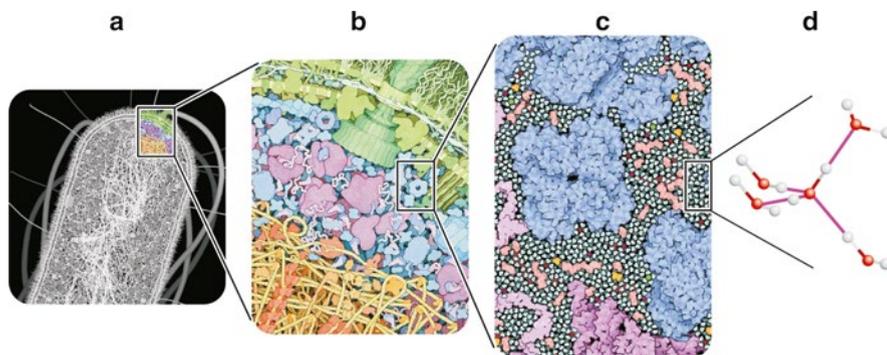


## Chapter 2

# The Chemistry and Physics of Life

Our idea of the interior of a cell at the molecular scale is often rather naïf. If one could see the interior of a cell with molecular resolution, one would not see an aqueous solution of molecules with the cellular organelles suspended. The molecular crowding, in particular the macromolecular crowding, inside a cell is such that the interior of a cell is more like a gel than a solution. Molecular packing is so dense that it is hard for macromolecules to diffuse freely. The ubiquitous presence of the cytoskeleton and macromolecular assemblies in a space that is highly restricted due to cellular organelles makes the interior of cells tightly packed (Fig. 2.1). Nevertheless, it is a highly hydrated environment, where solvation is made by water molecules (Fig. 2.1) and voids are filled by water that solubilizes ions and small molecules. Thus, virtually all exposed molecules in a cell are under the chemical and physical influence of water. The interior of a cell is not an aqueous solution, but the chemical reactions of the living cells are typical chemical reactions of aqueous solutions.

Life started in water and, chemically speaking, it is still dominated by water. Even the elemental composition of the molecules in a cell was determined by water. With few exceptions, the abundance of elements in a cell reflects the abundance of the same elements in the oceans (Fig. 2.2). Iron, phosphorus, or nitrogen is among the exceptions: they are more abundant in cells on average than in sea water due to their so-called chemical utility. The electronic structure of nitrogen (Fig. 2.2) makes it appropriate as an electron donor to establish dative covalent bonds, also known as dipolar or coordinate bonds. Phosphorus is an amazing element for its capabilities in coordination chemistry (Fig. 2.2). The chemistry of phosphate,  $\text{PO}_4^{3-}$ , is so useful to cells that phosphorylation/dephosphorylation is an ubiquitous mechanism to activate or inhibit enzymes or determine the reaction a molecule will undergo in a metabolic sequence. Iron may have once been abundant as soluble  $\text{Fe}^{2+}$  that was afterward oxidized to  $\text{Fe}^{3+}$  upon the appearance of molecular oxygen,  $\text{O}_2$ , in the Earth's atmosphere.  $\text{Fe}^{3+}$  formed oxides and hydroxides that precipitated, making iron less abundant in seawater. Nonetheless, iron was already being used by cells, and its “chemical utility” determined that cells kept this element at higher levels in their composition.

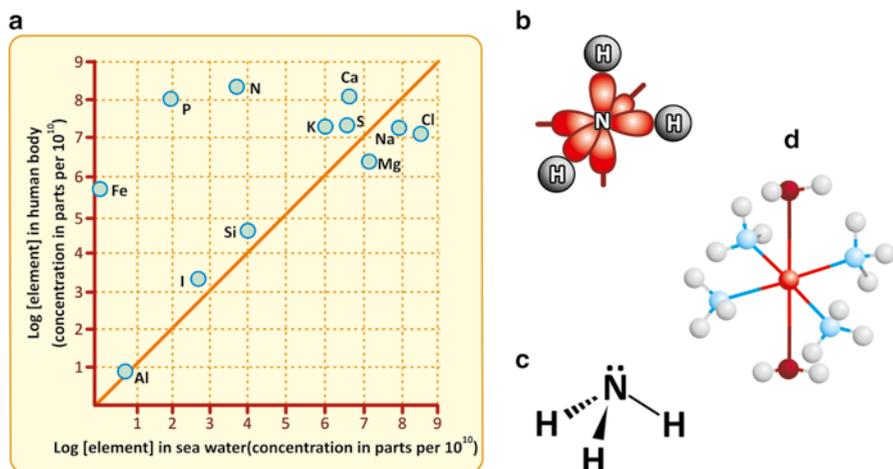


**Fig. 2.1** Crowding in the molecular organization of cells. Even in simple cells such as bacteria (e.g., *E. coli*, **a**), cytoplasm is a dense tight packing of macromolecules (e.g., proteins and nucleic acids) and smaller molecules such as water, nucleotides, and amino acids (**b**). This situation is frequently referred to as “molecular crowding.” The cell interior is thus more similar to a gel than to a solution. Nevertheless, virtually all the external surface of macromolecules, small molecules, and ions are in direct contact with water (**c**). Amino acids, saccharides, ATP, and many other small organic molecules are shown in *pink*. Metal ions are represented in *red*, phosphate ions are *yellow* and *orange*, and chloride ions are in *green*. Water molecules are *colored turquoise*. Although in many cases water molecules may be confined to the solvation shells (immediate layers of water surrounding other molecules), they impose many chemical and physical constraints to molecular organization and reactivity in cells. Water molecules are polar and prone to establish hydrogen bonds (**d**). Although the cell interior is not a solution, its chemistry and physics is dominated by water. Panels (**a**–**c**) are reproduced with permission from Goodsell, *The Machinery of Life*, 2009

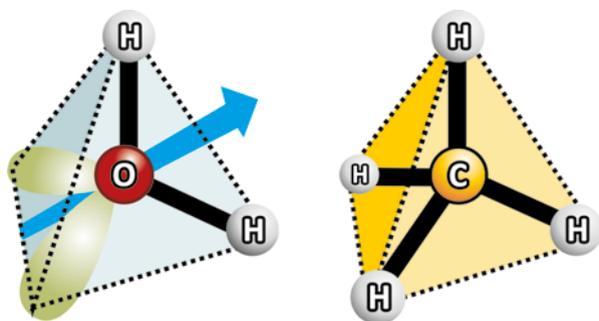
Iron is able to participate in coordination chemistry organometallic complexes, hemoglobin being an example.

Elements that are very abundant on Earth’s crust but not in the oceans such as aluminum (8.2 % of the atoms) or silicium (28 % of the atoms!) are also not abundant in cells because they are mostly part of insoluble oxides. Only 1 % of the total atoms in cells are not hydrogen (62.8 %), oxygen (25.4 %), carbon (9.4 %), or nitrogen (1.4 %). Yet, many elements that are only present in trace amounts may be part of molecules or processes essential to life, such as boron (B), cobalt (Co), copper (Cu), manganese (Mn), or molybdenum (Mo).

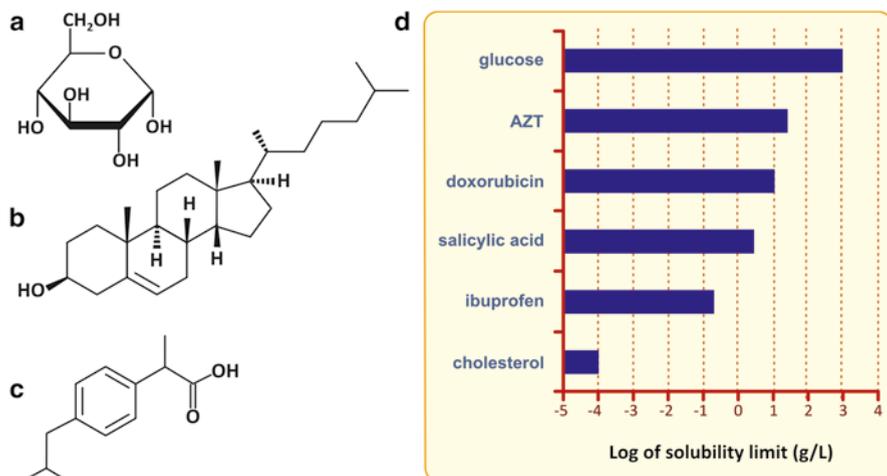
So, water imposed severe constraints on the molecular evolution of cells. And still does! Water is a small but amazing molecule. In spite of its simplicity and abundance, water still fascinates chemists. In particular, its polarity and ability to establish hydrogen bonds are determinant to influence chemical reactions in regions where water serves as a major solvent, which means almost the whole cell (lipid membranes are the main exception). If it weren’t for hydrogen bonding, for instance, water would not be a liquid at temperature and pressure ranges humans and most living beings are adapted to. Methane, with a molecular structure that can be compared to water, is not as nearly important as water in the history of life: methane boiling point is  $-162\text{ }^{\circ}\text{C}$  (Fig. 2.3).



**Fig. 2.2** Panel (a) shows that the abundance of the chemical elements in the human body matches the abundance of such elements in seawater, with few exceptions (reproduced with permission from Dobson et al., *Foundations of Chemical Biology*, 2001). Iron, phosphorus, and nitrogen are among such exceptions because they are very abundant in the human body. Elements such as carbon, hydrogen, and oxygen are not represented in the graph because they are extremely abundant. Nitrogen binds three hydrogens in ammonia ( $\text{NH}_3$ ). The covalent bonds are formed with the  $s$  orbitals of H (gray) and a mix of the  $s$  (not shown) and the three  $p$  orbitals of N (red) (b). A fourth mixed  $sp$  orbital has electrons that are available to participate in dative (coordination) bonds. A simplified representation of the molecule represents these electrons as  $\bullet\bullet$  over N (c). Oxygen in water or dioxygen ( $\text{O}_2$ ) molecules also have electrons available to participate in dative bonds, for instance, with metal ions. Panel (d) shows the tetraammineaquacopper(II) cation,  $[\text{Cu}(\text{NH}_3)_4(\text{H}_2\text{O})_2]^{2+}$ , in which a central  $\text{Cu}^{2+}$  ion accepts electrons from two water and four ammonia molecules. Binding of dioxygen to iron in hemoglobin follows the same principle



**Fig. 2.3** Water vs. methane: similar molecular geometry but different polarity (charge asymmetry axis represented in blue from the slightly higher electronic density in the nonbonding O orbitals in green to lower electronic density in H) and different hydrogen bonding capabilities, which determines very different ebullition points:  $100\text{ }^\circ\text{C}$  to water,  $-162\text{ }^\circ\text{C}$  to methane



**Fig. 2.4** Very hydrophilic molecules are polar, such as glucose (a). Like water, glucose can establish several hydrogen bonds per molecule. Its solubility in water is therefore very high, and glucose can be distributed freely in the human body when solubilized in the blood plasma. In contrast, cholesterol (b), having only a single polar group, the alcohol ( $-\text{OH}$ ), is very hydrophobic (nonpolar) and is not soluble in aqueous media, such as blood plasma. Distribution of cholesterol and other nonpolar molecules in the human body requires special structures that emulsify these hydrophobic molecules in the blood, the lipoproteins. Some biological molecules and drugs, such as ibuprofen (c), for instance, may be intermediate cases and have limited solubility due to the simultaneous presence of hydrophobic and hydrophilic groups in the same molecule (ibuprofen has a phenyl ring and a carboxylic group), which is very important for their ADME: absorption, distribution, metabolism, and excretion by the human body. Solubilities of selected biological molecules and drugs are presented in (d)

Molecules that are polar, mainly those that can establish hydrogen bonding with water, can be distributed in the human body by simple diffusion, such as in blood and/or cerebrospinal fluids, while nonpolar molecules have low solubility and can only be distributed by special carrier systems, such as lipoproteins (see Sect. 3.1.2). Glucose (Fig. 2.4), for instance, distributes in the human body without a carrier, as its concentration in blood can reach very high values with no solubility problems. Cholesterol is the opposite as its solubility in aqueous environment is very low, so it would form crystals and precipitate if it was not kept in nonpolar environments such as the hydrocarbon core of lipid membranes or the interior of lipoproteins. Some pathological situations such as gallstones relate to the low solubility of cholesterol-related molecules, which leads to formation of aggregates (due to the so-called entropic effect; see Sect. 3.1) that in turn nucleate small crystalline structures that grow into “stones” that can measure up to a few centimeters.

Solubility is as important for pharmacology as it is for physiology. A drug that precipitates and crystallizes in the blood, or even in the stomach after being swollen, is hardly effective as it cannot be absorbed and/or distributed in the body.

Nonpolar drugs are usually mixed with other molecules in formulations that prevent drug aggregation in aqueous environment. Solubility is one of the key parameters considered to devise drug development strategies.

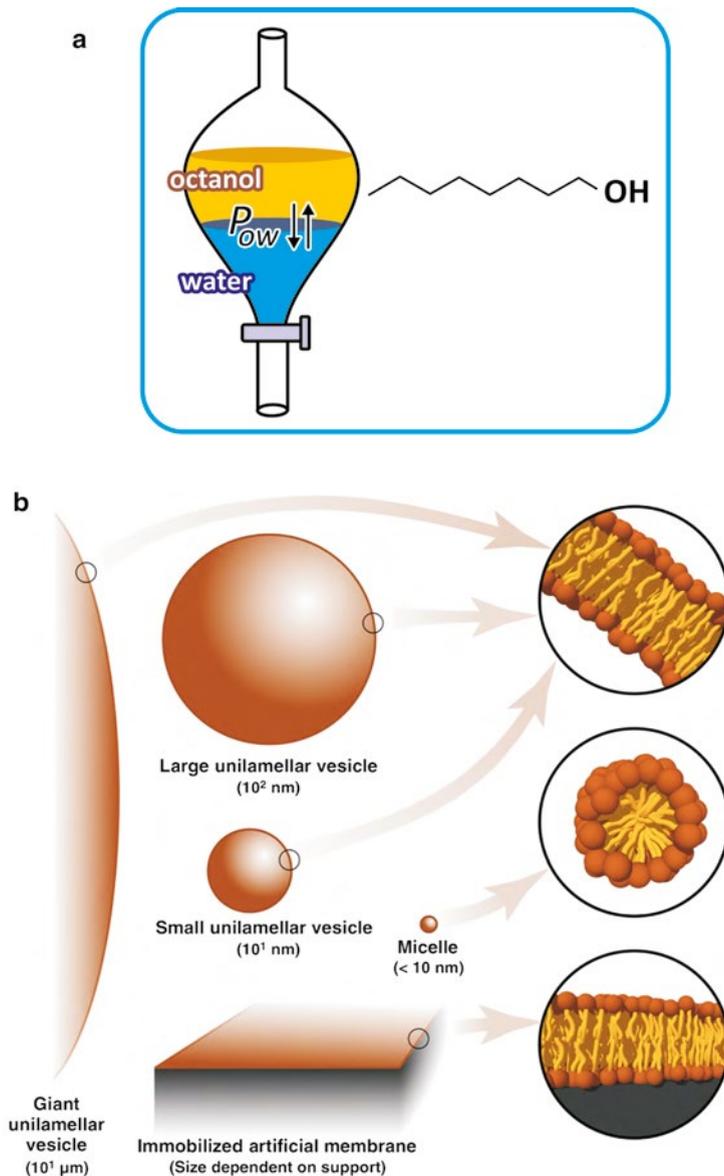
The tendency that drugs have to locate in aqueous or hydrophobic regions of tissues, such as lipoproteins, lipid bilayers, or adipose depots, is studied in pharmacology by measuring the partition of the drug between octanol and water, two immiscible solvents. Octanol is an organic solvent, largely apolar. In spite of the popularity of this method, octanol is still hardly similar to lipidic environments and more modern alternatives exist, like working with aqueous suspensions of lipid vesicles (Fig. 2.5).

The polarity of a given molecule is also determinant for its excretion. Polar molecules are easier to excrete for their solubility in blood and urine. The main strategy of the human body to eliminate xenobiotics (molecules that are not natural constituents of human tissues) consists in grafting hydroxyl (OH) groups, so that they become more polar and, therefore, more soluble in aqueous fluids. This is an efficient method that can be applied to a wide diversity of molecules, serving the purpose of low specificity for a broad protection of the human body against multiple toxic molecules. The molecular complex responsible for polyhydroxylation of different compounds is cytochrome P450, a big proteic complex that contains iron (Fig. 2.6).

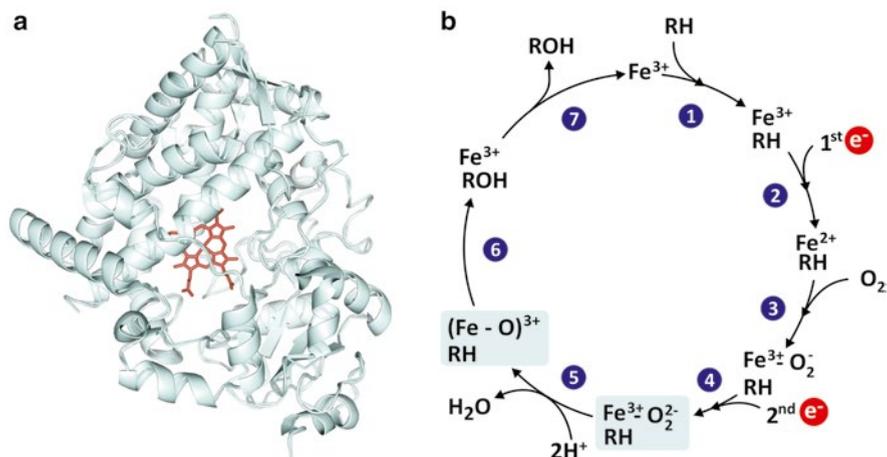
Hydrophobicity (more accurately one would say the “entropic effect”) is important not only for the absorption, distribution, and excretion of molecules, as discussed in this section, but also for holding together the most ubiquitous of all supramolecular non-covalent structures of cells: the lipid bilayer membranes. The importance of this issue is such that it will be kept for detailed explanation in a later section (Sect. 3.1)

## 2.1 The Basics of Chemistry in Cells and Tissues

The boundaries between chemistry and physics at the molecular scale are hard to establish. The interface between both is a rich scientific field referred to as physical chemistry or chemical physics. These disciplines deal typically with molecular structure and the way reactivity is affected by it. A functional distinction that is very practical for those working with molecules is to consider chemical reactions of all transformations of matter that involve formation or breakdown (or both) of covalent bonds. Transformations that do not involve alteration of covalent bonds are considered physical processes. Thus, light being absorbed by the molecules on the skin surface by a protective “sunscreen” cream constitutes a physical process, while UV radiation reaching the skin cells and damaging DNA due to covalent bond cleavage is chemistry. On a macroscopic scale, this functional frontier between chemistry and physics may lose intuitive sense: a plumber cutting a metallic or PVC tube would then be doing chemistry as he is actually destroying chemical bonds in doing so.



**Fig. 2.5** (a) Octanol is an amphiphilic molecule with an acyl chain of eight carbons and a polar alcohol group ( $-\text{OH}$ ). Its hydrophobicity prevents its miscibility with water. Depending on their own polarity, solutes will distribute more extensively to octanol or water. The ratio of the equilibrium concentrations of the solute in both phases is constant, regardless of the total amount of solute or the volume of each phase, which is the reason why this ratio is referred to as partition constant,  $P_{ow}$ . This parameter is used to estimate the tendency molecules under study (e.g., a drug candidate for a future medicine) have to interact with membranes and other lipidic structures, such as lipoproteins or lipid droplets. (b) More recent techniques use different approaches (artificial lipid bilayers in suspension). Despite the ubiquity and simplicity of the octanol/water approach, octanol is a poor replica of lipids. Lipid vesicles in suspension are lipid bilayers of very well-defined composition. Although they lack many characteristics of biological membranes (receptors, transporters, cytoskeleton anchorage, clustering of specific lipids, etc.), they are much more realistic as biological membrane mimetics than octanol. Figure b reproduced from Ribeiro et al., Trends Pharmacol Sci 31:449–454, 2010, with permission of Elsevier

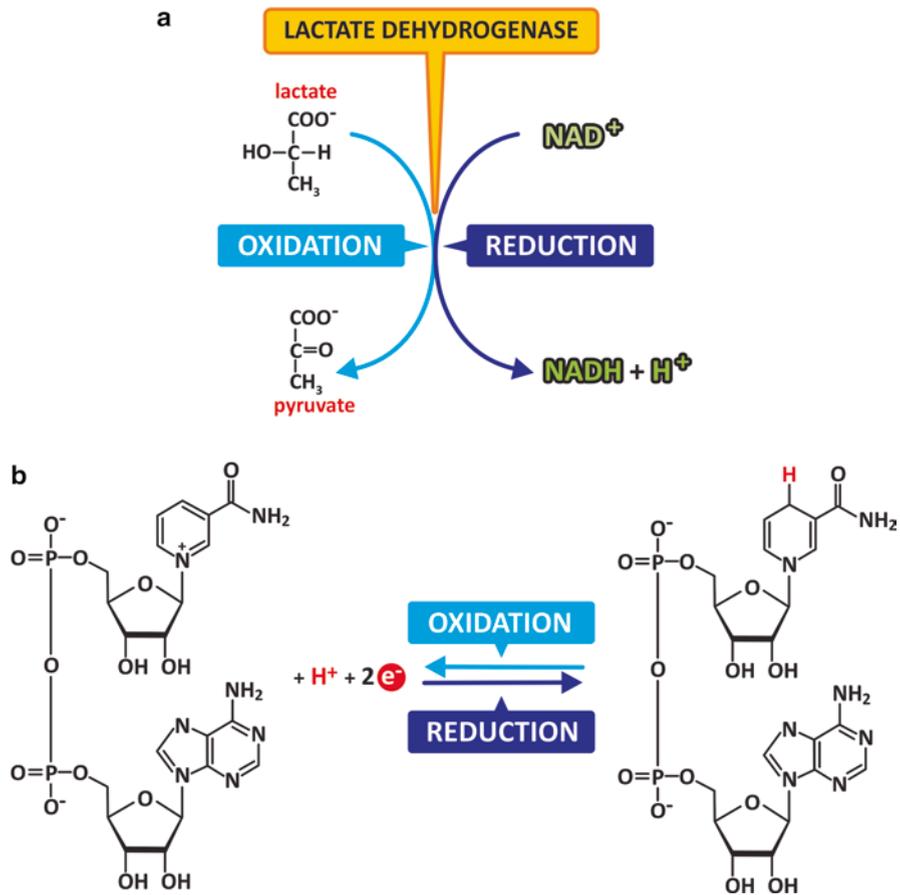


**Fig. 2.6** The P450 cytochrome complex (**a**; PDB 1W0E) uses iron and oxygen to add an hydroxyl (OH) group to organic molecules (generically represented by RH in panel **b**) rendering it more water soluble, suitable to be excreted through urine. This is an important detoxifying mechanism in the human body

In practice, a clear definition of what is chemistry by opposition to what is physics is not needed or useful. Many professionals use both and do not really mind or think about naming what they are doing in terms of chemistry vs. physics classification.

The “chemical life” of cells is very rich and diverse. Many different kinds of reactions may occur. Probably all kinds of reactions occurring in aqueous medium that are described in the most complete organic chemistry textbooks can be found in cells. In this chapter, we will focus only on those most important to understand human metabolic regulation, the current core of human integrative biochemistry (i.e., biochemistry in relation to other disciplines such as histology, physiology, pharmacology, and even anatomy, so that a global perception of human body homeostasis is achieved). It should be stressed that such reactions are favored by the presence of water as solvent. As interaction with solvent molecules affects the electronic distribution of molecules, their reactivity is affected by the solvent. If natural evolution at molecular level was based on a different solvent (e.g., octanol), the “portfolio” of the chemical reactions of life would be different.

Oxidation–reduction reactions are among the most important reactions of the living world. As the name implies, oxidation–reduction reactions are those in which electrons are donated (oxidation) or received (reduction). Because electrons in cells do not remain isolated, individually, as they would in vacuum in outer space, they are transferred between molecules or ions and therefore oxidation and reduction coexist. They are thus referred to as oxidation–reduction reactions. Many different molecules may be oxidized or reduced. Some are particularly well adapted

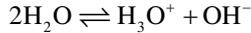


**Fig. 2.7** Example of an oxidation–reduction reaction. Lactate is oxidized to form pyruvate (a). Its  $-\text{OH}$  group is transformed in a  $\text{C}=\text{O}$  (carbonyl) group, and electrons were transferred to  $\text{NAD}^+$  in the process, which was transformed in  $\text{NADH}$  (b)

as reducing agents, such as  $\text{NADH}$  (Fig. 2.7); others are particularly well adapted to be part of a chain of successive electron transfers, such as some metalloproteins. Metalloproteins have metallic elements in their composition that facilitate reception and donation of electrons. The electron transport system, for instance, has several of these proteins (see Sect. 6.2.3).

Acid–base reactions constitute another class of extremely important and ubiquitous reactions in the “living world.” These are reactions in which a proton ( $\text{H}^+$ ) is either donated (by an acid) or received (by a base). In aqueous environment, such as in the almost totality of the cell,  $\text{H}^+$  does not exist as such because water molecules capture the proton forming  $\text{H}_3\text{O}^+$  or donate a proton, forming  $\text{OH}^-$ , the

hydroxide anion. These chemical species ( $\text{OH}^-$ ,  $\text{H}_3\text{O}^+$ , and  $\text{H}_2\text{O}$ ) are all related and equilibrium among them may be reached:



The equilibrium constant for this reaction is:

$$K_{\text{eq}} = \frac{[\text{H}_3\text{O}^+][\text{OH}^-]}{[\text{H}_2\text{O}]^2}$$

Because  $[\text{H}_2\text{O}]$  (molar concentration of water) is constant at any given temperature and pressure, the so-called ionic product of water,  $K_w$ , is used instead for its simplicity:

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$$

Nearly at 25 °C,  $K_w = 1 \times 10^{-14} \text{ mol}^2 \text{ dm}^{-6}$  (i.e.,  $1 \times 10^{-14} \text{ M}^2$ ). This may seem rather pointless at a first glance, but it is from here that one can conclude that in pure water, pH value is 7. In pure water one ion of  $\text{H}_3\text{O}^+$  is formed for each  $\text{OH}^-$ , therefore:

$$\begin{aligned} [\text{H}_3\text{O}^+]_{\text{eq}} [\text{OH}^-]_{\text{eq}} &= 1 \times 10^{-14} \text{ M}^2 \Leftrightarrow \\ [\text{H}_3\text{O}^+]_{\text{eq}}^2 &= 1 \times 10^{-14} \text{ M}^2 \\ [\text{H}_3\text{O}^+]_{\text{eq}} &= 1 \times 10^{-7} \text{ M} \end{aligned}$$

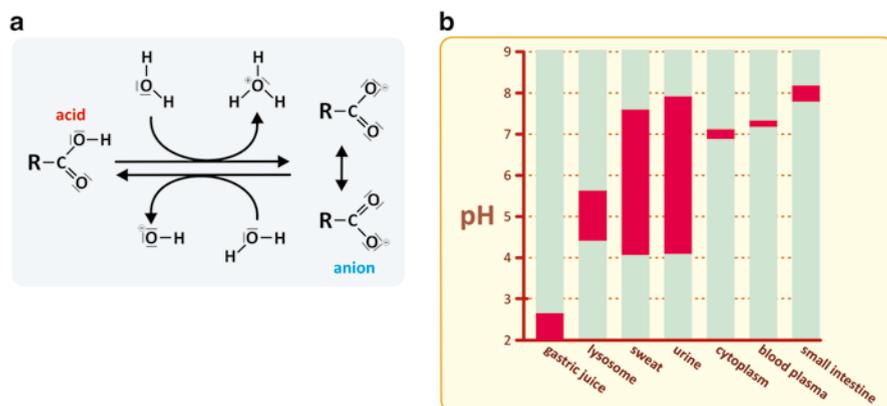
Hence:

$$\begin{aligned} [\text{H}_3\text{O}^+]_{\text{eq}} &= 1 \times 10^{-7} \text{ M} \Leftrightarrow \\ -\log [\text{H}_3\text{O}^+]_{\text{eq}} &= 7 \Leftrightarrow \\ \text{pH} &= 7 \end{aligned}$$

So, in pure water, at temperature nearly 25 °C, the pH is 7.

The cytoplasm of cells is approximately at pH 7, although the pH could vary in certain cellular organelles. In the human body as a whole, the pH values of different environments are very diverse, from the extremely acidic gastric juice to the basic intestinal lumen medium (Fig. 2.8).

Other kinds of reactions such as addition elimination or nucleophilic substitutions are also very frequent, but acid–basic will continue to be the focus of our attention for the importance the control of pH has in homeostasis. Variations in pH cause variations in the protonation/deprotonation of proteins and other biological molecules, which in turn affect their function. Take the example of enzymes: protonation or deprotonation of chemical groups on the structure of the protein



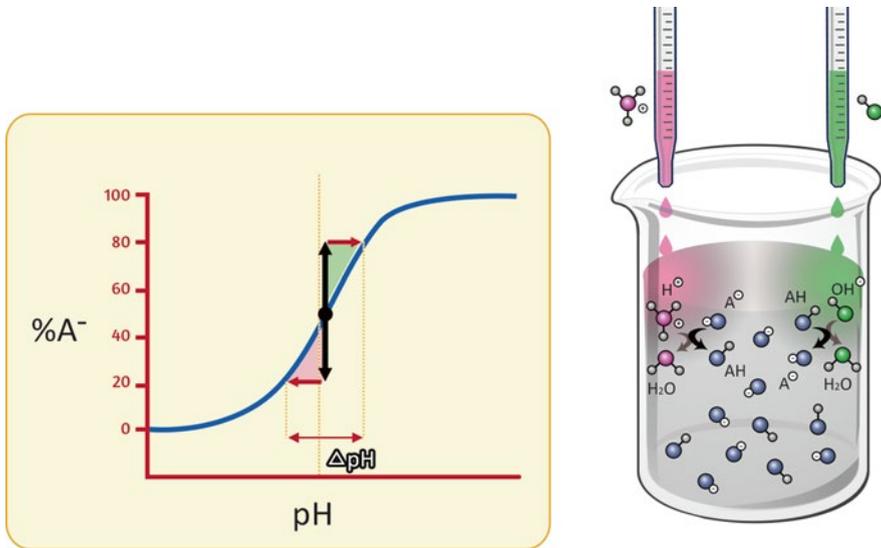
**Fig. 2.8** (a) Protonation (right to left) and deprotonation (left to right) of a carboxylic acid. The pH of the medium depends on this kind of reactions. (b) A high variety of environments, having different pH can be found in the human body

causes variation in charge, leading to new sets of attractions and repulsions between different parts of the molecule that may cause that some segments of the protein contract and others become looser, impairing or facilitating the optimal function of enzymatic catalysis (this will become more clear in Sect. 3.3, where we address the structure of proteins in detail). The same impact on molecular structure and function applies for other biological molecules, such as polysaccharides. Therefore, stabilizing pH in order to guarantee proper structure and function of biological molecules is very important. This is not saying that the pH should be the same in all tissues or in all cells of the same tissue or in all organelles of the same cell. pH is actively controlled in different anatomical, histological, and cellular environments, but it is not the same in all cases. Blood plasma pH, for instance, is very strictly controlled and only allowed to vary in a very restricted range around 7.4. This is not surprising because the efficiency with which hemoglobin transports  $O_2$  is very much dependent on pH. Nevertheless,  $CO_2$ , which is a molecule that has the potential to largely impact on pH (see next reaction scheme), diffuses freely in the blood.



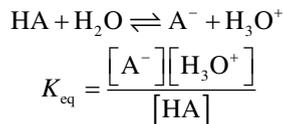
Then, how is it possible for our body to cope with  $CO_2$  diffusion and still maintain a blood plasma pH tightly controlled, centered at pH 7.4? The answer resides on a deceptively simple mechanism of pH control named “pH buffering” (Fig. 2.9).

pH buffers are no more than mixtures of weak acids or weak bases with their conjugated bases or acids, respectively. In practice, an aqueous solution of a weak acid or weak base in equilibrium is a pH buffer because these chemical species dissociate to a moderate extent forming mixtures that are pH buffers. The reason why these mixtures work in a way to maintain pH constant is related to the basic

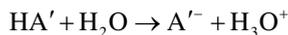


**Fig. 2.9** How a pH buffer works. A pH buffer solution is a mixture of a weak acid (HA) with its conjugated base ( $A^-$ ) or vice versa. When an acid is added to the solution, the deprotonated species ( $A^-$ ) reacts with the added acid to form the protonated form (HA). Part of the  $H_3O^+$  is thus consumed and the pH drop is thus attenuated. When the added solution is a base, part of the  $OH^-$  is consumed by reaction with HA, the acidic form of the buffer, and the rise in pH is thus attenuated. Additions of significant amounts of acids or bases to buffered solutions result in modest variation in pH as long as protonated (HA) and deprotonated ( $A^-$ ) buffer species coexist

principles of chemical equilibrium. Consider a generic weak acid, represented by HA for the sake of simplicity, in aqueous solution:



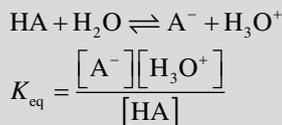
Upon the addition of a strong acid,  $HA'$  (by definition strong acids have nearly complete dissociation), the concentration of  $H_3O^+$  raises:



The newly formed  $H_3O^+$  have an impact on the equilibrium of the weak acid, which will progress in the reverse order (formation of HA) in order to consume part of the  $H_3O^+$ . The extent of this consumption of  $H_3O^+$  can be calculated based on the equilibrium constant, which remains unaltered: the concentrations of  $H_3O^+$  and HA increase and the concentration of  $A^-$  decreases down to the point where  $K_{eq}$  is kept (see Box 2.1).

### Box 2.1: pH Buffers and the Origin of the Henderson–Hasselbalch Equation

The initial situation, before strong acid addition is:



After strong acid ( $\text{HA}'$ ) addition  $[\text{H}_3\text{O}^+]$  raises:



But HA dissociation equilibrium is the same in both cases, so:

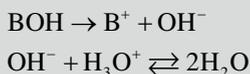
$$K_{\text{eq},i} = K_{\text{eq},f} \Leftrightarrow \frac{[\text{A}^-]_i [\text{H}_3\text{O}^+]_i}{[\text{HA}]_i} = \frac{[\text{A}^-]_f [\text{H}_3\text{O}^+]_f}{[\text{HA}]_f} \Leftrightarrow$$

$$\frac{[\text{H}_3\text{O}^+]_f}{[\text{H}_3\text{O}^+]_i} = \frac{[\text{A}^-]_i}{[\text{HA}]_i} / \frac{[\text{A}^-]_f}{[\text{HA}]_f}$$

(i and f stand for the initial and final equilibria, respectively.)

This equation shows that as  $\frac{[\text{H}_3\text{O}^+]_f}{[\text{H}_3\text{O}^+]_i} > 1$ , the weak acid equilibrium is perturbed in a way to favor formation of HA, thus consuming  $\text{H}_3\text{O}^+$ . All  $\text{HA}'$  is converted to  $\text{H}_3\text{O}^+$ , and in absence of the weak acid, the concentration of  $\text{H}_3\text{O}^+$  would increase correspondingly. In the presence of the weak acid, part of the  $\text{H}_3\text{O}^+$  is consumed by  $\text{A}^-$  to form HA, thus attenuating the drop in pH caused by the strong acid. This is the molecular mechanism of pH buffering (see also Fig. 2.9 for a pictured explanation).

If a strong base is used, the same principle applies, this time with the “intermediation” of the ionic product of water:



Also, if a weak base is used instead of a weak acid, the same buffering capacity exists.

The Henderson–Hasselbalch equation is a very simple and robust way to show how pH is expected to evolve when  $[\text{A}^-]$  and  $[\text{HA}]$  are perturbed and change when new equilibria is formed upon the appearance of new acids or bases in solution. The Henderson–Hasselbalch equation

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

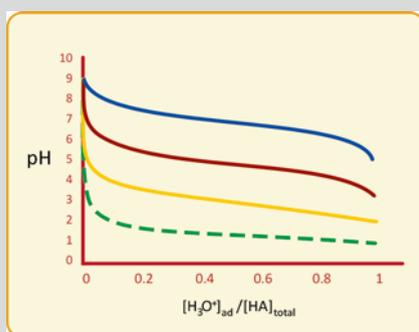
**Box 2.1** (continued)

is no more than a simple rewriting of the equilibrium constant (here named  $K_a$  to stress it refers to an acid):

$$K_a = \frac{[A^-][H_3O^+]}{[HA]} \Leftrightarrow [H_3O^+] = K_a \frac{[HA]}{[A^-]} \Leftrightarrow$$

$$-\log[H_3O^+] = -\log K_a - \log \frac{[HA]}{[A^-]} \Leftrightarrow \text{pH} = \text{p}K_a + \log \frac{[A^-]}{[HA]}$$

A graphical schematic representation of this equation (see figure) shows that pH varies very little when strong acids or bases are added to buffers, mainly when pH is within the  $\text{p}K_a \pm 1$  range. This range is centered around the point where  $[A^-] = [HA]$ , as implied by the Henderson–Hasselbalch equation when  $\text{pH} = \text{p}K_a$ . Naturally, the efficiency of the buffer increases with  $[HA]$  because higher  $[HA]$  imply that more  $H_3O^+$  or  $OH^-$  can be added to the solution before  $[A^-]$  reaches zero, a point where the buffer mechanism becomes exhausted.



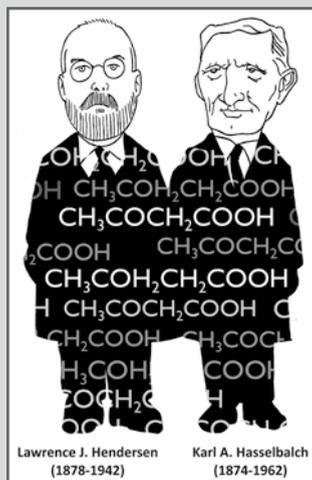
Changes in pH in a buffered solution are much less severe than they would be in the unbuffered solution. Examples of pH variation in a solution initially at pH 9 upon addition of a strong acid, with a total concentration of HA of 0.1 M, when the  $\text{p}K_a$  of HA is 7 (*blue*), 5 (*red*), or 3 (*yellow*). The *dashed green curve* indicates the pH drop if HA was absent (unbuffered solution,  $0 < [H_3O^+]_{\text{ad}} < 0.1 \text{ M}$ ). The concentration of the  $H_3O^+$  added to the solution is  $[H_3O^+]_{\text{ad}}$  and is represented relative to the total concentration of HA used in the buffer ( $[HA]_{\text{total}}$ ). The buffering zone (nearly horizontal pH variation lines) is mainly in the pH range that lies within  $\text{p}K_a \pm 1$ , and the buffering capacity (i.e., the limits of  $[H_3O^+]_{\text{ad}}$  in the buffer zone) depends on  $[HA]_{\text{total}}$ . This example illustrates how big amounts in added  $H_3O^+$  cause modest changes in pH in the range  $\text{p}K_a \pm 1$ . The case in which a base is added to an acidic solution is similar.  $OH^-$  concentration raises, which is concomitant with a decrease in  $H_3O^+$ , and the situation is symmetrical to the one obtained here (raise in pH instead of drop)

Although the Henderson–Hasselbalch equation is central in understanding fundamental pH buffer chemistry, its historical root is in medicine. Lawrence

(continued)

**Box 2.1** (continued)

Henderson was a doctor interested in pH plasma alterations in pathology. He created the concept of acid–base equilibrium. Karl Hasselbalch studied the effect of  $\text{CO}_2$  in hemoglobin with Christian Bohr (whom the Bohr effect was named after—see Sect. 3.3.3). The works of both doctors opened the way for the scientific study of respiratory and metabolic perturbation of blood plasma pH equilibrium, a significant breakthrough in the early twentieth century physiology.



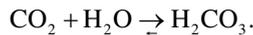
### 2.1.1 Principal Biological Buffers

Generalization of the previously mentioned concepts and equations should be made cautiously for several reasons:

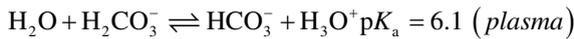
- From the point of view of scientific accuracy, activity coefficients should be used in addition to concentrations in all the abovementioned equations. Activity coefficients are used in thermodynamics to account for deviations from ideal behavior in a mixture of chemical substances. For practical reasons, it is assumed that activity coefficients do not alter equilibrium constants in the experimental conditions addressed.
- Water dissociates to form  $\text{H}_3\text{O}^+$ ; therefore,  $\text{H}_3\text{O}^+$  ions are always present in aqueous solutions. In the abovementioned equations and reasoning, the ionic product of water was never considered, which is valid in circumstances where the acidic species are present in concentration much higher than those involved in the ionic product of water ( $[\text{H}_3\text{O}^+] = 10^{-7} \text{ M}$ ).

(c) We have explored the concept of buffers in situations in which chemical equilibrium exists. This is not always the case in biological situations.

In spite of all these limitations, the general concept of buffer still applies to cells and organs. Equations should be applied judiciously to chemical problems in living systems, but the concept of pH buffer *in vivo* is still valid. Wherever weak acids or bases are present in cells or body fluids, they contribute to form a buffer. Ions such as  $\text{H}_2\text{PO}_4^- / \text{HPO}_4^{2-}$  ( $\text{p}K_a = 6.8$ ) are very important to buffer the cytoplasmic pH (notice that the pH of cytoplasm is about 7.0, well within the buffering range of  $\text{H}_2\text{PO}_4^- / \text{HPO}_4^{2-} = \text{p}K_a \pm 1 = 6.8 \pm 1$ ) but not plasma.  $\text{H}_2\text{CO}_3 / \text{HCO}_3^-$  is much more important to buffer plasma pH. The  $-\text{COO}^-$  and  $-\text{NH}_3^+$  groups present in plasma proteins also contribute to pH buffering but not as much as the hydrogenocarbonates ( $\text{H}_2\text{CO}_3 / \text{HCO}_3^-$ ): while proteins contribute to 24 % of the buffering capacity in plasma,  $\text{H}_2\text{CO}_3 / \text{HCO}_3^-$  contribute with 75 % (the remaining 1% is a modest contribution of  $\text{HPO}_4^{2-} / \text{H}_2\text{PO}_4^-$ ). The prevalence of the  $\text{H}_2\text{CO}_3 / \text{HCO}_3^-$  is not surprising because  $\text{CO}_2$  diffuses freely in plasma, regardless of a certain ability hemoglobin has to bind  $\text{CO}_2$ . Carbon dioxide is extensively converted to di-hydrogen carbonate by enzyme carbonate–dehydratase in erythrocytes:

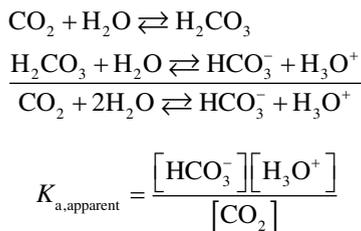


$\text{H}_2\text{CO}_3$  is then present in plasma, where it is in equilibrium with hydrogen carbonate (also known under the popular name of “bicarbonate”),  $\text{HCO}_3^-$ :



$\text{HCO}_3^-$  exists in plasma with typical concentrations in the 22–26 mM range.

It should be stressed that the  $\text{H}_2\text{CO}_3 / \text{HCO}_3^-$  equilibrium *in vitro* has  $\text{p}K_a = 3.8$ , quite different from the  $\text{p}K_a$  in plasma (6.1), which is said to be an apparent  $\text{p}K_a$  because it is under the influence of the enzymatic production of  $\text{H}_2\text{CO}_3$ . To account for the change in pH due to fluctuation in the plasma levels of  $\text{CO}_2$ , one can apply the Henderson–Hasselbalch equation to the multiple equilibria.



The water concentration is constant and is thus incorporated in  $K_{a,\text{apparent}}$ ; the variables are on the right-hand side of the equation, and the left-hand side of the equation is constant. It would be an unnecessary complication to have  $[\text{H}_2\text{O}]$ , which is constant, in the right-hand side of the equation.

Now the question arises as to what is the most appropriate way to express  $[\text{CO}_2]$  because, at the pressure of 1 atmosphere and usual temperatures,  $\text{CO}_2$  is a gas and molar units are not well suited for gases.  $[\text{CO}_2]$  should then be converted to partial pressure,  $\text{pCO}_2$ :

$$[\text{CO}_2] = 0.03 \times \text{pCO}_2$$

for  $[\text{CO}_2]$  in mM and  $\text{pCO}_2$  in mmHg. Thus:

$$K_{\text{eq,app}} = \frac{[\text{HCO}_3^-][\text{H}_3\text{O}^+]}{0.03\text{pCO}_2} \Leftrightarrow$$

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{0.03\text{pCO}_2}$$

This equation helps by anticipating the drop in pH when  $\text{pCO}_2$  increases, but it should be emphasized that an increase in  $\text{pCO}_2$  also causes an increase in  $[\text{HCO}_3^-]$ , mitigating the impact of the fluctuation of plasma levels of  $\text{CO}_2$  in pH.

## 2.2 More than Only Chemistry: There Is Physics Too

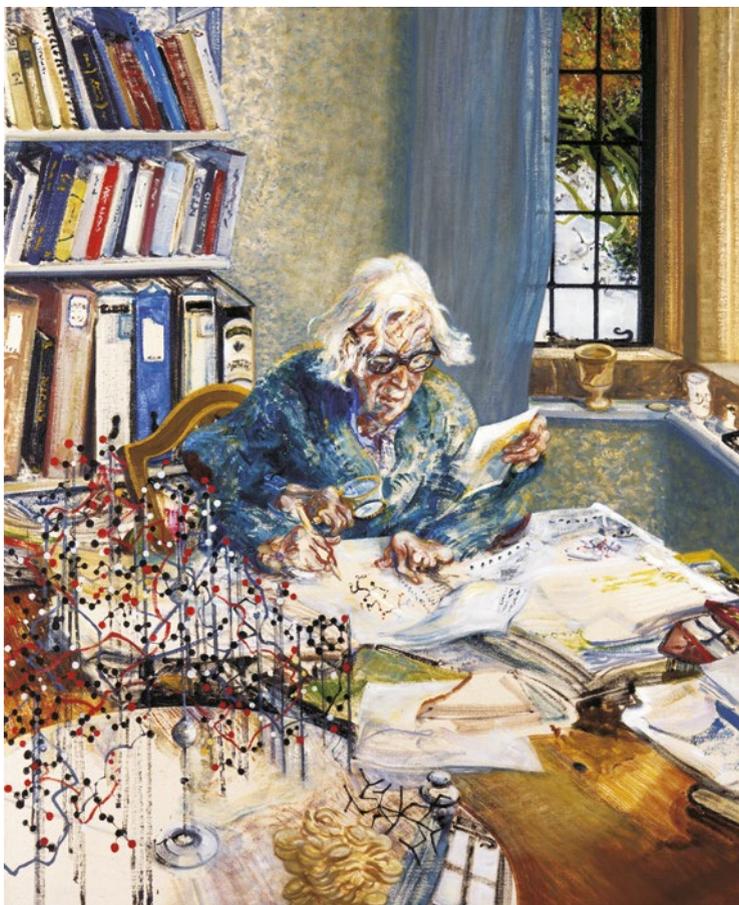
Given the importance of chemical reactions in cells and in the organisms as a whole, one tends to forget the importance of physical processes in life. They are also important to understand life at the molecular level. Apart from all the issues related to the solubility of molecules in aqueous media, which were discussed in the beginning of this chapter, the interaction of light and biomolecules, for instance, is of utmost importance. This is clear when studying the vision of animals, the photosynthesis in plants or the bioluminescence of microorganisms, just to name a few examples. Regarding human biochemistry in the health sciences field, the topics of election are the early events of vision and the effects of ultraviolet (UV) light in tissues such as skin and hair. Other topics, such as the effects of radioactivity and other nonoptical radiation on humans, are left out of this book, although they are interesting and very relevant in particular situations.

When radiation interacts with matter, different kinds of phenomena may occur, such as absorption, scattering, or diffraction. Absorption implies that the energy of radiation matches the energy needed to change molecular states. This means that the energy of radiation is used in the change of the molecular configuration of nuclei and/or electrons so that the radiation extinguishes in the process (it is “absorbed”). This happens when a photon enters our eyes and triggers a radical change in the conformation of retinol in the retina, thereby initiating the physiological process that ends with visual perception. Scattering relates to the radiation that interacts with molecules and is not absorbed, but causes the molecules to emit secondary

radiation, having the same energy or not. This is what happens when light impinges on matter and is not absorbed: the radiation causes oscillations on the electronic cloud of molecules that in turn causes simultaneous emission of radiation, the scattered light. Scattering is responsible for the white color of milk, blue color of the sky, or reddish color of the sunset. Excessive light scattering in human eyes causes blurring in vision. Diffraction is a particular kind of scattering that occurs when radiation impinges on matter that has voids of size comparable to wavelength of the radiation. X-rays, for instance, are diffracted by molecular crystals because the distances between the nuclei, the chemical bonds length, are similar to its wavelength, i.e., in the order of tenth of nanometer. The spatial pattern of diffracted X-rays can be analyzed to reveal the 3D structure of crystallized molecules, even if they are quite big and complex, such as proteins (Fig. 2.10).

The complete range of frequencies (i.e., energies) of radiation known so far constitutes the so-called electromagnetic spectrum (Fig. 2.11a). It includes low-energy (low frequency, long wavelength) radiation, such as radio and television radiation, as well as high-energy (high frequency, short wavelength) radiation, such as X- and gamma rays, capable of ionizing molecules and disrupting covalent bonds. Very low-energy radiation hardly causes changes in molecules and therefore is not prone to produce effects in living organisms. High-energy radiation (starting from the far, high-frequency, UV radiation) is able to disrupt molecules by destroying chemical bonds and is therefore a potential hazard to the chemistry of life (an amazing exception is presented in Box 2.2). It is not surprising, therefore, that natural phenomena in living beings involving radiation occur in a limited range of energies, from microwaves to near (lower frequencies) UV. Even so, one has to wonder why many animal eyes, including human, uses a much smaller fraction of radiation, from about 400 nm (blue light) to about 800 nm (red light). The answer is simple and can be found in molecular evolution toward the optimized use of environmental resources: the 400–800 nm range accounts for the most abundant sun radiation reaching the surface of planet Earth (Fig. 2.11b). In addition, this radiation penetrates water down to tenths of meters. Naturally, this dictated the course of evolution of vision. The same happened with photosynthetic organisms.

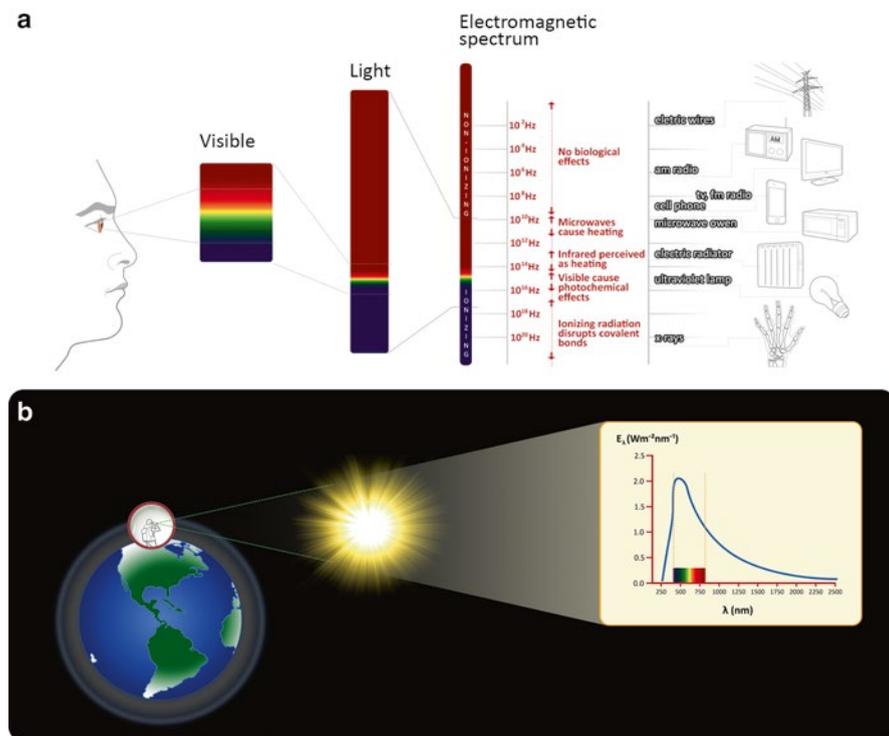
In the human eye, rhodopsin, a membrane protein (opsin) that exists in retina rod cells, has a covalently bound retinal molecule, which adopts two stable isomeric forms: *cis* and *trans* (Fig. 2.12). When the protein-attached *cis*-retinal residue absorbs light, it converts to *trans*-retinal, this being the triggering event of vision. Not only retinal changes in shape, from a bent structure to a nearly linear arrangement, but it also detaches from opsin. All-*trans*-retinal (i.e., the “linear” retinal, with all double bonds in the *trans*-configuration) does not fit the pocket formed by the opsin transmembrane helices as the *cis* isomer does, causing tensions in the protein structure, which has to change conformation (shape) to adapt. A reduction in cyclic GMP results from this process, which in turn triggers a series of reactions that cause an electric current along the cell. The intensity of this current is proportional to the intensity of light that reached the retina. So, an electric impulse is created that is detected by ganglion cell and then the optic nerve. Nerve fibers reach the



**Fig. 2.10** Dorothy Mary Crowfoot Hodgkin in her study at home portrayed by Maggi Hambling (oil on canvas, 1985). The four-handed scientist representation symbolizes her unusual working and entrepreneurial capacity. Dorothy Hodgkin made extremely valuable contributions to the advancement of biochemistry. She revealed the structure of cholesterol, penicillin, insulin, and vitamin B<sub>12</sub>, among other molecules. She also contributed to the refinement of X-ray diffraction spectroscopy to unravel the structure of proteins. A structural model of insulin is on the *left*. Reprinted with the permission of the National Portrait Gallery, London, UK

back of the brain (occipital lobe), where images are created. This region is called the primary visual cortex. Some of the visual fibers extend to other parts of the brain to help to control eye movements, the responses of the pupils and iris, and behavior.

The effects of UV light on the skin and hair constitute other examples on how the radiation–matter interaction is important in natural processes. In practical terms, UV is divided in three categories, A to C, according to their biological effects. UV-A is the lowest energy class (320–400 nm). UV-C is the highest energy range (200–290 nm) and is, to a great extent, filtered by the ozone layer in the atmosphere.



**Fig. 2.11** (a) The electromagnetic spectrum with the typical frequencies of radiation, devices that use them, and their typical biochemical/physiological effects. The narrow range of optical radiation (in practical terms, the radiation that can be conducted using lenses and mirrors), to which human eyes are sensitive, is highlighted. (b) Spectrum of total solar radiation power that reaches Earth surface. The maximum power range is coincident with optical radiation one calls “visible light,” the radiation many animal eyes, including human, have adapted to. This radiation penetrates water down to tenths of meters, which was also important for the evolution of vision

A part of the UV-B radiation is also absorbed by ozone. Having sufficient energy to break chemical bonds in the nucleic acids of exposed cells in the skin layers near the surface, UV-C and, to a less extent, UV-B are serious health hazards regarding skin tumors, mainly in prolonged exposure to sun and other UV sources. Whenever the ozone layer is threatened, the risk of skin cancer raises. Nevertheless, the immediate, most noticeable effects of UV-B are erythemas, such as those associated to “sunburns” in short-term solar overexposure. Being moderate to high-energy radiation, UV-C and UV-B interfere with many molecular processes. The consequence of this is that their penetrance is shallow in the skin (they are totally absorbed in the outer layers of skin—epidermis and nearby dermis; Fig. 2.13), where they are responsible for some naturally occurring biochemical processes, such as the conversion of the amino acid tyrosine in melanin (a sun radiation–protection pigment that colors the skin), but also by some lesions such as the detachment of epidermis from dermis (“sunburn blisters”). UV-A penetrates deeper in the skin because it is not so

**Box 2.2: The Toughest Cell on Earth**

In 1956 Arthur Anderson, working in Oregon (USA), was studying if canned food could be sterilized using high doses of gamma radiation. Despite the fact that he was using doses enough to destroy all forms of life known to man to sterilize meat, the food subsequently spoiled. A bacterium, *Deinococcus radiodurans*, was isolated and found to be responsible for this result. Later studies revealed that it is extremely resistant to ionizing radiation of different frequencies, desiccation, and oxidizing agents. While a bacterium like *E. coli* can stand up to 200–800 Gy, *D. radiodurans* can stand up to 5000 Gy. Human cells have much lower tolerance levels: below 5 Gy.

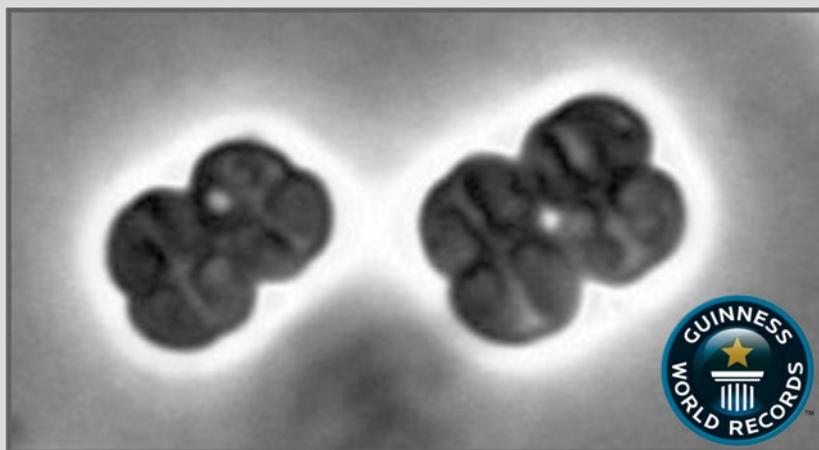
From the chemical point of view, the DNA composition of *D. radiodurans* is not different from that of other organisms. The key to resistance is not the chemical nature of DNA but the efficacy of the repair mechanisms. Radiation interacts with the chemical bonds of the DNA similarly in all cells, causing occasional breakings in these bonds when its energy is high enough. It is impossible for the vast majority of cells to cope with very frequent and simultaneous breaking of the DNA covalent bonds. *D. radiodurans*, however, has its DNA packed in toroids and multiple copies of the genome, usually 4–10. The whole repair machinery enables the reconstruction of the complete genome from shattered bits in hours. Bacterium-to-bacterium transfer of DNA may also play a role in increased resistance.

One interesting question that arises from the amazing properties of *D. radiodurans* is “Could a bacterium like this have developed in a planet where there are no environments with high doses of ionizing radiation?” It has been suggested that the origin of *D. radiodurans* is extraterrestrial because it has acquired resistance to a set of very harsh physical–chemical environmental conditions, like the ones expected in Mars. However, the bacterium is genetically, biochemically, and microbiologically very similar to other bacteria, and there is no other evidence for Martian forms of life. As dehydration and radiation cause very similar types of DNA damage, it is possible that resistance to radiation is a side effect from selective pressure toward resistance to dehydration. *D. radiodurans* is extremely well adapted to dryness.

The remarkable biochemical properties of *D. radiodurans* may make it a powerful ally to clean contaminated radioactive areas, in which the bacterium can survive and operate. This kind of approach is named bioremediation. Radioactive toxic waste would be processed by bacteria that would naturally colonize high risk areas.

**Box 2.2** (continued)

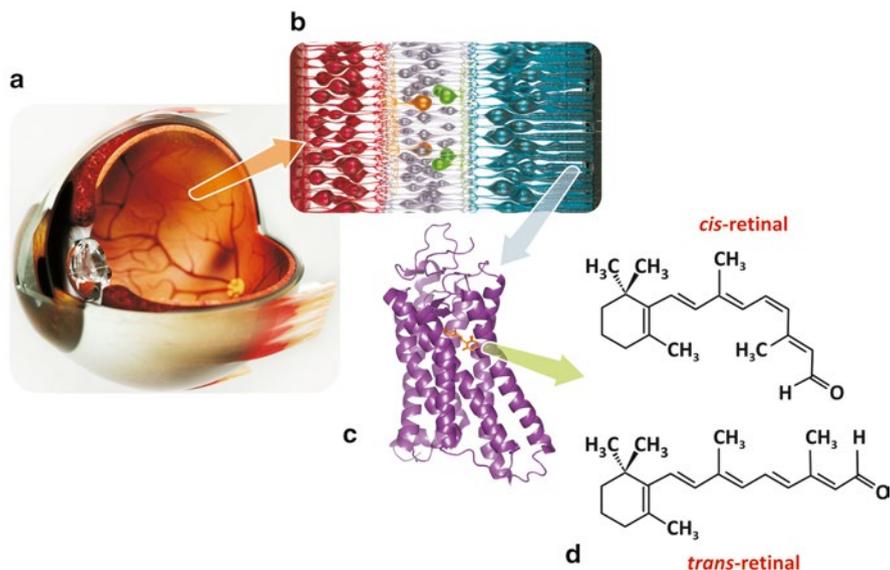
The extreme resistance of *D. radiodurans* inspired researchers and science communication professional, who frequently call it “Conan bacterium.” In 1998 the Guinness Book of World Records listed *D. radiodurans* the “most radiation resistant lifeform....”



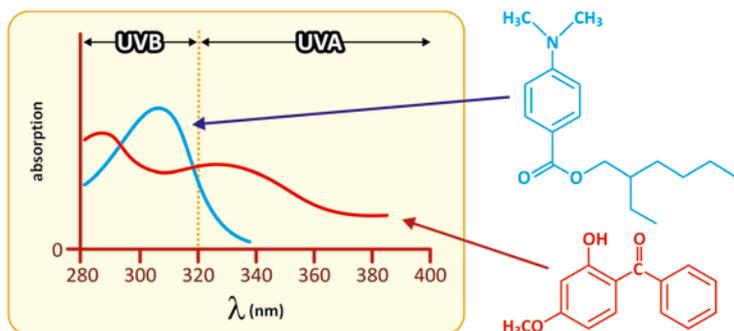
*Deinococcus radiodurans* (credits: Sandra P. Santos and Célia Romão, ITQB-UNL, Portugal)

energetic, reaching the core of the dermis. It also stimulates the formation of melanin (“suntan”), which provides some protection to the skin against the effects of solar radiation. Nevertheless, contrary to popular belief, the extent of this protection is very limited (Sun Protection Factor, SPF, of about 2 to 4, far below the minimum recommended SPF of 15 for direct skin exposition to sun radiation).

The SPF is determined from the UV exposure time that is needed for the appearance of a minimal erythema (i.e., erythema after 24 h). Specifically, it is the ratio of the time needed for a minimal erythema in protected skin over the time needed in unprotected skin. It is determined indoors with a light source that is meant to reproduce the noontime sun. The SPF is mainly useful to have a quantitative scale of UV-B protection when sunscreens are used. A sunscreen with an SPF of 10 filters 90 % of the UV-B light, for instance (in other words, a person that would have sunburn after a 20 min exposition to sun, using a sunscreen of SPF 10, has the same effect after 200 min). Sunscreen creams, lotion, sprays, gels, or other topical formulations have organic molecules in their composition that absorb UV light. Notice that the most common of these compounds have aromatic rings in their structure (Fig. 2.13), which are groups that absorb UV light, and/or inorganic metallic compounds (zinc oxide, for instance) that scatter UV light (the scattering efficacy is for UV than for visible light).

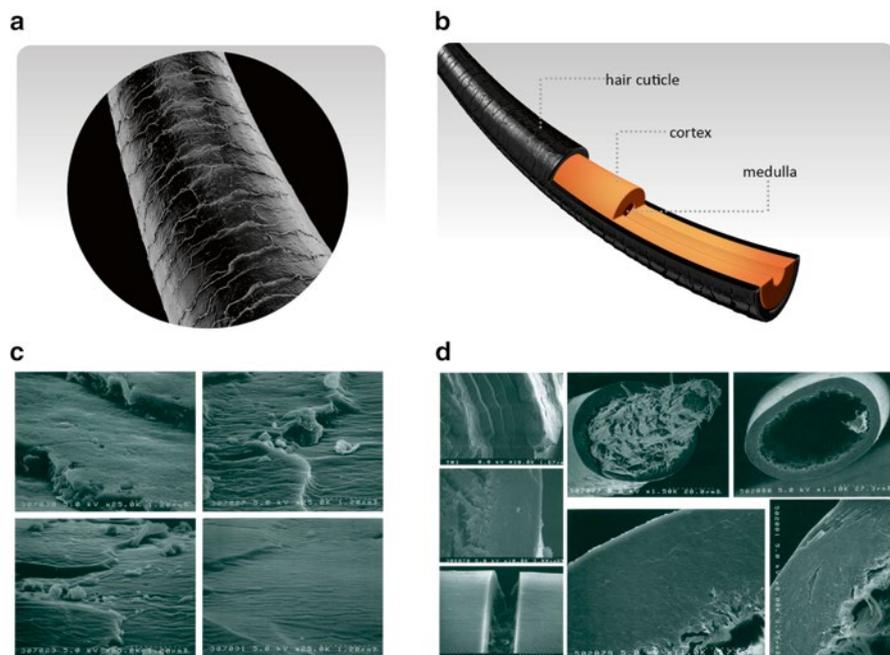


**Fig. 2.12** Anatomical cross-section of the eye (a), highlighting the retina (b), where rod cells are located. Sensory rhodopsin (c; PDB 2I35) binds retinal (d), which changes conformation with light absorption



**Fig. 2.13** Two UV-absorbing molecules commonly found in sunscreen formulations: octyldimethylaminobenzoate (OD-PABA), in blue, and 2-hydroxy-4-methoxybenzophenone (HM-BZP) in red. The chemical group responsible for the absorption of radiation (“chromophore”) is the aromatic (benzenic) ring, which is present in both molecules and many other components of sunscreens as well

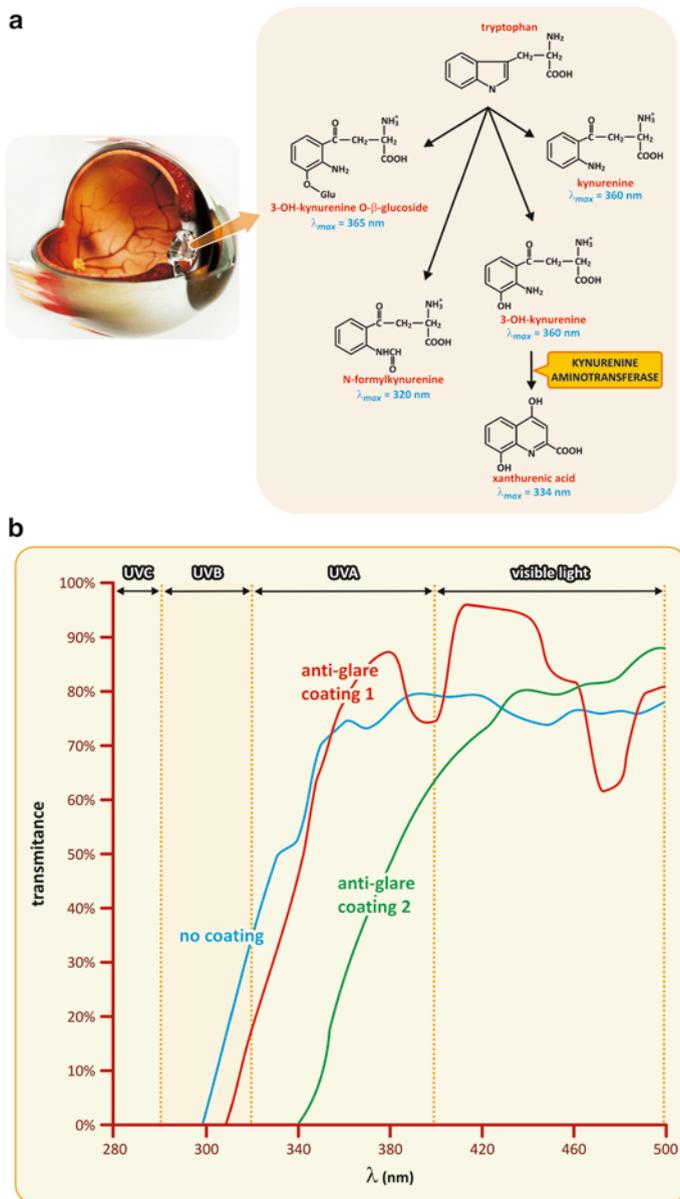
Although the effects of UV radiation on the skin usually monopolize one’s attention, the biochemistry involved in the damage caused by UV radiation on other structures such as hair and eyes is worthy of consideration. Hair is an extremely well-adapted cellular organization: it conjugates flexibility and strength. The hair has an external proteic cuticle, an inner core named medulla, and an intermediate



**Fig. 2.14** Hair surface as seen under the electronic microscope (a) reveals scales. The external layer of the hair shaft, named cuticle (b), is formed of proteic plaques that slide over each other due to the lubrication of lipids, which makes the hair flexible. Intense UV exposure leads to “fusion” of the plaques in the whole cuticle (c, d). “Fused” cuticles lose flexibility and break easily (d). Panels (c) and (d) were reproduced from Ruetsch et al., in *Comprehensive Series in Photosciences* 3:175–205, 2001, with permission from Elsevier

layer called cortex (Fig. 2.14). The cuticle is formed of proteic plaques that are lubricated by the presence of lipids. The plaques are rigid, but lubrication allows flexibility. Exposure to UV light, mainly UV-B, causes “fusion” of these plaques, turning the cuticle in a rigid non-flexible structure as a whole. The hair then becomes prone to breakage. Hair with broken ends loses its natural appearance, and UV protection of the hair is one of the main topics in cosmetic research. Loss of coloration due to oxidation of melanin pigments caused by the UV radiation is another factor that contributes to the loss of natural appearance.

While the solar UV-B radiation can be blocked and prevented from entering and damaging our eyes using glasses (not necessarily using dark lenses because UV rays are colorless, so transparent materials may be efficient in blocking UV light), one should ask ourselves how natural molecular evolution coped with the unavoidable exposure of eyes to UV radiation. The answer lies in the same concept as chemical sunscreens one uses to protect skin. All light enters the eye through the crystalline (also named “lenses,” Fig. 2.15). There are natural molecules that absorb UV light that are present in the crystalline. To ensure an efficient filtering of the whole range



**Fig. 2.15** Natural eye preventive protection against UV consists in the presence of a family of tryptophan derivatives in the crystalline (**a**). Each of these molecules absorbs UV light at a slightly different wavelength range. Acting together, they are able to filter a broad range of UV light entering the eye. Wearing glasses may be a form to reinforce the protection of eyes against UV radiation. Depending on the materials used in their manufacture, lenses may absorb UV and/or visible light. Transparent lenses do not interfere significantly with visible light but may filter, at least partially, UV light. Sunglasses having good quality lenses usually filter a substantial fraction of visible light (therefore the term “dark glasses” also used for sunglasses) and most UV light. Panel (**b**) shows the example of light filtering by vitreous material having two different possible coatings (transmittance is the fraction of light intensity that crosses the material)

of UV light, human eyes' molecules evolved to create a family of compounds derived from tryptophan, an amino acid that enters the constitution of proteins. All these molecules absorb UV light at slightly different wavelength ranges. The effect of the sum of all the spectra is a generalized blocking of UV radiation at the entrance of the eye. This is the natural barrier to UV radiation that protects the retina.

It should be stressed that cells have biochemical processes that repair the damage caused by the UV radiation on nucleic acids and other molecules. As this section focuses on light–matter interaction, this subject will not be further addressed, but it is important to keep in mind that even when molecular and cellular lesions occur due to the action of radiation, repair mechanisms exist. Pathological situations arise when both preventive and repair mechanisms fail to meet all the insults radiation exposure imposes.

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