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THE BIRTH OF OXYGEN[1]

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PROLOGUE

This paper discusses a quintessential problem in the field of geobiology. Geobiology can be defined in a single sentence: Evolution can only be understood in the context of geology…and vice versa. I am a biochemist but I have been a student of geobiology for the past five years and as President of the Agouron Institute, a patron of the field.

A word about the Agouron Institute: From 1968 to 1982 I was in the Department of Chemistry at the University of California, San Diego. When the recombinant DNA revolution occurred in the 1970s, my friend and colleague Mel Simon and I responded, not by forming a biotech company as some of my colleagues did, but by forming a non-profit institute, the Agouron Institute. Later a for-profit company, Agouron Pharmaceuticals, was spawned from the Institute to exploit advances we had made in the area of rational drug design. Agouron Pharmaceuticals was eventually a success. We discovered and marketed Viracept, an HIV protease inhibitor. This drug helped to save many lives. In 1998 Agouron Pharmaceuticals was sold to Warner Lambert, now a part of Pfizer. In the process the Agouron Institute obtained a significant endowment. We have used this money to support new fields. Geobiology is one of them. (see www.agi.org*). For the past seven years we have supported a course in geobiology. The course has included a geology field trip led by John Grotzinger of Caltech and Andy Knoll of Har-*

[1] This talk was given at a meeting of the Pontifical Academy of Sciences in Rome on November 1, 2008. I have also given the talk at a meeting of the American Academy of Arts and Sciences and a version of the paper, similar to this one, was published in the Bulletin of the American Academy of Arts and Sciences.

vard. I have been on all of the field trips. We have also carried out a drilling project in South Africa in which some 3000 meters of core were obtained that cover the period about 2.5 billion years to 2.2 billion years. It was during that period that oxygen first appeared in the atmosphere. In 2007 we sponsored an interdisciplinary meeting, 'Oxygen' in Santa Fe, New Mexico. About 40 chemists, biochemists, geologists, and microbiologists discussed the problem of the origin of oxygenic photosynthesis. This report represents my attempt to synthesize the ideas expressed in this exciting meeting.

We take it for granted that our atmosphere contains oxygen but we and most other animals would die within minutes if it were removed. It is not widely appreciated that for half of the earth's history there was virtually no oxygen in the atmosphere. Then 2.45 billion years ago oxygen appeared and has been present ever since though not always at its present level of 21%. More than 99% of the oxygen in the atmosphere is produced biologically, by photosynthesis. Arguably the biological invention of photosynthesis was, after the origin of life itself, the most important development in the history of our planet. About 12 times as much energy is derived from the aerobic metabolism of a molecule of glucose compared to the energy obtained from anaerobic metabolism. Without the invention of oxygenic photosynthesis multi-cellular organisms could not have evolved. Furthermore, the presence of oxygen in the atmosphere leads to an ozone layer that protects life from the lethal effects of ionizing radiation and allows life to flourish on land.

After life originated on earth there has been a continuous interplay between geological and biological evolution. The closely linked evolution of photosynthesis and the evolution of the atmosphere is perhaps the best example of the interdependence of geological and biological processes.

In chronicling the rise of oxygen, I will first describe photosynthesis and its origins. Then I will turn to a discussion of the state of the earth and its atmosphere before and during the rise of oxygen. After the rise of oxygen the atmosphere and the oceans went through some initial cataclysmic and finally very slow changes. Finally 540 million years ago, almost 2 billion years after the initial rise of oxygen, roughly the present levels of oxygen in the atmosphere and in the ocean were attained. It was only then that multi-cellular life began to flourish.

The story of oxygen and its effects takes place over a vast expanse of time – see the geologic time scale below. I will refer to the archean, the proterzoic and phanerozoic eons and sometimes to the Precambrian (all time up to 544 million years ago) and to the Cambrian (the 39 million years after). In geology 1 billion years is abbreviated Ga and 1 million years Ma (see Figure 1, p. 596).

One way to comprehend this vast expanse of time is to compare it with the time it takes the continents to completely rearrange themselves via plate tectonics. 225 million years ago all of the continents were together in the super-continent, Pangaea. In 225 million years the continents separated and the Atlantic and Indian oceans were formed. This is about 5% of earth's history and about one tenth of the time period we chronicle here.

It is also useful to consider how much biological change can take place in 2 billion years. A heritable and selectable change, a mutation, can take place at every cellular division. The earth's oceans contain about 4x 10^{24} ml. of water. If we conservatively assume a steady state of 1000 cells/ml in the ocean and a division time of one week (during this period most cells are unicellular microorganisms), then in two billion years something like 10^{39} divisions could take place. Specific mutations in bacteria take place at a frequency of about 10^{-8}. Even more rapid changes can occur when genes are transferred between different organisms. In 2 billion years, there is an enormous potential for evolutionary change.

PHOTOSYNTHESIS

In photosynthesis, the energy of light is used to extract electrons and protons from a donor molecule H_2A which are then used to reduce carbon dioxide, in the reactions:

$$2H_2A \rightarrow 4H^+ + 4e^- + 2A$$

$$CO_2 + 4H^+ + 4e^- \rightarrow (CH_2O) + H_2O$$

The donor molecule, H_2A can be a variety of reduced compounds including H_2S, Fe^{++}, H_2, various organic compounds and H_2O. Use of the former group of donors probably pre-dated the use of water in photosynthesis. The cellular machinery for oxygenic photosynthesis (in which water is used as the donor) is in part derived from it predecessors.

In oxygenic photosynthesis the electrons from water are extracted and used to generate energy and to reduce carbon dioxide to a carbohydrate according to the equation:

$$H_2O + CO_2 \rightarrow (CH_2O) + O_2$$

It has been known since the work of Martin Kamen and Samuel Ruben more than 50 years ago that the O_2 generated in photosynthesis is entirely derived from H_2O so water is dissociated in photosynthesis according to the equation:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

It takes an enormous amount of energy to extract an electron from water because oxygen has a high affinity for electrons. One photon of light is required to extract each electron so photosynthesis is a four electron process.

Oxygenic photosynthesis takes place in one class of bacteria, cyanobacteria. It also takes place in a number of eukaryotic organisms, e.g. algae and plants but photosynthesis in eukaryotes and in cyanobacteria is almost exactly the same because photosynthetic eukaryotes are all derived from a symbiotic event in which a primitive eukaryote captured a cyanobacterium, so in discussing photosynthesis and its origin it is appropriate to focus on cyanobacteria.

In cyanobacterium the photosynthetic machinery is located in a system of layered *thylakoid* membranes. The membranes enclose an interior space, the *lumen*. The machinery consists of many pigmented proteins, many of them extending across the thylakoid membrane to the exterior space, the *stroma*. Some of the proteins and pigments in the thylakoid membrane serve as antennae to funnel light energy into the reaction center.

The reaction center consists of two complex multi-protein assemblies, termed Photosystem I and Photosystem II (PSI and PSII). At the heart of both PSI and PSII is a cofactor chlorophyll molecule.

The figure below is complex but successfully depicts the major multi-protein complexes involved in photosynthesis.

There isn’t sufficient space here to discuss photosynthesis in depth. A book is required to do it justice. Instead I will focus only on the mechanisms of oxygen synthesis. This reaction takes place in photosystem 2 (PSII). The active site for di-oxygen synthesis is called the Oxygen Evolving Center (OEC). This site contains four manganese atoms and one calcium atom, coordinated mainly to one core PSII protein. The mechanism of water splitting is unique and so far, at least, a related metallo-protein has not been identified. The OEC allows for the integration of a one elec-

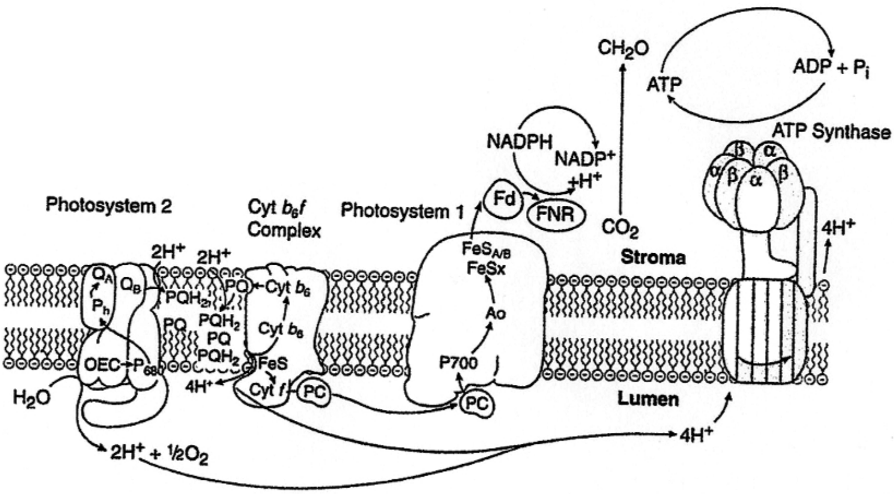

Figure 2. From *Molecular Mechanisms of Photosynthesis*, Robert E. Blankenship.

tron process, the excitation of cytochrome P680, with a four electron process, the splitting of H_2O to form O_2. A beautiful experiment done 50 years ago independently by Pierre Joliot and Bessel Kok proves that the OEC abstracts protons and electrons step-wise from water to evolve oxygen. Alternative models, ruled out by this experiment, include the cooperation of four reaction centers to cleave a single molecule of H_2O or that one center accumulates four oxidizing equivalents prior to oxidizing water in a single concerted step.

The OEC can now be understood more clearly because a 3.5A crystal structure has been obtained of PS II by J. Barber in London and a higher resolution structure of the OEC manganese oxide core by K. Sauer and W. Saenger *et al.* determined by x-ray absorption spectroscopy on single crystals of PSII (see Figure 3, p. 596).

In photosynthesis the manganese oxide cluster binds two molecules of H_2O. The energy of one quanta of light abstracts one proton and one electron. Thus this structure is the integrator of four electron transfer reactions resulting in the synthesis of one molecule of di-oxygen from two molecules of water. The invention of this mechanism was a unique event in evolution. When did it happen?

A Date for the Evolution of Oxygenic Photosynthesis?

Oxygen first appeared in the atmosphere 2.45 billion years ago and I will summarize the geological evidence for that below. Oxygenic photosynthesis must have evolved by that time but how much earlier did it evolve? There is a single piece of data that suggests that it had evolved by 2.7 billion years ago, 250 million years before the appearance of oxygen in the atmosphere.

Roger Summons, an Australian now working at MIT, has developed powerful analytical techniques (gas chromatography and mass spectrometry) for detecting minute traces of biological compounds (termed biomarkers) in ancient rocks. Rocks formed billions of years ago have gone through cycles of heating (termed diagenesis). The preservation of organic chemicals in ancient rocks is rare and when they are found they are limited to hydrocarbons.

In samples derived from black shales deposited in northwestern Australia 2.7 billion years ago a class of hydrocarbons called stearanes were found. Stearanes are derived diagenetically from steroids now found almost exclusively in eukaryotic cells. Cholesterol, e.g. is a steroid.

Steroid synthesis involves a number of steps requiring molecular oxygen. For example in the synthesis of cholesterol starting with squalene, eleven separate steps require molecular oxygen. It seems very unlikely to me that all of these steps would have used some other oxidant and different enzymes prior to the advent of oxygen and then been altered with the advent of oxygen. Thus the presence of stearanes in the Australian black shales argues for the presence of molecular oxygen in the ocean at 2.7 billion years.

Although the rocks from which these samples were extracted are correctly dated, it is more difficult to be sure that the biomarkers were deposited in the rocks at that date. They could have been the result of ground water penetration from the surface or penetration of oils from younger rocks into the older rocks. Or they could have been contamination from the drilling fluid. Great precautions are taken to avoid the latter artifact. The exterior surface of the drill cores is shaved off and the sample is taken from the interior of the core. But the cores used in this experiment were drilled with organic fluids and given the importance of this sort of result it is now considered imperative to drill with only water as a lubricant and this is being done (for example in our South Africa cores). It is also important, insofar as it is feasible, to investigate biomarkers in yet older rocks. The possibility that oxygenic photosynthesis evolved 300 million years before the advent of oxygen in the atmosphere poses the obvious question of why it took so long. We need to know what the earth was like prior to the appearance of oxygen in the ocean and what events might have triggered its rise in the atmosphere.

The Archean Earth and the Rise of Oxygen

In the Archean eon prior to 2.5 billion years ago, the atmosphere was reducing; the major components being N_2, CO_2, and perhaps CH_4, methane. The argument for methane is that at the origin of the earth the sun was 30% fainter than it is now and it can be calculated that without a greenhouse gas the earth would have been frozen until 2 billion years ago. The geological record shows that liquid water was present during the Archean eon and that the temperature was likely warmer than now. Certainly carbon dioxide would have provided a greenhouse effect but without oxygen in the atmosphere, methane, likely produced by methanogenic bacteria, could have accumulated to 1000 ppm; it is present at about 2 ppm now. The composition of the Archean ocean is less certain but geological evidence suggests that there was much less sulphate than now and there was certainly very little dissolved oxygen because there was abundant dissolved iron, Fe^{++}. In the Archean world organisms only lived in the ocean and the primary producers were likely the non-oxygenic photosynthesizers (although remember, we do not know for certain how early oxygenic photosynthesis evolved).

Geologists have known for more than 50 years that oxygen appeared in the atmosphere about 2.3 billion years ago. Preston Cloud and Dick Holland were the first to make this observation. What they realized early on and could see at many places around the world can perhaps most simply be chronicled in the Huronian Supergroup in southern Canada.

In the Matinenda formation (2.45 Ga) conglomerates can be seen that contain uraninite and pyrite. These conglomerates are detrital deposits meaning that were washed into the sea by ancient rivers. Uraninite, UO_2, is insoluble whereas unlike for iron the more oxidized form, UO_4 is soluble. If oxygen had been present in the atmosphere, UO_2 would have been oxidized and solubilized. Pyrite (FeS_2) is rapidly converted to hematite, Fe_2O_3 in the presence of oxygen. Pyrites and uraninites are not seen in the sediments above the Matinenda formation in the Huronian and they are not generally seen anywhere in detrital deposits younger than 2.3 billion years.

Between the Matinenda and the Lorraine formation in the Huronian can be found evidence for three glaciation events. We shall return to these glaciations later but when we reach the Lorrain formation (2.2 Ga) we first encounter red beds. These are sandstone beds, deposited by rivers or sand blown dust. Red crystals of hematite coat the sandstone grains. The presence of red beds is indicative of an oxidizing atmosphere. The earliest red beds were formed about 2.2 billion years ago. Oxygen must have appeared in the atmosphere after the deposition of the Matinenda formation, 2.45 Ga and before the deposition of the Lorrain formation, 2.2 Ga.

A more recent result has firmly pegged the rise of oxygen at 2.45 Ga. In order to understand this result we must briefly review the use of atomic isotopes in geochemistry. Four isotopes of sulfur occur naturally ^{32}S (94.9%), ^{33}S (.76%), ^{34}S (4.29%) and ^{36}S (.02%). In biological processes, for example SO_4 reduction to SO_2, ^{32}S is used preferentially to the other isotopes. ^{33}S is discriminated against by about half as much as ^{34}S. Starting with the work of Farquhar and Thiemens at the Scripps Institution of Oceanography, the isotopic abundances of the sulfur isotopes in various rocks has been meas-

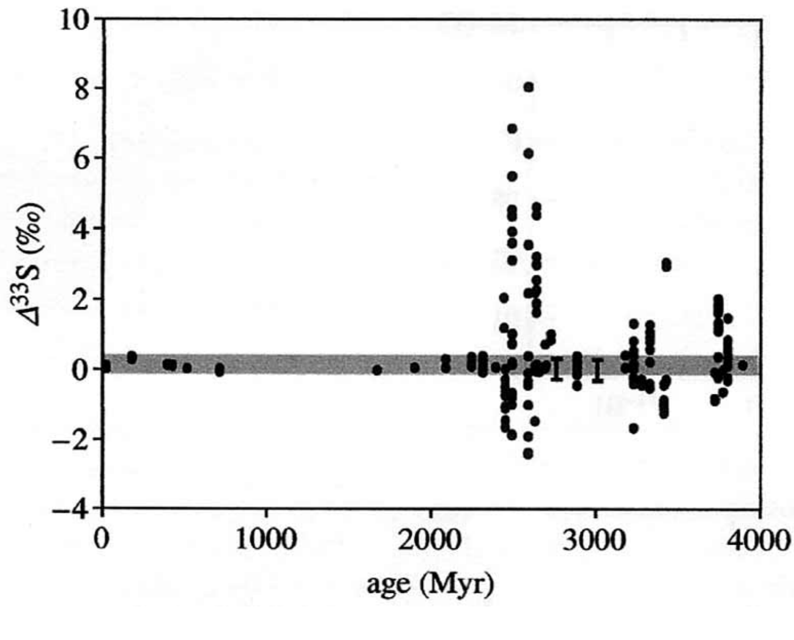

Figure 4. See J. Farquhar *et al., Science* 289: 756 (2000) (updated by S. Ohno).

ured. All modern rocks contain the same ratio of ^{33}S to ^{34}S because in modern rocks the ratio has been determined by the preferential use of ^{33}S to ^{34}S in biological processes. A quantity ^{33}S is a measure of the deviation of the abundance of ^{33}S from that ratio. In all modern rocks ^{33}S is zero. The figure below shows a recent compilation of the data.

In rocks before 2.45 Ga, the value of ^{33}S is zero; in rocks older than 2.45 Ga the value is different from zero – it is negative if the sulfur is derived from barite ($BaSO_4$) and positive if the sulfur is derived from pyrite (FeS_2). The variation of ^{33}S from zero is called *mass independent fractionation*. One is led to the conclusion that non-biological processes were at work on sulfur before 2.45 Ga. These processes were photochemical and the change that occurred at 2.45 Ga was the creation of an ozone shield due to the appearance of oxygen in the atmosphere. Ozone absorbs ultraviolet light, active in a number of photochemical processes in the atmosphere. For sulfur these could include reduction or oxidation of SO_2 or H_2S, leading to elemental sulfur or H_2SO_4 both of which can be incorporated into rocks. In the modern ocean all atmospheric sulfur is protected from photochemistry by the ozone layer and in the ocean is subjected to *mass dependent fractionation*. A level of oxygen in the atmosphere that is 1/100 the present level would lead to an effective ozone shield.

The sulfur isotope data fairly precisely determine the time for the rise of oxygen at some level. The biomarker data suggest that oxygenic photosynthesis originated at least 300 million years earlier. What prevented oxygen from appearing in the atmosphere earlier? Though this question has been frequently asked there is as yet no universally accepted answer. There could be either geological or biological reasons for the delay or both. Perhaps the level of reductants supplied to the atmosphere and the ocean by vulcanism decreased because of altered chemistry in the mantle. Or perhaps oxygenic photosynthesis, though it evolved earlier, had only become effective enough to alter the atmosphere at 2.45 Ga.

Interestingly the appearance of oxygen in the atmosphere had some relatively near term effects on the geology of the earth but did not markedly influence the biology at least as seen in the fossil record for another 1.8 billion years.

The Proterozoic Earth After the Rise of Oxygen

In the Huronian Supergroup evidence can be seen for three separate glaciation events between the anoxygenic uraninite conglomerates at 2.45

Ga and the oxygenic red bed deposits at 2.2 Ga. The glaciation events are seen as large dropstones, left behind in the sediment as the glacier recedes or as scratches in bed rock made as the glacier moves over it. Evidently in the period between 2.45 Ga and 2.2 Ga the earth went through a pronounced cooling period.

In South Africa evidence is seen in the Makganyene formation of another glaciation event at 2.2 Ga. Joe Kirschvink at Caltech has shown by paleomagnetism that the Makganyene glacial event took place when the Transvaal Craton was near the equator. This means that the entire earth was glaciated, a ‘snowball earth’ event. The most plausible cause of the cooling is that the rise of oxygen in the atmosphere destroyed the methane and thus the greenhouse effect that was warming the earth. As the earth cooled and ice formed, more and more solar radiation was reflected (ice reflects eight times as much radiation as water). Once ice had covered the poles to the thirtieth latitude north and south a positive feedback loop insured that a sheet of ice about two kilometers in depth would cover the earth.

Why did the earth not remain in a frozen state? How could life have survived? Likely through vulcanism; life would have been confined to heated regions near vents. Carbon dioxide escaping into the atmosphere would accumulate because it could not dissolve in the ocean and be lost in weathering processes as it is normally. It could have taken 30 to 50 million years for a sufficient level (350 times the current level) of carbon dioxide to accumulate providing a greenhouse level that would melt the ice. When a sufficient greenhouse had been attained, the reverse positive feedback loop would occur and melting of the ice could have taken place in a few hundred years.

In the aftermath of the snowball earth the intense greenhouse is predicted to have raised the surface temperature to 50°C – a hothouse earth. Carbon dioxide dissolved in the ocean and a massive precipitation of $CaCO_3$ and $MgCO_3$ (dolomite) occurred. These precipitates are called cap carbonates and they can be as much as 400m thick.

The post snowball earth ocean was rich in nutrients and cyanobacteria flourished, raising the level of oxygen in the ocean and in the atmosphere. Dissolved iron precipitated as hematite and manganese as MnO_2. South Africa possesses some of the richest manganese deposits in the world as a result of this event.

The Makganyene was the first snowball earth event (there were earlier regional glaciations) but it was not the only one. Two more snowball earth events took place in the period between 800 million years and 600 million years ago. In the intervening billion years the earth was relatively quiet. Geologists call this period the 'boring billion'.

The Boring Billion

Following a proposal made by Don Canfield in 1998 consensus is building among geologists that except for the likely spike after the Makganyene glaciation, the level of oxygen in the atmosphere remained low for more than one billion years and did not rise to present levels until the end of the proterozoic eon at 540 mY (see Figure 1, p. 596).

The modest levels of oxygen in the atmosphere could have led to an ocean that while weakly oxygenated at the surface was anoxic below and like the Black Sea today sulfidic. It is not possible here to review all of the geological data supporting this conclusion but one line of evidence from Ariel Anbar and Tim Lyons involving the level of molybdenum in black shales of the proterozoic strongly supports this model. In an oxic atmosphere, molybdenum is washed into the ocean by rivers as the soluble MbO_4^{2-} anion. Molybdenum is thus abundant in today's oceans.

A survey of molybdenum in black shales through time reveals that molybdenum is low during the archean, slightly elevated in the mid proterzoic and abundant in the phanaerozoic period. The relatively anoxic ocean of the mid-proterozoic could not have supported multi-cellular life and it would have been a poor environment for eukaryotes. There is plenty of evidence for single cell eukaryotes in the proterozoic, but Cyanobacteria would have dominated the shallow oceans and tidal flats. Beginning in the late proterozoic, as oxygen levels rose the multi-cellular eukaryotes make a modest appearance in the fossil record. It is the end of the boring billion.

The Rise of Multi-cellular Eukaryotes

The end of the proterozoic eon is punctuated by two snowball earth events: one at 750Ma and the other at 600Ma. These were not caused by oxidation of methane in the atmosphere but likely by a fall in carbon dioxide levels. At this time all of the land mass of the earth was near the equator and so none of it would have been covered with ice as Antarctica is today. Thus the entire land mass of the earth would have been available for removing CO_2 from the atmosphere by atmospheric weathering leading to a gradual cooling of the planet. The rich aftermath of the snowball earth events could have oxygenized the oceans and led to the initial rise of multi-cellular animals. Fossils from this period (called the Ediacaran or Vendian period) can be seen in many parts of the world. At the boundary between the Cambrian and the Precambrian at 542 Ma a mass extinction occurred. The Ediacaran animals disappeared and the modern world followed.

However we have to look at rocks deposited some 40 million years later to see the blossoming of animal life in the Cambrian as seen in the Burgess shales. The Burgess shales record a wonderful zoo of animals that have clearly developed many of the body plans seen later in evolution as well as mind boggling creatures that we never see again. Here are some of my favorites from Steven Gould's book *Wonderful Life* (see Figure 5, p. 597).

In an artist's rendering we see the entire community just before it was entombed for 500 million years by a mud slide.

By the Cambrian period, oxygen was near its present level in the atmosphere and the ocean. Animal evolution was on its way.

Epilogue

The unique and powerful process of oxygenic photosynthesis nearly resulted in the extinction of all life in the Makganyene glaciation. The earth itself with its molten core came to the rescue. After a period of nearly 2 billion years, however, photosynthesis made possible the evolution of multi-cellular animal life, a process still going on today.

Although it is in its infancy from a geological perspective, human intelligence may be as unique and potent a force for change on earth as photosynthesis was. Will human intelligence lead to a flowering of the earth as photosynthesis did or will it lead to the extinction of life? It is too early to say. Geology tells us that we will have to wait 2 billion years to know.

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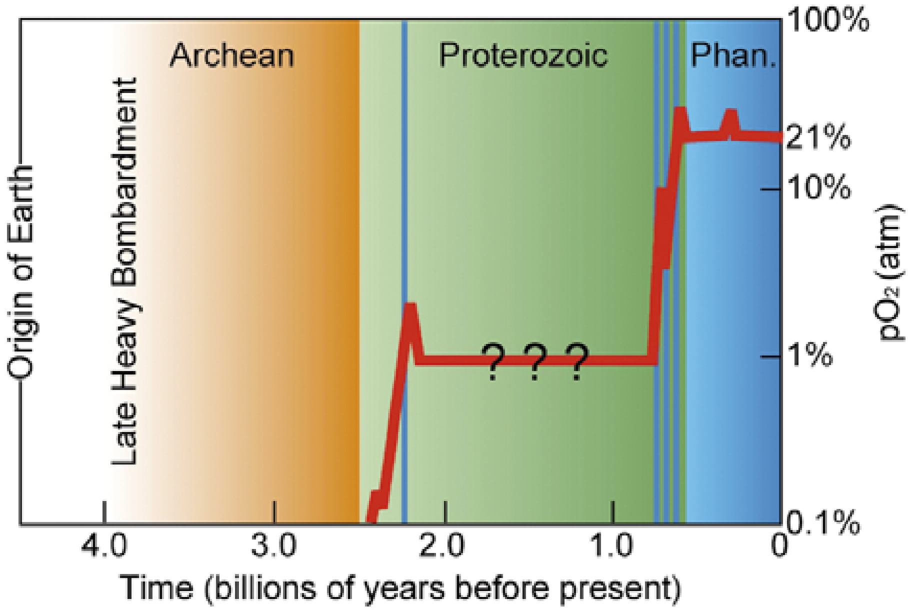

Figure 1.

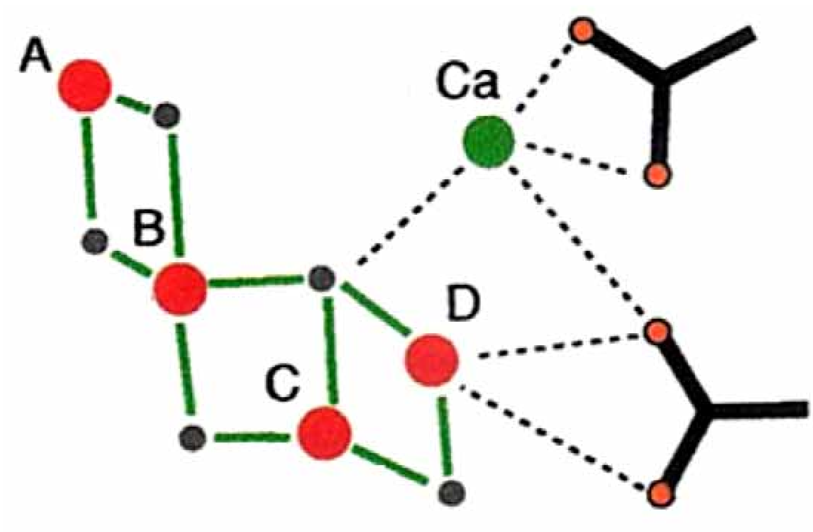

Figure 3. From Yano *et al.*, *Science* 3 November 2006, 314: 821-825

Figure 5.