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## Searching for life in the Universe: unconventional methods for an unconventional problem

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**Abstract** The search for life, on and off our planet, can be done by conventional methods with which we are all familiar. These methods are sensitive and specific, and are often capable of detecting even single cells. However, if the search broadens to include life that may be different (even subtly different) in composition, the methods and even the approach must be altered. Here we discuss the development of what we call non-earthcentric life detection – detecting life with methods that could detect life no matter what its form or composition. To develop these methods, we simply ask, can we define life in terms of its general properties and particularly those that can be measured and quantified? Taking such an approach we can search for life using physics and chemistry to ask questions about structure, chemical composition, thermodynamics, and kinetics. Structural complexity can be searched for using computer algorithms that recognize complex structures. Once identified, these structures can be examined for a variety of chemical traits, including elemental composition, chirality, and complex chemistry. A second approach involves defining our environment in terms of energy sources (i.e., reductants), and oxidants (e.g. what is available to eat and breathe), and then looking for areas in which such phenomena are inexplicably out of chemical equilibrium. These disequilibria, when found, can then be examined in detail for the presence of the structural and chemical complexity that presumably characterizes any living systems. By this approach, we move the search for life to one that should facilitate the detection of any earthly life it encountered, as well as any non-conventional life forms that have structure, complex chemistry, and live via some form of redox chemistry.

**Keywords** Life detection · Mars · Systems complexity · Chemical analyses · Life's thermodynamics · Life's kinetics

### Introduction

For centuries, people have looked at the stars with inquisitive and imaginative minds. Sailors and farmers have found guidance; poets and lovers, inspiration; scientists and scholars, answers. Many who have ever looked heavenward have also found questions in the cosmos. Perhaps the most common question pondered over thousands of years is: Are we alone? The search for life in the universe is at best an inexact science, and at worst a seemingly impossible task. If one does not know for sure that life will be similar to earthly life, then it is almost farcical to begin the search with earthcentric tools. Yet, given that the only example of life we now have is our own, it is hard to break out of the earthcentric mode. To a certain extent, the non-earthcentric approach has been outlined for us by James Lovelock in his Gaia hypothesis – proposing that one could identify a living planet simply by the atmosphere it produced, an atmosphere that would be remarkably out of equilibrium if life was producing and consuming gases to its own end [9]. The non-earthcentric approach demands that we shed all of our earthly life-detection “crutches”, learning to detect life without any of our usual aids. Having said that, in searching for life one still has to rely on some general features that should be fundamental to any kind of life, should we encounter it. What can we learn from studying the Earth that will aid us in finding life of any sort, if and when we encounter it? What features does life have that may be useful in searching for any kind of life in any environment? When we ask these questions, we see that our view of life provides us with a few general rules.

Our understanding of life has changed drastically in the past two decades, as our eyes have been opened to the limits of life and the ways in which life interacts with the geosphere. With this in mind, the “rules” we develop

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must be universally applicable. For example, life as we view it today is much “tougher” than we might have imagined 20 years ago, and our appreciation for the ability of life to survive extremes, and even thrive in them, has changed our view of habitability to include a much wider range of conditions (pH, salinity, temperature, aridity, etc.) [7, 19]. Intra-terrestrial life has been found at kilometer depths in subsea-floor sediments and in the basement crust beneath the sediments, up to 2,800 m deep in continental sedimentary rocks. This toughness or “extremophily”, as we call it, is, arguably, one of the major discoveries about life made in the twentieth century. The sun was considered to be the ultimate source of energy for all ecosystems on Earth until deep-subsurface microorganisms were discovered [5, 10]. Second, life is tenacious; it is now routine to isolate living microbes that have been entombed and/or dormant for thousands and even millions of years [2, 18, 19, 22]. Finally, we know that life is metabolically very diverse, apparently able to “eat anything or breathe anything” [17]. These insights into earthly life can be used to begin to frame the search for life both in extreme environments on Earth, and on extraterrestrial sites, in situ and in samples returned to the Earth.

Astrobiology has as one of its goals, the search for life in other places in the universe. Such an endeavor requires the joining of many sciences including physics, chemistry, mathematics and, of course, biology; it is decidedly interdisciplinary. Thus, one of the challenges of astrobiology is to determine how to recognize the signature of life on other worlds, and how to detect present or past life. In this article we discuss this challenge, introducing some of the approaches we use now for life detection.

## The Red Planet: Mars

To a great extent, the challenge of life detection is also one of searching for habitable sites (and life, should it exist) elsewhere in the universe. In terms of where life might be, we have the planets, and moons in our own solar systems and we have other solar systems. In terms of upcoming solar system exploration, Mars is the focal point of the projected efforts. It is “easy” to get to (only

about one year of transit time), and provides an excellent site in which to test methods for both in situ life detection and sample return. Mars, the fourth planet from the Sun, is about half the size of the Earth, and, in the absence of an ocean, has a surface area approximately the same as the land area of Earth. Mars appears reddish because much of it is coated with iron oxide minerals (the material that forms rust on Earth). With regard to factors important for life, Mars lacks a magnetic field, and is thus susceptible to the solar wind and cosmic radiation. It lacks an atmosphere to protect it from ionizing radiation (Mars’ atmosphere is only a few millibars of pressure, primarily CO<sub>2</sub>). Mars’ geologic history has been much simpler than the Earth’s, with apparently little or no plate tectonics, although it has some of the largest volcanoes known in the solar system. The combination of the lack of a magnetic field and the low gravity of Mars may account for the loss of water (ionization of water combined with the escape of hydrogen) and the present dry state of the planet. Table 1 compares some physical and chemical properties of the three neighbor planets, Venus, the Earth, and Mars [12].

Nineteenth century astronomers, who looked at Mars through telescopes, saw canals on its surface; they imagined mysterious canal builders made by intelligent green Martians. The *Mariner* spacecraft (*Mariner 4*, the first successful spacecraft launched to Mars in 1963) destroyed this vision of intelligent Martians when it sent back images of an arid landscape, without any sign of life. Nonetheless, there was still consensus that the Martian surface offered the best chance of discovering extant life on another planet. In 1976, the *Viking* landers carried three biology experiments to the surface of Mars to search for microbial life. No convincing evidence for life was obtained in these surface experiments [8], although the orbital images obtained from *Viking* strongly suggested that liquid water flowed on the surface of ancient Mars [13]. The lack of weathering of impact craters on Mars suggests that water flow took place long ago (> 3.5 billion years). Subsequent orbital images by the Mars Global Surveyor have supported this view, but have also raised the possibility of episodic local water flows having occurred much more recently (i.e. millions of years ago) [11].

**Table 1** Some physical and chemical features of three planets of the Sun (at present) [12]

	Venus	Earth	Mars
Diameter ( $\times 10^3$ m)	12,104	12,756	6,794
Mass ( $\times 10^{27}$ g)	4.8689	5.9742	0.64191
Density (g cm <sup>-3</sup> )	5.24	5.52	3.93
Mean distance from Sun ( $\times 10^9$ km)	108.2	149.6	227.9
Sidereal period (days)	224.7	365.3	686.9
Pressure (atm)	90	1	0.0064
Temperature (K)	750	290	220
Water (m) <sup>a</sup>	0.003	3000	0.00001
Carbon dioxide, atmosphere (%)	98	0.04	95
Nitrogen, atmosphere (%)	1.7 (Ve) <sup>b</sup>	79	2.7 (Vi) <sup>c</sup>
Oxygen, atmosphere (%)	Traces (Ve)	21	0.13 (Vi)
Satellites	0	1	2 (very small)

<sup>a</sup>Depth of water in meters over the planet if all vapor precipitated out of the atmosphere

<sup>b</sup>*Venera* spaceship (USSR)

<sup>c</sup>*Viking* spaceship (USA)

If Mars was indeed “wet and warm” in its early history, then, many have argued, life may have originated on Mars at that time. However, we have little knowledge of the surface of Mars in its history – apart from orbital images – and most of this knowledge comes from our present collection of Martian meteorites. Only one of these, ALH 84001, is of ancient age, and it has been reported to contain possible traces of ancient Martian life [4,14]. This meteorite, which was collected in the Allan Hills area of Antarctica (in 1984), has been the source of immense interest since these early reports, which remain controversial and unresolved.

NASA’s program has a major focus on Mars, the most accessible place for learning how to do in situ analysis and sample return. While we think of it as “nearby”, the distance to Mars is still such that it takes on the order of 20 min to get a radio message there, making real time (virtual reality) communication with landers and research vehicles impossible, and necessitating the development of smart landers and rovers that can make their own “decisions”. There are several Mars missions planned for the next decade, culminating with a sample return sometime after 2011. With luck, we could have extensive knowledge as well as pristine samples from Mars within the next 15–20 years. Many things need to be learned during this time period: precision landing, hazard avoidance, and autonomous operation to name a few. With regard to this, some of the orbiters we are sending will be used not only for remote-sensing studies, but also to establish a communications and navigation network around Mars – if all goes well – to provide future researchers with the equivalent of a primitive GPS system to enhance “landing” accuracy. Presently the *Mars Odyssey* orbiting spacecraft is gathering data at Mars. One of the instruments it carries is called Themis, a thermal emission imaging spectrometer. It is an infrared spectrometer that not only measures the surface temperature of Mars, but, by utilizing various infrared wavelengths, gathers information about the mineralogy of the surface, allowing us to assess the kinds and amounts of minerals. Many minerals (carbonates, sandstones, metal oxides, etc.) on Earth are almost universally colonized by microorganisms, and provide a friendly habitat when conditions are extreme [17]. Finding such minerals could have substantial impacts on site selection for in situ exploration missions as well as sample return missions.

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### Fundamental features of life

What have we learned studying the Earth that might be valuable for asking if there is life on Mars? We know what life here looks like. Therefore, can we develop general methods that always detect life on Earth and then apply them to Mars or anywhere else? What are the fundamental features of life that one might be able to measure to look for the evidence of present or past life?

Biology books are full of definitions of life, many of them converging on many of the same key properties, complex chemistry, metabolism, self-replication, and evolution. As we have considered the various aspects, and tried to ask which might be quantified and relatively easily measured, we have converged on a few general properties that we feel should be common to all life:

1. Life will of necessity have some structure (and thus some structural complexity) that can be observed and quantified. Life will consume energy, and will convert it from one or more types of environmental energy to chemical energy that can be used by the cell. Such energy conversions require physical mechanisms (energy transducers) and thus some sort of complex structures. The size scale of such structures can not be specified in advance, they could be nm in scale, like some bacterial energy transducers, or quite large, but they must be there.
2. The chemistry of the structures that make up life will have complexity and unique features that make them recognizable. These may include elemental composition, molecular composition, repeating polymeric structures, specific types of unusual chemical bonds, stable isotopic fractionations, and chirality. Once structures are found, the chemistry of these structures will provide the clues as to whether the structures are simply geological or chemical imposters, or perhaps the structures of life itself.
3. Life will show faithful replication, making, to its best ability, copies of itself that lead to success in terms of numbers of a given type of life. While measuring and quantifying replication will probably be of great difficulty, it is very likely that, if life is present, the ability to replicate will be easily inferred from the number of copies (of molecules, polymers, or cells) that can be observed. Thus, while the process itself can not be observed, its presence can be easily inferred.
4. Life will show some type of evolution (alteration of forms to new forms, and selection of the fittest), leading to multiple types of life. This will almost certainly include both chemical and morphological evolution, neither of which can be measured directly, although both of them can be inferred from environmental measurements. As with replication, while the process may be difficult to measure, it will be rather straightforward to infer.
5. Life will use energy to make copies of itself, and create energy disequilibrium that should be measurable as nutrient and/or energy depletion zones. These may be measurable only in physically stratified environments such as sediments, and at a variety of spatial scales.
6. Alternatively, as metabolism progresses, life will produce products – the waste products of the living machines. As wastes accumulate at rates greater than diffusion, they too will form detectable disequilibrium signals – signals that would not be there in the absence of life.

7. Finally, and probably in response to utilizing nutrients and/or producing wastes, life will show non-random movement. This may take the form of individual cells showing directed movement (bacteria swimming or amoebae crawling and engulfing prey), or the cytoplasmic streaming seen within larger cells, but for a variety of reasons probably all life shows non-random movement of some kind.

Based on molecular phylogenies, which are established by 16S rRNA sequence comparison [1, 24], the major genetic diversity on the planet (Earth) resides in the prokaryotes, whereas the genetic diversity of the eukaryotes is actually quite limited [6]. This view is consistent with what we know about evolution of the biota. In fact, the planet is believed to have been inhabited only by prokaryotes for more than 3.5 billion years, whereas eukaryotes (those organisms with chromosomes, nuclei, nuclear membranes, and many visible internal structures) are in comparison relatively recent inhabitants. Prokaryotes have remained small and apparently simple throughout evolution, although even the smallest cells must have a certain size to allow for the minimal number of molecules need for the simplest kinds of metabolism (Table 2). Nevertheless, prokaryotic diversity is expressed in terms of physiology and metabolism [15, 17, 23], whereas that of eukaryotes is expressed in terms of structures and behavior [16, 17].

Given these general features, a general non-earth-centric strategy is proposed:

1. *Complexity and chemical analyses.* When we have found the structures, then we can measure their properties, chemical composition, chirality, isotope fractionation, etc.
2. *Thermodynamics and kinetics.* The system may be defined in terms of energy sources, electron donors, and electron acceptors, and then temporal and spatial extents of energy disequilibrium can be looked for.

This is often manifested in the formation of chemically layered communities, which may be the best biosignatures on the planet.

Of course, there is one thing that would probably be very exciting on its own: non-random movement. Non-random directed movement presumably occurs very rarely without life. So, its detection by any method would be a cause for excitement with regard to the presence of life.

### First approach: complexity and chemical analyses

What kinds of measurements might be used for the initial analyses of rock samples – the indications of the haystacks in which the needles of life might be found? These questions are being investigated now, but some possibilities loom as hopeful. One approach that continues to be intriguing is that of X-ray tomography of the type used for medical scanning. Such computerized tomographic (CT) approaches have now been miniaturized to the laboratory-bench scale, and are capable of “seeing” colonies of bacteria residing within lithic environments [21]. CT machines keep getting smaller and in the near future it should be possible to send such instrumentation to Mars via spacecraft for in situ analyses of the Martian surface. A picture in three dimensions taken with the CT scan of an Antarctic rock shows an area of different density about 2 mm under the surface (Fig. 1).

Since the rock has a density much higher than that of living cells, it is relatively easy to detect life, the major component of which is water. This may be a reasonable first step for life detection: simply looking for areas of fundamentally different densities. Of course, none of this proves the existence of life – rather it tells one to stop and take a look, break the rock open, and do some more studies. And this is the point of this strategy, that no single test will tell you there is life. Instead, it sends a

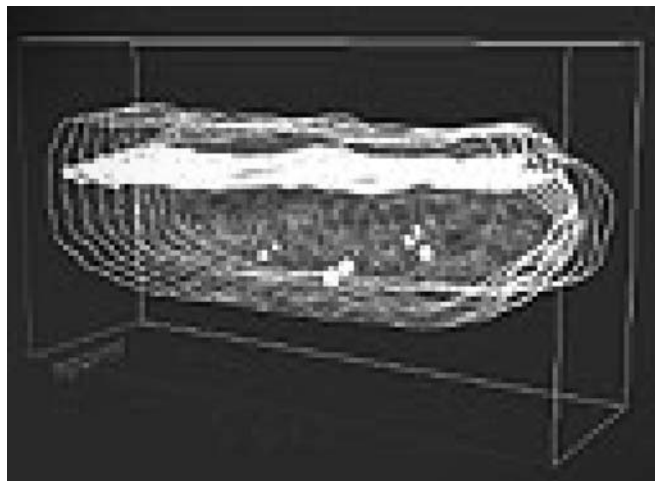
**Table 2** Size considerations for bacterial life and metabolism [16]

Cell size (μm) <sup>a</sup>	Radius (μm) <sup>b</sup>	Volume (μm <sup>3</sup> )	molecules/cell <sup>c</sup>				
			1 M	10 mM	1 mM	10 μM	1 μM
1	0.495	0.12	3.06·10 <sup>8</sup>	3.06·10 <sup>6</sup>	3.06·10 <sup>5</sup>	3058	305.8
0.5	0.245	0.015	3.71·10 <sup>7</sup>	3.71·10 <sup>5</sup>	3.71·10 <sup>4</sup>	371	37.1
0.2	0.095	8.57·10 <sup>-4</sup>	2.16·10 <sup>6</sup>	2.16·10 <sup>4</sup>	2.16·10 <sup>3</sup>	21.6	2.16
0.1	0.045	9.11·10 <sup>-5</sup>	2.29·10 <sup>5</sup>	2.29·10 <sup>3</sup>	2.29·10 <sup>3</sup>	2.29	0.229
0.05	0.02	8.0·10 <sup>-6</sup>	2.02·10 <sup>4</sup>	202	20.2	0.202	0.0202
0.02	0.005	1.25·10 <sup>-7</sup>	315	3.15	0.315	3.15·10 <sup>-3</sup>	3.15·10 <sup>-4</sup>

<sup>a</sup>For the purposes of this discussion, the cell is assumed to be spherical. Smaller bacteria are on the order of 500 nm, while those that pass through 0.2-μm filters are referred to as ultramicrobacteria. Bacteria-like particles in the 20–50 nm range are referred to as nanobacteria

<sup>b</sup>Radius is assumed to be half the diameter, and then 5 nm are subtracted for the width of the double membrane. No space is assumed for the rigid cell wall, so these estimates are conservative in the direction of high volumes

<sup>c</sup>These calculations are meant to show the number of molecules of any given compound (substrate, solute, etc.) within the spherical volume specified. As shown, when bacteria get to a size of 50 nm, there is space for only about 20 molecules when concentrations reach 1 mM. At 10-μM concentration, a 100-nm-sized sphere has only two molecules per cell! Clearly, there must be some lower limits that chemistry sets for a metabolizing cell

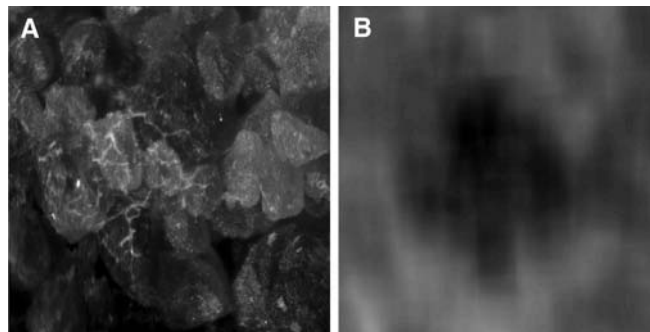


**Fig. 1** Three-dimensional image taken with the computerized tomograph (CT) of the endolithic layer of microorganisms inside Antarctic rock using X-ray CT [22]

message not to leave until you have done some chemical analyses of the suspicious structures. Thus, a density disparity (which is what the X-ray methods see) does not prove the presence of life, it simply sends a message that more sampling should be done, and indicates where such efforts should be focused. Once such information is available, it would be relatively easy to drill into the rock and analyze the drill fragments or core contents by a variety of other approaches, each of which could verify or deny the presence of potential life in that particular location.

Energy will indeed flow through life, and if chemical energy is involved, almost certainly some redox transformations will occur. Furthermore, double bonds will probably (almost certainly) be involved in the energy transformations. Double-bonded compounds interact well with deep UV irradiation, revealing, without the need for exogenous stains, the presence of potential life-containing materials. Deep UV (224 nm) radiation induces virtually no rock (mineral) fluorescence, thus removing the primary interference encountered with UV fluorescence methods in lithic environments [20]. Once again, it is stressed that as structures do not prove life, neither do structures that fluoresce; their discovery simply suggests that more chemical analyses (of those structures) should be done.

Defining complexity as information content and abrupt changes in information content allows one to apply standard data-compression algorithms to images, looking for areas that qualify as complex. Such analyses have been used to classify the complexity of galaxies by the astrophysics community with great success. Our experience with such approaches is that they work quite well for “seeing” the complexity of biological structures in images. One advantage of this approach is that several sets of “eyes” can be used to examine the same scene, thus looking for complexity that at one wavelength might be cryptic, and at another rather

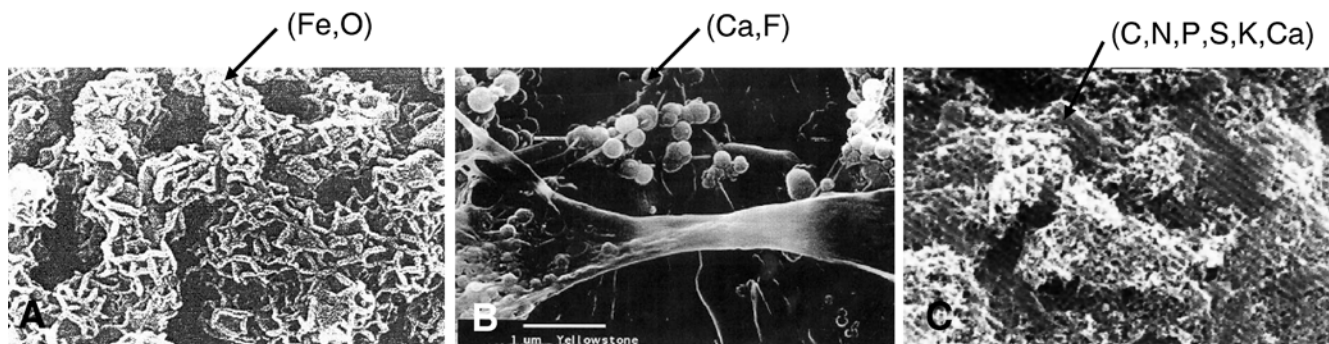


**Fig. 2A,B** Microbial community living inside an Antarctic sandstone. **A** Antarctic sandstone. **B** The central region containing the fluorescing organisms appears dark in the complexity map because the gra-scale encoding of the information content in the image codes information-rich areas as dark gray, while the areas containing less information appear white. [For more information on the complexity analysis contact Michael C. Storrie-Lombardi (mcs1@jpl.nasa.gov). For information on the LabView software implementation of the algorithm contact Rohit Bhartia (rbhartia@jpl.nasa.gov)]

obvious. Figure 2 shows an example of such an approach. A microbial community living inside an Antarctic sandstone rock was detected using the native fluorescence signature released when the proteins and genetic material in the organisms were excited with a 224 nm ultraviolet laser. The original image on the left was then transformed into the complexity map on the right.

To put this another way, physical shapes or complexities alone are insufficient. Once you have the shapes, you must determine the chemistry. We can find many things that look like bacteria, as shown in Fig. 3, where two different “life-shaped” objects are shown to be chemical artifacts. Figure 3A is a laboratory-formed artifact that is composed of iron and oxygen, and Fig. 3B is a sample from an evaporitic environment that is made of calcium fluoride. From morphology alone, it is easy to be deceived – these forms appear to be bacteria. Chemical analysis, however, reveals that there is nothing there but calcium and fluorine. Given that both of these compositions are well-known mineral compositions, the excitement about life will fade rapidly. In contrast, Fig. 3C shows morphological forms that, when examined, have the chemical composition of no known mineral. In this case, its composition is compatible with earthly life, but any unusual (i.e. unexpected composition with regard to known minerals) would be grounds for demanding more measurements of the morphologically complex forms.

That is, following this approach on Earth would soon lead to the identification of similarly shaped structures that were composed of C, H, O, N, P, S, Ca, K, Fe, and several trace elements. If you were a Martian who came to Earth and you were doing this kind of analysis, every time you came across one structure made of these elements, you immediately would send a note back to



**Fig. 3A–C** Shapes that look like microorganisms (bacteria); chemical analyses reveal they are chemical precipitates. **A** Iron and oxygen. **B** Crystals commonly found in evaporitic environments made of calcium fluoride. **C** Environmental scanning electron microscope (ESEM) image of *Shewanella oneidensis* cells on surface of manganite. [Images kindly supplied by: **A** Jillian Banfield (UC Berkeley), **B** Carlton Allen (Johnson Space Center)]

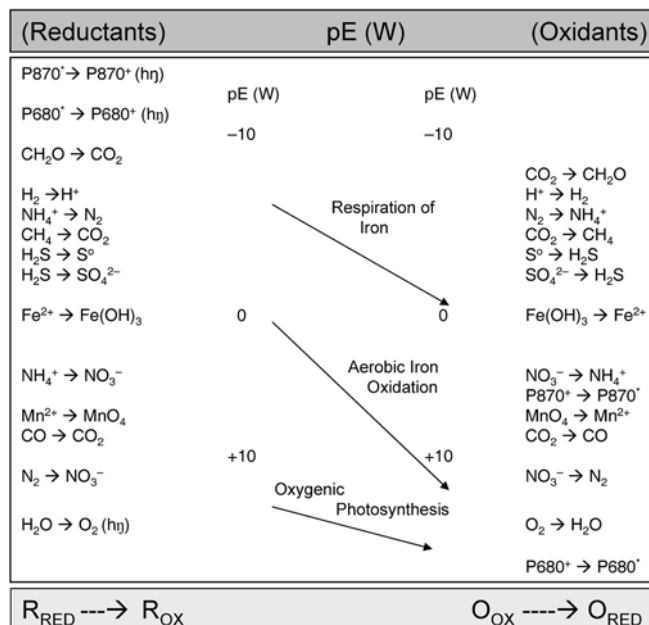
Mars saying: “we may have discovered life and this is what it is made of”. Because this particular mixture of elements does not appear commonly in any known minerals, it becomes a good candidate for further analysis. It is a feasible approach to use when we go to Mars. We do not demand to hunt for specific Earth-centric mixtures of elements, but instead, we look for something that should not be there. Something that is different from the standard minerals. If such an approach is used, candidates for further analyses will be identified by simple chemical measurements – candidates that, when examined for complex organic chemistry, chirality, isotopic fractionation, etc., may well yield the evidence for the presence of life [17].

## Second approach: energetics (thermodynamics and kinetics)

Earth is a light-dominated planet; 178,000 terawatts (TW) of light energy are estimated to reach the Earth annually, in comparison to the 30 TW of all the volcanic activity, with all other energy sources being trivially small [3]. Not surprisingly, the planet is “tuned” to these energy sources, particularly light, using them to generate a wide variety of reduced compounds (both organic and inorganic) that serve as energy sources for our diverse life forms. This is particularly true with regard to the metabolically diverse microbial life, which is able to (nearly) “eat anything and breathe anything” [17]. Observing this on Earth leads one to predict that any form of energy that could be used to generate a variety of chemically reduced species could, in theory, supply the components for a metabolically diverse planet.

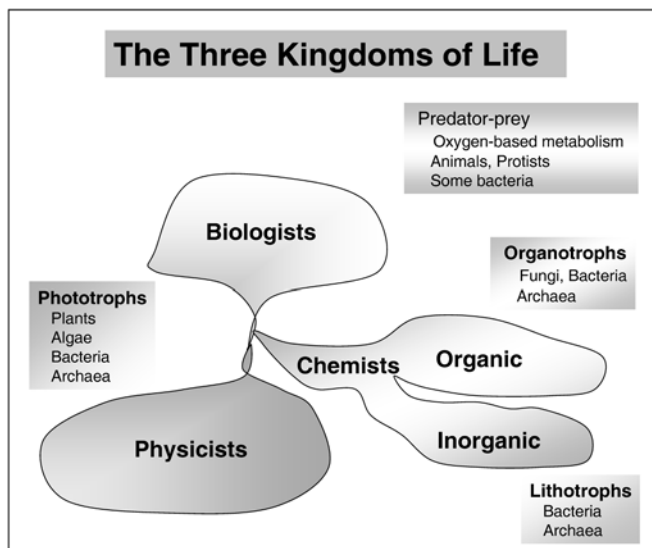
### Thermodynamics

With regard to the tremendous redox versatility of life (Fig. 4), we have put many of the fuels (reduced organics



**Fig. 4** Thermodynamics of life. This drawing shows the energy levels of reductants (left) that can be used by microbial life. This is expressed on a scale of pE (electrode potential) in which the most electronegative values (i.e. best electron donors) are at the top, and the least good fuels are at the bottom. The various oxidants that microbial life can use to burn the fuels are shown on the right. If any reductant is connected to an oxidant, and the slope of the arrow is negative, then energy is available, and some microbe on Earth is almost certain to exist that can utilize this “redox pair” for energy

and inorganics) that are abundant on Earth onto an energetic scale (electrode potential, or pE), with the most energetic at the top, and the least energetic at the bottom (left side), and all of the oxidants (oxidized organics or inorganics) on the same scale on the right side. In the case of oxidants, the best oxidant used by life – oxygen – is at the bottom, whereas the worst – CO<sub>2</sub> – is at the top. If one simply connects any fuel on the left with any oxidant on the right, and the arrow slopes downward, energy is available between this “redox couple”, and there will probably be a bacterium on this planet that grows by that energy. This remarkable statement suggests that virtually every source of redox energy on the planet that is available and abundant has been “discovered” by life. This is a very important perspective



**Fig. 5** The kingdoms of life. This cartoon, which began as an effort to simplify our view of the tree of life, has become a way of thinking with regard to searching for life. Here we view the vast array of life in terms of processes and energy, with the physicists being the photosynthetic organisms, the chemists being the organotrophs and lithotrophs, and the biologists being those organisms evolved for behavior and predation, which have adapted to take advantage of all three kingdoms via predation

with regard to the search for life elsewhere – perhaps the most important first set of measurements that need to be made are those connected with available fuels and oxidants: no sense to look for oxygen-utilizing life, or any other kind, if the oxidant is not present.

Such a way of thinking allows one to propose a process-related “new taxonomy” for the search for life: one that looks at the types of processes rather than the types of organisms (Fig. 5). While the humor of such an approach may seem evident, we believe that it can be used in a profitable way to frame the search for life. Using this system, the importance of hunting for types of energetic processes, rather than the organisms that catalyze them, is stressed. In this context, we divide the living world into three kingdoms: the physicists that use physical sources of energy (on this planet, the phototrophs); the chemists that use chemical energy, either organic or inorganic (the organotrophs and lithotrophs), and the biologists that feed on other organisms of all three kingdoms (while they are in essence organotrophs, they prey on other organisms rather than utilizing their waste or excreted products). On Earth, the only successful members of the physicist kingdom are the phototrophs, but one can easily imagine planets where light is not the dominant energy source, and we should not be so close-minded to think that there could not be other types of “physicists” capable of utilizing other energy sources. The kingdom of the chemists is divided into two subkingdoms, organic and inorganic, both of which can be expected in abundance on Earth, and presumably on any planet where carbon-based life has evolved. However, the extent of the redox scale that can

be used for life may be severely impacted by the availability of electron acceptors present. Finally come the biologists, predators who live via the demise of their prey. They are optimized for behavior rather than metabolism, often increasing in size, speed, predation specialization, etc.

## Kinetics

While thermodynamic considerations such as those above allow one to frame the search for certain types of activities, the real indicator of life is that of kinetics – at what rates these processes occur, and whether these rates are indicative of extensive catalysis. This is most true in low-temperature geochemical reactions, where natural rate constants are often very slow (turnover times of days to years, or even longer). In such cases, living processes, catalyzed by biological enzymes (often with turnover times of  $10^3/\text{s}$  or greater), are easily spotted as kinetic anomalies. In general, the enhancement of rates between non-living and living systems is on the order of six to ten orders of magnitude in rate. A good example is glucose, which is readily consumed by bacteria, often at very fast rates. Given that glucose under sterile conditions is a very stable molecule, observation of its rapid oxidation is a first-order biosignature because this simply will not happen without life. Such thinking allows one to begin to plan strategies for finding life – when you visit another site, you ask not only what is there (fuels and oxidants), but what their rates of consumption are. This type of approach allows one to frame the search for life in a useful kinetic approach that may (if Earth is any example) be quite easy to implement.

The utilization of substrates at rapid rates has the possible outcome that gradients are produced. That is, if the rate of consumption is greater than the rate of replenishment via diffusion, then a gradient will form. Such gradients are extremely common on Earth, as oxygen diffuses downwards into stratified environments such as sediments, where mixing is minimal (i.e. in mixed lakes or oceans, such gradients are difficult to form because of convective mixing). Such an approach limits the search for extant, and active, biomass. While living active bacteria will produce perfectly good gradients, once they are inhibited or killed, the gradients begin to dissipate, and after some days disappear.

One might argue that the production of a gradient could be done by inorganic catalysts as well as living catalysts, and thus that, like other methods of searching for life, this one is not definitive. On Earth, however, gradients are a common feature of stratified ecosystems including sediments, lakes, fjords, mat communities, and even biofilms; they are found in tandem to each other, each being the result of microbial activities, and each depending on the layers above and below. Stratified ecosystems are predominant on the planet, and can range from hundreds of meters to mm or less in spatial scale. They may be the best “biosignatures” available to



us. Given the natural rate constants of the reactions occurring at the interfaces between the layers, there is simply no way that most of these gradients would exist at all. The challenge is: (a) to develop methods to visualize and measure such gradients over a large range of spatial scales, searching for them as an indicator of extant life, and (b) to understand what signals might be left behind by such layered communities so that they might be recognized after the fact, as fossil communities indicative of past life.

### Concluding remarks

If we are going to search for life beyond Earth, we need to use innovative, non-Earth-centric approaches. By definition, these will be interdisciplinary, requiring the best currently available technologies and the development of new technologies. Unless something swims by in front of your microscope, no single result will ever indicate the presence of life. This approach can also be applied to unknown areas on our own planet, and we should not miss the opportunity to use our non-earth-centric methods. Never forget to first determine the physical and chemical properties as well as the geological setting before looking for life, because this can provide clues as to what kind of life may exist there. If no life is detectable, then this information can very likely provide important clues as to its absence. Finally, the most important piece of advice for young investigators is: keep an open mind. If you do not have an open mind, life could be standing in front of your eyes and you would miss it.

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