,” which narrowed their options to one specific model of GoPro camera, which was attached to the “flushers,” without Wi-Fi connection as it equaled another explosive hazard. The “autopsy” itself (i.e., dividing samples of fatberg mass into components and identifying the components) was a different matter; no one had ever done one before, so it was harder to plan. A decontamination tunnel was set up (it looked like something from ET). In addition, there was the risk of biological

\(^{10}\) Khomami N (2018) Fatberg ‘autopsy’ reveals growing health threat to Londoners. The Guardian. 
\(^{11}\) (2017) London’s monster fatberg FINALLY defeated after two months of ‘gut-wrenching’ work. Evening Standard website. https://www.standard.co.uk/news/london/londons-monster-fatberg-finally-defeated-after-two-months-of-gutwrenching-work-a3675866.html.
contamination with bacteria living in the sewers. Everyone entering had to put on a hazmat suit and gloves, and some had to wear face masks. Every time anyone left, for whatever reason, they had to strip off the suit, be sprayed with a bleach-like substance, and wash their hands with alcohol gel. The hurdles of documentary production are fun and enticing but we will focus on the outcome of the so-called autopsy.

The fatberg itself was a surprise. It wasn’t some gelatinous lump of fat, but solid yellow-colored matter that was so hard the “flushers” had to hack it out of the sewer with pick axes and shovels. It looked more like wax or soap than feces. Typical items found in the fatberg included condoms, sanitary towels, nappies, wet wipes, and cotton buds. But it was the in-depth analysis by the specialist team of scientists which revealed new insights into a growing urban crisis, as described in the “The Guardian” report cited in the footnote 10, and briefly transcribed with adaptations in the following text.

**Cooking Oil Forms the Matrix of the Fatberg**

The fatberg samples were analyzed. A small quantity of fats come from personal hygiene and beauty products. Topically applied creams and gels, which may contain oils and fats, can make their way into the sewer from bathing and washing. However, it is fats and grease from cooking that make up the largest proportion of the fatberg. Just under 90% of the sample is comprised of palmitic acid, commonly found in cooking oil, and oleic acid, found in olive oil (see next table).

| Trivial name and typical source | Structure | % content |
|--------------------------------|-----------|-----------|
| **Palmitic acid**<br>Palm oil, meats, and dairy products | ![Structure](image) | 80.865 |
| **Oleic acid**<br>Olive and other vegetable oils | ![Structure](image) | 5.870 |
| *trans*-Oleic acid | ![Structure](image) |
| *cis*-Oleic acid | ![Structure](image) |
| **Stearic acid**<br>Meats and lard, detergents, soaps, cosmetics | ![Structure](image) | 4.129 |

(continued)
### Trivial name and typical source

|          | Structure | % content |
|----------|-----------|-----------|
| **Myristic acid**<br>Dairy products, vegetable oils | ![Myristic acid structure](image1.png) | 3.278 |
| **Erucic acid**<br>Some plants | ![Erucic acid structure](image2.png) | 0.849<br>Identification uncertain due to low abundance |

Water companies continue to advise customers and commercial food outlets to not dispose of oils and fats down the drain.

### Cultivating Superbugs

Being rich in organic matter that may serve as growing media for microbials, the fatberg was tested for dangerous bacteria. The tests found potentially infectious bacteria including *Listeria*, *Campylobacter*, and *E. coli*. More importantly, the results discovered bacteria which were able to thrive in antibiotic environment in vitro. Antibiotic-resistant bacteria, sometimes known as “superbugs,” are a serious concern for public health. Bacteria like these pose an immediate severe risk to the operatives who work inside the sewers and the public at large could be at risk in the event of a sewer blockage, as contents of the sewers, including these harmful superbugs, could come back up through domestic or commercial pipes causing flooding to homes and businesses.

Thames Water waste networks manager Alex Saunders, quoted above, also said: “*For the sake of our sewer workers like Vince and the other guys who feature in the show please only flush the three Ps (pee, poo and toilet paper) and don’t feed the fatberg.*”

### Unraveling the Hidden Dimension of Drug (Ab)Use

The fatberg analyses uncovered evidence indicating people’s contact with street drugs and other pharmaceuticals, e.g., intact drugs-related items, including small plastic “baggies,” needles, and syringes. Presenter Rick Edwards described the finds as “*a sobering window into the lives of people living above the sewer*”10. Dr. John Wilkinson, from the University of York, collaborated with a team from Cambridge using mass spectrometry to identify different chemicals inside the fatberg. The teams analyzed the sample for traces of pharmaceutical chemicals and discovered a high proportion of salicylic acid, commonly found in analgesics and topical creams for acne, and paracetamol. The tests also discovered evidence of hordenine and
Ostarine, which is used for muscle gain, is on the World Anti-Doping Agency’s prohibited list and is not licensed for medical use in the UK. Hordenine and ostarine represented over half the proportion of pharmaceuticals found in the tested sample.

Other hard drugs found in the tested sample include cocaine, MDMA (3,4-methylenedioxymethamphetamine, commonly known as ecstasy), ketamine, morphine (which also accounts for heroin), and amphetamines (see next figure). Dr. John remarked that chemicals like these could make their way into the sewers when they aren’t broken down by the human body: “You wake up in the morning have your cup of coffee. Not all of the caffeine in that coffee you drink is going to be broken down by your body. In the case of caffeine, somewhere around 60% of that caffeine is broken down into metabolites, the remaining 40% is excreted as caffeine.” There’s no way of knowing if the chemicals have been consumed or directly flushed down the toilet, but these results revealed insights into the drugs that people interact with in modern society.

![Graph showing concentrations of various pharmaceuticals in the fatberg](image)

**Results of chemical analyses to pharmaceuticals found in the fatberg**

**Fatberg by the Sea**

A 64 m fatberg—greater in length than the Tower of Pisa—was discovered in Sidmouth, a tourist resort town situated on the English Channel coast. A team of scientists from the University of Exeter (23 km from Sidmouth) were asked to carry out an extensive “autopsy” of the fatberg to try and help solve the mystery of how it was constructed and whether it posed any environmental risks. The challenge is
well elucidated in the local university news. The scientists were given four samples from the fatberg, each weighing around 10 kg, as workers were removing it from the sewer. As in the London fatberg, the team found that the samples they received were mostly made of animal fats—consistent with domestic food preparation—combined with household hygiene products such as wet wipes and sanitary products, as well as natural and artificial fibers from toilet tissues and laundry (see next figure). Crucially, the team lead by Professor John Love found the fatberg contained no detectable levels of toxic chemicals—meaning its presence in the sewer, while increasing the risk of a blockage, did not pose a chemical or biological risk to the environment or human health. The Sidmouth fatberg was simply a lump of fat aggregated with wet wipes, sanitary towels, and other household products that should had never been put down the toilet. The microfibers found were probably from toilet tissue and laundry; the viruses and bacteria found were those normally associated with a sewer. At variance with London fatberg, the chemicals were those found in personal care products, rather than pharmaceuticals. The autopsy carried out at the University of Exeter was covered in an episode of BBC’s Blue Planet UK, broadcasted March 2019.

Questions

1. Fatty acids shown in the previous table are all long chain. Propose an explanation.
2. Morphine and heroin are both accounted for as morphine in the chemical analyses shown in the previous figure. Propose an explanation.

12 (2019) Autopsy reveals Sidmouth fatberg’s dirty secrets. University of Exeter news. https://www.exeter.ac.uk/news/research/title_756108_en.html.
3. Lipid-driven clogging of arteries is the main cause of stroke. Bridge the findings in this case with the general biochemical determinants of atherosclerosis and stroke.

4. Many toxins accumulate in the adipose tissue of humans and other animals. Bridge the findings in this case with the general biochemical determinants of toxicology associated with fast body mass losing diets (“yo-yo diets”).

5. Why is not adipose tissue commonly used in clinical biochemistry to search for toxins? Many toxins also accumulate in hair, but hair is also not commonly used to probe for toxin poisoning or drug abuse. Why?

6. Cholesterol is not part of the molecules found in fatberg. Propose an explanation.

7. Check which molecules detected in the chemical analyses shown in the previous figure can be assigned to drugs or cosmetic/hygiene products. Formulate your own opinion about illegal, prescription, and nonprescription (“over-the-counter”) drug abuse.

**Biochemical Insights**

1. Long-chain fatty acids have stronger adhesion forces among them and pack with each other easily. Their solubility is lower and they tend to have higher melting temperatures. In addition, fatty acid chains having 14–20 carbons are the most common in nature, therefore most abundant in food. Altogether, these factors (low solubility, high melting temperature, and abundance) concur to cause massive deposition of long-chain lipids in fatbergs. An additional important factor contributes to the compact nature of fatbergs: saponification. Ionized forms of fatty acids bind counterions forming salts that have the consistency of soaps. This soapy matter forms trapping other molecules inside.

2. Morphine and heroin have very similar chemical structures (see next figure). Depending on the analytical technique used to discriminate them, the separation between them may be total or not. If separation is not wide enough, it may not be possible to quantify them individually. This probably occurred in the reported study. Curiously, codeine, which is also very similar to morphine, could be discriminated. Small details in chemical structure sometimes dictate different outcomes for analytical procedures.
Chemical structures of morphine, codeine, and heroin. Morphine is an alcohol, codeine is an ether, and heroin is an ester, but the global structure is very similar.

3. Atherosclerosis is addressed in Sect. 3.1. It is a pathological condition in which deposition of lipid-rich structures (atheromatous plaques) deposit in the walls of arteries. Surface fixation of lipid-rich layers facilitates deposition of additional layers; a potential clogging site slowly forms. In the myocardium, this condition may cause a heart attack (myocardial infarction); in the brain, it may cause stroke. Both are very serious life-threatening conditions. Clogging halts blood irrigation to tissues. Without oxygen, the myocardium (see next figure) and brain cells die within minutes. Stroke and myocardial infarction are both medical emergencies. Early clinical action can minimize heart and brain damage and potential complications.

Clogging of a heart artery with lipid-rich materials may cause total interruption of blood flow and consequent supply of oxygen and nutrients to tissues. Cells die forming a lesion in the heart’s muscle. Cellular contents are released in extracellular space diffusing to local blood vessels; these molecules serve as identifiers and time course markers of heart failure (Sect. 3.3.4.1).

One important difference between lipid deposition in fatbergs and atheromatous plaques is the presence of cholesterol. Atheromatous plaques are rich in cholesterol, which is present in lipoproteins together with triacylglycerols so also depositing as lipid mixtures.

4. Hydrophobic (non-polar) molecules coexisting with lipids incorporate lipid depots when formed. This is a physically driven process dominated by entropy (see Sect. 3.1). This driving force occurs in lipid assemblies of any kind, such as the adipose tissue, which consists of lipid-loaded cells. Lipid disassembly releases the hydrophobic “contaminants.” In the case of adipose tissue, these “contaminants” are released in the bloodstream and distributed. Some of them are toxins and are released in significant amounts in case of severe dieting.
5. Lipophilic molecules accumulate in the adipose tissue for a long time. It is difficult to establish a robust correlation between toxin levels in the adipose and intake doses or time of intake. In contrast, in blood and urine, clearance makes this correlation trustworthy. “Contamination” of hair with toxic molecules or elements has the advantage of the straightforward correlation between height of contamination in hair and time of intake. Constant hair growth makes contaminants trapped in the protein structure of hair (see Sect. 2.2) deviate from the epidermis with time. Yet, a quantitative correlation between abundance in hair and intake doses is faint because the affinity of different “contaminants” to hair components is widely diverse. Moreover, hair protein assemblies (mainly keratin) are hard to untangle to efficiently extract the “contaminants.” For these reasons, despite the advantage of simplicity in handling relative to blood or urine, hair is not, in practice, a real alternative matrix in analytical biochemistry.

6. Eventually cholesterol may have not been tested. It may also have been tested but not present in sufficient amounts to be detected by the techniques that were used. Cholesterol is present in animals, therefore not present in vegetable oil such as palm oil or olive oil. Assuming the vast majority of cooking oils is from vegetables, low levels of cholesterol are not surprising.

7. Only dexpanthenol and mephedrone are clearly part of cosmetics. Salicylic acid is formed by metabolic transformation of acetylsalicylic acid (“aspirin”) in the body, but it is also an ingredient in topical anti-acne products. Theobromine is one of the products of caffeine processing in the human body and is naturally present in cocoa, therefore also present in chocolate. The relative abundance of pharmaceuticals/cosmetics in the fatberg depends on total quantity disposed and lipophilicity. So, a direct correlation between abundance in the fatberg and levels of consumption is not straightforward. Nevertheless, differences in the lipophilicity of molecules such as dexpanthenol or mephedrone and drugs in Fig. 3.6c cannot account for the huge differences in abundance; therefore, a broad conclusion on levels of consumption from abundance in the fatberg seems reasonable.

**Final Discussion**

Following the discussion of this case study, one additional topic is pertinent: the impact of domestic drug waste in the environment. A letter of Michael Depledge to *Nature* journal\(^\text{13}\) is quite eloquent:

*Low-cost pharmaceuticals are increasingly accessible to the global population, which is predicted to exceed 8 billion by 2050. Rising drug use is also driven by ageing populations. Widely used preventative medication — such as statins and anti-hypertensives — and cheap generic drugs add to the problem. The UK Office of National Statistics predicts that the country’s medicine usage*

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\(^{13}\)Depledge M (2011) Reduce drug waste in the environment. Nature 478:36.
will more than double by 2050. Agricultural soils and rivers are contaminated with a range of pharmaceuticals, including antibiotics, antidepressants, analgesics and cancer-chemotherapy agents. The effects are already evident: they include the feminization of fish by residues of the contraceptive pill, and the deaths of millions of vultures on the Indian subcontinent following ingestion of the anti-inflammatory drug diclofenac. Antibiotic overuse has led to the emergence of resistant pathogenic bacteria in the wider environment, and not just in medical settings.

Expired or unneeded drugs that are flushed unused down the toilet are not the biggest issue. Massive quantities of metabolites resulting from drug metabolization (i.e., chemical processing in the body) are excreted in the urine. Wastewater treatment plants are not always well equipped to deal with all kinds of pharmaceuticals and pharmaceutical by-products present in household sewage.

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14 European Environment Agency (2005) EEA technical report No 1. https://www.eea.europa.eu/publications/technical_report_2005_1/file.

15 Scudellare M (2015) Drugging the environment. The Scientist, p 23–28.