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Singlet Dioxygen $^1O_2$, its Generation, Physico-chemical Properties and its Possible Hormetic Behavior in Cancer Therapy

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Abstract. Singlet dioxygen $^1O_2$ is one excited state among the three other possible spectroscopic states of molecular oxygen. Here, we first describe the use of published spectroscopic data and thermodynamic modeling based on irreversible entropy production. Such concepts are further applied to the synthesis of singlet dioxygen and its reactions with crucial biological molecules. In a last section, we suggest that singlet dioxygen and ozone may be responsible for the success of radiation therapy, that has been used to treat cancer successfully for over 120 years. Its precise mechanism of action remains controversial. We thus aim to clarify the role of singlet oxygen in radiotherapy and chemotherapy. A partial conversion of ionizing radiation in the body into thermal photons could be assumed. The antitumor effect may involve these thermal photons, such as the one delivered by red/infrared sources. Thermal photons (wavelengths of 635 nm and 1270 nm) convert triplet dioxygen into singlet dioxygen by changing the spin of its outer electrons. Despite its short half-life, Singlet dioxygen is responsible for the activation of multiple free radicals (such as hydrogen peroxide), which may target proteins and DNA, induce either apoptosis or oxidative phosphorylation. At moderate concentrations, thermodynamic data suggests that singlet dioxygen may readily react with water to form a potent pro-apoptotic molecule (ozone), thus decreasing cancer growth. However, at high concentration cytotoxic effects against all kind of cells occurs. This strongly suggests a non-linear hormetic behavior of singlet dioxygen. It is also proposed that cytotoxic chemotherapy induces the same free radicals that singlet dioxygen does. There are also other ways to enhance the production of singlet dioxygen, such as phototherapy using Methylene Blue for instance. As a source of reactive oxygen species (ROS), singlet oxygen could thus be a common agent active both in radiotherapy and chemotherapy. It is probable that the activity of radiation therapy and chemotherapy may be mediated by the conversion of triplet to singlet oxygen. This may explain the oxygen effect such as described in radiotherapy and chemotherapy.

Keywords: cancer, phototherapy, Warburg's effect, radiation therapy, singlet oxygen, chemotherapy, oxygen effect.

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INTRODUCTION

Less than two months after the discovery of X-rays by Wilhelm Röntgen in 1895, Leopold Freund treated, successfully, a child with a large nevus, a benign skin lesion[1]. In the following months, there were multiple reports of the efficacy of radiation therapy in the treatment of both benign and malignant lesions. Radiation therapy (RT) is a therapy using ionizing radiation to control or kill inflammatory and cancer cells. RT has been extensively used for the treatment of inflammation, but this indication is slowly disappearing because of the risk of radiation-induced malignancies[2]. RT may be curative in several types of cancer if they are localized to one limited area of the body. Several shaped radiation beams coming from several angles of exposure intersect at the tumor to spare normal tissues (such as skin or organs that radiation must pass through to treat the tumor). This provides a much higher absorbed dose there than in the surrounding healthy tissue. RT kills normal cells, and every radiation oncologist knows the dose not to trespass to the normal tissues.

Ionizing radiation has been published to target the DNA leading to cell death. In the laboratory setting, the damage caused by ionizing radiation to the DNA is immediate and consists of single or double-strand breaks and mutations[3]. A correlation exists between the toxicity of RT to the normal cells and the damage to the DNA[4].

Unlike the laboratory setting, there is no immediate sign of death for cancer cells in clinical practice. In the minutes following a cardiac infarct, there is an increased level of cardiac enzymes, such as troponin, in the blood plasma[5]. Assessment of the treatment response after RT occurs, not minutes or even days but weeks after the inception of treatment[6].

Recently, Radman demonstrated that the prime target of radiation is not the DNA as previously thought but the proteasome. The cell dies because of oxidative damage to its proteins[7]. The polymerases may repair the concomitant damage to the DNA.

This paper aims to suggest existence of a link between RT and production of singlet dioxygen mediated by thermal photons. Radiation may also affect the activity of water around proteins or DNA and change mitochondria activity. Herein, we will not try reviewing the past 50 years in mechanistic, spectroscopic, computational, and biological studies of singlet oxygen. This topic is covered in great details in a recent textbook[8]. Our interest is rather to work in an historical perspective with focus on rather old concepts that will be revisited through the lens of the entropy concept[9-11]. Moreover, we are perfectly aware that singlet dioxygen reacts by two distinctive pathways (Type I and Type II mechanisms), and causes damage to biomolecules, materials such as polymers, food, paints etc. Amino acids, nucleic acids, unsaturated molecules (e.g.; membranes) also react with singlet oxygen to yield decomposed products and consequently to cause cell death. But, all these important properties, which rationalize the toxic and fatal behavior of $^1O_2$ to organisms completely neglects the fact that many biological processes, display a biphasic or triphasic response to exposure to increasing amounts of a substance or condition such as radiation. Even if this hormesis model of dose response is still vigorously debated[12], it seems worth investigating if it could apply to the biological response of singlet dioxygen. Moreover, in the spirit of putting more physics in biological or medical thinking, part of the article will be devoted to a reminder of the electronic structure and spectroscopic properties of these species deriving from molecular oxygen. Finally, we will focus mainly on healing cancer, even if the ideas exposed here could be extended to other diseases.

THERMAL PHOTONS ARE EFFECTIVE AGAINST CANCER AND INFLAMMATION

The metabolism of the cancer cells has been extensively studied since the seminal work of the German scientist Otto Warburg[13]. Warburg's effect is, in fact, the cause of every hallmark of cancer, such as the proliferation of cells, angiogenesis, or immortality[14]. Cancer is not the only disease involving Warburg's effect, as this effect is also crucial in inflammation[15] or Alzheimer's disease[16]. Alleviating Warburg's effect decreases cell proliferation[14].

Non-ionizing radiation has been developed successfully in the treatment of both benign and malignant tumors. Delivery of hyperthermia is possible using non-ionizing radiation such as ultrasound, microwave, or most commonly infrared (thermal) photons. Red and infra-red photons have also been used in the treatment of inflammation.

It is a well-accepted fact that a practice does not need total mechanism clarity to operate. More than 6500 publications registered on PubMed from LLLT keyword (*Low-Level Light Therapies*) covering cancer[17] wound healing[18], inflammation and pain management[19], muscles and joints injuries[20] as well as nerve regeneration[21], traumatic brain injuries[22], depression and anxiety[23] and more recently neurodegenerative such as Alzheimer and Parkinson diseases[24] as well as Age-Related Macular Degeneration[25].

THE THREE FORMS OF MOLECULAR DIOXYGEN

Dioxygen is a very peculiar molecule whose chemical behavior cannot be explained using conventional octet's rule[26]. Such rule generally applies to any molecule built from atoms belonging to the second period of the periodic table of the elements. Let N be the total number of atoms, E, the total number of valence electrons, Q the number of atoms other than hydrogen, and C, the number of cycles. It then mathematically follows that the number of single bonds should be S = N + C – 1, the number of lone pairs should be L = E – 3×Q – N, and the number of multiple bonds should be M = 3×Q – E/2 – C + 1 – E%2[27]. Here E%2 = 0 or 1 if E is respectively even or odd. For dioxygen O_2 characterized by E = 6 + 6 = 12, N = Q = 1 + 1 = 2, C = 0, the rule predicts that S = 2 + 0 – 1 = 1 (one single bond), L = 12 – 3×2 – 2 = 4 (four lone pairs) and M = 3×2 – 12/2 – 0 + 1 = 1 (one double bond). This corresponds to the classical notation :O:=:O: found in every elementary chemistry textbook. The trouble is that such a formula is utterly wrong as it predicts that dioxygen, having an even number of electrons, should be a diamagnetic molecule in its ground state. Experiments, on the other hand, show that dioxygen is rather a paramagnetic molecule in its ground state, diamagnetic states corresponding to excited states.

Such a deep mystery could be resolved by writing Lewis's structures after the removal of two electrons ($O_2^{2\oplus}$ ion with E = 10) or the addition of two electrons ($O_2^{2\ominus}$ ion with E = 14)[28]. For the dication, Langmuir's rules predicts that the number of single bonds does not change (S = 1), but that L = 10 – 3×2 – 2 = 2 (two lone pairs) and M = 3×2 – 10/2 – 0 + 1 = 2 (one triple bond). This corresponds to the classical notation, $^{\oplus}$:O≡O:$^{\oplus}$, meaning that the two electrons in the highest occupied energy level are of anti-bonding character, as removing them leads to the apparition of an additional chemical bond. Concerning, the dianion, the same rules predicts that L = 14 – 3×2 – 2 = 6 (six lone pairs) and M = 3×2 – 14/2 – 0 + 1 = 0 (no multiple bond, i.e. $^{\ominus}$:Ö:–:Ö:$^{\ominus}$). This means that the lowest unoccupied energy level is also of anti-bonding character, as adding 2 electrons there leads to the transformation of the double bond into two lone-pairs and a single bond. So, using just the well-established octet's rule, it could be anticipated that the states of the highest energy (occupied and unoccupied) in dioxygen are of similar nature (anti-bonding character). This strongly suggests that these two states have the same energy (degeneracy) with a single unpaired electron in each state, •:O: —:O:•. This explains the observed paramagnetism of dioxygen in its ground state.

Further development of quantum mechanics and group theory has confirmed the validity of such a picture. Accordingly, owing to its high symmetry ($D_{\infty h}$ cylindrical symmetry), molecular orbital (MO) theory predicts that dioxygen has a doubly degenerated HOMO (highest occupied molecular orbital) or LUMO (lowest unoccupied molecular orbital). In other words, writing structures obeying the octet's rule is an easy graphical way to get good solutions for Schrödinger's equation.

From MO-theory, we also learn that, owing to the phenomenon of resonance, obeying octet's rule can be of a dynamic nature. Thus, starting from the static solution (S = 1, L = 4, M = 2), we get the dynamic solution (S = 1, L = 5, M = 1), after transformation of the double bond into a delocalized lone pair:

:Ö:$^{\ominus}$—:O:$^{\oplus}$ ↔ $^{\oplus}$:O: —:Ö:$^{\ominus}$

Here, violation of the octet's rule occurs at a given time. However, after averaging in time, there is no possibility of distinguishing between the two oxygen atoms owing to the $D_{\infty h}$ cylindrical symmetry. This restores octet's rule, but in a dynamic sense.

Consequently, for a good understanding of dioxygen chemistry, it appears necessary to consider three main forms for this molecule:

One apolar resonant paramagnetic bi-radical (triplet dioxygen): $^3O_2$ ($^3\Sigma_g^-$):

$^{\bullet a}$:O: —:O:$^{\bullet b}$ ↔ $^{\bullet b}$:O: —:O:$^{\bullet a}$

One polar resonant diamagnetic molecule (singlet dioxygen) $^1O_2$ ($^1\Delta_g$):

:Ö:$^{\ominus}$—:O:$^{\oplus}$ ↔ $^{\oplus}$:O: —:Ö:$^{\ominus}$

One apolar static diamagnetic molecule (singlet dioxygen) $^1O_2$ ($^1\Sigma_g^+$):

:O:=:O:

The Greek symbols in brackets are the rigorous notation using labels derived from the symbols of the irreducible representations of $D_{\infty h}$ point group symmetry. Without such a notation, it would be impossible to distinguish between the two different forms of singlet dioxygen $^1O_2$. This is a crucial point, as these three forms do not have the same energy.

Solving Schrödinger's equation thus shows that the ground state is $^3O_2$ ($^3\Sigma_g^-$) followed by the first excited state $^1O_2$ ($^1\Delta_g$) located at an energy ΔE = 153 zJ (1 zJ = 10^{-21} J) above the ground state. To reach this state, the dioxy-

gen molecule should absorb a photon of wavelength λ = h·c/ΔE, where h is Planck's constant and c the celerity of light in the vacuum. With h·c = 198 645 nm·zJ, the $^1O_2$ ($^1\Delta_g$) state could be reached with a photon of wavelength λ = 198 645/153 = 1298 nm (infrared light). Reaching the second state $^1O_2$ ($^1\Sigma_g^+$), located at an energy ΔE = 259 zJ above the ground state, will involve a photon of wavelength λ = 198 645/259 = 767 nm (red light).

It is then quite unfortunate that most biology textbooks treat dioxygen as a single species, generally written as :O:=:O: or O_2 in short, which is not the ground state formulation. The fact that the spin state (upper left digit before the chemical symbol) is usually not even mentioned is also quite unfortunate. Accordingly, it is worth recalling that spin conservation is one of the most fundamental laws of physics expressed by Witmer-Wigner for chemical transformation[29]. These rules state that if S_A is the spin of reactant A and S_B the spin of reactant B, a reaction will be spin-allowed if the total spin of the products is included in the series: $|S_A + S_B|$, $|S_A + S_B - 1|$, $|S_A + S_B - 2|$,…, $|S_A - S_B|$.

Let us consider, for instance, one of the most exothermic reactions known in chemistry:

$2\ ^1H_2\ (g) + {}^3O_2\ (g) \rightarrow 2\ ^1H_2O\ (l)$
$\Delta_rG° = -2\times237 = -474\ kJ\cdot mol^{-1}$

As indicated by the superscripts showing spin multiplicities, such a direct reaction is spin-forbidden, as we have $S(^1H_2) = ½(1 - 1) = 0$ and $S(^3O_2) = ½(3 - 1) = 1$. It then follows that the total spin of the reactants is S = 2×0 + 1 = 1. For the products, we have $S(^1H_2O) = ½(1 - 1) = 0$, meaning that the total spin of the reactants is S = 2×0 = 0. There is thus a violation of spin conservation in water synthesis from dihydrogen and dioxygen taken in their ground state. This is the reason why nothing happens upon mixing a powerful reductant (H_2) with a powerful oxidant (O_2). However, it is a well-known fact that the reaction is immediate and explosive after the introduction of sparkle in the mixture. The role of sparkle is to bring enough energy to transform triplet oxygen $^3O_2$ ($^3\Sigma_g^-$) into singlet oxygen $^1O_2$ ($^1\Delta_g$). The reaction then becoming:

$2\ ^1H_2\ (g) + {}^1O_2\ (g) \rightarrow 2\ ^1H_2O\ (l)$
$\Delta_rG° = -2\times237 - 95 = -569\ kJ\cdot mol^{-1}$

As now ΔS = 0, the reaction can proceed easily without any catalyst. It is worth recalling here that water synthesis is at the heart of complex-IV (CcO). This complex of the electron transport chain (ETC) in mitochondria has a catalytic site allowing direct reduction of triplet dioxygen into the water using separated fluxes of protons and electrons. The separation of protons from electrons is thus mandatory, as upon mixing them together, we would obtain singlet dihydrogen that is unable to react with triplet dioxygen to form water.

This means that quantum chemistry should be at the heart of biological thinking. The fact that it does not have deleterious consequences, particularly for medicine, as prevention of water synthesis from triplet dioxygen in mitochondria leads to Warburg's effect, a common source for many kinds of diseases[14].

SPECTROSCOPIC PROPERTIES OF SINGLET DIOXYGEN

As explained above, thermal photons may interact with triplet dioxygen ($^3O_2$) to form singlet dioxygen ($^1O_2$). Switch from triplet to singlet state necessitates energy. The most common way to switch to the singlet form is irradiation by visible photon (red at 635 nm), allowing reaching the $^1O_2$ ($^1\Sigma_g^+$) state or infrared ones at 1270 nm, leading to the $^1O_2$ ($^1\Delta_g$) state. The average lifetime of singlet oxygen is 1-50 μs in aqueous systems[30]. In the gas phase, both singlet states may relax towards the triplet state $^3O_2$ ($^3\Sigma_g^-$) by two different mechanisms. The $^1O_2$ ($^1\Delta_g$) state may use collisions with other molecules M according to:

$^1O_2(^1\Delta_g) + n\ M \rightarrow {}^3O_2\ (^3\Sigma_g^-) + n\ M^* + heat$

The notation M* means that, after the collision, the molecules M are left in a rotating state of higher energy. The intrinsic electronic spin has thus been transformed into an extrinsic spin (rotations), ensuring spin-conservation. The generated heat corresponds to the energy difference ΔE = 153 zJ existing between the first excited state and the ground state. The following relationship allows estimating the expected temperature increase ΔT after dissipation of an energy ΔW into heat:

$$\Delta T(K) = \frac{\Delta W(zJ)}{0.0069[6 \times (N - n_C) - 5 \times n_L]}$$

Here, we have used the equipartition theorem of statistical physics $\Delta W = ½k_B\times\Delta T\times\Sigma(df)$, where k_B is Boltzmann's constant and Σ(df), the total number of degrees of freedom concerned by the relaxation process. Now, for a non-linear molecule made of n atoms, one may expect three degrees for the translation of the center of mass, three degrees for the rotation around the center of mass, and 2×(3n – 6) degrees for the normal modes of

vibration. Factor 2 considers that each vibration mode has two degrees, one associated with the position and the second one to speed. Each non-linear molecule will then contribute $6\times½k_B + (3n\text{-}6)\times(½k_B + ½k_B) = ½k_B\times(6n - 6)$. For a linear molecule, the rotation around the molecular axis cannot be used to store energy and thus corresponds to a vibration mode. Each linear molecule will then contribute to $½k_B\times(6n -5)$. It then follows that if n_L stands for the number of linear molecules and n_C for the number of non-linear ones and if N is the total number of atoms, we have $\Sigma(df) = 6\times(N - n_C) - 5\times n_L$.

Water being the most abundant molecule in a cell, we have $N = 3\times n_W$, $n_L = 0$ and $n_C = n_W$, leading to $\Delta T(K) = 12\times\Delta W(zJ)/n_W$. Consequently, in order reaching a temperature T = 310 K (or 37°C) from a temperature T = 288 K (or 15°C, the average temperature of the earth), the total number of concerned water molecules involved in the relaxation of one $^1O_2(^1\Delta_g)$ molecule characterized by ΔW = 153 zJ is estimated as $n_W = 1849/(310 - 288) = 84$ molecules. Furthermore, the average volume v of a molecule having a molecular weight M (Da) in a liquid of density ρ ($g\cdot cm^{-3}$), assuming a random packing efficiency ξ = 0.6366, is given by:

$$V = \xi \times \frac{M \times 10^{-3}}{N_A \times \varrho \times 10^{-3}} \Rightarrow v/Å^3 = 1.057 \times \frac{M(Da)}{\varrho(g \cdot cm^{-3})}$$

For water (M = 18 Da, ρ ≈ 1 $g\cdot cm^{-3}$, i.e. v = 19 $Å^3$), the thermal relaxation volume around one $^1O_2(^1\Delta_g)$ molecule is about $19\times84 = 1598$ $Å^3$, corresponding to a sphere of radius R = 7.3 Å. As the average diameter of isolated water is a molecule is $D = (19\times6/\pi)^{⅓} \approx 3.3$ Å, this corresponds to 2 shells of water molecules. This shows how we may relate a biological number, the average body temperature, to a molecular quantum process relaxation of $^1O_2(^1\Delta_g)$ towards the $^3O_2$ ($^3\Sigma_g^-$) ground state through heating, using well-known physical laws.

Besides this thermal relaxation process involving water molecules, there is a radiative mechanism involving infrared photons:

$$^1O_2(^1\Delta_g) \rightarrow {}^3O_2\ (^3\Sigma_g^-) + {}^3\gamma_{IR}$$

Here, nature uses the fact that a photon is a particle of spin S = 1, allowing photonic relaxation with the emission of a photon spinning in one direction (m_S = +1), leaving the dioxygen molecule in its ground state with the two electrons spinning in the same direction opposite to that of the photon ($m_S = -1/2 - ½ = -1$) to conserve the initial null spin (0 = 1 – 1). Heisenberg's uncertainty relationship drives the timescale associated with such photonic relaxation. It allows relating the intrinsic lifetime τ of the excited state having energy ΔE to the reduced Planck's constant ħ ≈ 106 zJ·fs: $\Delta E\times\tau \approx ħ$. Consequently, with ΔE = 153 zJ, it comes that $\tau \approx 106/153 = 0.7$ fs. This lifetime should be compared with the average rotation time τ_c of a water molecule at a given temperature, needed for allowing thermal non-radiative relaxation. Stokes-Einstein relationship gives this correlation time that depends on absolute temperature T, viscosity η and molecular volume v: $\tau_c = \eta\times v/(k_B\times T)$, i.e. $\tau_c(ps) = 72.4\times\eta(mPa\cdot s)\times v(Å^3)/T(K)$[31]. For liquid water (v = 19 $Å^3$) at T = 310 K, we have η = 0.69 mPa·s, meaning that $\tau_c \approx 3$ ps. This shows that for one molecule undergoing thermal relaxation from the excited state to the ground state, about 5,000 molecules undergo photonic relaxation in the near-IR part of the electromagnetic spectrum.

As the second excited state $^1O_2$ ($^1\Sigma_g^+$) is much higher in energy (ΔE = 259 zJ), its thermal relaxation towards the ground state will mobilize a much more number of water molecules, typically $n_W = 3128/(310 - 288) = 142$ molecules. This forms a relaxation volume of 2,702 $Å^3$, corresponding to a sphere of radius R = 8.6 Å, i.e., nearly 3 shells of water molecules around one $^1O_2$ ($^1\Sigma_g^+$) molecule. The average lifetime of this second excited state being shorter, $\tau \approx 106/259 = 0.4$ fs, about 7,300 molecules undergo photonic relaxation as a characteristic, red-colored visible light when one relaxes using the thermal channel.

FORMATION OF SINGLET DIOXYGEN

Singlet dioxygen cannot be formed by direct optical excitation of triplet dioxygen by infrared or red photons. Accordingly, from Fermi's Golden rule and group theory, such transitions are both spin-forbidden and orbital-forbidden. This is the reason why a photosensitizer should be used[30]. Another completely different way of forming singlet dioxygen is to use a chemical reaction releasing a large amount of entropy. Reasons for using entropy and not Gibbs' free energies have been analyzed elsewhere[9-11]. Shortly, a single criterion of spontaneous evolution in nature is that entropy of the universe should always increase in any kind of transformation, whether chemical or biological. In other words, biological systems are fully compliant with the second law of thermodynamics with no need to introduce alternate notions such as negentropy, for instance. The observed complexity of biological systems is a consequence of large entropy flux towards the universe, in compliance with the laws of irreversible, far from equilibrium, thermodynamics. From a technical viewpoint to each

transformation of matter corresponds to a change in the standard irreversibility potential $\Delta\pi_i°$ (T = 25°C, p = 1 atm) that cannot be negative. Rules for computing an irreversibility potential $\pi_i°$ for each substance involved in the transformation have been presented elsewhere[10]. For biological systems, such standard irreversibility potentials are transformed to $\pi'_i°$ values considering that biology occurs in water (pH = 7) in the presence of ionic species (ionic strength I ≈ 250 mM). We have used generalized Legendre's transformation, a mathematically straightforward procedure[32]. All the computational details are available as supplementary information (SI).

Irreversibility potentials (IrPs) are useful for comparing two substances according to their entropy content relative to the whole universe. Basically, substances that have strongly negative IrPs are reducing substances. They present a spontaneous tendency to be irreversibly transformed through oxidation into substances having a strongly positive IrP. One may thus notice that singlet dioxygen has a significantly more negative IrP than triplet dioxygen. This automatically means that combustion with $^1O_2$ leads to larger entropy production than combustion with $^3O_2$. Moreover, the burning of a combustible substance existing in a singlet spin-state with $^1O_2$ is spin-allowed, whereas its combustion with $^3O_2$ is spin-forbidden, needing the presence of a catalyst.

In biology, singlet dioxygen plays a key role in photosynthesis. Generation of $^1O_2$ from water molecule has been widely reported during photosynthesis in plants, using energy from the sunlight. Photosensitizers are generally necessary for producing singlet through light absorption. This is particularly true in plants where $^1O_2$ is generated by chlorophyll and other cofactors of the photosystem[33].

In plants exposed to excess light, the increased production of singlet dioxygen can result in cell death[34]. Various substances such as quinones, carotenoids, and tocopherols contained in chloroplasts quench singlet dioxygen and protect against its toxic effects.

In humans, transportation of the dioxygen molecule to the target cell occurs through the triplet state. It is used in the mitochondria together with electrons and protons at the level of the complex IV of the mitochondria, producing water as a non-toxic waste together with some heat and biomolecules with very negative IrPs such as NADH ($\pi_i'° = -6.23829$ zJ·K^{-1}) or NADPH ($\pi_i'° = -1.32422$ zJ·K^{-1}) for instance. It is worth noticing the large difference in irreversibility potentials between NADH and NADPH. However, when considering oxidized forms $NAD^⊕$ ($\pi_i'° = -5.89863$ zJ·K^{-1}) and $NADP^⊕$ ($\pi_i'° = -0.98399$ zJ·K^{-1}), we get almost the same standard oxidation potential:

$NAD^⊕ + H^⊕ + 2\ e^⊖ = NADH \Longrightarrow \Delta\pi_i'° = -0.34534$ zJ·K^{-1} $\Longleftrightarrow E'° = -321$ mV

$NADP^⊕ + H^⊕ + 2\ e^⊖ = NADPH \Longrightarrow \Delta\pi_i'° = -0.34534$ zJ·K^{-1} $\Longleftrightarrow E'° = -321$ mV

Therefore, IrPs are much more useful for biological thinking than oxidation potentials. Accordingly, NADH appears in catabolism for glycolysis, for β-oxidation, by pyruvate dehydrogenase (PDH), by tricarboxylic acid cycle (TCA), in the electron transport chain (ETC), and by nicotinamide nucleotide transhydrogenase (NNT) (35). This simply stems from its negative IrP much lower than any of the non-metallic species. Accordingly, biosynthesis of NADH needs absorption of a large positive entropy flux, such as the one generated at the level of the TCA or the ETC. In deep contrast, NADPH is used in anabolism for performing reductive biosynthesis, in the pentose phosphate pathway (PPP), by isocitrate dehydrogenase (IDP), by the malic enzyme (ME), by aldehyde dehydrogenase (ALDH), and by NADPH-oxidase (36). Owing to its much lower IrP, biosynthesis of NADPH needs a much smaller positive entropy flux than the one required for NADH. It follows that NADPH is more able to drive biosynthetic pathways and is also involved in redox sensing and as a substrate of NADPH oxidases for generating reactive oxygen species. So, we have here a good example of two remarkably similar reductants having quite contrasted entropy content, explaining the observed strong compartmentalization of redox functions in a living cell.

It is also worth noticing that at the mitochondrion level, there is the orientation of the positive entropy flux towards the synthesis of biomolecules displaying large positive IrPs. Such molecules play the role of "canned entropy" for driving molecular machines, just like batteries act as "canned electricity" for driving electrical motors. The best candidates are polyphosphates such as adenosine diphosphate (ADP with $\pi_i'° = +7.93486$ zJ·K^{-1}) or adenosine triphosphate (ATP with $\pi_i'° = +12.76803$ zJ·K^{-1}). Accordingly, the positive entropy flux for making ATP from ADP appears too small relative to their entropy content:

$ADP + P_i = ATP + H_2O \Longrightarrow \Delta\pi_i'° = -0.2007$ zJ·K^{-1} $\Longleftrightarrow$ pK = 6.3

At a temperature T = 298.15 K, such a pK-value corresponds to a free energy change $\Delta G' = +36.0$ kJ·mol^{-1} = 60 zJ. Conversely, this is just the amount of heat that would be generated upon the hydrolysis of ATP into ADP. Here, it is worth using our relationship $\Delta T(K) \approx 12\times W(zJ)/n_W$ allowing converting an amount of heat W into a temperature change ΔT after spreading such heat

among n_W water molecules. Choosing ΔT = 1 K for W = 60 zJ leads to n_W = 720 or $R_W = 1.66 \times n_W^{1/3}$ = 14.9 Å, in terms of radius of the hydration shell surrounding the spatial location of the reaction. Now, on average, four shells of water molecules surround each biomolecule in a living cell[37]. This translates into a radius of hydration $R_h = 4 \times 3.3$ = 13.2 Å, a value close to the radius of conversion of entropy into heat R_W. As explained in previous papers (9,10), the main role of ATP in a living cell is not to provide energy but rather to play the role of a powerful hydrotrope[35]. ATP has thus the crucial double role of being both an entropy sink and avoids by its presence the irreversible coagulation of proteins.

There is obviously not enough entropy liberated through hydrolysis of a single ATP molecule to convert triplet dioxygen into singlet dioxygen. From the relative IrPs of $^1O_2$ and $^3O_2$ and with $\Delta\pi_i'^\circ$ = 0.2007 zJ·K^{-1} for ATP hydrolysis, the formation of singlet dioxygen from triplet dioxygen would require the simultaneous hydrolysis of at least n(ATP) = sup(0.53256/0.2007) = 3 molecules. As this is very unlikely on the statistical ground or as it would involve a huge protein, it may seem that singlet dioxygen would have a negligible role to play in a living cell favoring triplet dioxygen. This is, of course, the conventional biological thinking putting the exclusive focus on the ground state $^3O_2$ ($^3\Sigma_g^-$) with very few references to the first excited state $^1O_2$($^1\Delta_g$). Owing to its quite negative IrP, very few substances can create singlet dioxygen as a waste. Among them, we have, for instance, ozone $^1O_3$. It is easy checking that water has entropy high enough to resist oxidation into hydrogen peroxide by ozone:

$^1O_3 + {}^1H_2O = {}^1O_2 + {}^1H_2O_2$
$\Delta\pi_i'^\circ$ = -0.22868 zJ·K^{-1} ⟺ pK = 7.2

This is not the case of hydrogen peroxide that is easily reduced into the water by ozone with singlet dioxygen as a by-product:

$^1O_3 + {}^1H_2O_2 = 2\ {}^1O_2 + {}^1H_2O$
$\Delta\pi_i'^\circ$ = +0.29663 zJ·K^{-1} ⟺ pK = -9.3

Suppose the reaction leading to triplet dioxygen is much more favorable, it is, however, spin-forbidden, allowing singlet dioxygen to be the main kinetic product in the absence of a catalyst. The trouble is that if ozone is an important compound in the atmosphere owing to its irradiation by the sun, its occurrence in a living cell is not so obvious.

Singlet dioxygen may also be produced in a living cell subjected to an oxidative stress upon annihilation of oxygen-based radicals:

$^2O_2^{\bullet\ominus} + {}^2OH^\bullet = {}^1O_2 + {}^1OH^\ominus$
$\Delta\pi_i'^\circ$ = +0.80038 zJ·K^{-1} ⟺ pK = -25

$^2HO_2^\bullet + {}^2HO_2^\bullet = {}^1O_2 + {}^1H_2O_2$
$\Delta\pi_i'^\circ$ = +0.03602 zJ·K^{-1} ⟺ pK = -1.1

However, such reactions are in competition with formation of triplet dioxygen and a much larger entropy release:

$^2O_2^{\bullet\ominus} + {}^2OH^\bullet = {}^3O_2 + {}^1OH^\ominus$
$\Delta\pi_i'^\circ$ = +1.33282 zJ·K^{-1} ⟺ pK = -42

$^2HO_2^\bullet + {}^2HO_2^\bullet = {}^3O_2 + {}^1H_2O_2$
$\Delta\pi_i'^\circ$ = 0.56846 zJ·K^{-1} ⟺ pK = -18

Catalysis of this last reaction *in vivo* involves the well-studied enzyme superoxide dismutase (SOD) that is not affected by the presence of singlet oxygen[35].

As triplet dioxygen has higher irreversibility potential than singlet dioxygen, it will always play the role of the thermodynamically favored species. This means that the production of singlet dioxygen using the annihilation of inorganic radicals is, as a rule, quite difficult to control. This is no more the case by using singlet species, as even if the formation of triplet dioxygen is still more favorable, it becomes slow as the reaction is now spin-forbidden. Here is a good example that readily occurs in neutrophils, for instance:

$^1ClO^\ominus + {}^1ClO^\ominus = {}^1O_2 + 2\ {}^1Cl^\ominus$
$\Delta\pi_i'^\circ$ = +0.39840 zJ·K^{-1} ⟺ pK = -12.5

However, such a reaction requires a high concentration of the rather unstable hypochlorous ion. This is the reason for the extensive use of phagosomes by neutrophils. Under diluted conditions, there is the possibility of using hydrogen peroxide, forming as by-products water and chloride ions:

$^1H_2O_2 + {}^1ClO^\ominus = {}^1O_2 + {}^1Cl^\ominus + {}^1H_2O$
$\Delta\pi_i'^\circ$ = +0.46186 zJ·K^{-1} ⟺ pK = -14.5

It is worth noting that use of the hypochlorous ion is mandatory, as the entropy difference between the chlorous and hypochlorous species is not high enough for allowing the production of singlet dioxygen:

$^1H_2O_2 + {}^1ClO_3^\ominus = {}^1O_2 + {}^1ClO_2^\ominus + {}^1H_2O$
$\Delta\pi_i'^\circ$ = -0.09802 zJ·K^{-1} ⟺ pK = 3.1

TRAPPING OF SINGLET DIOXYGEN

Singlet dioxygen could be very harmful to normal cells. The first reason stems from the fact that there is no spin restriction for reacting with other singlet molecules. A second reason is that it has a quite negative IrP. It is worth recalling here its Lewis' structure:

$$:\ddot{O}:^{\ominus}—:O:^{\oplus} \quad \leftrightarrow \quad {}^{\oplus}:O:—:\ddot{O}:^{\ominus}$$

A most prominent feature is the formal positive charge on one of the two oxygen atoms, meaning that singlet dioxygen has a high affinity for any electron-rich centers. Among them, carbon atoms engaged in a C=C double bond are sites for preferential attack owing to their complementary dynamic Lewis' structure:

$$>C=C< \quad \leftrightarrow \quad >C:^{\ominus}—C^{\oplus}< \quad \leftrightarrow \quad >{}^{\oplus}C—C:^{\ominus}<$$

The reaction of singlet dioxygen with C=C double bonds often leads to the formation of endoperoxides (figure 1).

For a single C=C double bond, the resulting endoperoxides have a quite strained four-membered ring, leading to a highly unstable addition compound. This is not the case when oxidation leads to a rather stable six-membered ring, a situation encountered in any molecule containing at least two conjugated C=C double bonds.

Singlet dioxygen may react rapidly with other singlet molecules forming species such as hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) or superoxide radical ($^{\bullet}O_2^-$). These reactive oxygen species will oxidize DNA (mutation and DNA breaks), proteins and lipids. Here is a list of favorable reactions with ubiquinol (H_2CoQ_{10}), ascorbic acid (vitamin C, $AscH_2$), reduced cytochrome-c, dihydrolipoic acid (DHLA), reduced glutathione (GSH) and free iron (II):

$$^1O_2 + {}^1H_2CoQ_{10} = {}^1H_2O_2 + {}^1CoQ_{10}$$
$$\Delta\pi_i{}'^{\circ} = +0.850\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -27$$

$$^1O_2 + {}^1AscH_2 = 2\ {}^2HO^{\bullet} + {}^1DHA$$
$$\Delta\pi_i{}'^{\circ} = +0.680\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -21$$

$$^1O_2 + {}^1AscH_2 = {}^1H_2O_2 + {}^1DHA$$
$$\Delta\pi_i{}'^{\circ} = +1.719\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -54$$

$$^1O_2 + \text{cyto-}{}^1Fe^{2\oplus} = {}^2O_2{}^{\bullet\ominus} + \text{cyto-}{}^2Fe^{3\oplus}$$
$$\Delta\pi_i{}'^{\circ} = +0.326\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -10$$

$$^1O_2 + {}^1DHLA = 2\ {}^2HO^{\bullet} + {}^1ALA$$
$$\Delta\pi_i{}'^{\circ} = +0.195\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -6$$

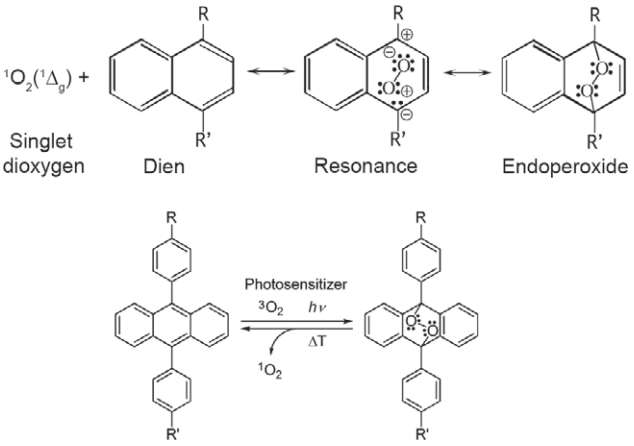

Figure 1. Affinity of singlet dioxygen for conjugated double bonds leading to the formation of an endoperoxide bridge. Endoperoxides may also be formed from triplet dioxygen in the presence of a photosensitizer. One may speak of endoperoxides as "canned singlet dioxygen" owing to their ability to release $^1O_2$ upon heating.

$$^1O_2 + 2\ {}^1GSH = 2\ {}^2HO^{\bullet} + {}^1GSSG$$
$$\Delta\pi_i{}'^{\circ} = +0.173\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -5$$

$$^1O_2 + Fe^{2\oplus} + = {}^2O_2{}^{\bullet\ominus} + {}^2Fe^{3\oplus}$$
$$\Delta\pi_i{}'^{\circ} = +0.049\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -2$$

It is worth noticing the mandatory generation of hydrogen peroxide with ubiquinol, as there is, in this case, not enough entropy for generating two hydroxyl radicals:

$$^1O_2 + {}^1H_2CoQ_{10} = 2\ {}^2HO^{\bullet} + {}^1CoQ_{10}$$
$$\Delta\pi_i{}'^{\circ} = -0.190\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = 6$$

SINGLET DIOXYGEN, OZONE, AND RADIATION THERAPY

The above reactions explain why singlet oxygen ($^1O_2$) is widely used in photodynamic therapy of cancer. During photodynamic therapy, photosensitizers excited by light react with ground state oxygen $^3O_2$, which leads to the generation of this major cytotoxic agent. After generation, singlet dioxygen oxidizes all the molecules responsible for the redox homeostasis of the cell rapidly, killing the surrounding tissues and cells[38].

It has been more than 60 years since the discovery of the oxygen effect that empirically demonstrates the direct association between cell radiosensitivity and oxygen tension, important parameters in radiotherapy. However, no real understanding of the mechanisms underlying this principle tenet of radiobiology is yet available[39].

Photons react with water to form free radicals, including singlet oxygen. Singlet oxygen interacts with the mitochondria to cause the permeabilization of the mitochondrial outer membrane, leading to the cytosolic release of pro-apoptotic proteins and to the impairment of the bioenergetic functions of mitochondria and resulting apoptosis[40].

About twenty years ago, it was shown by Wentworth *et al.* that antibodies catalyze the generation of ozone by a water oxidation pathway[41]. It was first postulated that dihydrogen trioxide [H_2O_3] was a key intermediate. However the direct formation of this intermediate is not thermodynamically favorable:

$$^1O_2 + {}^1H_2O = {}^1H_2O_3$$
$$\Delta\pi_i'^\circ = -0.509\ zJ\cdot K^{-1} \Longleftrightarrow pK = 16$$

It is worth noticing that adding another singlet dioxygen cannot oxidize water into ozone O_3 according to:

$$2\ {}^1O_2 + {}^1H_2O = {}^1O_3 + {}^1H_2O_2$$
$$\Delta\pi_i'^\circ = -0.297\ zJ\cdot K^{-1} \Longleftrightarrow pK = 9$$

However, upon generation of at least three singlet dioxygen molecules, water oxidation becomes possible with the release of triplet dioxygen as waste:

$$3\ {}^1O_2 + {}^1H_2O = {}^1O_3 + {}^1H_2O_2 + {}^3O_2(aq)$$
$$\Delta\pi_i'^\circ = +0.236\ zJ\cdot K^{-1} \Longleftrightarrow pK = -7$$

However, such a reaction is spin-forbidden. Hence, we propose this final scheme, which is spin-allowed:

$$4\ {}^1O_2 + {}^1H_2O = {}^1O_3 + {}^1H_2O_2 + 2\ {}^3O_2(aq)$$
$$\Delta\pi_i'^\circ = +0.768\ zJ\cdot K^{-1} \Longleftrightarrow pK = -24$$

Owing to the liberation of ozone, any tumor would be burnt with the generation of only gases as wastes. Moreover, one of the reactants is the water molecule, the most abundant chemical species in a living cell. The crucial point is that water no more acts here as a solvent whose activity is equal to one, owing to its huge abundance. It is a well-established fact that the status of water in tumors is quite different from that of water in a normal cell. In thermodynamics language, this translates into the fact that water activity cannot be the same in a tumor and in a normal cell[42–46]. As the above equilibrium is sensitive to water activity, one may expect different yields of ozone according to the status of water in the cell exposed to radiations able to generate singlet dioxygen in the large amount. In other words, there is a possibility of targeting any $^1O_2$-treatment towards cancer cells, leaving normal cells relatively unaffected.

The radiation therapist knows that soft tumors like lymphomas and seminoma are more sensitive to radiation than harder ones. Accordingly, doses needed to eradicate seminoma and lymphoma is smaller, and the treatment is shorter than the treatment of squamous cell carcinoma or adenocarcinoma. The earlier sign of tumor response during radiation therapy is the change of consistency (harshness) of the tumor. This is in line with a change in the activity of water (see above).

CONCLUSION

It is possible that ionizing radiation such as produced by modern linear accelerators act at the cellular level by the mean of thermal photons. These photons will induce, in turn, the synthesis of singlet dioxygen. In such a scheme of thought, high-energy photons are just a way to deliver thermal photons to deep-seated tumors. Infrared photons are not powerful enough to reach these lesions. Absorption of over 90% of the dose occurs in the first cm[47].

Cytotoxic chemotherapy activates the concentration of free radicals such as the ones induced by singlet dioxygen or radiation therapy. This is evident by the elevation of lipid peroxidation products; the reduction in plasma levels of antioxidants such as vitamin E, vitamin C, and β-carotene; and the marked reduction of tissue glutathione levels that occurs during chemotherapy. Those agents that generate high levels of ROS include the anthracyclines (e.g., Doxorubicin, Epirubicin, and Daunorubicin), alkylating agents, platinum coordination complexes (e.g., Cisplatin, Carboplatin, and Oxaliplatin), epipodophyllotoxins (e.g., Etoposide and Teniposide), and the Camptothecins (e.g., Topotecan and Irinotecan) [48]. One other option to improve the efficacy of infrared photons is to activate a photosensitizer such as methylene blue[49].

Moreover, an often-overlooked fact is that water activity is higher in cancer cells than in normal cells. As demonstrated just above this could mean that in a cancer cell, singlet dioxygen may react with water yielding ozone, a powerful oxidant. Such a possibility opens the road to a non-linear hormetic behavior of singlet dioxygen. Typically, we expect a harmful increase of oxidative stress at low concentration, a healing effect against cancer at moderate concentration (due to selective in-situ formation of ozone) and a well-documented cytotoxic effect towards any kind of cell at high concentration. Future experimental research is needed to confirm

or reject such a putative behavior suggested by available thermodynamic data.

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ANNEX

Table 1 gives irreversibility potentials (IrPs or π_i'°) values in ascending order for species discussed in this work. As the whole universe is by definition a closed system, this allows, in compliance with the second law, to identify three kinds of processes in nature:

i) Irreversible processes that are spontaneous being such that $\Delta\pi'_i{}^\circ > 0$.
ii) Fully reversible processes are characterizing equilibrium situations as $\Delta\pi'_i{}^\circ = 0$.
iii) Non-spontaneous processes, such that $\Delta\pi'_i{}^\circ < 0$, thus requiring to be coupled with another spontaneous process characterized by $\Delta\Pi_i'^\circ > -\Delta\pi'_i{}^\circ > 0$.

Moreover, owing to their definition, irreversibility potentials changes may be related to equilibrium constants K, or to standard oxidation potentials E'°, using the following conversion relationships (T = 298.15 K):

$$\begin{cases} pK = -log_{10}K = -31.456 \times \Delta\pi_i'^\circ(zJ \cdot K^{-1}) \\ E^\circ(V) = \dfrac{1.860906}{n} \times \Delta\pi_i'^\circ(zJ \cdot K^{-1}) \end{cases}$$

Conversion into standard oxidant potentials are for transformations involving electrons and requires the knowledge of the number of electrons n that should be added to an oxidant to transform such species into its conjugated reduced form.

Let us consider for instance the two-electrons reduction of protons into dihydrogen ($2\ H^{\oplus} + 2\ e^{\ominus} = H_2$) or the four-electrons reduction of dioxygen into water ($^3O_2 + 4\ H^{\oplus} + 4\ e^{\ominus} = 2\ H_2O$). From table 1, we evaluate that:

$$\begin{cases} E'^{\circ}(H^{\oplus}/H_2) = \dfrac{-1.860906 \times 0.55211}{2} = -0.51\ V \\ E'^{\circ}(O_2/H_2O) = \dfrac{1.860906 \times (2 \times 0.86694 + 0.09134)}{4} = +0.85\ V \end{cases}$$

This allows classifying dihydrogen as a reductant ($E'^{\circ} < 0$) and dihydrogen as an oxidant ($E'^{\circ} > 0$). But, one may also consider reacting dihydrogen with dioxygen in order to produce water ($2\ H_2 + O_2 = 2\ H_2O$). Electrons being eliminated, the irreversibility potential change is now expressed as equilibrium constant K:

$$pK = -31.456 \times [2 \times (0.8667 + 0.55218) + 0.09135) = -92.1$$

As at T = 298.15K, we have $\Delta G'^{\circ}(kJ{\cdot}mol^{-1}) = RT{\cdot}\ln(10){\times}pK = 5.708{\times}pK$, the highly positive $\Delta\pi_i'^{\circ} = 2.92943\ zJ{\cdot}K^{-1}$ variation responsible to the quite negative pK, corresponds to a large negative change of the so-called "Gibbs' free energy," viz. $\Delta G'^{\circ} = -526\ kJ{\cdot}mol^{-1}$. With such a pK value, one may conclude that water synthesis is a spontaneous quasi-quantitative process. The reason for such a huge release of entropy is obvious after noticing that on the right of the equation, a substance with a large positive irreversibility potential appears, whereas, on the left, two substances with negative irreversibility potentials disappear.

Leading the left column, we find species with large negative potentials (reductants), thus providing the largest entropy production upon their transformation into species located on the right column (oxidized forms). Consequently, such species are to be considered as useful low entropy "food." Reciprocally, species at the bottom of the right column are generally end products in a chemical transformation, owing to their high entropy content. Consequently, they may be qualified as "waste" that will be eliminated in order to maintain the largest entropy gradient in the living organism. Another crucial point is that we find in both columns radical species holding unpaired electrons. This means that some radicals should be considered as food and others as waste. Moreover, some radicals may be strong reductants, such as atomic hydrogen:

$$H^{\oplus} + e^{\ominus} = H^{\bullet}(aq) \Longrightarrow \Delta\pi_i'^{\circ} = -1.46898\ zJ{\cdot}K^{-1} \Longleftrightarrow E'^{\circ} = -2.31\ V$$

On the other hand, the hydroxyl radical $HO^{\bullet}$ behaves as a strong oxidant:

$$HO^{\bullet} + H^{\oplus} + e^{\ominus} = H_2O \Longrightarrow \Delta\pi_i'^{\circ} = +1.24046\ zJ{\cdot}K^{-1} \Longleftrightarrow E'^{\circ} = +2.73\ V$$

Table 1. Irreversibility potentials $\pi_i'^{\circ}$ and corresponding standard free energies of formation $\Delta_fG'^{\circ}$ for chemical species considered in this work. Values computed at T = 398.15 K, pH = 7 for an ionic strength I = 0.25 M.

Species	$\pi_i'^{0}$/zJ·K-1	$\Delta_fG'^{\circ}$/zJ	$\Delta_fG'^{\circ}$/kJ·mol^{-1}
CoQ10	-25.28740	7539	4540.36
$CoQ10H_2$	-25.22108	7520	4528.45
DHLA	-1.77480	529	318.67
$H^{\bullet}$(aq)	-1.46898	438	263.75
ALA	-1.45613	434	261.45
O_3	-0.96965	289	174.10
$^1O_2$	-0.62378	186	112.00
H_2(aq)	-0.55211	165	99.13
$^1O_2$(g)	-0.52665	157	94.56
H_2(g)	-0.45409	135	81.53
$HO^{\bullet}$	-0.37352	111	67.07
H_2O_3 (C_2-symmetry)	-0.26618	79	47.79
$HO_2^{\bullet}/O_2^{\bullet\ominus}$	-0.18370	55	32.98
$^3O_2$(aq)	-0.09134	27	16.40
$^3O_2$(g)	-0.00000	0	0.00
Cytc-[$Fe^{3\oplus}$]	0.04059	-12	-7.29
$[ClO_3]^{\ominus}$	0.04879	-15	-8.76
$Fe^{3\oplus}$(aq)	0.06676	-20	-11.99
Cytc-[$Fe^{3\oplus}$]	0.15455	-46	-27.75
$HOCl/ClO^{\ominus}$	0.22429	-67	-40.27
H_2O_2	0.29239	-87	-52.50
$Fe^{2\oplus}$(aq)	0.45747	-136	-82.14
$Cl^{\ominus}$	0.73538	-219	-132.04
H_2O	0.86694	-258	-155.66
GSH	1.52051	-453	-273.01
$AscH_2$	3.03142	-904	-544.29
GSSG	3.33710	-995	-599.18
DHA	3.83413	-1143	-688.42
P_i	5.90080	-1759	-1059.49
NADH	6.10589	-1820	-1096.31
$NAD^{\oplus}$	6.44221	-1921	-1156.70
ADP	7.93486	-2366	-1424.71
NADPH	11.08026	-3304	-1989.46
$NADP^{\oplus}$	11.40756	-3401	-2048.23
ATP	12.76803	-3807	-2292.50