1 The energy around us

1.1 Forms of energy

We are all subject to the laws of physics. Every process, living or not, obeys the laws of thermodynamics. Biophysical systems in living organisms must have a constant input of energy to remain alive, but will reach thermal equilibrium after death. Sufficiently small subsystems within an organism will be at thermal equilibrium, even if the organism as a whole is not at thermal equilibrium with its environment. Biophysical systems can neither create nor destroy energy, but they can manipulate energy by doing work or altering the internal energy of the system. Biophysical processes removed from equilibrium will produce an increase in entropy. When biophysical systems, including physiological ones, have an increase in energy produced by ordering the local environment, there must be a greater decrease in the energy of the universe. The difference in these energies is the change in entropy. These principles of the zeroth, first and second laws of thermodynamics appear all the time in discussing biophysics. Understanding these general principles will make understanding energy absorbance, bond formation, ion diffusion, fluid flow, muscle contraction and dozens of other processes possible.

Everyone has a basic idea of muscle contraction or blood flow. These are biophysical, and physiological, processes. A process is a transition between state functions. State functions are thermodynamic quantities, and are therefore, in the absence of external energy input, at equilibrium. Processes describe the quantitative transition between state functions or, more simply, different states. The internal energy of a system is the sum of the different states comprising that system. In the world of chemistry, there are multiple ways in which the internal energy of a system can be subdivided, including the enthalpy and the Helmholtz free energy. In discussing the energy of a physiological system, the Gibbs free energy is most relevant.

At its most fundamental level, the Gibbs free energy is

$$G = H - TS \tag{1.1}$$

where H is the enthalpy, T is the absolute temperature and S is the entropy. The exact value of S cannot be known. The change in the Gibbs free energy $\mathrm{d}G$ is more commonly used:

$$\mathrm{d}G = -S\mathrm{d}T + V\mathrm{d}p + F\mathrm{d}l + \sum_{i=1}^{m} \mu_i \mathrm{d}n_i + \psi \mathrm{d}q \tag{1.2}$$

where V is the volume, dp is the change in pressure, F is the mechanical force, dl is the change in length, μ_i is the chemical potential, dn_i is the change in the number of molecules, ψ is the electric potential and dq is the change in electric charge. This daunting equation is more complex than the molar Gibbs free energy of reaction, familiar to many students from their biochemistry classes:

$$\Delta G = -RT \ln K_{eq} \tag{1.3}$$

where R is the molar gas constant and K_{eq} is the equilibrium constant.

The difference between the equations is illustrative. The smaller free energy of reaction equation is a molecular subset of the larger Gibbs free energy equation, the reaction equation being derived by assuming that temperature, pressure, length and charge are all constant, reducing the free energy equation to a statement only relating to the chemical potential of the system. In practical terms, this is the goal of experimental science: to hold all variables constant except the one we are interested in measuring. Studies of thermal regulation focus only on dT; respiration depends on dp; muscle contraction measures dl; and electrophysiology depends on dq. All of these elements are always present, but using logic and control conditions we try to minimize the effect of outside forces that would alter our results. These forces manifest themselves as system "noise." Noise is nothing but the unintended input of one of those other elements, whether biological, such as the heating of muscle during contraction, or mechanical, such as a faulty recorder switch.

Other processes that have little influence on normal physiological systems come into play when the body is exposed to unusual energetic input, such as magnetic fields during magnetic resonance imaging (MRI). In this case, the term BdM would have to be added to the free energy equation, with B being the external magnetic field and dM the change in magnetization. Outside of the magnetic resonance magnet, this term is negligible because B is so small that there is no significant change in this term. When you analyze an MRI, consider the other terms of the free energy equation: does the temperature change during the input of radio-frequency pulses used to alter the magnetization? Does the pressure change (unlikely)? Does the person move (dl)? Does the person have a cardiac pacemaker whose performance could be altered by the magnetic field? And, importantly, is there a difference in chemical potential in different areas, such as the more hydrophobic white matter and more hydrophilic gray matter of the brain, the hydrogen nuclei of which respond differently in the magnetic field? It is the interaction between the magnetic field and the chemical potential that is used to produce contrast in the MRI.

The energy of biophysical systems, then, takes on many forms. Even as we focus on individual elements, it is important to bear in mind that other elements can sometimes play a role, even unanticipated ones. The best scientists have so much familiarity with their equipment they know that when they make an unusual finding, the *sine qua non* for all new knowledge, that finding is not due to the limitations of their equipment, but because they have uncovered something novel. True creativity, true genius, requires both technical expertise and inspired insight.

1.2 Ambient energy

We live in a world where our body temperature is 98.6° F, 37° C or 310 K. We regulate this temperature closely, no matter the temperature around us. With the exception of a few molecules near our body surfaces, the constant temperature of the body produces an average energy that the molecules in the body are exposed to. This energy is the absolute temperature T (in K) times the Boltzmann constant, k, 1.38×10^{-23} J/K · molecule. Except for those occasions when a molecule is involved in a reaction, molecules will be in equilibrium with the energy of the environment around us, E_o,

$$E_o = kT = 310\,\mathrm{K} \cdot 1.38 \times 10^{-23}\mathrm{J/K} \cdot \mathrm{molecule} = 4.28 \times 10^{-21}\mathrm{J/molecule}. \quad (1.4)$$

This is the equilibrium energy of a single molecule. When we deal with an ensemble of molecules, we measure molecules on the molar scale, using Avogadro's number, N_A, to convert Boltzmann's constant to the gas constant, R,

$$\begin{aligned} R &= N_A \cdot k = 6.02 \times 10^{23}\mathrm{molecule/mol} \cdot 1.38 \times 10^{-23}\mathrm{J/K} \cdot \mathrm{molecule} \\ &= 8.31\,\mathrm{J/K} \cdot \mathrm{mol} \end{aligned} \quad (1.5)$$

and the molar equilibrium energy to

$$E_m = RT = 8.31\,\mathrm{J/K} \cdot \mathrm{mol} \cdot 310\,\mathrm{K} = 2.58\,\mathrm{kJ/mol}. \quad (1.6)$$

Many of the traditional physical chemical measurements of molecule activity use the kT scale. We will find the RT scale useful when dealing with cellular energy levels, as they are routinely measured in kJ/mol, so that it is important to be familiar with both scales.

Like all physical systems, biological systems will go to the lowest energy state, maximizing entropy. No matter which scale is used, kT or RT, the environmental energy is the lowest energy state that living physiological systems will go toward. This energy is elevated above global thermal equilibrium (GTE), the energy of the world around us. We exist at a steady-state minimum removed from global thermal equilibrium (Figure 1.1).

All systems will try to maximize entropy, always tending to the lowest energy state possible. All living systems on the earth will tend toward the energy of the environment around them, global thermal equilibrium. All beings will reach GTE when they die. Until then, living beings must have a constant input of energy in order to counter entropy.

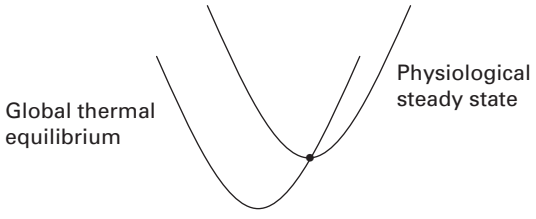

Figure 1.1 All living systems exist at a steady state whose minimum energy position (•) has a higher energy than the global equilibrium it is part of. To maintain this steady state, there must be a constant input of energy to offset entropy.

The ● in Figure 1.1 represents the steady-state point at which the vectors for energy input and entropy exactly balance one another. When death occurs, there is no longer energy input, and the ● will slide to the GTE minimum, and the being will be at equilibrium with the earth. (One could make further nestings as the earth is in a steady state relative to the sun, the sun is in a steady state relative to the galaxy, and the galaxy is in a steady state relative to the universe.) Within any substructure in the body, the molecules will be at equilibrium with the environment around them, at 2.58 kJ/mol, unless they are involved in a reaction, such as absorbance of light by melanin or 11-cis-retinal. Melanin will spontaneously return to thermal equilibrium but, as we will see below, the new all-trans state of retinal does not spontaneously return to the 11-cis state without an enzymatic reaction.

Every ensemble of molecules at equilibrium will have the same average energy, but each individual molecule within the ensemble will not have the same energy. The Boltzmann function of energy distribution shows the number (n_i) of molecules that have a given energy level (E_i), according to the relationship

$$n_i = Ce^{-E_i/kT} \tag{1.7}$$

where C is a normalization constant. Because the exponent is negative, there will be fewer molecules with a given energy as E_i increases. Each molecule in the ensemble will be subject to local conditions, such as collisions with other molecules, which will constantly change its velocity and thus its energy. Maxwell developed the equation of velocity distribution:

$$\frac{dn(v)}{n_o dv} = \frac{4}{\sqrt{\pi}}\left(\frac{m}{2kT}\right)^{3/2} v^2 e^{-\frac{mv^2}{2kT}} \tag{1.8}$$

in which the fraction of molecules (dn/n_o) is within a particular velocity range (dv). The velocity distribution is nearly symmetrical, as shown in the dashed line of Figure 1.2. Look at the exponential term: $mv^2/2$ represents the kinetic energy of the molecule, divided by kT. Since the molecules are at equilibrium, there is no potential energy, only kinetic energy, and the energy of a molecule E_m is

$$E_m = \frac{mv^2}{2}. \tag{1.9}$$

The Maxwell velocity distribution equation can be modified, multiplying the non-exponential part by $2/m \cdot m/2$ and converting $mv^2/2$ to E_m to give the energy distribution:

$$\frac{dn(E)}{n_o dE} = \frac{8}{m\sqrt{\pi}}\left(\frac{m}{2kT}\right)^{3/2} E_m e^{-\frac{E_m}{kT}}. \tag{1.10}$$

This allows us to plot the energy distribution of the molecules in Figure 1.2.

The important concept here is that even at equilibrium there will be a distribution of the energy of the molecules. They will not all have the same energy. The range of the energy distribution will be determined by the physical conditions surrounding the molecule. While its mass will not change, the molecular interactions with its surroundings will affect its velocity, and thus alter its particular energy. As can be seen in Figure 1.2, the

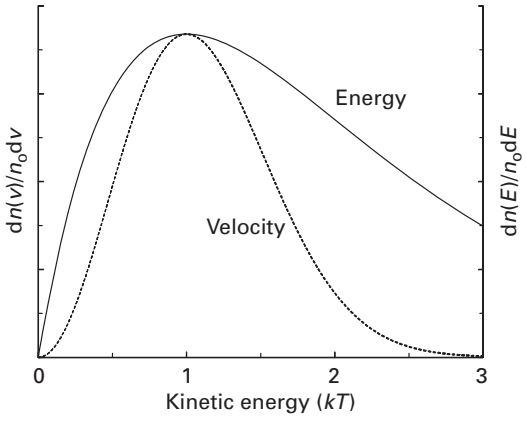

Figure 1.2 The energy and velocity distributions of molecules at equilibrium. The abscissa is plotted in units of kT relative to the ambient energy. The velocity distribution will have its peak when the $mv^2/2kT = 1$. The energy distribution will have its peak when $E_m/kT = 1$.

energy distribution will not be a normal distribution, with the most common energy occurring at a lower energy value than the average energy value.

1.3 Molecular energy

The energy associated with each atom and each bond is not continuous, but quantal, based on the electron shells occupied in the electron cloud around the nucleus. For a given atom, there would be a quantal energy distribution, with the lowest energy configuration being the most common, as Boltzmann demonstrated. Within each quantum domain small variations in thermal excitation exist. In a molecule, however, not every bond will be at its lowest energy: instead, the molecule as a whole will seek its lowest overall energy, out of the many possible configurations of attractions and repulsions that will alter bond angles and the energy in the bonds. The larger the molecule, the greater the number of potential configurations and energy levels that are possible. Because of this, the energy distributions of a molecule will appear to be continuous, as seen in Figure 1.2, but if magnified sufficiently the digital nature of molecular configurations would be revealed. For those special atoms which respond to a particular electromagnetic frequency of radiation, they may have an electron raised to a higher electron orbital. This occurs in fluorescent and phosphorescent systems and for molecules in which a particular bond is sensitive to a particular electromagnetic frequency.

The thermal energy of molecules must be distinguished from the chemical energy of molecules. All molecules, regardless of chemical structure, will see the same thermal energy kT. The different chemical structure of molecules means that different amounts of energy are trapped within the chemical bonds of different molecules. The organic part of a molecule will have its atoms connected by covalent bonds. Each of these bonds has an energy associated with it: the dissociation bond energy necessary to break the bond.

Table 1.1 Average bond dissociation energies at 25 °C

Bond	Dissociation energy (kJ/mol)
C—C	344
C═C	615
C—H	415
C—N	292
C—O	350
C═O	725
N—H	391
O—O	143
O—H	463
O_2	498

Source: Tinoco *et al*., 1995

Some of the most common covalent bond dissociation energies are listed in Table 1.1. The ambient energy in the human body is 2.58 kJ/mol. This energy is far below that of any covalent bond in the body. This means that it is statistically unlikely that any covalent bond would spontaneously break due to the random thermal fluctuations around it.

1.4 Molecular energy absorbance

Despite the thermal stability of covalent bonds in physiological systems, some of these bonds are sensitive to energy input from external sources. When energy is absorbed by a molecule, it will either release the energy as heat, returning to its original configuration, or trap some of the energy within the molecule by altering its structure, as shown in Figure 1.3. In the first case, the molecule can absorb heat from the environment without changing its chemical structure, as will occur when there is a local temperature increase. The molecule will have a higher energy. If the increase in energy is above kT (i.e., the entire environment has not increased its temperature), the molecule will come to thermal equilibrium with the environment around it, and return to its original energy state. This scenario is shown in the upper part of Figure 1.3. The absorbance of radiant energy by protein in the skin, for instance, would be an example of this. This is the most common type of energy absorbance in physiological molecules.

In the second case, shown in the lower part of Figure 1.3, a molecule will absorb energy, alter the electrons of the bonds of the molecule, and change its chemical structure. The new structure, on the right, will have its own energy minimum. It may be possible for the molecule to revert to its original structure, but this will be determined by the height of the energy barrier between the two states. The greater the height of the energy barrier, the less likely a molecule will spontaneously revert due to random energy fluctuations in its environment. If the energy barrier is less than kT, then spontaneous reversion will occur. The equilibrium between the two states of the molecule will be determined by the relative basal energy states. The higher of the two minima will have fewer elements at

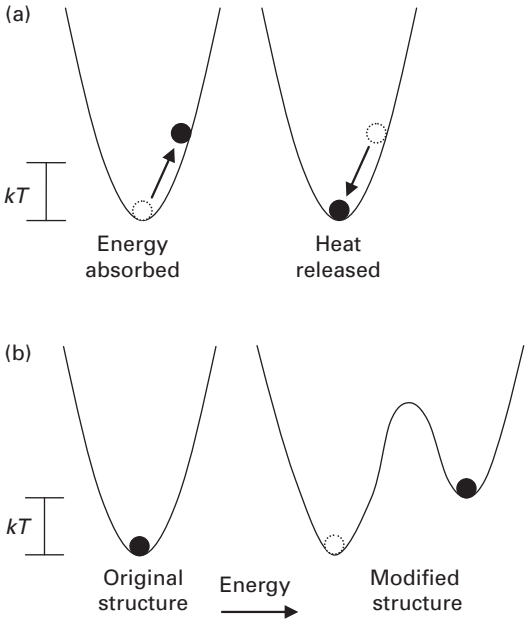

Figure 1.3 Energy absorbance within molecules. The molecule may absorb energy and radiate heat (a), or alter its chemical structure (b). The effect of kT on any state is measured from its local minimum relative to the lowest local energy barrier.

equilibrium than the lower minimum. If the two minima are equal, then at equilibrium there will be an equal number of both states. The absorption of energy may be so great that no reversion to the original state can occur. This is the case when a molecule absorbs large amounts of heat, destroying its three-dimensional structure, as occurs in the thermal denaturation of a protein. The energy barrier between the original and the new state is so great that no enzyme is capable of lowering the energy barrier sufficiently to return the molecule to its original configuration. When foods are cooked by increasing their temperature, whether by radiant heat, conduction (heating by contact) or by microwave radiation increasing the friction between water molecules and thus the internal heat, the molecules cannot revert to their original structure, as seen in the translucent to opaque conversion of egg whites. There is an energy barrier between the new and original states that greatly exceeds kT.

1.5 Molecular transduction

Between the cases in which the molecule that changes its structure can spontaneously revert to its original conformation and in which it is denatured so that no return is possible, there are particular alterations in physiological systems that can revert with the assistance of enzymes. In these cases, a particular bond can absorb energy from the surrounding environment and alter its structure. Unlike the case in which all parts of the

molecule see a higher local temperature, here a particular bond is sensitive to a particular wavelength of electromagnetic radiation, due to a match of the electron oscillation frequency of the bond and the external radiation frequency. The energy of an individual bond is not continuous, but has specific quantum energy levels. Planck postulated that the energy ε of the quantum is not fixed, but will increase as the frequency v of the oscillation increases, with Planck's constant h as the proportionality factor:

$$\varepsilon = hv. \tag{1.11}$$

The frequency of electromagnetic radiation is inversely proportional to the wavelength λ of the electromagnetic wave, with the product equal to the speed of light c in a vacuum:

$$c = \lambda v. \tag{1.12}$$

Thus, the energy, frequency and wavelength of electrons are all connected. The electromagnetic spectrum (Figure 1.4) has been subdivided from gamma rays to radio waves. Since gamma waves have the highest frequency, they will have the highest energy. Radio waves, with the lowest frequency, will have the lowest energy.

Quantum mechanics limits the states of electrons. (See the Pauli exclusion principle, not covered here, for the details of this.) The ground state for an electron is the S_0 singlet state, from which a photon can excite an electron to the S_1 singlet state (Figure 1.5).

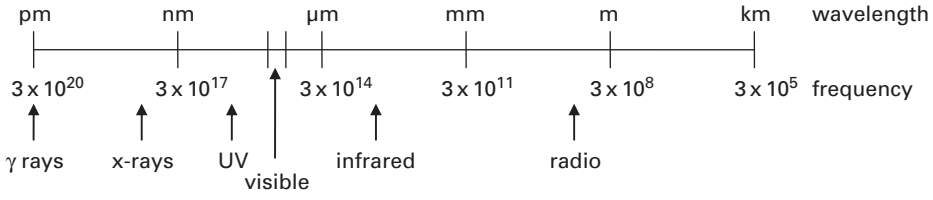

Figure 1.4 Electromagnetic spectrum. Wave energy is directly proportional to frequency and inversely proportional to wavelength.

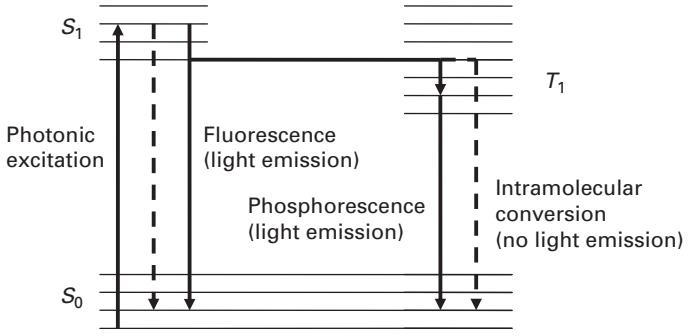

Figure 1.5 At the single atom level, the different quantum states (S_0, S_1, T_1) have thermal variations at each quantum level, indicated by the horizontal parallel lines. Fluorescence and phosphorescence do not play a role in human physiology. Intramolecular conversion occurs in phototransduction, and the destructive effects of ultraviolet and x-rays.

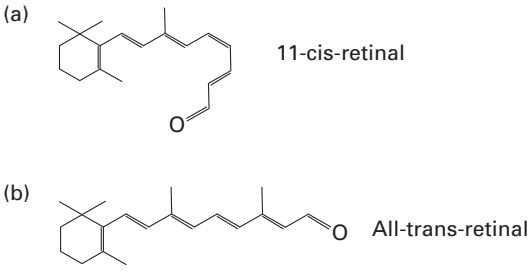

Figure 1.6 Structural forms of retinal: (a) 11-cis-retinal and (b) all-trans-retinal.

The electron can spontaneously release this energy as a photon of light: this is fluorescence. Or, there may be an energetic transfer to an adjacent triplet state T_1, from which a photon of light can be released at a different frequency: this is phosphorescence. Fluorescent radiation (10^{-9} to 10^{-5} s) is faster than phosphorescent radiation ($> 10^{-5}$ s) due to the greater stability of the T_1 state (Glaser, 2001). These mechanisms do not play a direct role in physiological systems, although innumerable laboratory methods use both in the study of biological molecules.

The third path, indicated by the dashed lines in Figure 1.5, shows an energy release after quantum absorption of energy without light emission from either the S_1 or T_1 state. In this case, a portion of the energy remains within the molecule and alters its structure. The retinal portion of rhodopsin is an example of this, as shown in Figure 1.6.

Upon exposure to light, with a maximum absorbance at 500 nm, the 11-cis form of retinal absorbs sufficient energy to be converted to the all-trans form of retinal. The all-trans form partially dissociates from the opsin portion of rhodopsin, triggering the cascade that produces phototransduction. The all-trans form does not spontaneously revert entirely to the 11-cis form, indicating that there is an energy barrier between the forms that exceeds ambient energy. An enzyme, retinal isomerase, is responsible for the conversion back to the 11-cis form. Since at equilibrium the 11-cis form predominates, it must have a lower energy minimum than the all-trans form.

DNA is particularly sensitive to UV-B radiation. Upon exposure to this wavelength, 280–315 nm, thymine–thymine and thymine–cytosine pyrimidine bridges may form. These mutations in the DNA can be corrected enzymatically, so that from an energetic perspective this change is analogous to the changes in retinal. In both cases, the product of the energy absorbance has lost its physiological function, the ability to absorb light or to pass on genetic information, respectively. But in both cases, there has been evolutionary development of enzymatic activity that can return the molecules to their original, functional state.

1.6 Ionizing radiation

DNA damage can also occur from exposure to x-rays. X-rays have wavelengths in the nanometer range, shorter than the visible and UV-B wavelengths in the cases above.

X-rays produce ionizing radiation, in which molecules are altered by ionization of one of their electrons. The usefulness of x-ray radiation for medical diagnosis lies in the ability of the x-rays to interact with tissue: if there were no interactions, all the x-rays coming from the x-ray source would uniformly pass through tissue, and the x-ray film would have no contrast. Damage to the tissue with diagnostic x-rays is slight, and in most cases can be repaired enzymatically. In contrast, radiation therapy to kill cancer cells is much more intense, with the goal of eliminating the cancerous tissue.

X-rays are highly energetic, and can ionize many different molecules. Every molecule has an energy of ionization: in the case of water, that energy is 1200 kJ/mol, 100–1000 times less than the energy of x-rays. The collision of an x-ray with a water molecule will result in the ionization of the water molecule. The ionization of water leads to the formation of the destructive radicals that are a product of ionizing radiation. There are multiple reaction sequences generated by this initial reaction. An example of one of these leading to a chain reaction of destructive reactions is as follows:

$$H_2O + h\nu \rightarrow H_2O^+ + e^- \quad \text{Ionization of water} \tag{1.13}$$

$$H_2O^+ + e^- \rightarrow H_2O^* \quad \text{Excited water molecule} \tag{1.14}$$

$$H_2O^* \rightarrow H^\bullet + OH^\bullet \quad \text{Dissociation to radicals} \tag{1.15}$$

$$R_1H + OH^\bullet \rightarrow R_1^\bullet + H_2O \quad \text{Generation of organic radical } R_1 \tag{1.16}$$

$$R_1^\bullet + O_2 \rightarrow R_1O_2^\bullet \quad \text{Generation of oxygen radical } R_1 \tag{1.17}$$

which leads to the chain reaction:

$$R_1O_2^\bullet + R_2 \rightarrow R_1O_2H + R_2^\bullet \quad \text{Covalent change in } R_1\text{, generation of radical } R_2 \tag{1.18}$$

$$R_2^\bullet + O_2 \rightarrow R_2O_2^\bullet \ldots \quad \text{Generation of oxygen radical } R_2\text{, etc.} \tag{1.19}$$

The presence of oxygen extends the destructive power of radical formation. Each organic radical has its covalent structure permanently altered, often in a manner that eliminates the normal function of that molecule. The chain reaction nature of radical formation means many molecules will be destroyed and the cell potentially killed. In the case of radiation therapy on a cancerous growth, this is the desired outcome; in the case of healthy tissue, it is not. Antioxidant molecules such as Vitamin C can stop this chain reaction. Non-lethal removal of radicals results in the production of H_2O, H_2, and hydrogen peroxide, H_2O_2. H_2O_2 is also a destructive molecule, but the enzyme catalase in peroxisomes converts hydrogen peroxide to H_2O and O_2 and stops the cycle of destruction.

X-rays are of course not the only imaging technology. Computerized tomography (CT) images produce two-dimensional images using Radon transforms of multiple x-ray scans. Closely related to CT is positron emission tomography scanning, or PET. PET scans use a metabolic tracer radio-labeled with a positron-emitting nuclide.