Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Citation: M. Henry, L. Schwartz (2019) Entropy export as the driving force of evolution. *Substantia* 3(2) Suppl. 3: 29-56. doi: 10.13128/Substantia-324

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Entropy export as the driving force of evolution

Marc Henry, Laurent Schwartz*

Assistance Publique des Hôpitaux de Paris, Paris, France
*Corresponding author: dr.laurentschwartz@gmail.com

Abstract. The entropy concept was forged by the middle of the nineteenth century to predict how a chemical system may undergo spontaneous evolution over time. At the dawn of the twentieth century, four principles of a new science called "thermodynamics" have been firmly established. Concept of thermal equilibrium (zeroth law), conservation of energy (first law), spontaneous increase in entropy over time (second law) and vanishing entropy at the absolute zero of temperature (third law also called Nernst's theorem). Among these principles, the most troublesome one was the second law that points to gases as the end final product of any evolution in a closed system. From direct observation, it seems that biological systems undergo spontaneous evolution from gases characterized by a maximum entropy to highly complex structures displaying considerably lower entropies, an apparent violation of the second law. However, it is also perfectly allowed to view the Earth as an open system able to undergo a spontaneous (local) decrease of entropy, provided that the excess entropy could be efficiently exported towards the whole universe through invisible infrared photons. Provided that the entropy exported as invisible infrared radiation is much higher than the entropy decrease observed on Earth, life apparition on this planet becomes fully compliant with thermodynamics laws. The consequences of such an enlarged viewpoint taking into account all kinds of processes (reversible as well as irreversible ones) are studied in depth in this paper. Here we advocate that the first requirement allowing spontaneous life apparition on Earth is the existence of a metabolism, taking the form of thermodynamic cycles able to generate a large output of entropy by degrading low entropy molecular systems (food) into high entropy molecular compounds (waste). Two possible cycles have been identified, relying on the very low entropy of Earth's metallic core for generating reducing gases (such as H_2, CO, NH_3, H_2S) coupled to the low entropy of sun's radiation (food) and producing minerals (such as serpentine, metallic sulfide, magnetite, goethite) as well as high entropy gases (water, carbon dioxide) as waste. The large entropy flux generated by such processes can then be used to build low-entropy molecular systems based on reduced carbon species and soluble phosphates that are observed in any living cell. Another consequence of this approach is stressing the ubiquitous role played by water in every feature of life, through the concept of "water activity".

Keywords. Entropy, Life, Water activity, Irreversibility, Thermodynamics, Biology.

INTRODUCTION

The apparition of life is a deep rooted mystery. Life appeared on earth over 3,6 billions years ago. Life is a robust phenomenon with similar concentrations of sodium, potassium and chloride in every living cell. Moreover, similar lipoproteins constitute the membranes and the nucleic acids are always built with the same bases.

Taking the problem from the biological side, focusing on protein-trafficking related to gene activation and transcription, as currently done is a daunting task. Putting numbers on the table, a back of the envelope calculation leads to an average concentration of 3 millions of proteins per μm3 of cell.[1] This translates into 3 millions of proteins for E. Coli ($V \approx 1$ fL), 150 millions for budding yeast *S. cerevisiae* ($V \approx 50$ fL)[2], and 3 billions for a mammalian cell ($V \approx 1$ pL).[3] Concerning the human genome, we are facing about 18,000 genes[4] able producing about 100,000 different types of proteins.[5] Nowadays, only super-computers are able to crawl in huge databases giving rise to new research domains such as genomics, proteomics or metabolomics, epigenomics, lipidomics, glycomics, etc.

An alternative line of research is to admit that such a complexity accessible only through high-speed computers is sustained by laws deep-rooted in physics and chemistry. If this is the case, then identifying some key physicochemical variables should be enough to control cell growth and proliferation.

In deep contrast with biology, physics does not look for detailed mechanisms, facts being the mere consequences of fundamental laws given a certain set of initial conditions. The goal of this paper is to suggest that the universality of only one form of life might be the simple consequence of the second law of thermodynamics.

Such an approach is obviously complementary to the –omics strategy and would allow physicians to act on diseases without the help of computers, would also considerably reduce the number of drugs for healing patient and most importantly would allow using very cheap chemicals instead of drugs characterized by sky-rocketing prices.

The paper is organized as follows. In a first section we will go back to abiotic Hadean times where only physical and chemical events were able to take place. This will allows us to identify key physicochemical parameters that should be still operational in modern cells. The second part of the paper will be the formulation of bioenergetics in terms of irreversible entropy variations instead of energy changes.

In a third part, this new quite general formalism will be applied to prebiotic chemistry of lipids, aminoacids as well as phosphate-based compounds. Finally, a last part will stress the important role played by the water activity in anabolism and catabolism processes.

HADEAN TIMES

Life seems to originate with the formation of liquid water on Earth about 4,4 Gyr ago.[6] First forms of life as prokaryotes seem to appear about 3,8 Gyr ago.[7] During this first period covering 600 Myr, we have to make a mapping between what we currently know about the last universal common ancestor of all cells (LUCA) and some basic chemistry. Using phylogenetic trees for 6.1 million protein coding genes from sequenced prokaryotic genomes has allowed reconstructing the microbial ecology of LUCA.[8] With its 355 identified genes, LUCA could be viewed as anaerobic, CO_2-fixing, H_2-dependent (elimination of acetate via the reduction of acetyl-CoA), N_2-fixing and thermophilic. LUCA's biochemistry was replete with FeS clusters and radical reaction mechanisms. Its cofactors reveal dependence upon transition metals, flavins, S-adenosyl methionine, coenzyme A, ferredoxin, molybdopterin, corrins and selenium. Its genetic code required nucleoside modifications and S-adenosyl methionine-dependent methylations.

Our reasoning is based on the hypothesis that entropy-driven networks of small molecules ruled by thermodynamics afford better odds as the initiators of life than a set of highly complex molecules such as RNA and proteins. Among all the scenario proposed for life apparition on earth, it appears, as explained thereafter, that the geochemical rocky route is the only one compatible with LUCA's portrait emerging from phylogenetic trees analysis. Starting from geochemistry occurring at hot, reduced, alkaline hydrothermal vents, the route leads to a prebiotic era, followed by the successive development of RNAs, RNPs, DNAs molecules as water becomes cooler, more oxidized and more acidic. This means that we may set aside the genetic definition of life to focus on a more water-based picture. The necessity of putting water at the front stage is well illustrated by figure 1 that describes the composition of the Gram-negative prokaryote *Escherichia coli* (E. coli).

The key point here is to focus on the molar fraction scale for quantifying the amount of matter. On such a scale, it should be obvious that life is a water-based phenomenon (99.3 mol%) with inorganic ionic species playing the major role (0.5 mol%) over organic matter (0.2 mol%). Basically, every event in a living cell appears to be orchestrated by water and ions, organic matter behavior being under the full control of these two kinds of chemical species.

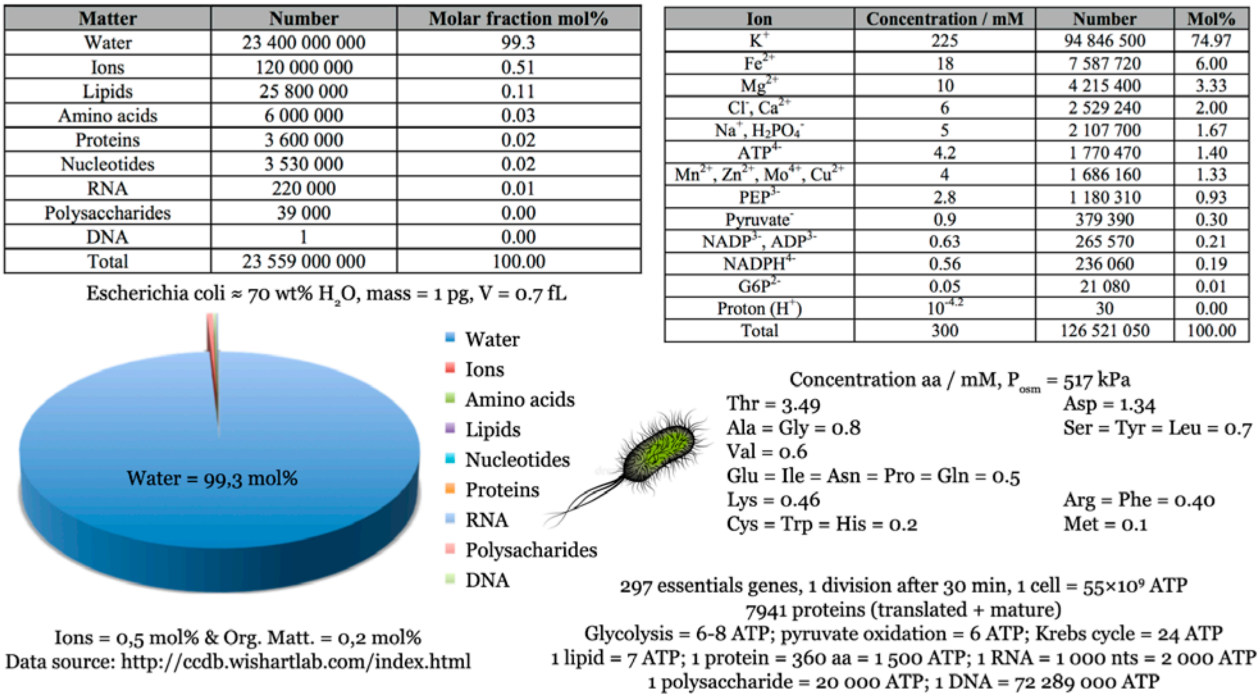
Shapiro stated five requirements for the emergence of life:[10]

1. Existence of a membrane allowing defining compartments able to sustain ionic concentration gradients.
2. Existence of an external energy source to drive the increase in complexity and biodiversity.
3. Existence of a growing and splitting mechanism for the network allowing information duplication.
4. Existence of a chemical network able to sustain circular transformations (cycles) permitting adaptation and evolution.
5. Existence of coupling mechanisms linking the release of energy with entropy exports.

In fact some of these points have already been answered. Point #1 is by no means mandatory, as it has been demonstrated that water adjacent to hydrophilic surfaces and submitted to radiant energy from the sun or from a geothermal vent, is perfectly able to produce coalescence, order, and even function at multiple levels of organization without any enclosing membrane.[11] Point #2 is thus automatically solved.

Point #3 is based on the trivial fact that a physical collection of small items holds the same information as a list that describes the items formally with a code. In other words there is no difference in terms of information between holding ten real metallic gold coins in your pocket and holding them virtually in a bank account. In both cases, you will be able to take the same decisions on how to spend such an amount of money despite the fact that one set is purely material and the other one is completely dematerialized. There is thus no need to store information in a single molecule such as DNA (playing in the cell the role of the bank account) that have to be duplicated and passed to the descendants as observed in a modern cell. Self-replicating reverse micelles[12] or mere aggregates of iron sulfide bubbles[13] are perfectly able to do such a job (equivalent of gold coins).

Concerning point #4, it should be clear that one cannot rely on the citric acid cycle or on its reverse version owing to the difficulty of achieving all of the required reactions using only mineral catalysts.[14] Accordingly, there is so many side reactions in the citric acid cycle that a large number of different mineral surfaces would be needed to close the cycle. A much simpler version with fewer steps is then required. Fortunately, the existence of a proto-metabolic analog of the TCA involving two linked cycles, which convert glyoxylate into CO_2 has been recently evidenced.[15] To initiate the two cycles, only glyoxylate H-CO-COO- and pyruvate CH_3-CO-COO- are needed with the help of hydrogen peroxide H_2O_2 all reactions proceeding under



Matter	Number	Molar fraction mol%
Water	23 400 000 000	99.3
Ions	120 000 000	0.51
Lipids	25 800 000	0.11
Amino acids	6 000 000	0.03
Proteins	3 600 000	0.02
Nucleotides	3 530 000	0.02
RNA	220 000	0.01
Polysaccharides	39 000	0.00
DNA	1	0.00
Total	23 559 000 000	100.00

Escherichia coli ≈ 70 wt% H_2O, mass = 1 pg, V = 0.7 fL

Ion	Concentration / mM	Number	Mol%
K^+	225	94 846 500	74.97
Fe^{2+}	18	7 587 720	6.00
Mg^{2+}	10	4 215 400	3.33
Cl^-, Ca^{2+}	6	2 529 240	2.00
Na^+, $H_2PO_4^-$	5	2 107 700	1.67
ATP^{4-}	4.2	1 770 470	1.40
Mn^{2+}, Zn^{2+}, Mo^{4+}, Cu^{2+}	4	1 686 160	1.33
PEP^{3-}	2.8	1 180 310	0.93
$Pyruvate^-$	0.9	379 390	0.30
$NADP^{3-}$, ADP^{3-}	0.63	265 570	0.21
$NADPH^{4-}$	0.56	236 060	0.19
$G6P^{2-}$	0.05	21 080	0.01
Proton (H^+)	$10^{-4.2}$	30	0.00
Total	300	126 521 050	100.00

Concentration aa / mM, P_{osm} = 517 kPa

Thr = 3.49
Asp = 1.34
Ala = Gly = 0.8
Ser = Tyr = Leu = 0.7
Val = 0.6
Glu = Ile = Asn = Pro = Gln = 0.5
Lys = 0.46
Arg = Phe = 0.40
Cys = Trp = His = 0.2
Met = 0.1

297 essentials genes, 1 division after 30 min, 1 cell = 55×10^9 ATP
7941 proteins (translated + mature)
Glycolysis = 6-8 ATP; pyruvate oxidation = 6 ATP; Krebs cycle = 24 ATP
1 lipid = 7 ATP; 1 protein = 360 aa = 1 500 ATP; 1 RNA = 1 000 nts = 2 000 ATP
1 polysaccharide = 20 000 ATP; 1 DNA = 72 289 000 ATP

Ions = 0,5 mol% & Org. Matt. = 0,2 mol%
Data source: http://ccdb.wishartlab.com/index.html

Figure 1. Molecular composition and basic energetic needs for the Gram-negative prokaryote Escherichia coli according to the CyberCell Database (CCDB).[9]

neutral aqueous conditions and at mild temperatures (figure 2). Here, the molecule oxaloacetate or 2-oxo-succinate (2-OS) is acting as a central hub allowing continuous production of either malonate (upper part) or 4-hydroxy-oxo-glutarate (lower part). So one, could speak of an 2OS-cycle fed by a GPH-flux.It was also shown that aspartic acid, a crucial aminoacid, might be produced by reacting malonate with α-hydroxy-glycine (α-HG). Moreover, the conversion of malate to oxaloacetate is an elementary step of the citric acid cycle. The problem now boils down to the emergence of only three chemicals: glyoxylate, pyruvate and hydrogen peroxide from inorganic species. As explained below, hydrogen peroxide is continuously produced using solar radiations, while glyoxylate and pyruvate precursors are continuously produced at the mouth of hydrothermal vents. Hydrogen peroxide plays here the role of the very first electron acceptor and glyoxylate and pyruvate, the very first electron donors. Please notice that there is no need here to invoke negative loops and regulation signals that occur in modern biological systems. Our point is that for the most primitive living cell, a few cycles and the laws of kinetics are all is needed for starting chemical oscillations, as shown by the work of Ilya Prigogine.[16] We should thus at this point speak a little bit of bioenergetics in order to write a plausible scenario in order to discuss point #5 more deeply. Here is a summary of the proposed reformulation of Shapiro's conditions:

1. Lipid membranes replaced by polarized multilayers of water (EZ-water) as proposed by Gilbert Ling in 1962 and Gerald H. Pollack in 2013.
2. External energy source may be of two kinds, sun or earth, as only IR radiations matter for EZ-water creation.
3. No mechanism for information duplication as only brute copying of a full set of molecules is needed.
4. Identification of a 2OS-cycle based on glyoxylate, pyruvate and hydrogen peroxide precursors (GPH-flux) for the first chemical oscillations.
5. Coupling mechanisms concerns only entropy exports as only one kind of energy (IR radiations) is used by the cell for building water layers.

BIOENERGETICS: THE KEY ROLE OF ENTROPY

Bioenergetics is a domain based on thermodynamics, a science that is often misinterpreted. The name "bioenergetics" is by itself quite misleading as it could make believe that evolution is ruled by energy exchanges with states of high energy evolving spontaneously into states of low energy. In fact, this cannot be, as energy being a conserved quantity has nothing to say about spontaneous evolution. The entity giving a preferential direction to energy fluxes is in fact entropy, a state variable characterizing how energy may spread among all the available degrees of freedom. Accordingly, the second law states that entropy should always increase or remain constant in any spontaneous chemical reaction. So, faced with any chemical reaction, we should always refer to entropy variations. But referring to entropy and not to energy is not the attitude adopted by most scientists. In order to speak only in terms of energy and never refer explicitly to entropy, scientists have masqueraded the entropy concept under various expressions such as "internal energy" for adiabatic and isochoric processes, "enthalpy" for adiabatic and isobaric changes, "Helmholtz's free energy" for isothermal and isochoric changes and "Gibbs's free energy" for isothermal and isobaric processes. For open systems able to exchange both heat and matter, it is necessary to introduce chemical potentials, yet another masqueraded form of entropy. If electrons are exchanged during a transformation, entropy is again masqueraded as a redox potential.

As stated by the second law of thermodynamics, any spontaneous irreversible evolution corresponds always to an *increase* in entropy inside the system. However, the price to be paid for any internal increase in entropy is always a loss of *structure*. The reason behind such a loss of structure is merely that energy may be spread either on positions of matter particles (potential energy) or on the velocities of these matter particles (kinetic energy). In such a context, increasing its entropy means that the system spread its energy more on velocities (more kinetic energy) than on positions (less potential energy). When kinetic energies becomes larger than potential energies, chemical bonds responsible for the existence of solid as well as liquid structures break down, the volume occupied by the system becoming delimited by the container and no more by the chemical bonds.

Here, another pitfall should be avoided by speaking of loss of structure upon any entropy increase and not of a loss of *order*. A liquid has always a much smaller entropy than a gas despite the fact that both systems are completely disordered. The lower entropy of the liquid comes from the fact, positions above the liquid/gas interface are accessible only to a very small number of the constituting matter particles (vapor pressure). Similarly, a solid will always have an entropy smaller than a liquid, as constituting matter particles are doomed to remain at fixed points in space, thus being unable to occupy all the available positions in the proper volume. In a liquid, any constituting particles may be found *everywhere* in the proper volume, meaning a much greater entropy. Consequently, there is absolutely no need to invoke order/disorder arguments for discussing entropy changes.

Recognizing that thermodynamic potentials are in fact masqueraded irreversible entropies of variation and definitively not energies, leads to a considerable simplification of thermodynamics. Accordingly, instead of dealing with a multitude of different kind of energies (mechanical, thermal, chemical, electrical, magnetic…), it remains only two basic types of *real* energies. First, the energy stored in electromagnetic waves or in massless photons (radiant energy). Second, the energy associated to particles having a non-zero rest mass (gravitational, kinetic and nuclear energies). All the other forms of “energies” are in fact irreversible entropy variations ΔS_i that can be translated into energetic equivalents after multiplication by the temperature at which the transformation occurs. One may thus formally write, $\Delta G = -T{\cdot}\Delta S_i$, with a criterion of spontaneous evolution $\Delta G \leq 0$ owing to the fact that according to the second law one should always have $\Delta S_i \geq 0$. As explained above, one is thus obliged to add the term “free” to the term “energy” to remember that we are dealing here with an irreversible entropy change and not with an energy change.

We thus propose here to adopt such a very convenient convention sticking closely to what is in fact really occurring during a spontaneous evolution within a system. The only fundamental law is thus that everything proceeds from a state of low entropy towards a state of high entropy. If the system is not able to export this excess of entropy towards its surroundings the entropy irreversibly grows inside the structure and at some point, the structure breaks down. Such a behavior is typical of transformations occurring in a beaker or in the universe where inert structures are doomed to disappear irreversibly after breaking down into smaller and smaller units of matter. At the end, solids and liquids evaporates leaving only a gas, which is always the state having the largest entropy for a given composition.

The situation is different, if it exists inside the system a mechanism able to export entropy from its interior towards its exterior: this means that the various components of the system should now cooperate in order to absorb compounds having the lowest possible entropies and reject in its environment compounds having the highest possible entropies. As shown by the laws of irreversible phenomena, any system being crossed by

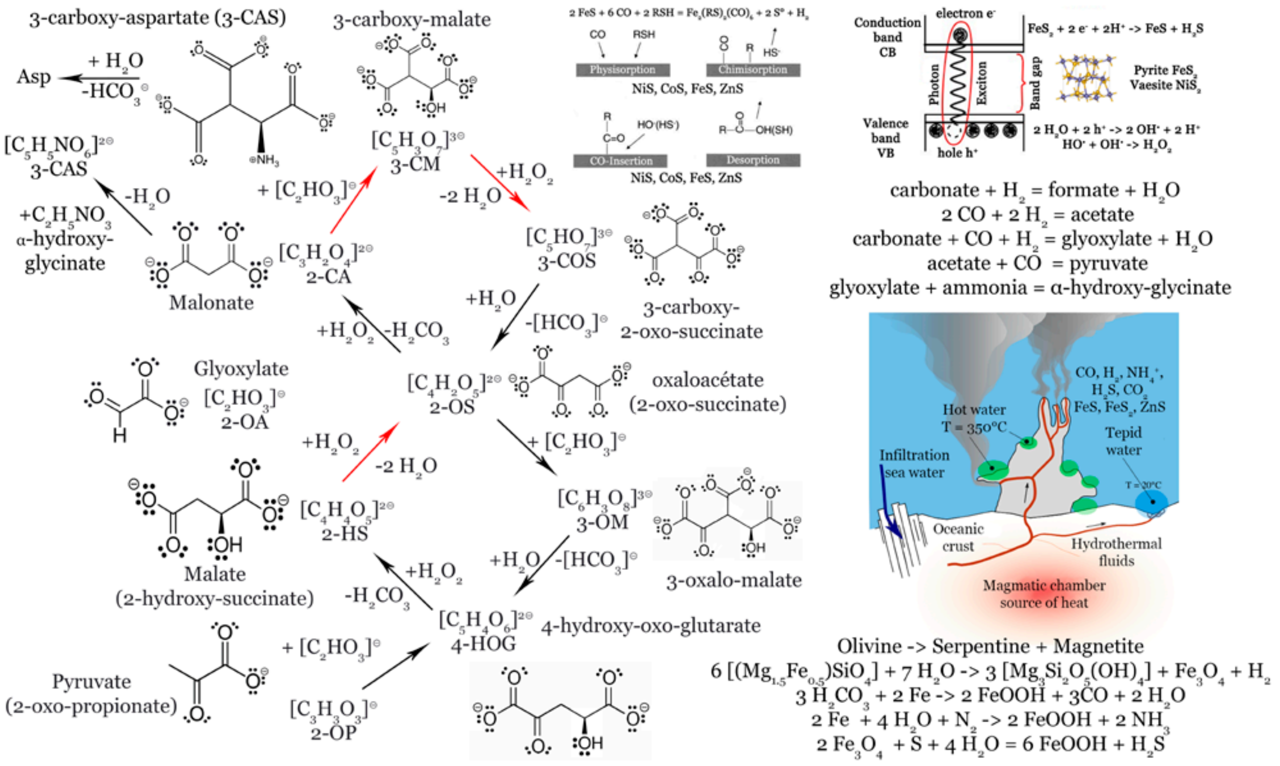


Figure 2. Two abiotic cycles each oxidizing glyoxylate into carbon dioxide CO2 with the regeneration of oxaloacetate, a key intermediate of the modern tricarboxylic acid cycle. Such reactions progress significantly in hours at pH values 7–8.5 at 50 °C (red arrows) or 23 °C without the help of enzymes.[15]

an irreversible flow of entropy, becomes automatically self-organized.16 The higher the entropy flux, the higher the number of functional links between different the parts of the system. The key point here is the unavoidable existence of fluctuations that could be organized into spatial and temporal coherent behavior (dissipative structures) when the system is brought far enough from equilibrium. Mathematically speaking, one may write the following fundamental equation for entropy variations, $dS = d_iS + d_eS$, where d_eS is the variation of entropy due to exchanges with the surroundings and diS the entropy increase due to irreversible processes occurring inside the system, such as diffusion, chemical reactions, heat conduction and so on.

Basically, one has $d_eS = 0$ for an isolated system, meaning that $dS = d_iS \geq 0$, according to the second law. This means that if a system in evolution is not able to export any irreversible internal increase in entropy towards its surroundings, it is doomed to land in a state of maximum entropy, where all available energy is spread among all the possible degrees of freedom (positions and velocities). In other words, any kind of structural arrangement observed at a given time is doomed to become a gas at an infinite time. This is what is expected for any kind of inert matter.

The situation is completely different as soon as a mechanism is available for exporting the newly generated entropy in order to avoid entropy accumulation. The most interesting case occurs for open systems that are allowed to exchange both heat and matter towards their surroundings. Here, during evolution, ejection of wastes of sufficiently high entropies or release of a sufficient amount of heat, allow reaching a state where entropy is smaller than at the beginning, meaning that $d_eS \leq 0$. Such a state which is highly improbable from a statistical viewpoint and doomed to disappear in a closed or isolated system, could in this case survive indefinitely in a steady state (dissipative structure) provided that $dS = 0$ or $d_eS = -d_iS \leq 0$. This basically means that highly improbable states need just highly efficient mechanisms of entropy export, and that there is no upper limit in complexity. The price to pay for being quite complex is that the higher the entropy export, the higher the destruction of the surroundings that have to sustain such a huge flow of entropy coming from the complex system.

It is worth noticing that here we are considering the whole earth as a living system exporting entropy towards the whole universe through infrared radiations centered on a wavelength of about 10 µm. The fact is that the universe has very few structures by itself and is in continuous expansion, meaning that it is able to absorb an almost infinite amount of entropy from the earth. And if we have so many structures on earth, it is precisely because the universe is almost totally devoid of structures. This obviously does not apply to the earth that is not in expansion. Being on earth, living systems should export their entropy first to the earth putting the burden on the earth for exporting entropy towards the universe, the ultimate garbage. However, the rate of export towards the universe is finite: $\Delta S \approx 1\ W{\cdot}m^{-2}{\cdot}K^{-1}$. If living systems release entropy in their immediate surroundings at a rate higher than this critical value, the earth will begin to accumulate entropy with the immediate consequence of breaking down structures. Natural means for such breakdowns are tornadoes, hurricanes, floods, fires, volcanoes and earthquakes.

Consequently, when the surroundings becomes saturated with wastes, entropy export is doomed to be reduced, leading to the disappearance of the dissipative structure with a breaking down into smaller components as explained above. Here is the thermodynamic basis for aging and death of any kind of living system. Obviously one of the most efficient ways of exporting entropy is to release gases, explaining why most complex living systems always hold an internal machinery for manipulating gases. Another very efficient way of exporting entropy, is by releasing heat (i.e. infrared radiation), explaining why complex living systems are systematically warmer that their surroundings.

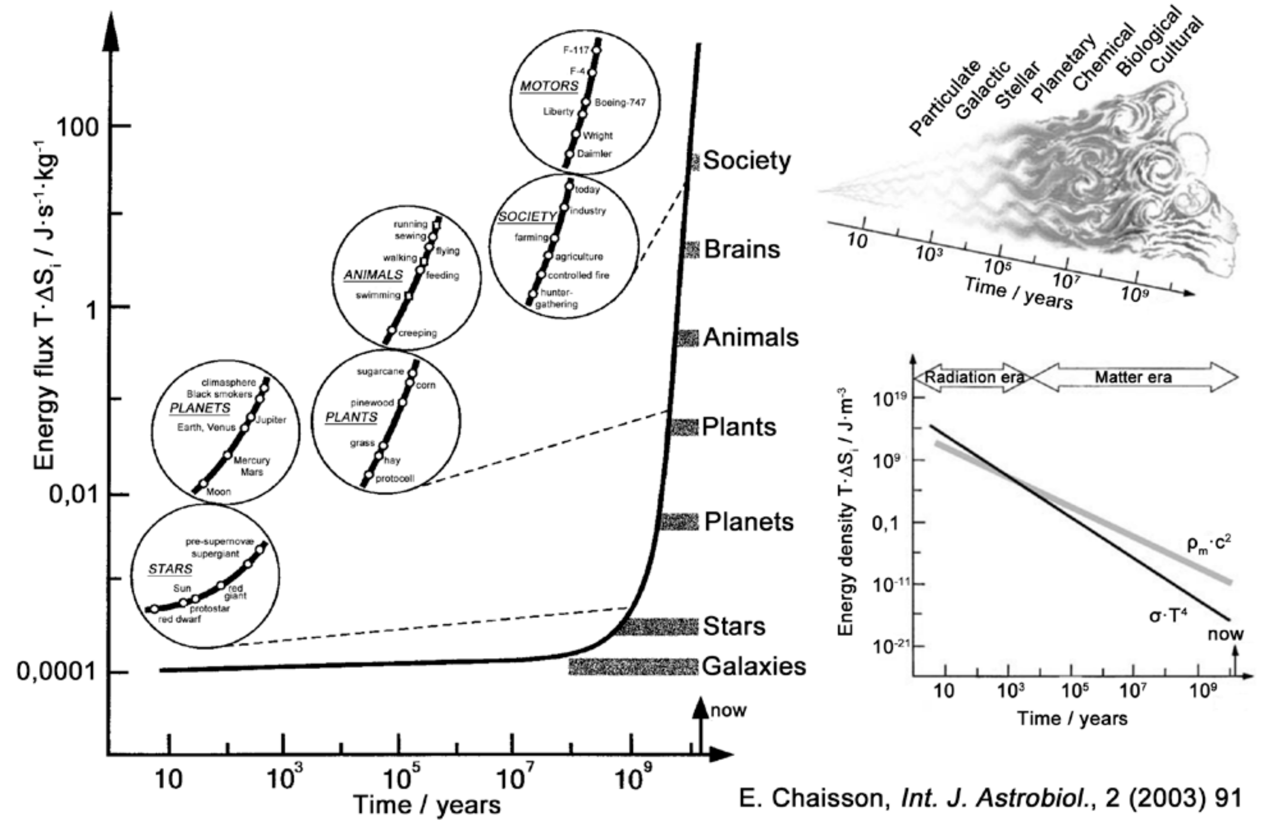
A basic equation for life should then be: foods (in) = biomass (in) + heat (out) + wastes (out), where "in" and "out" refer to a system surrounded by a containment able to exchange heat and matter between the inside and the outside. Now in the following, we will assume that each substance carries within its structure a certain amount of a so-called irreversibility potential noted Πi hereafter. Such a new term is here introduced for stressing the fundamental role played by irreversible processes and also stressing the fact that it is a thermodynamic entity ruling a possible evolution that may occur or not depending on kinetic factors. Its main role is to put on a quantitative basis the ability of substance of being a food (low irreversibility or entropy-poor substance) or a waste (high irreversibility potential or entropy-rich substance). As shown below, for systems evolving at constant temperature and pressure, the irreversibility potential is measured by the ratio of the opposite chemical potential -µ divided by the constant temperature T, that is to say $\Pi_i = -\mu/T$. The irreversibility potential has thus the dimension of an entropy. The reason for changing the name is that such a correspondence between irreversibility potential and chemical potential exists only if pressure and temperature remain constant during the evolution. For other experimental conditions, the numerical values are changed, but the rule remains the same. For instance for an isolated system unable to exchange heat and matter with its surroundings Π_i may now be identified directly with entropy and no more with the chemical potential.

Consequently, for a system absorbing one type of food F having an irreversibility potential Π_i^F, used to create one type of molecule M having an irreversibility potential Π_i^M and generating an amount of heat Q with ejection of one type of waste W having an irreversibility potential Π_e^W, a fundamental law of existence should be:

$$\frac{dS}{dt}=\frac{d_iS}{dt}+\frac{d_eS}{dt}= \\ -\Pi_i^F\cdot\frac{dn_F}{dt}+S\Pi_i^M\cdot\frac{dn_M}{dt}+\frac{1}{T}\cdot\frac{dQ}{dt}+\Pi_e^W\cdot\frac{dn_W}{dt}\geq 0 \qquad (1)$$

If several types of foods are needed or several types of wastes produced, one should subtract a term $\Pi_i(k)\cdot dn_k/dt$ or add a term $\Pi_e(j)\cdot dn_j/dt$ for each type of food (k) or waste (j), n being the number of entities absorbed (food), created (biomass) or ejected (wastes) by the system. Systems with a low dS/dt are simple, complexity increasing rapidly with any increase in dS/dt.

Figure 3 gives an illustration of such a relationship.[17] According to such a law, in order to have the largest dS/dt, one should have on input foods of very low irreversibility potentials ($\Pi_i^F \approx 0$) that should be metabolized at the smallest possible rate ($dn_F/dt \approx 0$) in order to create large entities ($\Pi_i^M >> 0$) at the highest possible rate ($dn_M/dt >> 0$). On output, one should evacuate the largest amount of heat at the lowest temperature and/or eject wastes having the highest irreversibility potentials ($\Pi_e^W >> 0$) at the highest admissible rate ($dn_W/dt >> 0$). Any increase in dn_F/dt (overfeeding), decrease in dn_M/dt (starvation), increase in temperature surroundings (heating) or decrease in dnW/dt (clogging) would, below a critical threshold, lower dS/dt and brings the system closer to its death.



E. Chaisson, *Int. J. Astrobiol.*, 2 (2003) 91

Figure 3. The exponential rise in complexity as a function of irreversible energy flux (left). Top-right: arrow of time and major evolutionary phases that have produced the visible universe. Bottom-right: the crossover between evolution in time of radiation energy density (Stefan-Boltzmann $\sigma\cdot T^4$ law) and matter energy density ($\rho m\cdot c^2$) leading to neutral atoms formation some ≈ 105 years after the big-bang. Adapted from Eric J. Chaisson's work.[17]

It is worth noting that these are very general rules that apply to any kind of living system whatever its internal machinery and complexity. More particularly, the higher is dS/dt, the higher the complexity and the higher the dependence on the surroundings for survival. We will now apply such a universal recipe to prebiotic life as represented in figure 2. To do this, we have to know reasonable values measuring irreversibility potentials. In fact, as explained above, for systems evolving at constant temperature and pressure, we should have $-T\cdot\Pi_i = \mu$, where μ is Gibbs 's chemical potential, or partial molar Gibbs free energy. So, all the data we need are contained in tables of Gibbs's free energies. As such tables give data for a particular reference state (usually T° = 298.15 K and p° = 100 kPa), it is worth knowing how the chemical potential varies with temperature, pressure and composition. We will use the following expression easily derivable from the definition of a chemical potential:[18]

$$\Pi_i(M,T,p,n) = \Pi_i^0(M) + S_m^0(M)\cdot\left[\frac{T}{T^\circ}-1\right] - V_m^0(M)\cdot\left[\frac{p}{T^\circ}-\frac{p^\circ}{T^\circ}\right] - R\cdot\ln a(M) \tag{2}$$

Here, $S_m°(M)$ and $V_m°(M)$ are the molar absolute entropy and molar volume of substance M respectively while a(M) is the activity of the substance when not in pure state, that is to say mixed with other substances. For a pure substance a(M) = 1 by definition, meaning that $S_i°(M)$ is the standard irreversibility potential at T = T°, p = p° of the pure substance, a(M) = 1. Choosing the elements in their standard state as setting the level zero of irreversibility potential leads to $\Pi_i°(M) = -\Delta_f G°(M)/T$.

Table 1 gives some selected values that we should use to follow the irreversible flux of entropies associated to a possible abiotic metabolism represented formally in figure 2. Concerning units, the usual choice for energy in to use kilojoule per mole ($kJ\cdot mol^{-1}$) in chemistry and electron-volt (eV) in physics. In fact, these two units have been arbitrarily chosen as respectively convenient for calorimetry where heat fluxes are expressed in joules and for electrochemistry or nuclear physics where charges are measured in coulombs and potentials in volts. In order to switch from the macroscopic world (joules, volts) to a microscopic world, one uses respectively the Avogadro constant $N_A = 6.02214129\times10^{23}$ mol^{-1} and the elementary charge $e = 1.602176565\times10^{-19}$ C. The drawback of such units is that they are not related to biological conditions that are centered on a characteristic temperature T ≈ 300 K. A much better unit of energy for biology should thus be $E = k_B\cdot T \approx 4$ zJ, where $k_B = 0.013806488$ $zJ\cdot K^{-1}$, is Boltzmann's constant and 1 zepto-joule (zJ) = 10^{-21} J. This explains why table 1 uses the zepto-joule as a fundamental unit of energy, with 1 $kJ\cdot mol^{-1}$ = 1.66 zJ and 1 eV = 160.2 zJ as conversion factors between the new unit (zJ) and the old ones. Similarly, the standard volumes are directly expressed in nm^3 (1 nm^3 = 10^{-21} cm^3) in order to compare the standard entropy S° with a state where all atoms are just mixed together without making any chemical bonds. For such an "ideal" state where the only source of entropy increase Sid is diffusion through translation in space , one may use the Sackur-Tetrode equation allowing establishing a direct link between S^{id} and elementary quantum processes:[19]

$$\frac{S^{id}}{k_B} = 0.83 + 1.5\cdot\ln\{(M/Da)\cdot(T/K)\} + \ln\left(V/nm^3\right) \tag{3}$$

PREBIOTIC CHEMISTRY

Let's now show how such a table should be used for understanding what is happening during any irreversible evolution. First, the table has been organized by ascending order of standard irreversibility potential $\Pi_i°$ in order to see at once what substance could be a food (top of the table) and what substance would be a waste (bottom of the table). One thus immediately see that metals and gases are the most interesting foods, while liquids and solids are better qualified as wastes. This explains the basic scheme of most complex living systems: breathing gases (air) and rejecting liquids (urine) and solids (feces).

A fundamental point of living systems is that even a waste can be used as a food by rejecting another waste, provided that such a waste has a higher irreversibility potential than the waste used as a food. A good example is provided by the bacteria *Shewanella oneidensis*, that is able to "respire" from goethite α-FeOOH, the iron ore responsible for the brown color of the soil, and reject black magnetite Fe_3O_4 as a waste.[20] As can be seen in table 1, this is possible because the irreversibility potential of magnetite is higher than the one of goethite. This shows that when speaking of living system, solids minerals should not be discarded as possible source for food. This explains, why after its apparition, life may be found in different ecological niches.

On such a ground, metallic iron or nickel owing to their very small irreversibility potentials should be

Table 1. Irreversible standard potentials Π_i° (T = 298.15 K, p = 100 kPa), standard absolute entropies S° and standard molecular volume V° for some selected compounds involved in the prebiotic metabolic cycle shown in figure 2. Values being given per molecule or motif, the unit of energy corresponds to the zepto-joule (zJ), with 1 zJ = 10^{-21} joules and 1 kJ·mol^{-1} = 1,66 zJ. See annex for the derivation of this table from literature data. (s) = solid, (liq) = liquid, (g) = gas and (aq) = aqueous solution.

Substance (state)	Formule	Π_i° / zJ·K^{-1}	S° / zJ·K^{-1}	V° / nm^3
Nickel (g)	Ni	-2.14146	0.30255	41.164
Iron (g)	Fe	-2.06460	0.29973	41.164
Iron (liq)	Fe	-0.55808	0.16550	0.01321
Iron (s)	ε-Fe (hcp)	-0.02507	0.05525	0.01132
Iron (s)	γ-Fe (fcc)	-0.02490	0.05694	0.01151
Iron (s)	α-Fe (bcc)	0	0.04533	0.01178
Nickel (s)	Ni	0	0.04965	0.01094
Sulfur (s)	S	0	0.05322	0.02576
Dihydrogen (g)	H_2	0	0.21700	41.164
Dinitrogen (g)	N_2	0	0.31816	41.164
Di-oxygen (g)	O_2	0	0.34066	41.164
Ammonia (g)	NH_3	0.09134	0.32010	41.164
Hydrogen sulfide (g)	H_2S	0.18602	0.34174	41.164
Troilite (s)	FeS	0.56419	0.10013	0.03022
Hydrogen peroxide (aq)	H_2O_2	0.74648	0.23895	-
Carbon monoxide (g)	CO	0.76358	0.32762	41.164
Pyrite (s)	FeS_2	0.89167	0.08784	0.03975
Water (g)	H_2O	1.27386	0.31351	41.164
Ice I_h (s)	H_2O	1.31769	0.07434	0.03193
Water (liq)	H_2O	1.32097	0.11624	0.03000
Carbon dioxide (g)	CO_2	2.19660	0.35502	41.164
Goethite (s)	α-FeOOH	2.73907	0.10030	0.03457
Carbonic acid (aq)	H_2CO_3	3.47090	0.30670	-
Hematite (s)	α-Fe_2O_3	4.14592	0.14513	0.05027
Magnetite (s)	Fe_3O_4	5.64021	0.24267	0.07393
Fayalite (s)	Fe_2SiO_4	7.68086	0.25074	0.07690
Forsterite (s)	Mg_2SiO_4	11.43747	0.15626	0.07248
Serpentine (s)	$Mg_3Si_2O_5(OH)_4$	22.45835	0.36748	0.17851
Apatite (s)	$Ca_5(PO_4)_3OH$	35.29432	0.64827	0.26502

considered as the ultimate source of all foods on Earth. This stems from the fact that these two elements are formed from the four most tightly bound nuclides in the universe, with an average binding energy decreasing in the order ^{62}Ni > ^{58}Fe > ^{56}Fe > ^{60}Ni.[21] Both species form the core of any telluric planet such as the earth. Consequently, any substance on Earth is to doomed to encounter sooner or later metallic nickel or iron during its recycling by the plate tectonics. The importance of nickel and iron for early forms of life is represented nowadays by two crucial metallo-enzymes.[22] First, carbon monoxide dehydrogenase (CODH) using $NiFe_3S_4$ and Fe_4S_4 clusters for transforming carbon dioxide CO_2 into carbon monoxide CO. Second, acetyl-CoA synthase (ACS) combining the CO generated by CODH with a methyl group to form the key metabolite intermediate acetyl-CoA using a Ni_2-Fe_4S_4 cluster. Table 2 shows other important (Ni,Fe)-based metallo-enzymes demonstrating the crucial role played by these two metals in biology,[23] in geochemistry and in astronomy.

Accordingly, Ni-Glx(I) protects the bacteria E. Coli against the damages made by methylglyoxal, a reduced form of pyruvic acid, to the arginine-bearing proteins and to nucleic acids. ARD is an enzyme involved in the ubiquitous methionine salvage pathway. Urease protects bacteria, archaea, plants, algae and fungi against harmful acidification of the living medium. Ni-SOD is an enzyme that protects cells from an excess of the superoxide ion, a free radical byproduct of aerobic metabolism. (Ni-Fe)-hydrogenase manage the reversible interconversion of hydrogen gas with protons and electrons in archaea, bacteria and selected eukaryotes. MCR play

Table 2. The "two" most tightly bound nuclides in the universe, nickel (Ni) and iron (Fe), are at the heart of many crucial biological processes involving at least nine different Ni-based enzymes used by 80% of the archaea and 60% of the eubacteria. Glx = Glyoxalase, ARD = Acidreductase dioxygenase, SOD = Superoxide dismutase, Hyd = Hydrogenase, CODH = CO dehydrogenase, ACS = Acetyl-CoA synthase, MCR = Methyl-coenzymeM reductase, Lar = Lactate racemase, DHK-MTPene = 1,2-dihydroxy-3-keto-5-methylthiopentene, KMTB = 2-keto-4- methylthiobutyrate, FeSP = corrinoid/Fe–S protein.

Enzyme	Metals	Reaction
Ni-GlxI	2 Ni^{2+}	Me-CO-CHO + ½ O_2 = Me-CHOH-COO^- + H^+
ARD	Fe^{2+} or Ni^{2+}	DHK-MTPene + O_2 = KMTB + $HCOO^-$ + $2H^+$
Urease	2 Ni^{2+}	$CO(NH_2)_2 + 2\ H_2O = 2\ NH_4^+ + CO_3^{2-}$
Ni-SOD	Ni^{3+}	$2\ O_2^{\bullet -} + 2\ H^+ = O_2 + H_2O_2$
[Ni-Fe]-Hyd	$NiFeS_2$	$H_2 = 2\ H^+ + 2\ e^-$
CODH	$NiFe_3S_4$	$CO_2 + 2\ H^+ + 2\ e^- = CO + H_2O$
ACS	Ni_2-Fe_4S_4	Me-Co(III)-FeSP + CO + CoASH = MeCO-SCoA + Co(I)-FeSP
MCR	Ni(I)	Me-SCoM + CoB-SH = CH_4 + CoM-S-S-CoB
Lar	Ni^{2+}	(D)-Me-CHOH-COO^- = (L)-Me-CHOH-COO^-

a major role in the global carbon cycle by controlling the biological emission of methane. Finally lactic acid (L- and D-isomers) is an important and versatile compound produced by microbial fermentation being involved in the energy metabolism of many prokaryotic species, as a product of sugar fermentation or as a carbon and electron source to sustain growth.[24]

In other words, if something is complex relative to its inner constituents, this means that it is the result of a huge flow of outgoing entropy. Wastes are to be found in its immediate surroundings. If not, the waste may have been exported far away, for example in the form of far-infrared photons that may have escaped towards the intergalactic space. This distant export of entropy may give the false impression, at first glance, that such a complex thing having an incredible low probability of formation from simpler elements apparently violates the second law. In fact, there is no violation at all, the point being that wastes are just invisible photons, i.e. heat for instance.

Another possibility is that wastes generated by a given species have been used as foods for another one, the observable result being a large increase in chemical as well as biological diversity. This is exactly what happens when plants generate O_2, a waste then breathed by animals. Owing to recycling, the amount of waste may appear, at first glance, to be lower that what it really is. Figure 2 show that the two purely abiotic spontaneous cycles can synthesize complex molecules such as glyoxylate, pyruvate and hydrogen peroxide. In accordance with the second law, a spontaneous process is such that the total irreversibility potential after reaction (B) should have increased relative to its initial value (A), or $\Delta\Pi_i^\circ = \Sigma n_i \cdot \Pi_i^\circ(B) - \Sigma n_i \cdot \Pi_i^\circ(A) > 0$.

SPONTANEOUS FORMATION OF REDUCED CARBON, NITROGEN AND SULFUR SPECIES

It is highly probable that life begins with water encountering a hot magma made of olivine somewhere on the oceanic floor.[25] Upon mixing, olivine will reduce water into hydrogen H_2 leaving as wastes serpentine. Assuming that olivine is made as 75% of forsterite Mg_2SiO_4 and 25% of fayalite Fe_2SiO_4 we will assume from table 1 that $\Pi_i^\circ(\text{olivine}) = 0.75\times 11.43747 + 0.25\times 7.68086 = 10.49832\ zJ\cdot K^{-1}$. Before transformation, the total irreversibility potential is thus $\Pi_i^\circ(A) = 6\times 10.49832 + 7\times 1.32097 = 72.23671\ zJ\cdot K^{-1}$, while after transformation we get $\Pi_i^\circ(B) = 3\times 22.45835 + 5.64021 = 73.01526\ zJ\cdot K^{-1}$. The difference $\Delta\Pi_i^\circ = \Pi_i^\circ(B) - \Pi_i^\circ(A) = +0.77855\ zJ\cdot K^{-1}$ being positive, this explains the great amount of hydrogen gas escaping from the hydrothermal vent on the oceanic floor where the waste, magnetite, accumulates with time, the other waste, serpentine, remaining within the oceanic crust. In fact, the vent should be viewed a very powerful entity able transforming a waste H_2O (low position in table 1) into a valuable food (high position in table 1). Such a reduction that corresponds to a strong decrease in entropy from water's viewpoint is nevertheless possible because wastes high in entropy (serpentine and magnetite) have been produced simultaneously, pushing the overall entropy balance in the good direction.

However, in order to make organic matter, one should also dispose of a valuable source of carbon able to act as a food. Carbon dioxide, which was an abundant molecule in early Earth's atmosphere being a waste rather than a food, one should again makes use of the powerful regenerating power of Earth's core containing metallic iron in order to transform it into carbon monoxide. After hydration by a water molecule to form carbonic acid H_2CO_3, we have $\Pi_i^\circ(A) = 3\times 3.47090 + 0 = 10.4127\ zJ\cdot K^{-1}$. After transformation into carbon monoxide producing goethite and water as wastes, we get $\Pi_i^\circ(B) = 2\times 2.73907 + 3\times 0.76358 + 2\times 1.32097 = 10.4108\ zJ\cdot K^{-1}$. With a difference $\Delta\Pi_i^\circ = -0.0019\ zJ\cdot K^{-1}$, we have an almost equal repartition of carbon between carbonic acid and carbon monoxide in such a transformation. However, carbon monoxide being a gas is able to escape from the location of the transformation provoking by its departure more transformation of carbonic acid into carbon monoxide in order to restore the equilibrium fixed by the relative irreversibility potentials in presence.

Concerning nitrogen, a mandatory element for synthesizing aminoacids, table 1 shows that it may already be classified as a food, being in higher position than carbon monoxide and at equal level with hydrogen. However, dinitrogen is a completely apolar molecule with nitrogen atoms engaged into very a stable triple bond that is very difficult to break spontaneously. But table 1 shows that another nitrogen-based molecule, ammonia NH_3, is almost at the same level of irreversibility potential without the problem of the triple bond. Here one may use again Earth's core to perform the transformation of N_2 into ammonia NH_3 with as always the help of the ubiquitous water molecule. Before transformation we have $\Pi_i^\circ(A) = 4\times1.32097 + 0 + 0 = 5.28388$ zJ·K^{-1}, while after transformation into ammonia producing goethite as a waste, we get $\Pi_i^\circ(B) = 2\times2.73907 + 2\times0.09134 = 5.66082$ zJ·K^{-1}. With a difference $\Delta\Pi_i^\circ = +0.37694$ zJ·K^{-1}, the reaction thus proceeds quite easily. Accordingly, in the laboratory it was possible to convert 17 mol% of nitrogen gas into ammonia by reaction with iron and water at a temperature of 700°C and a pressure of 0.1 GPa according to: $3(1\text{-}x)\ Fe + N_2 + 3\ H_2O = 3\ Fe_{(1\text{-}x)}O + 2\ NH_3$.[26] Ammonia was also obtained by with a mixture of nitrogen and formic acid at the surface of magnetite.

Table 3. Group contributions to aqueous irreversibility potentials $\Pi'_i{}^\circ$ at a pressure of 100 kPa, pH = 7 and three different ionic strengths I. This table is based on the convention that $\Pi_i^\circ = 0$ for species H^+, adenosine, NAD^- and $NADP^{3}$- at zero ionic strength.

Group	I = 0 M	I = 0.1 M	I = 0.25 M
Adenosine	-2.89295	-2.93729	-2.95160
$C\text{-}CH_2\text{-}NH_3^+$	-0.94837	-0.96876	-0.97538
$C_2\text{-}CH\text{-}NH_3^+$	-0.88070	-0.89780	-0.90326
Methyl: $C\text{–}CH_3$	-0.56235	-0.57254	-0.57588
Methylene: $C\text{-}CH_2\text{-}C$	-0.47396	-0.47736	-0.47842
Ammonium: $C\text{-}NH_3^+$	-0.38602	-0.39621	-0.39950
$\text{-}CH_2\text{-}OH$	0.23876	0.22852	0.22517
$C_2\text{-}CH\text{-}OH$	0.37594	0.36909	0.36692
Aldehyde: C-CHO	0.44634	0.44294	0.44183
$C_3\text{-}C\text{-}OH$	0.45915	0.46706	0.47006
Amide	0.59248	0.57889	0.57438
Keto: -CO-	0.67892	0.67324	0.67886
Ol: C-OH	0.80111	0.80106	0.80106
Carboxyl: $C\text{-}COO^-$	1.95160	1.95500	1.95606
$C\text{-}O\text{-}P\text{-}O\text{-}P\text{-}O\text{-}PO_3^-$	4.81002	4.82640	4.83302
$C\text{-}O\text{-}P\text{-}O\text{-}PO_3^-$	4.82796	4.83976	4.84450
$C\text{-}O\text{-}PO_3^-$	4.93973	4.95338	4.95823

What have been said for N_2, applies also to sulfur that exists as a solid and not as a gas under standard conditions. Here also, transformation of sulfur into hydrogen sulfide having a position rather close to sulfur in table 1 could be envisaged with the help of water as before and of a previous waste, magnetite, acting now as a food. This would allow disposing of the last important organic element necessary for building living matter as a gas and not as a solid. Accordingly, before transformation we have $\Pi_i^\circ(A) = 2\times5.64021 + 0 + 4\times1.32097 = 16.5643$ zJ·K^{-1}, while after transformation into H_2S producing goethite as a waste, we get $\Pi_i^\circ(B) = 6\times2.73907 + 0.18602 = 16.62044$ zJ·K^{-1}. With a difference $\Delta\Pi_i^\circ = +0.05614$ zJ·K^{-1}, the reaction is thus in favor of H_2S over a mixture of water and sulfur.

According to the previous considerations we understand that a hydrothermal vent could be viewed as a powerful source of foods in the form of H_2, CO, NH_3 and H_2S gases. However, these gases are released in water, a protic solvent, they may exist under several forms depending on the pH of water. For instance, at pH = 7, hydrogen sulfide exists as a 50-50 mixture of H_2S and HS- species. For ammonia, it is the ammonium form NH_4^+ that is the predominant species at pH ≈ 7. It follows that a given irreversible process may be represented by several chemical reactions involving species differing by their number of H-atoms and that can bear a non-zero electrical charge. In order to deal with such situations, one should use an apparent irreversible potential $\Pi'_i{}^\circ(pH, I)$ that reflects all the possible protonated forms of a chemical species in water existing at a given pH at a specified ionic strength I. Specific procedures have been designed to retrieve such apparent potentials from thermodynamic data of each chemical species at pH = 0 and I = 0 M.[27] When the necessary thermodynamic data is not available, one may decompose a given chemical species into various characteristic functional groups and add all the contribution to retrieve the corresponding apparent potential.[28] Table 3 gives such group contributions for pH = 7 and three ionic strength, I = 0 M, 100 mM and 250 mM adapted to our entropy units (zJ·K^{-1}). As with table 1, we have listed irreversibility potentials in ascending order in order distinguishing between potential foods (top of the table) and potential wastes (bottom of the table).

In order to convince of the usefulness of such a table, we will try to see if fatty acids may synthesized inside the mouth of a hydrothermal vent:

$$n\,CO + 2(n-1)\,H_{2,aq} = CH_3\text{-}(CH_2)_{n-2}\text{-}COO^-_{aq} + H^+_{aq} + (n-2)\,H_2O$$

The resulting fatty acid being composed of 1 methyl, 1 carboxyl groups and (n-2) methylene groups, one should expect from table 3 for an ionic strength I = 0.25 M the following irreversibility potential:

$$\Pi'_i{}^\circ(CnH_{2n+2}) = 1.95606 - 0.57588 - (n-2)\times 0.47842 = (2.33702 - 0.47842\times n)\ zJ\cdot K^{-1}$$

The prime symbol is a remainder that such a value is an apparent irreversibility potential valid for pH = 7 and I = 0.25 M. Its negative value above n ≈ 5 means that formation of fatty acids with long hydrocarbon tails involves a large decrease in entropy relative to its constituting elements (carbon C and hydrogen gas H2). Consequently, the higher n, the less the probability of seeing such a species forms spontaneously. However, we are not interested here by the formation from the constituting elements, but rather by the formation from carbon monoxide (food) reacting with hydrogen to produce the fatty acid with elimination of water as a waste. For the small molecules, we will use $\Pi'_i{}^\circ(CO) = 0.66778$ zJ·K-1, $\Pi'_i{}^\circ(H2) = -0.55210\ zJ\cdot K^{-1}$ and $\Pi'_i{}^\circ(H_2O) = 0.86695\ zJ\cdot K^{-1}$ valid for pH = 7 and I = 0.25 M,[28] leading to:

$$\Delta\Pi'_i{}^\circ = (n-2)\times 0.86695 + (2.33702 - 0.47842\times n) - 0.66778\times n + 0.55210\times(2n-2) = (0.82495\times n - 0.50108)\ zJ\cdot K_{-1}$$

This now shows that fatty acid formation is always a spontaneous process ($\Delta\Pi\cdot_i{}^\circ > 0$) for all values of n larger than n = 1.

SELF-ASSEMBLY OF LIPIDS INTO MICELLES

This is an important result, as every living cell has a membrane made of long-chain fatty acids. A crucial point, here, is that as soon as the number of carbon atoms becomes large enough, such fatty acids are able to self-assemble spontaneously into micelles, vesicles and membranes. Accordingly, a long-chain fatty acid is basically a small rigid pinhead welded on a flexible whip strap. In water, these long chain fatty acid assembles to form cavities not accessible to water molecules. This translates into a prohibitive entropy cost explaining the very low solubility of such species.

However, provided that the hydrocarbon tail is long enough, it becomes possible to have a large gain in entropy by creating in water large cavities holding several tails instead of a single one.[29] For instance, for a spherical cavity of radius R able holding N tails of volume V of a fatty acid, one should have $N\cdot V = 4\pi\cdot R^{3}/3$. But the fatty acid has also a head-group sweeping an area A, so that one has simultaneously $N\cdot A = 4\pi\cdot R^2$, leading to $R\cdot A = 3\cdot V$. Now, R cannot be greater than the maximal length L of the fully stretched hydrocarbon chain. With $R = 3V/A \leq L$, it comes that a criterion for making a spherical micelle is to have a packing parameter $PP = V/(L\cdot A) \leq 1/3$. Fatty acids having a packing parameter such that PP > 1/3 should then look for another less curved topology. Let us then consider a cylinder of arbitrary length D and radius R. Here, one should have $N\cdot V = \pi\cdot R^2\times D$ and $N\cdot A = 2\pi\cdot R\times D$, leading to $R\cdot A = 2\cdot V$ or $R = 2V/A \leq L$ as before. It follows that a criterion for making a cylindrical micelle is to have a packing parameter $PP = V/(L\cdot A) \leq 1/2$. If PP > ½, the fatty acid is not able to aggregate as a cylinder and should look again for another topology. A possibility is to make closed spherical bilayers having a radius equal to twice the hydrocarbon chain length R. For such a vesicle one may write that $N\cdot V = 4\pi\cdot(2R)^{3}/3$ to be compared to $N\cdot A = 4\pi\cdot(2R)^2$, leading to $R = 3V/2A \leq L$ that is to say $PP = V/(L\cdot A) \leq 2/3$. Finally, if PP > 2/3, the solution is to make a planar double layer having a thickness equal to 2R. For a square having an arbitrary area equal to D^2, one may the write that $N\cdot V = 2R\times D^2$ and $N\cdot A = 2D^2$, leading to $R = V/A \leq L$ that is to say $PP = V/(L\cdot A) \leq 1$. Finally, if PP > 1, the area of the head-group is too small to pack into bilayers and inverse micellar structures are formed (figure 4).

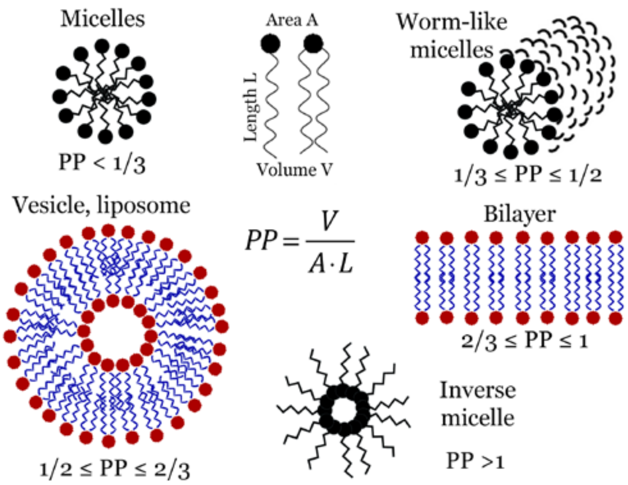


Figure 4. Spontaneous self-assembly in water of fatty acids characterized by the volume V of the hydrocarbon tail, the area A of the head-group, the maximum chain length L and observed topologies as a function of the packing parameter PP.

The final topology of the self-assembly process is thus controlled by a simple geometric criterion PP = V/(A·L). Now, for saturated hydrocarbon chains C_nH_{2n+1}, one has V ≈ (0.0274 + 0.0269×n) nm3 and L = (0.150 + 0.1265×n) nm, leaving only the area of the head-group A as a variable. Depending on the number of water molecules associated with the head-group, a given fatty acid may adopt any of the topologies represented in figure 3. The head-group area in the bilayer phase may be measured from force-area isotherms of squeezed monolayers giving an average value $A_0 = 0.247\ nm_2$ for fatty acids (n = 16-22).[30] Now, the average volume V_W occupied by a single water molecule may be computed from the observed density of liquid water $\rho \approx 1\ g{\cdot}cm^{-3}$, water's molecular weight $M_W = 18.0015$ Da and Avogadro's constant $N_A = 0.602214129\times1024$ mol,^{1} leading to $V_W = M_W\times10^{-3}/(N_A\times\rho) \approx 0.03\ nm^3$. It follows that hydrating the carboxyl head-group with N water molecules would lead to an effective area $A = (A_0^{3/2} + k\times N\times V_W)^{2/3}$, with k a shape factor equal to $6\times\pi^{1/2} \approx 10.635$ for spherical head-groups. For instance, for stearic acid (n = 18), we get $V = 0.5116\ nm^3$ and L = 2.427 nm. With two water molecules per head-group (N = 2), we get an effective area $A = 0.833\ nm^2$, leading to PP = 0.25 < 1/3, pointing to spherical micelles formation when ordinary soap is dissolved in water. For N = 1, the area is reduced to A = 0.580 nm2, leading to PP = 0.36 > 1/3, that is to say formation of worm-like micelles responsible for the high viscosity of concentrated soap solutions. Finally, for a fully dehydrated head-group $A = A_0 = 0.247\ nm^2$, leading to PP = 0.85 > 2/3, that is to say formation of stacked planar bilayers typical of solid soap.

This shows that the same fatty acids are able to form spherical, worm-like or planar bilayers depending on the fatty acid molar fraction relative to water. In other words, the shape of a cell is dictated by the amount of water molecules available in the medium. Obviously, the trouble with fatty acids is that they form bilayers only when the polar carboxylate head-group is not solvated by water molecules. In order to get stable bilayers in a large amount of water, it is mandatory to select a polar head-group not heavily solvated by water molecules and find a trick to increase the hydrocarbon volume V without increasing too much the chain length L. For the polar head-group a smart solution was to use a quaternary ammonium moiety, such as $-NMe_3^+$, as methyl groups are not able to be hydrogen-bonded with water molecules, explaining the ubiquitous choline head-group $HO\text{-}CH_2\text{-}CH_2\text{-}NMe_3^+$, in modern living membranes.

As shown in table 4 giving irreversibility potentials at pH = 7 as a function of ionic strength for prebiotic species, choline is a most valuable low entropy food with a quite negative irreversibility potential. Reaction (a) and (b) in table 5 shows that its abiotic synthesis within a hydrothermal vent may occur spontaneously by reducing carbon monoxide or dioxide with hydrogen gas in the presence of ammonia and with an appropriate mineral surface for catalyzing the reaction.

Concerning the chain length, the trick was to use 2 fatty-acids chains instead of one, allowing doubling the volume without changing the length of the hydrocarbon tail. In order to get a molecule with 2 tails and one head-group welded together to form a single entity, an obvious choice was to select glycerol molecules $HO\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}OH$ having three OH groups available for esterification. As ether-links -C-O-C- are much more difficult to synthesize than ester links –C(O)-O-C-, it was necessary for welding choline to glycerol to recruit a divalent weak inorganic acid for bridging two alcohol moieties together. Sulfuric acid H_2SO_4 being a strong acid and carbonic acid H_2CO_3 being too instable, a good candidate was phosphoric acid H_3PO_4 existing in aqueous solution around pH ≈ 7 under a di-hydroxy form $H_2PO_4^-$.

A convenient source of phosphate on earth is hydroxyapatite $Ca_5(PO_4)_3(OH)$, but this mineral appears to be definitively a waste and not a food as it is found at the very bottom of table 4. To realize the difficulty of extracting phosphate groups from apatite by water, we see that reaction (c) in table 5 leads to a negative irreversibility potential variation $\Delta\Pi_i°$, meaning that such a transformation cannot occur spontaneously. It should thus be coupled with another transformation releasing enough entropy to compensate for such a large decrease. As explained below, this is precisely the role of a metabolism to furnish such entropic compensation.

ABIOTIC ORGANIC CHEMISTRY

Concerning the formation of glycerol, another mandatory ingredient for having a double chain phospholipid, reactions (d) and (e) in table 5 shows that such a molecule may be formed spontaneously by reducing carbon monoxide or dioxide by hydrogen gas, as was the case for fatty acids or choline. Taking into account the fact that phosphate groups were not available for welding using a choline head-group, a possibility could be to use glycerate, $HO\text{-}CH_2\text{-}CHOH\text{-}COO^-$, obtained according to reactions (f) or (g) in table 5. This shows that abiotic glycerate synthesis is less favorable than that of glycerol but nevertheless still possible.

The apparition of double chain amphiphiles was an important step for evolution because it has make pos-

Table 4. Aqueous irreversibility potentials $\Pi'_i{}^\circ$ ($zJ \cdot K^{-1}$) at a pressure of 100 kPa, pH = 7 and three different ionic strengths I. This table is based on the convention that $\Pi_i{}^\circ = 0$ for species H^+, adenosine, NAD^- and $NADP^{3}$- at zero ionic strength.

Compound	I = 0 M	I = 0.1 M	I = 0.25 M
NADH	-6.13461	-6.21303	-6.23831
NAD^+	-5.78590	-5.87117	-5.89867
Choline ($C_5H_{14}NO^+$)	-3.39955	-3.44411	-3.45820
Adenine ($C_5H_5N_5$)	-2.84297	-2.85942	-2.86550
Cyanide	-0.88917	-0.89251	-0.89357
Adenosine	-1.80969	-1.85402	-1.86834
Methane-thiol	-0.84734	-0.86099	-0.86539
Dihydrogen	-0.54308	-0.54993	-0.55210
Ammonia	-0.44845	-0.45865	-0.46193
Valine	-0.45040	-0.48789	-0.50003
Proline	-0.28861	-0.31930	-0.32921
Sulfide	-0.28037	-0.28338	-0.28416
Dinitrogen	-0.10128	-0.10128	-0.10128
Dioxygen	-0.09134	-0.09134	-0.09134
Thioacetate	0.08442	0.07742	0.07519
Hydrogen peroxide	0.30142	0.29418	0.29240
Formaldehyde	0.34470	0.33784	0.33567
Alanylglycine	0.49758	0.46349	0.45246
Alanine	0.50855	0.48467	0.47697
Carbon monoxide	0.66778	0.66778	0.66778
Glyceraldehyde	0.68510	0.67146	0.66700
Methane	0.69897	0.71256	0.71696
Water	0.87597	0.86912	0.86695
Glycylglycine	0.94848	0.92125	0.91239
Glycerol	0.99042	0.96313	0.95433
Acetate	1.38925	1.38246	1.38023
Formate	1.73233	1.73233	1.73233
α-HG	1.80434	1.78730	1.78173
Ribose ($C_5H_{10}O_5$)	1.88933	1.85525	1.84422
Pyruvate (2-OP)	1.96268	1.95589	1.95366
Glutamate	2.10426	2.08042	2.07268
Glyoxylate (2-OA)	2.38730	2.38730	2.38730
Aspartate (Asp)	2.54052	2.52347	2.51796
Glycerate	2.56630	2.55260	2.54815
Carbonate	3.04817	3.04734	3.04706
AMP	3.13027	3.09958	3.09011
Malonate (2-CA)	3.38556	3.43263	3.43369
Malate (2-HS)	3.80301	3.80301	3.80301
2-OS	3.97310	3.97995	3.98212
4-HOG	4.48410	4.47463	4.47947
Portlandite $Ca(OH)_2$	4.55633	5.54948	4.54731
3-CAS	4.58852	4.58502	4.58396
3-CM	5.84516	5.85190	5.85413
Phosphate P_i	5.89562	5.89902	5.90080
3-COS	6.14363	6.15605	6.16608
3-OM	6.52408	6.52514	6.52787
Ribose-5-phosphate	6.81676	6.79654	6.79025
ADP	7.95839	7.93957	7.93483
Pyrophosphate PP_i	10.7767	10.7999	10.8085
ATP	12.7686	12.7661	12.7680
Triphosphate PPP_i	15.6178	15.6665	15.6838
Hydroxyapatite	34.9609	34.9575	34.9564

sible the appearance of endocytosis and exocytosis. We will take the example of egg lecithin, that have the following geometrical parameters: V = 1.063 nm^3, A = 0.717 nm^2, L = 1.75 nm,[31] leading to PP = 0.84 favoring planar bilayers. However, by mixing such lecithin molecules with a fat such as stearic acid we get for a 50:50 mixture an average PP-value of (0.30 + 0.84)/2 = 0.57, i.e. formation of a closed spherical double layer. For a lecithin/fat ratio of 75:25, it comes <PP> = (0.30 + 3×0.84)/4 = 0.705 and now we are back again with a planar bilayer. We have thus here a very basic mechanism of exocytosis (PP < 2/3) or endocytosis (PP > 2/3) allowing such micelles to absorb foods and reject wastes. Another quite important molecule for membranes was cholesterol that have the following geometrical parameters: V = 0.630 nm^3,[31] A = 0.41 nm^2,[32] L = 1.03 nm,[33] leading to PP = 1.49 favoring inverse closed spherical double layer, allowing transport of water in oil and not oil in water as with direct micelles. Owing to such a property cholesterol may be used to favor very stable planar bilayers for molecules having a packing parameter not close to 1, the optimal value for such a topology. For instance by mixing egg-lecithin with cholesterol in a 75:25 ratio, one get <PP> = (1.49 + 3×0.84)/4 = 1,00.

Now, we should understand that extracting phosphate group from apatite was a crucial problem that has to be solved before the spreading of life on Earth. When a transformation is not thermodynamically allowed owing to a decrease of the total entropy ($\Delta\Pi i^\circ < 0$), a good solution is usually to make a coupling with radiant energy coming from the sun as visible light or from the Earth as infrared radiation. Characterizing radiations by their wavelengths λ, such a coupling may be expressed by the following relationship:

$$-\lambda \cdot T \cdot \Delta\Pi_i{}^\circ = h \cdot c = 198.645 \text{ zJ} \cdot \mu\text{m} \qquad (4)$$

Thus, for reaction (c) in table 5 characterized by $\Delta\Pi_i{}^\circ = -2.32000$ $zJ \cdot K^{-1}$, one should use at T = 298.15 K a photon having a wavelength less than λ = 287 nm. The problem is that apatite is a solid that could be used for storing radioactive nuclides generated by nuclear power

Table 5. Thermodynamic analysis of some abiotic reactions generating important compounds for the emergence of life on Earth. (a,b) Choline; (c) Phosphate; (d,e) Glycerol; (f,g) Glycerate; (h) Hydrogen peroxide; (i,j) Formate; (k,l) Acetate; (m,n) Methanethiol; (o,p) Thioacetic acid; (q,r,s) Glyoxylate; (t,u,v) Pyruvate. All values are given at pH = 7 and I = 0.25 M and reported in $zJ\cdot K^{-1}$.

Reaction	$\Sigma\ \Pi_i°$ (left)	$\Sigma\ \Pi_i°$ (right)	$\Delta\Pi_i°$
(a) $5\ CO + NH_4^+ + 9\ H_2 = C_5H_{14}NO^+ + 4\ H_2O$	-2.09193	0.0096	+2.10153
(b) $5\ H_2CO_3 + NH_4^+ + 14\ H_2 = C_5H_{14}NO^+ + 14\ H_2O$	7.04397	8.6791	+1.63513
(c) $Ca_5(PO_4)_3OH + 9\ H_2O = 5\ Ca(OH)_2 + 3\ P_i$	42.75895	40.43895	-2.32000
(d) $3\ CO + 4\ H_2 = C_3H_8O_3$	-0.20506	0.95433	+1.15939
(e) $3\ H_2CO_3 + 7\ H_2 = C_3H_8O_3 + 6\ H_2O$	5.27648	6.15603	+0.87955
(f) $3\ CO + 2\ H_2 + H_2O = C_3H_5O_4^-$	1.76615	2.54815	+0.78200
(g) $HCO_3^- + 2\ H_2CO_3 + 5\ H_2 = C_3H_5O_4^- + 5\ H_2O$	6.38068	6.88290	+0.50222
(h) $2\ H_2O + FeS_2 = H_2O_2 + H_2S + FeS$	2.62557	0.57243	-2.05314
(i) $CO + H_2O = HCOO^- + H^+$	1.53473	1.73233	+0.19760
(j) $HCO_3^- + H_2 = HCOO^- + H_2O$	-2.49496	2.59928	+0.10432
(k) $2\ CO + 2\ H_2 = CH_3COO^- + H^+$	0.23136	1.38023	+1.14887
(l) $HCO_3^- + H_2CO_3 + 4\ H_2 = CH_3COO^- + 4\ H_2O$	3.88572	4.84803	+0.96231
(m) $CO + 2\ H_2 + H_2S = CH_3\text{-}SH + H_2O$	-0.72058	0.00156	+0.72214
(n) $H_2CO_3 + 3\ H_2 + H_2S = CH_3\text{-}SH + 3\ H_2O$	1.10660	1.73546	+0.62886
(o) $CO + CH_3\text{-}SH = CH_3COS^- + H^+$	-0.19761	0.07519	+0.27280
(p) $HCO_3^- + H_2 + CH_3\text{-}SH = CH_3COS^- + 2\ H_2O$	1.62957	1.80909	+0.17952
(q) $HCOO^- + CO = C_2HO_3^-$	2.40001	2.38730	-0.01281
(r) $HCO_3^- + H_2CO_3 + 2\ H_2 = C_2HO_3^- + 3\ H_2O$	4.98992	4.98815	-0.00177
(s) $HCO_3^- + CO + H_2 = C_2HO_3^- + H_2O$	3.16274	3.25425	+0.09151
(t) $CH_3COO^- + CO = C_3H_3O_3^-$	2.04801	1.95366	-0.09435
(u) $HCO_3^- + 2\ H_2CO_3 + 5\ H_2 = C_3H_3O_3^- + 6\ H_2O$	6.38068	7.15536	+0.77468
(v) $3\ CO + 2\ H_2 = C_3H_3O_3^- + H^+$	0.89914	1.95366	+1.05452

plants during a geological period of time.[34] Consequently, if apatite remains stable upon heavy irradiation with gamma photons, there is no chance that a UV-C photon will be able to extract phosphate from apatite. It is at this point that a metabolic cycle being a perpetual source of entropy alimented by Earth plate tectonics and emerging at the mouth of hydrothermal vents could be very useful. From, figure 2 we know that to start the cycle, three ingredients should be able to meet upon a mineral surface: glyoxylate, pyruvate and hydrogen peroxide. A look at table 4 immediately shows that the most critical food will be hydrogen peroxide H_2O_2, as it is the compound that displays the smallest irreversibility potential. The problem is then how to reduce water H_2O into hydrogen peroxide H_2O_2. A possible solution is to consider that there was on the primitive Earth probably a lot of pyrite FeS_2 that could react according to the scheme (h) in table 5 and illustrated in figure 2. Such a reaction is characterized by a large decrease in total entropy, but could be compensated according to (6), by a UV-A photon having a wavelength less than λ = 325 nm, using pyrite's surface as a photo-electrode (figure 2). Here, the solid reacts only by its surface and does not need to be solubilized as in the case of apatite. The fact that all form of life uses extensively ferredoxins containing iron-sulfur clusters as source of electrons is thus probably a vestige of these Hadean times where pyrite and sun was the sole source of hydrogen peroxide. Such a reaction has been tested in the laboratory and it was shown that upon UV-irradiation water could be decomposed into hydrogen peroxide with steady-state concentration of 34 μM.[35] It was also shown that pyrite was not the most efficient photo-catalytic surface as a concentration of 400 μM was obtained in the presence of vaesite NiS_2, pointing to the crucial role played by nickel and iron for the apparition of life on a primitive Earth. On the other hand, it was also found that FeS and ZnS surfaces were not able to catalyze such a reaction.

With the help of the sun and pyrite, hydrogen peroxide could be absorbed by the primitive oceans and reach the mouth of hydrothermal vents located upon the oceanic floor. Now, the problem is to explain how α-keto-acids such as glyoxylate and pyruvate could also be present here in order to react with hydrogen peroxide. In fact, laboratory experiments has shown that metal sulfides could acts as catalysts for hydrocarboxy-

Table 6. Thermodynamic analysis of the 4-hydroxy-2-oxo-glutarate cycle represented at the bottom of figure 2. Such a cycle consume one glyoxylate molecule that is oxidized into carbon dioxide and water with an overall largely positive increase in entropy All values are given at pH = 7 and I = 0.25 M and reported in $zJ \cdot K^{-1}$.

Reaction	$\Sigma \Pi_i°$ (left)	$\Sigma \Pi_i°$ (right)	$\Delta\Pi_i°$
2-OP + 2-OA = 4-HOG	4.34096	4.47947	+0.13851
4-HOG + H_2O_2 = 2-HS + carbonate	4.77187	6.85007	+2.07820
2-HS + H_2O_2 = 2-OS + 2 H_2O	4.09541	5.71602	+1.62061
2-OS + 2-OA = 3-OM	6.36942	6.52514	+0.15572
3-OM + H_2O = 4-HOG + carbonate	7.39482	7.52653	+0.13171
$C_2HO_3^-$ + 2 H_2O_2 = H_2CO_3 + HCO_3^- + H_2O	26.97248	31.09723	+4.12475

Table 7. Thermodynamic analysis of the malonate cycle represented at the top of figure 2. Such a cycle consume one glyoxylate molecule that is oxidized into carbon dioxide and water with an overall largely positive increase in entropy. All values are given at pH = 7 and I = 0.25 M and reported in $zJ \cdot K^{-1}$.

Reaction	$\Sigma \Pi_i°$ (left)	$\Sigma \Pi_i°$ (right)	$\Delta\Pi_i°$
2-OS + H_2O_2 = 2-CA + H_2CO_3	4.27452	6.48075	+2.20623
2-CA + 2-OA = 3-CM	5.82099	5.85413	+0.03314
3-CM + H_2O_2 = 3-COS + 2 H_2O	6.14563	7.89998	+1.75435
3-COS + H_2O = 2-OS + HCO_3^-	7.03303	7.02918	-0.00385
$C_2HO_3^-$ + 2 H_2O_2 = H_2CO_3 + HCO_3^- + H_2O	23.27417	27.26404	+3.98987

lation (Koch's reaction) where a α-keto-acid is obtained via carbonyl insertion at a metal-sulfide-bound alkyl group in the absence of peptide-based enzymes.[36] It was found that the sulfides of nickel, cobalt, iron and zinc were able to catalyze such an insertion but not copper sulfides. Glyoxylate would thus be the result of inserting carbon monoxide into formate, while pyruvate would be the result of inserting carbon monoxide into acetate. As shown in table 5, formate could be formed according to scheme (i) or (j) and acetate according to scheme (j) and (k) with a moderate increase in entropy in the case of formate and a substantial one in the case of acetate.

Table 5 also shows that formation of methane-thiol through reactions (l) or (m) and thioacetate through reactions (n) or (o) proceeds with a neat increase of entropy, larger for the thiol derivative than the thiolate one. This basically means that alkyl sulfides were available around hydrothermal vents. However, scheme (p) in table 5 shows that direct insertion of carbon monoxide into formate yielding glyoxylate, proceeds with a slight decease in total entropy. The direct reduction of a mixture of carbon monoxide and dioxide by hydrogen gas according to scheme (q) is a better solution. However, the best way to form glyoxylate leading to a an increase in total entropy is scheme (r) proceeding by insertion of carbon monoxide into bicarbonate followed by reduction with hydrogen gas. The abiotic synthesis of pyruvate by insertion of carbon monoxide into acetate according to scheme (t) in table 5 appears to be not favorable. Direct reduction of bicarbonate by hydrogen gas according to scheme (u) is a much better solution, the best one being direct reduction of carbon monoxide following scheme v). Having checked the avaibility of glyoxylate, pyruvate and hydrogen peroxide, we should now focus on the 4-hydroxy-oxo-glutarate cycle shown at the bottom of figure 2 and analyzed for its feasibility in table 6. The result is impressive and shows that such a cycle is a totally abiotic "respiration" relying on carbon monoxide and hydrogen gas that are precursors of glyoxylate and pyruvate generated by the Earth that are burned by hydrogen peroxide generated by the sun, rejecting as waste carbon dioxide and water. On such a ground, there is no major difference between such a purely abiotic respiration supported transition metal sulfides and a plant capturing the energy from the sun to make organic matter that would be burned away releasing entropy at night. Moreover, by coupling the 4-hydroxy-2-oxo-glutarate cycle generating a large amount of entropy with the solubilization of apatite reaction (c) in table 5, it becomes possible extracting inorganic phosphate and release simultaneously 537 zJ of heat. These inorganic phosphate would be used to create phospholipids during the Hadean period, then coenzyme-A and its derivatives (NADH, NADPH, FAD, FMN, etc…) and most importantly nucleotides.

We could also have a look at the upper malonic cycle (table 7), that starts from 2-oxo-succinate (also called oxalo-acetate), an intermediate molecule synthesized during the revolving of the 4-HOG cycle. This cycle is not so efficient as the first one, but it also leads to the burning of glyoxylate by hydrogen peroxide with a large entropy increase available for coupling with a process associated to a decrease in entropy together with the eventual release of any excess entropy as heat. One may notice that a critical step in this cycle is the decarboxylation of 3-carboxy-2-oxo-succinate, which is the only step with a negative entropic contribution. However, as most of the irreversibility potentials for such intermediates has been estimated using Alberty's group contribution

Table 8. Thermodynamic analysis of the abiotic synthesis of the aspartate aminoacid from malonate –OOC-CH_2-COO-, carbon monoxide, ammonia and hydrogen gas. All values are given at pH = 7 and I = 0.25 M and reported in zJ·K^{-1}.

Reaction	Σ Π_i° (before)	Σ Π_i° (after)	ΔΠ_i°
HCO_3^- + CO + H_2 + NH_4^+ = α-HG + H_2O	2.70081	2.64868	-0.05213
α-HG + 2-CA = 3-CAS + H_2O	5.21542	5.45091	+0.23549
3-CAS + H_2O = $C_4H_6O_4N$ + HCO_3^-	5.45091	5.56502	+0.11411
$C_3H_2O_4^-$ + CO + H_2 + NH_4^+ = $C_4H_6O_4N$ + H_2O	13.36714	13.66461	+0.29247

method,[28] such a very small value is within the errors of the algorithm. But again, this does not really matters as there is enough entropy release in the other steps for keeping the cycle into a permanent running state.

As shown in figure 2, the malonate cycle is of crucial importance because as it allows formation of the important aminoacid aspartate by reacting malonate with alpha-hydroxy-glycine (see table 8).[15]

ABIOTIC AMINOACID SYNTHESIS

As shown in figure 5, five other aminoacids may be derived from aspartate: lysine, asparagine, threonine, isoleucine and methionine. Another important precursor of aminoacids, sugars, cofactors and lipids is pyruvate, that is an important hub metabolite that have been obtained from FeS surfaces.[37] Another study has shown that pyruvate could be transformed readily in the presence of transition metal sulfide minerals under simulated hydrothermal fluids at temperature T = 25-110°C.[38] Depending on the relative concentration of H_2S (3-110 mM), H_2 (0.1-50 mM), alanine could be obtained among nine other species with yields ranging from 0.1% to 60%. With 100 mM H_2, 100 mM CO_2 and 20 mM H_2S at T = 25°C on FeS pyrrhotite, it was also possible to perform aldol condensation of pyruvate to 2-hydroxy-2-methyl-4-oxo-pentane-dioate with a yield of 13%. On such surfaces, it was also possible to form various thio-derivatives.

Now experimental studies have shown that the full sequence complexity of naturally occurring proteins is not required to generate rapidly folding and functional proteins, i.e. proteins can be designed with fewer than 20 letters.[39] Using combinatorial mutagenesis in an

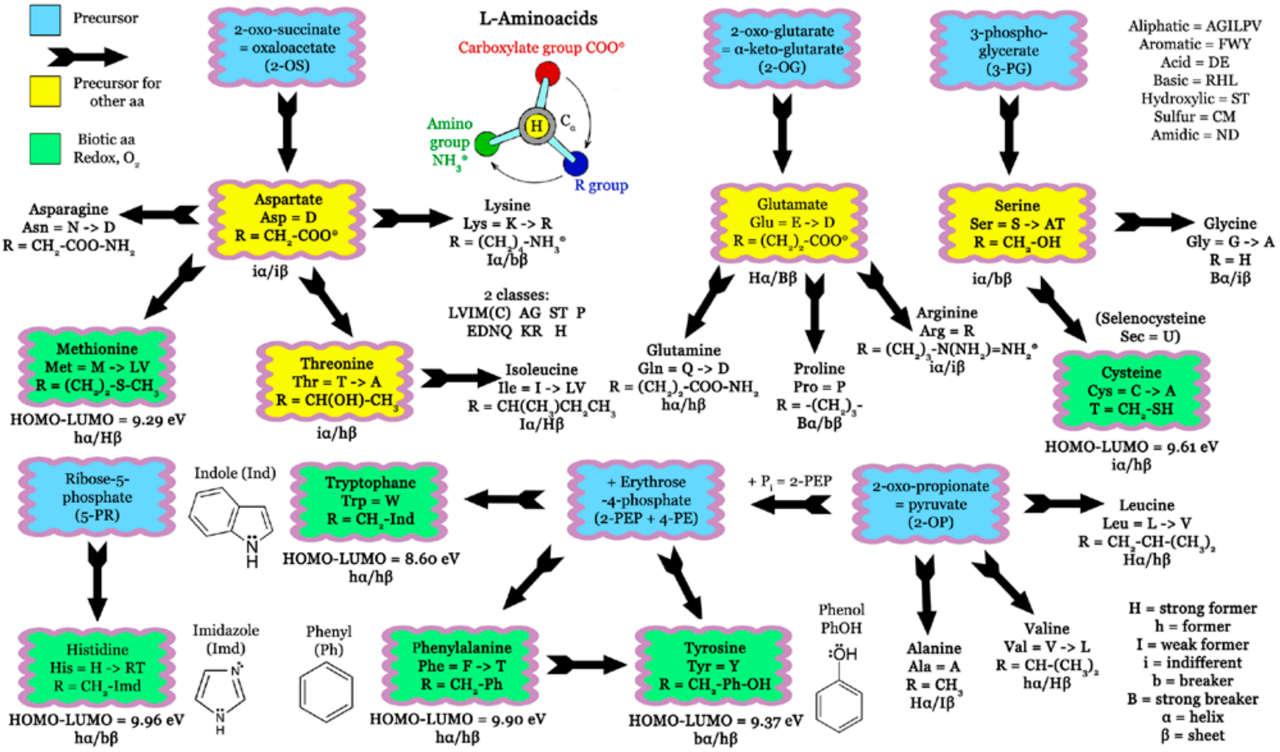


Figure 5. Relationships between the twenty aminoacid within their 3-letters and 1-letter codes, possible substitutions in proteins and secondary structure (helix or sheet) preference. Primary organic precursors are highlighted in blue, secondary aminoacid precursors of other aminoacids are highlighted in yellow. Aminoacids added lately to the universal genetic code under oxic conditions are highlighted in green.

enzyme of the bacterium *E. coli*, it has thus been shown that it was possible grouping the 20 aminoacids to a reduced set of 7 groups: Asp (Asn, Glu, Gln); Ala (Gly, Ser, Thr, Cys); Val (Met, Leu, Ile); Arg (Lys, His); Tyr (Phe), Trp and Pro.[40] Among these groups the four aromatic aminoacids (His, Tyr, Phe, Trp) and Met are characterized by an HOMO-LUMO gap smaller than 10 eV.[41] This indicates that in demanding building blocks with more versatile redox chemistry, biospheric molecular oxygen triggered the selective fixation of these last amino acids in the genetic code. Similarly, arginine may be discarded on the ground that it is essential only for catalytic activity and not for conformational stability.[42] Consequently, one may retain only four critical aminoacids for building prebiotic peptides precursors of proteins: Asp, Ala, Val, and Pro. In such a minimal set, we find a strong former of α-helix (Ala) and a strong former of β-sheet (Val). We have also a breaker of helices and sheets (Pro) and an indifferent aminoacid (Asp) bringing acidic properties. The next aminoacid that will be logically recruited upon evolution is also an indifferent aminoacid (Arg) bringing basic properties.

Table 9 shows that alanine, valine and proline may be formed from pyruvate with an increase in entropy in all cases. We have also considered the formation of glutamate, which is expected to be an important intermediate for the synthesis of arginine. Accordingly, glutamate has also been obtained under hydrothermal conditions at a concentration of about 4 μM.[43] Table 8 also shows that condensation of two amino acids to form a dipeptide is a non-spontaneous process that requires coupling with another process releasing at least +0.2 $zJ\cdot K^{-1}$ of entropy. As there is plenty of entropy release in the two abiotic cycles represented in figure 2, oligomerization of amino acids into polypeptides just needs an adequate mineral surface. Accordingly, it is a well-established fact that amino acids concentrate and polymerize on clay minerals or apatite to form small, protein-like molecules containing up to 12 amino-acids.[44] For instance, the sanidine feldspar not only favors the peptide bond formation from both a thermodynamic and kinetic viewpoint but also prevents their hydrolysis.[45] Moreover, some mineral surfaces are known to be chiral, an important requirement for explaining the chirality of biomolecules. It has also be demonstrated that formation of a dipeptide from two amino acids is about eight times more difficult than subsequent condensations of an amino acid to a dipeptide or longer chain.[46] The same study has shown that adding an amino acid to a peptide of any size is five times more difficult than joining small peptides together. This demonstrates that the rather small entropy decrease associated to formation of a peptide bond is not a real problem and could be easily overcome. According to (4), one could for instance make a coupling with the absorption of an infrared photon having a wavelength λ = 4.8 μm. According to Wien's displacement law the light emitted by a black body, $4.9651\cdot\lambda_{max}\cdot(k_BT) = h\cdot c$ i.e. $\lambda_{max}\cdot T = 2898$ μm·K, such photons are emitted in great amount by any surface heated to a temperature T ≈ 600 K ≈ 330°C. Such a temperature is typical of hydrothermal vents and experiments have confirmed that peptide synthesis was indeed favored in hydrothermal fluids.[47]

Table 9. Thermodynamic analysis of the abiotic synthesis of the some aminoacid from pyruvate and their condensation into di-peptides. Alanine = $C_3H_7NO_2$; Valine = $C_5H_{11}NO_2$; Proline = $C_5H_9NO_2$; Glutamate = $C_5H_9NO_4$; Glycine = $C_2H_5NO_2$; Glycylglycine = $C_4H_8N_2O_3$; Alanylglycine = $C_5H_{10}N_2O_3$. Also shown the analysis for the formation of inorganic as well as organic polyphosphates and polymers of formaldehyde (sugars) or cyanhydric acid (purine base). Ribose = $C_5H_{10}O_5$; Glyceraldehyde = $C_2H_4O_2$; Adenine = $C_5H_{10}O_5$. All values are given at pH = 7, I = 0.25 M and reported in $zJ\cdot K^{-1}$.

Reaction	$\Sigma\ \Pi_i°$ (left)	$\Sigma\ \Pi_i°$ (right)	$\Delta\Pi_i°$
$C_3H_3O_3^- + H_2 + NH_4^+ =$ alanine $+ H_2O$	0.93963	1.34392	+0.40429
$C_3H_3O_3^- + 2\ CO + 5\ H_2 + NH_4^+ =$ valine $+ 3\ H_2O$	0.06679	2.10082	+2.03403
$C_3H_3O_3^- + 2\ CO + 4\ H_2 + NH_4^+ =$ proline $+ 3\ H_2O$	0.61889	2.27164	+1.65275
$C_3H_3O_3^- + 2\ CO + 2\ H_2 + NH_4^+ =$ glutamate $+ H_2O$	1.72309	2.93963	+1.21654
$HCO_3^- + H_2CO_3 + 3\ H_2 + NH_4^+ =$ glycine $+ 4\ H_2O$	3.97589	4.44847	+0.47258
Gly + Gly = Gly-Gly + H_2O	1.96134	1.77934	-0.18200
Ala + Gly = Ala-Gly + H_2O	1.45764	1.31941	-0.13823
$2\ P_i = PP_i + H_2O$	11.8016	11.6755	-0.1261
AMP + P_i = ADP + H_2O	8.99091	8.80178	-0.18913
$3\ P_i = PPP_i + 2\ H_2O$	17.7024	17.4177	-0.2847
$P_i + PP_i = PPP_i + H_2O$	16.7093	16.5508	-0.1585
ADP + P_i = ATP + H_2O	13.8356	13.6350	-0.2006
$2\ H_2CO_3 + 4\ H_2 =$ glyceradehyde $+ 4\ H_2O$	3.88572	4.13480	+0.24908
$5\ H_2CO$ = ribose	1.67835	1.84835	+0.17000
5 HCN = adenine	4.46785	-2.86655	+1.60130
Ribose + P_i = Ribose-5-phosphate + H_2O	7.74502	7.65720	-0.08782
Ribose + adenine = Adenosine + H_2O	-1.02128	-1.00139	+0.01989

ABIOTIC PHOSPHATE-BASED COMPOUNDS

Our last concern will be the exact role played by ATP, a molecule intimately associated with any kind of life process. As shown in figure 6, this molecule is made of three main parts: a triphosphate, a C5-sugar and a heterocyclic base named adenine. Table 8 shows that formation of polyphosphates, whether inorganic or organic is always associated to a decrease of the total entropy. Consequently, such reaction should be coupled with an entropy-generating process. Here, coupling with an infrared photon is unlikely as this would require a wavelength $\lambda \approx 3.3$ µm generated by surfaces heated at $T = 870K \approx 600°C$, a temperature much too hot for hydrothermal vents. Here coupling with a metabolic cycle seems thus mandatory for the synthesis of such polyphosphate species. The same conclusion obviously applies to other phosphate-based species such as RNA or DNA.

The abiotic synthesis of the ribose and adenine moieties poses no particular problems, as a sugar is just an addition polymer of formaldehyde $(H_2C{=}O)_5$, while adenine is an addition polymer of cyanhydric acid $(HCN)_5$. Table 8 shows that formation of these polymers is associated to a significant increase in total entropy in both cases and may thus proceed without the need for an extra source of entropy. Obviously, such reactions need to be catalyzed by serpentinizing olivine and borate minerals for the formation of ribose from formaldehyde and small quantities of glycolaldehyde.[48] This last compound is necessary for catalyzing the first step of dimerization of formaldehyde that requires a high energy of activation. As shown in table 8, it could be easily formed in hydrothermal vents or derived from surface hydrogenation of CO molecules in interstellar dark cloud regions of the universe.[49] Similarly, the mechanism of formation of adenine, a HCN pentamer, under prebiotic conditions has been studied, and it seems that catalysis by water and ammonia molecules is needed.[50] From table 8, one may see that the condensation of the ribose moiety with an inorganic phosphate is associated with a small decrease of entropy. For the condensation between adenine and ribose to yield adenosine, we are within the errors (±0.02 $zJ\cdot K^{-1}$) of the method and nothing can be said about such a process.

Figure 5 also shows that the ATP molecule has a particularly good geometry for chelating divalent metallic ions[51] and has in particular a quite high affinity for magnesium ions.[52] Consequently, one should be cautious when computing irreversibility potential differences involving species bearing a strong negative charge such as ATP. But, the most interesting aspect of ATP is its position at the very bottom of table 4 giving irreversibility potentials in ascending order. One can see that the overall effect of phosphorylation is to strongly increase the irreversibility potential. It follows that ATP should be more assimilated to a waste than to a food. Moreover, one can see from table 8 that the energy released upon hydrolysis of ATP, about 60 zJ, is of the order of magnitude of at most two hydrogen bonds (about 40 zJ between two water molecules in liquid water). Speaking of the P-O-P linkage as a "high energy" bond seems thus quite exaggerated. One may also compare such an energy with the energy released by one turn of the two abiotic cycles shown in figure 2 that are 1330 zJ for the 4-hydroxy-2-glutarate cycle and 1190 zJ for the malonate cycle. This means that about 20-22 molecules of ATP would be needed for storing the energy liberated by the combustion of glyoxylate. The question is thus why con-

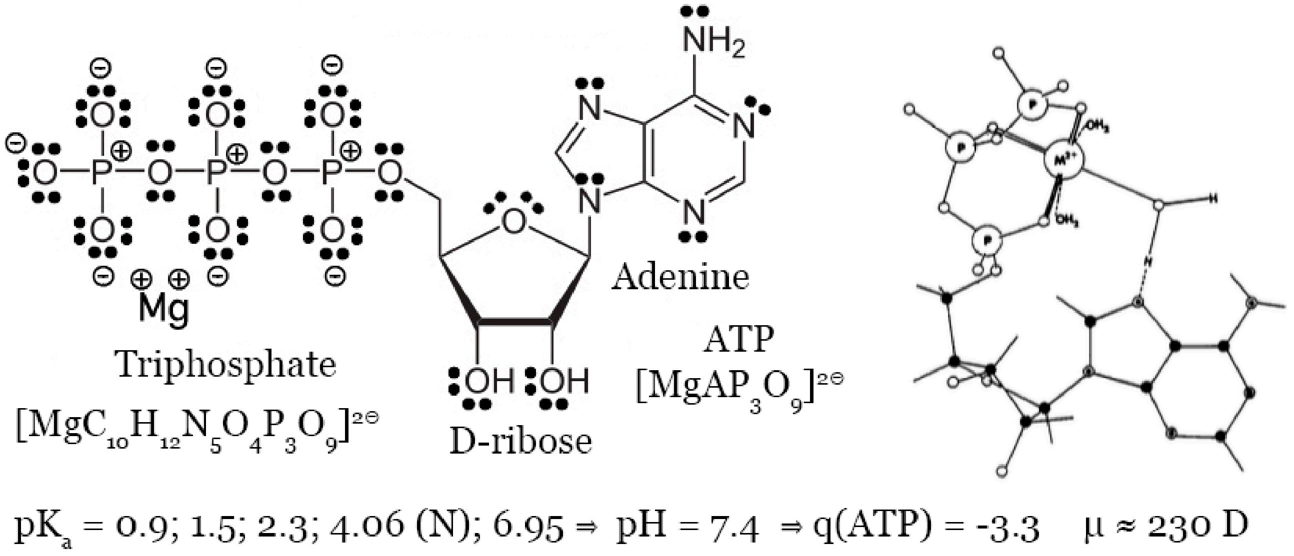


Figure 6. Lewis structure of adenosine triphosphate (ATP) shown here chelating a magnesium ion.

sidering a waste as a ubiquitous agent for storing energy? Table 4, shows that much better agents would be NAD^+ or NADH that are at the very top of the table. Such a position is perfect for "high energy" molecules and accordingly the $NAD^+/(NADH,H^+)$ couple is the universal energy pool in any kind of respiratory chain.

One of the reasons for focusing attention on ATP is merely that without a continuous production of such a compound, a cell is not able to survive. As a consequence, "high-energy" molecules such as NADH that are basically foods are systematically degraded to produce ATP, a very valuable waste indeed for the cell. A recent publication on the real role of ATP in the cell gives a clue.[53] It was shown that ATP at physiological concentrations 5-10 mM has properties of a biological hydrotrope, preventing the formation of protein aggregates and dissolving them if they happen to be formed. In other words, without ATP the intracellular medium is doomed to precipitate owing to its very high molecular crowding. Such a property of maintaining solubility is more in line with the position of ATP in table 4 and points to the importance of a systematically neglected factor, water activity a_W.

WATER ACTIVITY A_W

To understand this crucial point, we have to go back to equation (2) and see that an irreversibility potential for a given substance depends on temperature T, pressure p and on the activity a of this substance. Let's us recall that $a = 1$ for a species in a pure state and that $a < 1$ as soon as the substance is mixed with other substances. For condensed states such as liquids or solids, activity may be written as a product of two terms, $a = \gamma \cdot X$, where X is the molar fraction of the substance in the mixture and γ an activity coefficient taking into the deviations from ideal behavior of the mixture. Basically, an ideal mixture is a medium where constituents do not interact with each other. In other words, by considering an ideal mixture we implicitly assume that it exists only a single form of energy: kinetic energy. In such a case, one has $\gamma = 1$ and the activity is essentially ruled by molar fractions X, that is to say molecular composition. However, as soon as the constituents interact through potential energy, one has $\gamma \neq 1$ and activity is no more ruled by molar fractions alone. A situation where the molecular interactions between constituents are repulsive would lead to $\gamma > 1$, meaning that activity is increased relative to the ideal non-interacting case. Now, if molecular interactions are attractive then $\gamma < 1$, meaning that activity is decreased relative to the ideal non-interacting case. From a practical viewpoint, for species having a sufficiently high vapor pressure, activities may be defined as the ratio $a = p_{vap}(T)/p°_{vap}(T)$, where $p_{vap}(T)$ and $p°_{vap}(T)$ are the vapor pressure measured at saturation and temperature T for the mixture and the species in a pure state respectively.

As a substance ubiquitous in the cell, water has its own activity that depends on the composition and of the interactions between water molecules and all the species in contact with water. A common assumption is that in a cell one should have $a_W \approx 1$, on the ground that the molar fraction of water X_W is very high ($X_W > 0.99$) relative to all other constituents (see figure 1). By doing this, one automatically assume that $\gamma_W \approx 1$, meaning that water molecules do not interact at all with the solutes. Such an assumption is obviously utterly wrong, as water is a solvent that always interacts quite strongly with any kind of solute, meaning that one has necessarily $\gamma_W < 1$. As soon as this is posed, the immediate question is to quantify the deviation from unity for the activity coefficient of water. Obviously, there will be some substances provoking a large decrease of γ_W even at very low molar fraction and other substances needing high molar fractions to significantly decrease γ_W. A direct proof of the crucial importance of water activity for life is given by the fact that no microbial growth is possible below $a_W < 0.6$ for the three domains of life (prokaryotes, archaea and eucaryotes).[54] In fact most microbes living at room temperature and ambient pressure are able to grow only if $a_W > 0.9$. Such a finding is clear evidence of the importance of water activity for life processes.

Norrish's equation is the best approach to understand how water activity changes upon adding a solute:[55]

$$K = (KBI_{WW} + KBI_{SS} - 2 \cdot KBI_{SW})/2V_W = a_W = X_W \cdot \exp(K \cdot X_S)$$

Here KBI_{WW}, KBI_{SS} and KBI_{SW} are Kirkwood-Buff integrals (KBI) that are function of the radial distribution functions of water-water (WW), solute-solute (SS) and water-solute (SW) interactions. In practice, Norrish's equation applies up to moderate solute concentrations (ca 60 wt%, 5-10 M or 0.1-0.2 mole fraction) and may thus be applied to any kind of living cell.

As Norrish's constant K is generally negative, it follows that water activity always decreases when molar fraction of solutes increases. However, the variation of water activity with X_S appears to be modulated by the value of K that strongly depends on the chemical nature of the solute. In fact, owing to its very small size (ca 0.3 nm in diameter), it follows that the water-water integral KBI_{WW} does not change very much upon adding a solute. This is not the case of the two other integrals that are generally both negative owing to the existence of large excluded volumes. Generally speaking, the larger the solute species, the larger the exclusion volume and the more negative the Kirkwood-Buff integral. It thus follows that as a glucose molecule is bigger than a glycerol molecule, the decrease in water activity is stronger at the same concentration for glucose than for glycerol. This explains the phenomenon of cryptobiose where a cell produces a large amount of sucrose (K = -6.47) in order to protect itself against freezing, dehydration, osmotic stress or low pressure. By strongly lowering its water activity, the cell is no more able to grow but can survive under very harsh conditions waiting eagerly for better conditions. As soon as water activity rises again, the sucrose is exchanged for water and the cell become able to grow again, as soon as $a_W > 0.9$.

Now, we are in the position of understanding that ATP owing to its very large negative charge and its big size is in fact an agent able to strongly decrease water activity. Table 10 gives the water activity calculated at $X_W = 0.91$ for different substances soluble in water.[56] Values for Na_3ADP and Na_2ATP were derived from measurements of hydration isotherms of the solids.[57] It should be clear that the water activity coefficient γ_W is never equal to 1 and is strongly dependent on the nature of the solute. The water activity coefficient is in most cases less than one, pointing to attractive solute-water interactions. The smaller the activity coefficient, the stronger is the attraction. The only exception is urea that has an activity coefficient greater than one pointing to repulsive interactions with water molecules. We have also indicated in table 10, the equivalent temperature that should be applied to supercooled water to get the same water activity value.[58] The lower the temperature, the stronger is the interaction. Values in table 9 are sorted in ascending order in order to highlight the fact that Na_3ADP and Na_2ATP salts are quite efficient in decreasing significantly water activity for a concentration below 2M. All other compounds require concentrations higher than 2M.

It is worth recalling that as shown in figure 7, no life is possible below $a_W \approx 0.6$ corresponding to an osmotic pressure $P \approx 70$ MPa ≈ 0.7 kbar and to a temperature less than -40°C. The fact that Na_3ADP leads to a larger decrease in water activity than Na_2ATP at the same molar fraction comes from the fact that Na_3ADP has one additional negative charge relative Na_2ATP. One may then expect that the decrease in water activity for the salt Na_4ATP will be much larger than for Na_3ADP. Similarly, it may be expected that the complexation of ATP^{4-} by magnesium ions, yielding $MgATP^{2-}$ will be associated to large increase in water activity, allowing inducing conformational changes of many proteins. Accordingly, it was shown that the F_1 part of the ATP synthase present in the inner mitochondrial membrane was strongly dependent on water activity, the reaction

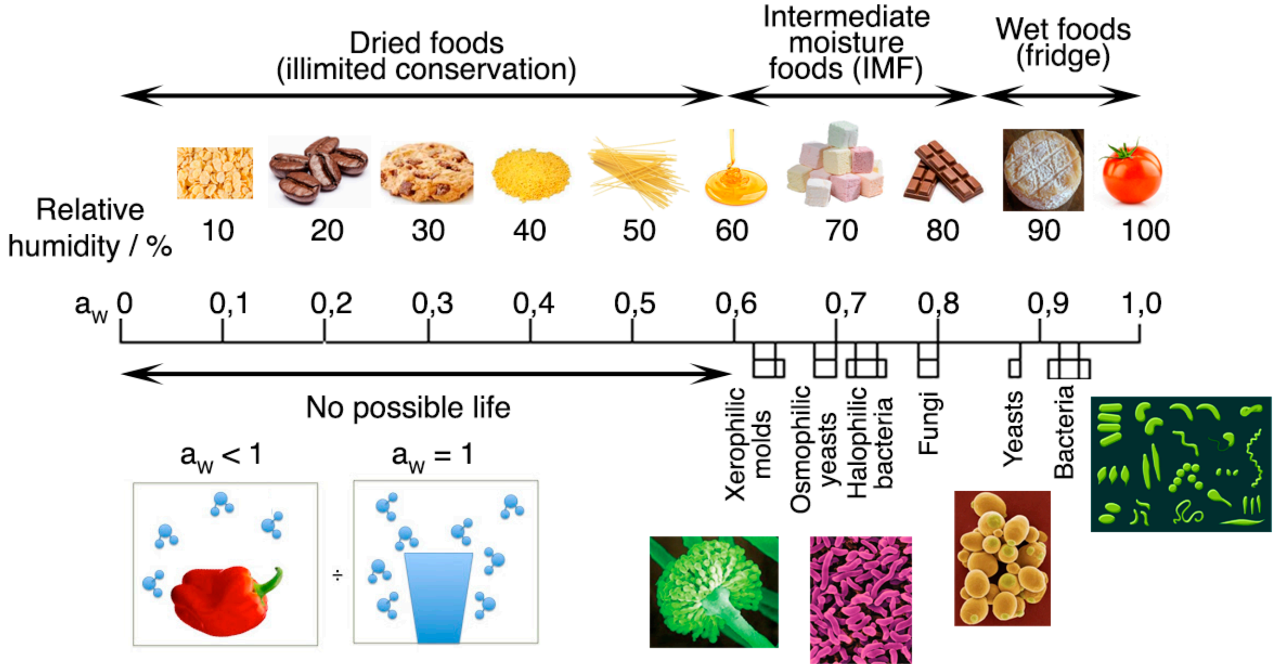


Figure 7. Water activity and growth of several kinds or microorganisms in relation woth food preservation.

Table 10. Water activity a_W, water activity coefficient γ_W and equivalent equilibrium temperature T relative to supercooled water for a constant molar fraction $X_W = 0.91$ of common solutes. One may convert water activities to equivalent osmotic pressures at T = 298.15 K through the relationship $P(MPa) = -137.15\times\ln(a_W)$.

Substance S	M.W. / Da	Concentration / M	a_W	γ_W	T / °C
PEG600	400.00	2.28	0.578	0.635	< -40
Na_3ADP	493.15	1.85	0.606	0.666	< -40
NaCl	58.44	15.57	0.790	0.868	-24
KCl	74.55	12.21	0.834	0.917	-19
Na_2ATP	551.14	1.65	0.838	0.921	-18
Lactose	342.30	2.66	0.838	0.921	-18
Lactulose	342.30	2.66	0.853	0.937	-16
Trehalose	342.30	2.66	0.862	0.947	-15
Sucrose	342.30	2.66	0.864	0.950	-15
Citric acid	192.12	4.74	0.866	0.952	-15
Tartaric acid	150.09	6.06	0.876	0.963	-14
Maltose	342.30	2.66	0.877	0.964	-14
Propylene glycol	76.09	11.96	0.881	0.968	-13
Fructose	180.16	5.05	0.889	0.977	-12
DMSO	78.13	11.65	0.889	0.977	-12
Glucose	180.16	5.05	0.894	0.982	-12
Galactose	180.16	5.05	0.894	0.982	-12
Malic acid	134.09	6.79	0.897	0.986	-11
Xylitol	152.15	5.98	0.898	0.987	-11
Sorbitol	182.17	5.00	0.898	0.987	-11
Lactic acid	90.08	10.10	0.898	0.987	-11
Xylose	150.13	6.06	0.899	0.988	-11
Arabinol	152.14	5.98	0.900	0.989	-10
Erythritol	122.12	7.45	0.900	0.989	-10
Glycerol	92.09	9.88	0.901	0.990	-10
Mannitol	182.17	5.00	0.903	0.992	-10
Urea	60.06	15.16	0.925	1.017	-8

$F_1{\cdot}ADP + P_i = F_1{\cdot}ATP$ being shifted to the right by adding DMSO, a molecule able to decrease water activity below $a_W = 0.80$ at a concentration of 40 vol%.[59] Similarly, it was observed that reduction in water activity upon addition of DMSO greatly retards the phosphoryl transfer from ATP to enzyme protein in the catalytic cycle of sarcoplasmic reticulum Ca^{2+}-ATPase.[60] Finally, synthesis of ATP after a single catalytic cycle of the same enzyme was promoted by water activity jumps.[61]

It follows that a cyclic activity of many enzymes could be related to cyclic variations in water activity. This is in fact quite logical when it is realized that most reactions in a living cell corresponds to the following reaction:

$$X\text{-}OH + H\text{-}Y = X\text{-}O\text{-}Y + H_2O$$

Where X and Y may be C, CO or P and Y may be O, S or NH. Proceeding to the right, we have anabolism, while proceeding to the left we have catabolism. In both cases, the presence of the water molecule means that changing water activity could easily shift such equilibria. As shown in table 9, any variations in salt concentrations or in small organic solutes (sugars, polyols, carboxylic acids or urea) will change more or less water activity and shift the above equilibrium either to the left if a_W increases or to the right if a_W decreases. As this concerns water, the most abundant species in a living cell, we have here a quite general binary code. More importantly, any cyclic variation in aW could be associated to the existence of a clock, a prerequisite for having coherent movements in a living cell.

CONCLUSION

The main result of our approach is the crucial role played by the entropy for understanding any kind of biological transformation. Most previous publications focused on energy not on entropy. Energy applies to the first law of thermodynamics, entropy to the second law. Our analysis is based on the second law and therefore on entropy.

Such a distinction between energy and entropy becomes obvious when one tries to understand the physical origin of the entropy concept. From Boltzmann's formulation of entropy, $S = -k_B \cdot \Sigma_i\ p_i \cdot \ln\ p_i$, we may link this concept to the number of indistinguishable microscopic configurations of a system corresponding to a single macroscopic state. Here, a clear link could then be established with Shannon's information content, $H = -\Sigma_i\ p_i \cdot \ln\ p_i$, of a message as both expression differs only by a universal scaling constant. In other words, if energy has something to do with mass or frequency (times a universal constant), entropy has something to do with information. As far as evolution is concerned, knowledge of the involved masses and frequencies is useless, relative to the information content. Accordingly, as energy should never increase or decrease during any evolution, the focus should be put on information that can be created at will and once created will never be destroyed. Variations of information contents (entropy) is the real driving force for any kind of evolution, available energy being here to set up the *speed* at which such an evolution may occur. As shown above, it is possible to describe the bioenergetics principles in terms of irreversibility potentials, leaving energy considerations to kinetics. On such a ground, one accounts for a fundamental splitting of the bioenergetics field into two distinct fields: biothermodynamics on one hand ruling evolution and focusing on entropy variations, and biokinetics on the other hand focusing on energy flux. Such a splitting is fully coherent with the fact that thermodynamics considerations are useless for discussing kinetics problems and vice-versa.

The main consequence of such a formulation is the existence of a single criterion of evolution in terms of irreversibility potentials $\Delta\Pi \geq 0$, instead of five different ones $\Delta S \geq 0$ (evolution for an isolated system), $\Delta U \leq 0$ (evolution at constant entropy and volume), $\Delta H \leq 0$ (evolution at constant entropy and pressure), $\Delta F \leq 0$ (evolution at constant temperature and volume) and $\Delta G \leq 0$ (evolution at constant temperature and pressure) when focusing on the energy/entropy duality. In other words, instead of putting experimental constraints on the thermodynamic potentials, a simpler approach is to have a single thermodynamic potential measuring irreversible power and distinct numerical tables according to the experimental constraints. For instance, the values of the $\Pi°_i$ potentials used here applies to transformations occurring at constant temperature and pressure and they should thus not be used for transformations occurring at constant entropy and volume for instance. For such transformations, other tables with different numerical values have to be used. In other words, the revolution for biologists is not the data (they are unchanged), but simply, putting entropy at the center of the equations. Life could be summarizing as able to export a large amount of entropy from the inside to the outside ($\Delta S >> 0$). It is the progressive decrease in entropy export ability that causes aging ($\Delta S > 0$), death ($\Delta S = 0$) and finally dispersion ($\Delta S < 0$) when entropy becomes imported from the surroundings, instead of being exported towards the surrounding ($\Delta S >0$).

By putting entropy at the forefront, the quest for survival is deep-rooted in the ability for exporting entropy rather than in the ability of finding energy that is always available. On such a ground, existence of a metabolism based on a food/waste balance appears to be a prerequisite for any form of life, even the most rudimentary ones. Defining life by entropy export establishes a natural link with information quest. A system could then be qualified as 'living" as soon as it is able to export information towards its environment, keeping the useful and pertinent one for maintaining its internal structure. As demonstrated by the DNA molecule, an information content of about 1 Gb is enough to encode in all details a whole human being. Another advantage of the proposed approach is to qualify the whole Earth as a living system through its plate tectonics allowing mixing of the high entropy upper materials in the atmosphere and the crust with the low entropy lower materials in the mantle and the core. Having a self-assembled membrane lipidic bilayer is thus not a mandatory condition for being qualified as a living system. Any rocky system could be a potential living entity as soon as an exporting mechanism of entropy becomes available. Again, the differentiation between a living rocking system and a living biological cell is just a matter of kinetics with a time scale of billions years for the rocks and of a few years for cells.

Finally, such a reformulation of the emergence of life has deep implications in medicine where illness could now be viewed by a decrease of the ability for exporting entropy. Restoring the entropy export ability at its optimum level, or preventing entropy importation could be very valuable tools for healing. The main characteristics of such new healing methods would be treat the body as a whole and to use very simple chemical compounds able to restore compromised entropy outputs (breath, sweat, urine, feces, heat for instance) by changing water activity. Time seems then to be ripe for stopping discussing biological events in terms of energy variations.

Any "energy" variation should be reformulated as an irreversible entropy increase, by changing the sign of the involved energy and dividing the result by the temperature of the medium. This is not at all just a mathematical game, but rather the clear acknowledgment that what drives evolution is entropy and not energy. Without a clear recognition that an expression such as "chemical energy" is meaningless, evolution of biology as a science will be deeply hampered.

APPENDIX A

At the root of the development of thermodynamics, two notions are usually introduced: energy that is always conserved (first law) and entropy that should never decrease (second law). Such a dichotomy has led to much confusion, with an opposition between processes occurring in inert matter piloted by an increase in entropy and processes occurring in living matter supposed to proceed with a decrease in entropy. It is thus quite strange that the notion of chemical potential introduced at the beginning of the XX century by John Willard Gibbs is seldom used in chemistry and biology. One of the big advantage of considering chemical potential rather than energy or entropy is that there is a single fundamental law stating that a spontaneous transformation is always associated to a *decrease* of the total chemical potential μ. Accordingly moving a weight up, increasing a speed, heating a body, decreasing volume of gases, increasing an area, increasing concentration of a solution, increasing an electrical potential difference are not processes occurring spontaneously. In our universe, weights always fall down, speeds always decrease, hot bodies always cool down, gases always expands, areas always decrease, solutions becomes always diluted, and potential differences always decrease. The fundamental reason behind such phenomena is always the same: a decrease in each case of an entity having the dimension of a mass times an area times the square of a frequency. In the previous examples, such an entity was the product of a force by a height, the product of a linear momentum by a speed, the product of an entropy by a temperature, the product of a volume by a pressure, the product of a number of moles by a chemical potential and the product of an amount of electrical charge by a voltage.

Confusion immediately arises as soon as such an entity is called "energy". This stems from the fact that in physics energy is defined as mass (m) times the square of the velocity of light in vacuum c (theory of relativity) or as frequency (f) times Planck's constant h (quantum theory). So, even if the propensity to spontaneous evolution in thermodynamics and energy in physics are quantified by the same physical unit (joule), they should not be confused. The consecrated name "Gibbs free energy" (symbol G) for measuring propensity for spontaneous evolution in thermodynamics is a first step, but has the drawback of still making a link with the energy concept of physics (E). We will use the symbol U and write according to the first law: $\Delta U = \Delta Q - p\cdot\Delta V$, where ΔQ is the amount of heat, p the pressure and ΔV the variation in volume. For energy we have on the other hand, $\Delta E = \Delta m\cdot c^2$ from relativity theory or $\Delta E = h\cdot f$, from quantum theory. One should easily understand that setting $\Delta E = \Delta U$ is quite absurd, despite the fact that both quantities share the same physical unit. The absurdity is that mass is assumed to be conserved in ΔU, but is allowed to change in ΔE. Similarly, ΔU refers to systems made of matter whereas $\Delta E = h\cdot f$ refers to photons that have no mass.

In order to unveil the real significance of ΔU, one may consider that pressure p is a kind "mechanical potential" associated to variations in volume. This fundamentally means that spontaneous changes are expected as soon as it exists pressure differences in a system, mechanical equilibrium being reached when pressure is the same everywhere (no more changes in volume). It was a quite brilliant idea of Rudolf Clausius, to introduce a state function named "entropy" S ruling reversible infinitesimal heat transfers between a system and its surroundings, associated to a "thermal potential", measured by the temperature T, $dQ = T\cdot d_eS$. As with pressure, this fundamentally means that spontaneous changes are expected as soon as it exists temperature differences in a system, thermal equilibrium being reached when temperature is the same everywhere (no more changes in entropy). But, it was also realized by Clausius, that entropy exchanges diS could also occur inside a system with the constraint that $d_iS \geq 0$. In other words, if deS is perfectly allowed to increase ($d_eS > 0$) or decrease ($d_eS < 0$), diS has the unique property of being a quantity that should always increase or remain constant.

Taking into account these two laws and writing the total entropy variation as $dS = d_eS + d_iS$, leads to $dU = T\cdot d_eS - p\cdot dV = T\cdot dS - p\cdot dV - T\cdot d_iS$. It follows that for any evolution occurring at constant entropy and volume ($dS = dV = 0$), one should have $dU = -T\cdot d_iS \leq 0$ as T and d_iS are both positive quantities. The drawback of such a formulation is that most transformations occurs at constant pressure ($dp = 0$) and constant temperature ($dT = 0$). However, one may introduce a new potential $G = U + p\cdot V - T\cdot S$, leading to $dG = dU + V\cdot dp + p\cdot dV - T\cdot dS - S\cdot dT = V\cdot dp - S\cdot dT - T\cdot d_iS$. Now, for any isobaric ($dp = 0$) and isothermal ($dT = 0$) evolution, we get $dG = -T\cdot d_iS \leq 0$. It is an easy matter to check that the same criterion of evolution could also be written $dH = -T \cdot d_iS \leq 0$ for isobaric ($dp = 0$) and adiabatic ($dS = 0$) evolution and $dF = -T \cdot d_iS \leq 0$ for isochoric ($dV = 0$) and isothermal ($dT = 0$) evolution. It thus appears that there is a single universal criterion of evolution, $d_iS \geq 0$, whatever the pair of variable chosen for controlling the system. Consequently, any spontaneous evolution always corresponds to an irreversible increase in entropy. For chemistry, one speaks of irreversible transformation while for biology one speaks of irreversible aging. In all cases, the thing that is decreasing has nothing to do with the energy of physicists related to mass content for material systems or to frequency of oscillations for radiations.

The above considerations apply rigorously for a closed system allowed to exchange only heat with its surroundings. If exchange of matter are also allowed, one may introduce a "chemical potential" μ in addition to the mechanical potential p and the thermal potential T and writes that $dG = V \cdot dp - S \cdot dT + \mu \cdot dN - T \cdot d_iS$. Consequently, any substance is characterized by a set of three thermodynamic properties, a molar volume $V_m = (\partial G/\partial p)_{T,\,N}$, a molar entropy $S_m = -(\partial G/\partial T)_{p,N}$ and a chemical potential $\mu = (\partial G/\partial N)_{T,p}$. Choosing a standard reference state $T° = 298.15$ K, $p° = 100$ kPa allows writing the chemical potential as $\mu(p,T) = \mu° - S°_m \cdot (T - T°) + V°_m \cdot (p - p°)$. A universal rule is then that if a substance is able to exist under different states, the observed state will always be the state having the lowest chemical potential. As $S°_m(\text{gas}) > S°_m(\text{solid})$ and $V°_m(\text{gas}) > V°_m(\text{solid})$, it directly follows that any pure substance should vaporize at a sufficiently high temperature($\Delta T > 0$) or low pressure ($\Delta p < 0$). Similarly one should expect a solid at a sufficiently high pressure ($\Delta p > 0$) or low temperature ($\Delta T < 0$). If a solid substance exists under several polymorphs, the observed polymorph at high temperature and pressure will be the one displaying respectively the highest entropy and the lowest molar volume.

Chemical potentials may also be defined for mixtures of substances, as $\mu_i(p,T,Ni) = \mu_i(p,T) + RT \cdot \ln a_i$, where $0 < a_i \leq 1$ measures the "activity" of each substance in the mixture. By definition, $a_i = 1$ for a pure substance. For mixture of liquids containing N molecules, the activity of each component is given by the product of the molar fraction $X_i = n_i/N$ by an activity coefficient γ_i characterizing how the various molecules interact together either trough attractive ($\gamma_i < 1$) or through repulsive ($\gamma_i > 1$) forces: $a_i = \gamma_i \cdot X_i$. For solutions, the activity corresponds to a molarity ratio, $c_i/c°$, or molality ratio, $m_i/m°$, times an activity coefficient γ_i : $a_i = \gamma_i \cdot c_i/c°$ or $a_i = \gamma_i \cdot m_i/m°$. Here, c° and m° refer to a reference state such that $c° = 1$M or $m° = 1$ mol·kg^{-1}. For gases, the activity is function of the partial pressure $p_i = p \cdot X_i$ relative to a standard pressure p° times an activity coefficient γ_i : $a_i = \gamma_i \cdot p_i/p°$, the reference pressure being $p° = 100$ kPa. Concerning activity coefficients, a general rule is that for low molar fraction $X_i << 1$, highly diluted solution $c_i << c°$ and low partial pressure $p_i << p°$ one may safely assume that $\gamma_i \approx 1$.

APPENDIX B

As the existence of irreversibility potentials should be quite new for most of the readers, we will give here some clues for decoding its physical significance. It should first be realized that such potentials results from the competition between two antagonistic kind of energies, the first one being kinetic energy favoring expansion and repulsion, the second one being electrical potential energy of attraction between positively charged nuclei and negatively charged electronic clouds. The stronger this attraction, the lower the irreversibility potential of the substance. But this is not the whole story, as these potentials are also dependent on the ability to liberate a large number of different kinds of chemical species during any transformation. This explains why elements, substances able to generate only a single kind of atom upon reaction and making strong covalent bonds under standard conditions, have the lowest $\Pi_i°$ values and may thus be considered as primitive foods. This also explain why complex substances made of a large number of different atoms have higher $\Pi_i°$ values. The larger the number of different constituting atoms, the larger the $\Pi_i°$ values.

Taking for instance the case of carbon dioxide CO_2 that appears from table 1 to be rather close to solid wastes, despite the fact that it is a gas. This stems from the fact that electrical interactions between CO_2 molecules are here very weak (absence of permanent dipole moment owing to a very high symmetry) and that carbon dioxide may be cleaved into 2 different chemical species carbon monoxide CO on the one hand and dioxygen O_2 on the other hand. Carbon monoxide CO, being more polar than CO_2 owing to its asymmetric structure and being more reluctant to give off its oxygen owing a triple bond between carbon and oxygen has logically a much lower Πi°-value. Finally dioxygen O_2 and dinitrogen N2 molecules are still less polar than CO and may give off upon dissociation only a single kind of atom, explaining their position near the top of the table.

Water H_2O, which is able as CO_2 to be cleaved into two gases (here H_2 and O_2), but is also a highly polar substance has thus a $\Pi_i°$-value intermediate between that of CO and CO_2. On the other hand hydrogen sulfide H_2S which is a gas and which may be also cleaved into two different substance, sulfur S and H_2, has a much lower $\Pi_i°$-value because one of these substance, sulfur, is made of molecules S8 that display attractive interactions strong enough to form a solid under standard conditions. As irreversibility potentials for compounds are all measured relative to the irreversibility potentials of the constituting elements, these elements all have the same zero $\Pi_i°$-value. In order to distinguish between them, one may use their absolute standard entropy S° or their volume V° under standard conditions of pressure and temperature. Here, the lower S°, the tighter the organization of atoms in space. For gases, the Sackur-Tetrode equation (3) shows that the higher the molecular weight, the higher the entropy. Consequently, one gets $S°(O_2) > S°(N_2) > S°(H_2)$ as $M(O_2) = 31.999$ Da, M(N2) = 28.013 Da and $M(H_2) = 2.016$ Da.

For condensed phases such as solids and liquids, it becomes more difficult to interpret changes in absolute entropy. However, the Sackur-Tetrode equation (3) shows that entropy always increases with temperature, meaning that we should always expect the order: S°(g) > S°(liq) > S°(s). Now, a general rule is that if a substance is able to exist under different phases the phase observed at given temperature and pressure will always be the one with the largest irreversibility potential. Consequently, from the definition of $\Pi_i°$-values given in equation 2, it follows that at sufficiently high temperature every substance should exist as a gas, as entropy is always maximized in the gaseous state. Similarly, if a solid substance may exist under several distinct crystalline polymorphs, the polymorph observed at high temperature below melting temperature would be the one with largest S°. For iron, for instance, we see from table 1 that Fe(bcc) is expected to transform into Fe(fcc) or Fe(hcp) as temperature is increased.

The Sackur-Tetrode equation predicts that entropy S° should also increases with volume, meaning that one may expect: V°(s) < V°(liq) < V°(g). From equation (2) it follows that at sufficiently high pressure any substance should be transformed into a solid, as owing to the negative sign before V°, it is phases with the smallest volume that would have the largest irreversibility potentials. For iron, this means again that Fe(bcc) is expected to transform into Fe(fcc) or Fe(hcp) as pressure is increased. One should also understand that with V°(liq) < V°(s) < V°(g), water appears as a quite strange substance, as it displays a lower volume than ice despite having a larger entropy. Basically, this means that upon heating, an increase in kinetic energy leads to a decrease in volume leading to a liquid being more dense than ice, the solid form of water. Similarly, by applying pressure on ice, one should get a liquid. Such "anomalies" for water has deep consequences for life on Earth. The fact that ice floats upon water, means that it is impossible to transform a large mass of liquid water into a block of ice. This is because below 4°C, the liquid always sink at the bottom of the container, thus escaping from the freezing. The fact that ice melts upon applying a pressure means that large masses of ice cannot remain static but should always flow downwards as observed with glaciers. Being able to break down rocks by its increase in volume upon freezing and to abrade them away by flowing under an icy form, water is thus the main shaper of Earth with time.

It is also worth noting that irreversibility potentials given in table 1 applies to a single particle of each concerned species. Consequently, if one has in one part of the system N(A) particles characterized by an irreversibility potential $\Pi_i(A)$ and in another part N(B) particles having irreversibility potential $\Pi_i(B)$, the irreversibility potentials of both parts should be $P(A) = N(A)\cdot\Pi_i(A)$ and $P(B) = N(B)\cdot\Pi_i(B)$ respectively. If both part are allowed to exchange particles and if P(A) = P(B), the system is said to be in equilibrium. In such a case the number N(A) and N(B) does not change with time. But if $P(A) \neq P(B)$, the system becomes out of equilibrium and changes in N(A) and N(B) will be observed until restoration of the equilibrium P(A) = P(B). A direct consequence is then that if $N(A)\cdot\Pi_i(A) = N(B)\cdot\Pi_i(B)$, then $N(A)/N(B) = \Pi_i(B)/\Pi_i(A)$. One may thus compute relative populations from the knowledge of irreversibility potentials.

Applying such considerations to a pure substance (a = 1) assumed to exist under two phases A and B means that equilibrium between both phases is expected as soon as N(A) = N(B) or $\Pi_i(A) = \Pi_i(B)$. At constant pressure (p = p° = 0.1 MPa), it comes from (2) that: $\Pi_i°(A) + S°(A)\cdot(T - T°) = \Pi_i°(B) + S°(B)\cdot(T - T°)$, leading to:

$$T = T°\cdot\left[1 + \frac{\Pi_i^0(A) - \Pi_i^0(B)}{S°(B) - S°(A)}\right] \qquad (B1)$$

Using equation (2) and table 1, it is thus possible to approximate melting (T_m) and vaporization (T_{vap}) temperatures of pure substances. For instance one may understand the very high cohesive energy of metallic iron as with A = solid phase (bcc) and B = liquid, (4) predicts that $T_m = 1682$ K versus $T_m(\exp) = 1811$ K, while with A = liquid and B = gas, it comes that $T_{vap} = 3644$ K versus $T_{vap}(\exp) = 3343$ K. The differences with experimental values may be accounted for by the fact that in (4) we have used S°-values at T = 298.15 K and neglected the variation of these standard entropies with temperature. Nevertheless, this clearly shows that iron atoms are in strong attractive interactions in the solid or in the liquid. One may also use table 1 to predict that upon heating α-Fe(bcc) should be transformed into γ-Fe(fcc) and not into ε-Fe(hcp). Now, applying (4) to water with A = ice and B = liquid leads to $T_m = 275$ K = 2°C, while with A = liquid and B = gas, it comes $T_{vap} = 369$ K = 96°C. Again, this shows the ability of irreversibility potentials to account approximately for observed melting and ebullition temperatures.

One may also consider what happens at constant temperature (T = T° = 298.15 K) with (2) giving a new equilibrium condition as function of applied pressure $\Pi_i°(A) - V°(A)\cdot(p - p°)/T° = \Pi_i°(B) - V°(B)\cdot(p - p°)/T°$ and leading to:

$$p(MPa) = p° + \frac{\Pi_i^0(B) - \Pi_i^0(A)}{V°(B) - V°(A)} \cdot T° \qquad (B2)$$

Consequently, one predicts that the transformation of A = α-Fe(bcc) into B = ε-Fe(hcp) should be observed at about p = 16.3 GPa, in good agreement with experiments revealing a transition pressure above 11 GPa (see L. Miyagi et al., *J. Appl. Phys.*, **2008**, *104*, 103510). These considerations shows the usefulness of such irreversibility potentials for a good understanding of the behavior of a pure substance as a function of temperature and pressure.

REFERENCES

1. R. Milo, *Bioassays*, **2013**, *35*, 1050.
2. P. Jorgensen, J. L. Nishikawa, B. J. Breitkreutz, M. Tyers, *Science*, **2002**, *297*, 395.
3. P. Echave, I. A. Conlon, A. C. Llyod, *Cell Cycle*, **2007**, *6*, 218.
4. F. Abascal, D. Juan, I. Jungreis, L. Martinez, M. Rigau, J. M. Rodriguez, J. Vazquez, M. L. Tress, *Nucleic Acids Research*, **2018**, *46*, 7070.
5. N. Savage, *Nature*, **2015**, *527*, 86.
6. D. L. Pinti, *Lectures in Astrobiology*, M. Gargaud & al. (Eds), Springer-Verlag, Berlin, **2005**, Vol. I, p. 83.
7. S. J. Mojzsis, G. Arrhenius, K. D. McKeegan, T. M. Harrison, A. P. Nutman, C. R. L. Friend, *Nature*, **1996**, *384*, 55.
8. M. C. Weiss, F. L. Sousa, N. Mrnjavac, S. Neukirchen, M. Roettger, S. Nelson-Sathi, W. F. Martin, *Nature Microbiology*, **2016**, *1*, 16116.
9. S. Sundararaj, A. Guo, B. Habibi-Nazhad, M. Rouani, P. Stothard, M. Ellison, D. S. Wishart, *Nucleic Acids Res.*, **2004**, *32* (Database issue): D293.D295.
10. R. Shapiro, *Scientific American,* **2007**, 12 Feb.
11. G. H. Pollack, X. Figueroa, Q. Zhao, *Int. J. Mol. Sci.*, **2009**, *10*, 1419.
12. P. A. Bachman, P. Walde, P. L. Muisi, J. Lang, *J. Am. Chem. Soc.,* **1990**, *112*, 8200.
13. W. Martin, M. J. Russell, *Phil. Trans. R. Soc. Lond. B*, **2003**, *358*, 59.
14. L. E. Orgel, *PLoS Biology*, **2008**, *6*, e18.
15. G. Springsteen, J. R. Yerabolu, J. Nelson, C. J. Rhea, R. Krishnamurthy, *Nature Commun.,* **2018**, *9*, 91.
16. I. Prigogine, G. Nicolis, A. Babloyantz, *Phys. Today*, **1972**, *11*, 23.
17. E. Chaisson, *The Scientific World Journal,* **2014**, 384912.
18. G. Job, F. Herrmann, *Eur. J. Phys.*, **2006**, *27*, 353.
19. W. Grimus, *Ann. Phys. (Berlin)*, **2013**, *525*, A32.
20. J. E. Koska, D. D. Dalton, H. Skelton, S. Dollhopf, J. W. Stucki, *Appl. Environ. Microbiol.*, **2002**, *68*, 6256.
21. M. P. Fewell, *Am. J. Phys.*, **1995**, *63*, 653.
22. M. Can, F. A. Armstrong, S. W. Ragsdale, *Chem. Rev.*, **2014**, *114*, 4149.
23. J. L. Boer, S. B. Mulrooney, R. P. Hausinger, *Arch. Biochem. Biophys.*, **2014**, *544*, 142.
24. B. Desguin, P. Goffin, E. Viaene, M. Kleerebezem, V. Martin-Diaconescu, M. J. Maroney, J.-P. Declercq, P. Soumillion, P. Hols, *Nature Commun.*, **2014**, *5*, 3615.
25. S. Guillot, K. Hattorin, *Elements*, **2014**, *9*(2), 95.
26. J. A. Brandes, N. Z. Boctor, G. D. Cody, B. A. Cooper, R. M. Hazen, H. S. Yoder Jr., *Nature*, **1998**, *395*, 365.
27. R. A. Alberty, *Thermodynamics of Biochemical Reactions*, **2003**, John Wiley & Sons, Hoboken, New Jersey.
28. R. A. Alberty, *J. Phys. Chem. A*, **1998**, *102*, 8460.
29. J. N. Israelachvili, S. Marcelja, R. G. Horn, *Quart. Revs. Biophys.*, **1980**, *13*, 121.
30. R. E. Heikkila, C. N. Kwong, D. G. Cornwell, *J. Lipids Res.*, **1970**, *11*, 190.
31. A. I. Greenwood, S. Tristram-Nagle, J. F. Nagle, *Chem. Phys. Lipids*, **2006**, *143*, 1.
32. I. Brzozowska, Z. A. Figaszewski, *Colloids Surf. B: Biointerfaces*, **2002**, *23*, 51.
33. A. Kessel, N. Ben-Tal, S. May, *Biophys. J.*, **2001**, *81*, 643.
34. R. C. Ewing, *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 3432.
35. M. J. Borda, A. R. Elsetinow, M. A. Schoonen, D. R. Strongin, *Astrobiology*, **2001**, *1*, 283.
36. G. D. Cody, N. Z. Boctor, J.A. Brandes, T. R. Filley, R. M. Hazen, H.S. Yoder Jr., *Geochim. Cosmochim. Acta*, **2004**, *68*, 2185.

37. G. D. Cody, N. Z. Boctor, T. R. Filley, R. M. Hazen, J. H. Scott, A. Sharma, H.S. Yoder Jr., *Science*, **2000**, *289*, 1337.
38. Y. Novikov, S. D. Copley, *PNAS*, **2013**, *110*, 13283.
39. K. Fan, W. Wang, *J. Mol. Biol.*, **2003**, *328*, 921.
40. S. Akanuma, T. Kiawa, S. Yokoyama, *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 13549.
41. M. Granold, P. Hajieva, M. I. Tosa, F.-D.Irimie, B. Moorsmann, *Proc. Natl. Acad. Sci. USA*, **2018**, *115*, 41.
42. R. Shibue, T. Sasamoto, M. Shimada, B. Zhang, A. Yamagishi, S. Akanuma, *Sci. Reports*, **2018**, *8*, 1227.
43. R. J. C. Hennet, N. G. Holm, M. H. Engel, *Naturwissenschaften*, **1992**, *79*, 361.
44. R. M. Hazen, *Am. Miner.*, **2006**, *91*, 1715.
45. A. Rimola, P. Ugliengo, M. Sodupe, *Int. J. Mol. Sci.*, **2009**, *10*, 746.
46. R. B. Martin, *Biopolymers*, **1998**, *45*, 351.
47. K. H. Lemke, R. J. Rosenbauer, D. K. Bird, *Astrobiology*, **2009**, *9*, 141.
48. S. A. Benner, H.-J. Kim, M. A. Carrigan, *Accounts Chem. Res.*, **2012**, *45*, 2025.
49. G. Fedoseev, H. M. Cuppen, S. Ioppolo, T. Lamberts, H. Linnartz, *Monthy Notices Royal Astronomical Soc.*, **2015**, *448*, 1288.
50. D. Roy, K. Najafian, P. R. Schleyer, *Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 12272.
51. T. A. Glassman, C. Cooper, L. W. Harrison, T. J. Swift, *Biochemistry*, **1971**, *10*, 843.
52. J. L. Bock, G. B. Crull, A. Wishnia, C. S. Springer Jr., *J. Inorg. Biochem.*, **1991**, *44*, 79.
53. A. Patel, L. Malinovska, S. Saha, J. Wang, S. Alberti, Y. Krishnan, A. A. Hyman, *Science*, **2017**, *256*, 753.
54. A. Stevenson, J. A. Cray, J. P. Williams, R. Santos, R. Sahay, N. Neuenkirchen, C. D. McClure, I. R. Grant, J. D. R. Houghton, J. P. Quinn, D. J. Timson, S. V. Patil, R. S. Singhal, J. Anton, J. Dijksterhuis, A. D. Hocking, B. Lievens, D. E. N. Rangel, M. A. Voytek, N. Gunde-Cimerman, A. Oren, K. N. Timmis, T. J. McGenity, J. E. Hallsworth, *The ISME Journal*, **2015**, *9*, 1333.
55. A. J. Maneffa, R. Stenner, A. S. Matharu, J. H. Clark, N. Matubayashi, S. Shimizu, *Food Chem.*, **2017**, *237*, 1133.
56. J. Chirife, C. Ferro-Fontan, E. A. Benmergui, J. *Food Technol.*, **1980**, *15*, 59.
57. M. Falk, *Can. J. Chem.,* **1965**, *42*, 314.
58. H. Sippola, P. Taskinen, *J. Chem. Eng. Data*, **2018**, *63*, 2986.
59. M. T. de Gomez-Puyou, G. Perez-Hernandez, A. Gomez-Puyou, *Eur. J. Biochem.*, **1999**, *266*, 691.
60. H. Suzuki, T. Kanazawa, *J. Biol. Chem.*, **1996**, *271*, 5481.
61. L. de Meis, G. Inesi, *J. Biol. Chem.*, **1982**, *257*, 1289.