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Chapter 9: Life as We Don't Know It

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Abstract

While Earth contains the only known example of life in the universe, it is possible that life elsewhere is fundamentally different from what we are familiar with. There is an increased recognition in the astrobiology community that the search for life should steer away from terran-specific biosignatures to those that are more inclusive to all life-forms. To start exploring the space of possibilities that life could occupy, we can try to dissociate life from the chemistry that composes it on Earth by envisioning how different life elsewhere could be in composition, lifestyle, medium, and form, and by exploring how the general principles that govern living systems on Earth might be found in different forms and environments across the Solar System. Exotic life-forms could exist on Mars or Venus, or icy moons like Europa and Enceladus, or even as a shadow biosphere on Earth. New perspectives on agnostic biosignature detection have also begun to emerge, allowing for a broader and more inclusive approach to seeking exotic life with unknown chemistry that is distinct from life as we know it on Earth. Key Words: Biosignatures—Bioenergetics—Astrobiology—Extraterrestrial life—Life. Astrobiology 24, S-186–S-201.

Throughout the Primer 3.0, when discussing life and its characteristics (Chapter 2), how it originated (Chapter 4), how it evolved (Chapter 5), its diversity (Chapter 6), where to look for it (Chapter 7), and what to look for (Chapter 8), we have focused on life as we know it. The primary reason for focusing on terran life is simple: it is the only example of life available and thus forms the base of any discussion. However, there is a growing recognition in the astrobiology community that using terran-based assumptions for the chemistry of life may not account for potential life-forms that differ from the familiar.

As discussed in Chapter 2.4, although there is no universally accepted definition of life, a working definition (operationally defined to aid in life-detection efforts) denotes life as "a self-sustaining chemical system capable of Darwinian evolution" (Joyce *et al.,* 1994). This definition is blind to the chemical composition of a system and attempts to be inclusive of potential forms of life as we don't know it (referred to as *exotic life* throughout; Cleland, 2019). Recent efforts to describe life *universally* focus on more abstract descriptions of life's processes: autocatalysis, dissipation, learning, and homeostasis (Bartlett and Wong, 2020). While these four pillars of life stem from observations of life as we know it (*i.e.,* terran life), their abstraction helps expand the definition to include other, yet undiscovered, forms of life. Furthermore, the concept of the grayness of life is another challenge on the path to defining life (see Chapter 2.3.2 and Chapter 8.5). While there is fascinating

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research into other, nonchemical forms of life (*e.g.,* digital life; Chapter 2.1.4), we limit our discussions in this chapter to life-forms based on chemistry.

How different life could be beyond Earth is still an open scientific question, and it is possible that the biochemistry of life beyond Earth could be constructed of (near-)identical building blocks to terran life (Pace, 2001). Nevertheless, it is reasonable to assume that distinct origins and evolutions of life in vastly different environments will yield life-forms with correspondingly different biochemistries. Recent efforts in astrobiology seek to understand what features of terran life are due to historical contingency (see Chapter 2.1) and what features are fundamental to life universally. This chapter begins (Chapter 9.1) with a discussion of how understanding the origins of life on Earth (Chapter 4) and natural selection (Chapter 2.2.4 and Chapter 5) might inform us about the universal properties of life. It further explores how exotic life could operate (Chapter 9.2), the most promising Solar System locations to search for exotic life (Chapter 9.3), and how to recognize life without looking for specific diagnostic molecules (*i.e.,* agnostic biosignatures; Chapter 9.4).

9.1. How the Origin of Life on Earth Can Inform Us About Exotic Life

Life on Earth emerged from its geochemical environment as a set of interacting molecules perpetuating their existence through space and time. Several hypotheses can explain how prebiotic molecular ensembles may have given rise to full-fledged life and how key properties (*e.g.,* complexity) and processes (*e.g.,* autocatalysis and phase transitions) may have played a role in life's onset and early evolution (Libby *et al.,* 2019 and references therein). Research is also beginning to shed light on what may constitute universal principles of biology and physical laws of biological organization (Ruiz-Mirazo *et al.,* 2004; West and Brown, 2004; Goldenfeld *et al.,* 2017; Kim *et al.,* 2019; Walker, 2019; Kempes *et al.,* 2021). However, the exact sequence of steps needed for a transition from a ''soup of weakly interacting molecules'' (Cronin and Walker, 2016) to robust biochemical networks is currently unknown, limiting our ability to resolve what makes life so fundamentally different from nonlife. Ultimately, recognizing exotic life will require distinguishing between true universal features of life and the historical features of life that we know from Earth. Indeed, life at its inception may have looked and behaved very differently than it does today. Understanding the differences can help us evaluate just how different life can be, both during the history of life on Earth and for potential life elsewhere.

Diverse theories related to life's emergence on Earth influence our concepts of exotic life. Some theories envisage life originating as un-encapsulated nucleic acid polymers capable of key life processes (*e.g.,* self-replication, information encoding, and adaptive evolution) that eventually become more complex and give rise to modern genetics (Gilbert, 1986). Other theories propose that alternative biopolymers may have preceded the advent of RNA/DNA-based genetics on Earth (see Chapter 9.2.5) or that life started with the emergence of evolvable autocatalytic systems of molecules (a series of chemical reactions that mutually catalyze each other's formation), typically on a mineral surface (Kauffman, 1986; Wächtershäuser, 1988; Segré and Lancet, 2000). Modern versions of the autocatalytic theory posit that the system could be evolvable without any genetic encoding (Baum, 2018; Vincent *et al.,* 2019). Heritable information would be encoded in the form of compositional inheritance (Segré and Lancet, 2000; Baum, 2018), where chemical composition is maintained as these ''surface organisms'' grow and disperse, and mutations arise in the form of compositional changes (see Chapter 9.2.5.3).

When discussing whether life is restricted to the molecular classes found in modern terran biochemistry, it is important to note that these models do not strictly assume the presence of specific kinds of chemical constituents. Weak autocatalysis is not exclusive to biologically relevant molecules (in fact, it is common in many areas of chemistry), and evolvability in some models does not depend on the appearance of specific molecular species (Segré and Lancet, 2000; Baum, 2015). This idea that important biological functions are not restricted to the appearance of specific molecular classes is further supported by the discovery that certain molecules can play roles that are very different from their typical role (*e.g.,* the discovery of ribozymes, catalytic RNA molecules; Cech, 1993). This molecular redundancy helps relax assumptions on what chemistry is needed for those functions in general. This suggests that key aspects of life, such as self-replication and evolution, could also be realized using very different chemistries, resulting in exotic forms of metabolism.

It is also important to consider whether the emergence of life could happen in other types of environments. One approach to understanding the likelihood of life arising elsewhere is to investigate the factors that influence the emergence process in an abstract manner; for example, by assessing whether life can arise multiple times on the same planet and considering the distribution of these potential abiogenesis events through time. Spiegel and Turner (2012) hypothesized that emergence of life events could be represented as a Poisson process, where the average time between two abiogenesis events can be calculated, yet the actual timing between two events is random and the probability of abiogenesis at any given time does not depend on previous abiogenesis events. In a Poisson model, it would be possible that multiple, and perhaps distinct, forms of life could emerge independently from the chemical environment at random intervals on a planet, leading to multiple biospheres (*e.g.,* a shadow biosphere on Earth, see Chapter 9.3.1.1). Some researchers have attempted to quantify the probability of familiar life emerging on other terrestrial planets based on the knowledge that life emerged rapidly on Earth (Lineweaver and Davis, 2002). Quantifying the probability for life as we know it to form and the external factors involved could allow a deeper understanding of alternative life-forms, or what other types of abiogenesis events might be possible.

It is difficult to discuss the emergence of life without considering open-endedness, emergence, and complexity (Chapter 2.2.5). Complex biotic and abiotic systems are all around us. These systems are made up of many components that interact together in various ways, thus leading to emergent properties. Understanding how these emergent properties differ in life and nonlife can help us build better conceptual frameworks and understand life as a general phenomenon, beyond life as we know it. An example of complex emergent properties is nonlinear interactions, in which the interactions within a system increase nonlinearly as the number of system components grows (Pérez-Mercader, 2002), resulting in more components and interactions the more complex the system gets. This phenomenon can be observed in terran life, where an evolutionary development (*e.g.,* energy harvesting metabolisms or self-replication) can lead to an explosion of new molecular components and biochemical interactions (Des Marais and Walter, 1999). Another example of emergent properties of complex systems, which life as we know it exhibits, is interactions between the system and the environment in which it exists. During the origin of life, chemicals had to persist despite fluctuations in the environment. This situation required a balance between selective chemistry and stochastic (random) behaviors of the environment. Selection and persistence of these molecules led to the evolution of nascent proto-biological systems and eventually to more complex living systems. Furthermore, interactions with the environment are key to biological evolution, where the diversity of life is driven by random changes in biological forms (*e.g.,* mutation) influenced by environmental factors and processes imposed by natural selection (Kauffman, 1996). Emergent properties may be critical to understanding life when considering unfamiliar or exotic biology, as they allow us to search for properties of life that are universally shared rather than those that are dependent on a particular environment or a particular biochemistry. By studying life through the lens of complex systems, expressed physically or chemically, prominent features of life and combinations thereof can be abstracted (*e.g.,* nonlinear dynamics, responses to the environment) and may be used to guide our search for exotic life elsewhere (see Chapter 9.4).

9.2. How Different Life Could Be

The general traits of terran life are derived from physical, chemical, and biological features of current or extinct terran organisms (see Chapter 2.2). These features are unlikely to be arbitrary and presumably arose from the specific distributions of elements and geochemical environments on Earth. For example, different planets (even within the Solar System) have different chemical cycles, as elements like sulfur, carbon, and hydrogen circulate (or not) through the atmosphere, hydrosphere, and lithosphere. Life may manifest completely different molecular machinery in these different environments (see Chapter 9.2.1). It is also possible that exotic life is based in solvents other than water, or even in nonliquid phases that could have dramatically different properties yet allow for the persistence of chemical reactions that could support life (see Chapter 9.2.2). In addition, while cellular compartmentalization is deemed to be vital for the development of life on Earth, such separation from surroundings could conceivably be achieved through other means (see Chapter 9.2.3). On Earth, life harnesses solar and chemical energy from the environment (see Chapter 6.2), but many alternative energy sources exist that could be used by exotic forms of life (see Chapter 9.2.4). Finally, terran life stores information about previous states using the genetic code and nucleic acids (DNA and RNA, see Chapter 2.2.3.1). However, such information storage mechanisms are not necessarily exclusive to specific biomolecules (see Chapter 9.2.5).

9.2.1. Alternative elemental building blocks: Is non-carbon-based life possible?

Carbon is the foundational element upon which all terran biochemistry is based. Carbon is an ideal element for life on Earth due to its chemical and structural functionality (see Chapter 2.2.2.2). However, researchers have long speculated that life in the universe may not be restricted to carbon-based biochemistry. Several alternative base elements are presented below.

9.2.1.1. Silicon-based life. Since silicon (Si) is directly underneath carbon on the periodic table of elements, it shares similar chemical properties. Like carbon, silicon can form four covalent bonds and stable structures with CHONPS elements, halogens, and trace metal species, thus allowing for functional diversity (Bains, 2004; Petkowski *et al.,* 2020). Silicon can also theoretically form Si-Si structure scaffolds, like C-C chains seen in terran biochemistry, and these can form macromolecular assemblies suitable for compartmentalization (Unno *et al.,* 2000). Silicon is the second most abundant element in Earth's crust, and the seventh most abundant element in the universe (Gill, 2015), making it widely available for potential life.

Despite their similarities, carbon and silicon have been called ''false twins'' (Petkowski *et al.,* 2020). There are notable differences in potential biochemistries based on silicon versus carbon. For example, because of its atomic radius, silicon forms longer bonds with different bond angles than carbon, which has effects on chemical structures that can be formed. Some carbon-based compounds that are central to terran biochemistry, such as alcohols and sugars, have no silicon-based analogs because the same functional groups (*e.g.,* -H or -OH) exhibit different chemical reactivities in silicon chemistry (*e.g.,* silane, SiH_4, and methane, CH_4). Although Si-Si bonds are far more reactive than C-C bonds in water, another polar solvent (*e.g.,* ammonia of sulfuric acid) could allow for a larger silicon-based chemical space (see Chapter 9.2.2.2). Finally, silicon is largely unavailable for biochemical reactions on Earth due to its propensity to form chemically stable minerals. While there are challenges for silicon-based life to exist in terran-like conditions, silicon chemistry could provide complex and diverse chemical functionality to support alternative forms of life.

9.2.1.2. Boron-based life. Boron (B) is also next to carbon on the periodic table and shares a similar ability to form a diversity of chemical species that range from chemically stable to highly reactive (Scorei, 2012). Although boron is a relatively rare element on Earth (Cleaves and Chalmers, 2004), borates (B_xO_y) potentially played an important role at the origins of life (see Chapter 4.2.3.6) and are still used to perform key biological functions in extant life (Scorei, 2012). Boranes (B_xH_y) can stabilize polymer linkages and function as chemical catalysts (Burg, 1965; Saladino *et al.,* 2011). Boranes can also form neutral analogs of carbocations (ions that contain a positively charged carbon atom) that are key components of modern biochemistry (Petasis, 2007). Potential barriers to boron life are its rarity in the Solar System and the relatively high stability of B-O bonds, which limits its chemical reactivity (Krogh-Moe, 1962). However, B-N bonds are much more like C-C bonds in terms of stability, and B-N structures are known to form aromatic structures that could form nucleic acid analogs (Schulze-Makuch and Irwin, 2018b).

9.2.1.3. Metal oxide-based life. Metal oxides show some promise as a potential basis for biochemistry. In laboratory experiments, polyoxometalates (POMs) have demonstrated biomimetic functions, such as the ability to form membranes, later termed iCHELLs (inorganic chemical cells; Cooper *et al.,* 2011). Additionally, iCHELLs can display similar characteristics to biological photosynthetic cells when coupled to light-sensitive dyes and other electron-mediating molecules (Nakanishi *et al.,* 2018). Some POMs have also exhibited weak autocatalytic behavior (Miras *et al.,* 2020) or formation of supramolecular structures akin to the 3D assembly of known biologically relevant molecules (*e.g.,* DNA helices; Kulikov *et al.,* 2017). While these complex metallic structures might not be largely available prebiotically, their properties suggest that lifelike chemistry can occur in unexpected ways.

9.2.1.4. Sulfur-based life. Sulfur plays an important role in the chemistry of life on Earth (Chapter 2.2.2.5), and it may also have been a key element at the origins of life (Chapter 4.2.2.5). Some researchers have proposed an ''iron-sulfur world'' or ''thioester world'' dominated by sulfur chemistry (Chapter 4.2.6.2). Sulfur is thought to have been far more bioavailable than phosphorus on early Earth, and sulfur-containing compounds (*e.g.,* thioesters or thioacetates) could have powered early biochemical reactions that today depend on phosphorus (Keller *et al.,* 2017). Additionally, sulfuric acid may serve as an alternative solvent for life (see Chapter 9.2.2.2).

Could sulfur chemistry be the basis for alternative living systems, and could it assume carbon's essential roles in modern biochemistry? Sulfur-containing molecules can drive protometabolic cycles (Keller *et al.,* 2017), suggesting that a sulfur-based analog of modern metabolism is possible. Sulfur can also form polymers in the same way carbon can (Schulze-Makuch and Irwin, 2018b), so information storage and genetics based on sulfur polymers seem chemically plausible. While sulfur-based polymers can form linear chains, they are unable to form higher dimensional conformations, which may place an upper limit on the possible configurations of sulfur-based life (Petkowski *et al.,* 2020).

9.2.2. Alternative media for life: How important is water?

All known life on Earth requires water in the liquid phase. Water (*i.e.,* H_2O) has numerous important properties that help sustain terran life (Chapter 2.2.2.1) and drives the functional assemblies that build our biochemical repertoire (Chapter 4.3.1). Water is regarded as an important solvent and has been at the forefront of NASA's search for life elsewhere (Chapter 7.1.1). However, the role that water can play depends on whether it is in a solid, liquid, or gas phase. The physical phase of water is controlled by the environmental conditions (*e.g.,* temperature ranges, atmospheric composition, or sources of energy dissipation), and these vary greatly in other planetary environments (see Chapter 7.2). The role of water in terran life could perhaps be a unique result of the geochemical processes that are particular to Earth. Thus, our understanding of life arising from alternative solvents can be boiled down to one fundamental question: Would alternative selective pressures promote the use of different liquid media that are capable of sustaining life?

For a liquid solvent to qualify as a suitable candidate for supporting life, it has to be both available and abundant. For example, hydrogen sulfide (H_2S), while relatively abundant in the universe, is a liquid over a temperature range of only 22°C (-82°C to -60°C, at 1 atmospheric pressure [atm]), making it largely unavailable as a liquid in terran-like conditions. Here, we focus our initial discussion on the most considered alternative solvents with differing chemical properties than water and the alternative biochemistries that could be supported in different media.

9.2.2.1. Ammonia. Ammonia (NH_4) is liquid at temperatures much colder than water (-78°C to -33°C at 1 atm) and could be present as a solvent for life on cold, outer Solar System bodies such as Europa, Enceladus, and Titan (see Chapter 9.3.2). Ammonia is a polar molecule, similar to water but with significantly lower electrical conductivity, weaker hydrogen bonds, and lower surface tension (Bains, 2004). Ammonia more readily dissolves some organic compounds and can disrupt the function of terran biomolecules and cellular structures. However, alternative chemistries based on NH substitution for O atoms in biomolecules or membrane compartments composed predominantly of nonpolar lipids could be possible in ammonia (Haldane, 1954; Firsoff, 1963; Raulin *et al.,* 1995).

While the prospect of life in ammonia seems plausible, the low temperature required for liquid ammonia would result in slower chemical reaction rates, potentially limiting the ability for life to develop and thrive. Mixtures of ammonia and water could also provide some advantages over either alone. For instance, several outer Solar System moons such as Titan and Enceladus are thought to have subsurface oceans composed of water and ammonia, which would help keep water liquid at colder temperatures (Schulze-Makuch and Irwin, 2018c).

9.2.2.2. Sulfuric acid. Sulfuric acid (H_2SO_4) is a highly reactive protic solvent (liquid from 10°C to 337°C at 1 atm), meaning that it readily exchanges protons with dissolved molecules and rapidly degrades many organic compounds (*e.g.,* sugars; Griebel *et al.,* 2016). Thus, life's biochemical and structural architecture would need to be fundamentally different in a sulfuric acid solvent and perhaps be based on elements other than carbon. Sulfuric acid is known to support a wider range of silicon chemistry than water and could potentially enable complex chemistry and the formation of Si-containing compounds (Petkowski *et al.,* 2020), some of which can even perform analog biological functions (Tokito and Okazaki, 1998). In addition, polymers containing a carbon-silicon backbone are stable in sulfuric acid, suggesting that polymer-based genetics could be possible (Schulze-Makuch *et al.,* 2004).

9.2.2.3. Formamide. Formamide (CH_3NO) has long been debated as a possible alternative to water for the main liquid solvent of the earliest life-forms on Earth (Saladino *et al.,* 2012, 2019). Many molecules important in modern biochemistry are degraded in the presence of water (*i.e.,* hydrolyzed), a situation that has come to be known as the ''water-paradox'' (see Chapter 4.2.4.3). Formamide might have been a better and more robust alternative solvent in prebiotic Earth (see Chapter 4.2.4.1), and it is thought to be a common molecule in the universe (Codella *et al.,* 2017). In other planetary scenarios, formamide could be a viable alternative solvent because it remains in a liquid state within a greater temperature range than water (2°C to 210°C at 1 atm), thus expanding the habitat for life-forms to hotter environments than even those of hyperthermophiles on Earth (see Chapter 6.3.1). Furthermore, formamide has similar solvation properties to water, such as a large dipole moment, and it is capable of dissolving nearly all chemical species that are dissolved in water. For example, dissolved polyelectrolytes could be important for biopolymers with a charged backbone (see Chapter 9.2.5.2). Due to its higher thermal stability, formamide would be capable of sustaining chemical species in a liquid state for longer, thus allowing for subsequent chemical reactions that otherwise would not be possible in the presence of water at those temperatures.

9.2.2.4. Methane. Methane is liquid from -182°C to -161°C at 1 atm, and it has gained considerable attention due to the presence of methane lakes on the surface of Saturn's moon Titan (Stofan *et al.,* 2007). On Titan, methane cycles through solid, liquid, and gas phases analogously to the terrestrial water cycle (Lunine and Atreya, 2008). Although many organic compounds suffer a low solubility in methane (McKay and Smith, 2005), it could still support life composed of small hydrocarbons and N_2 (McKay, 2016). Many organic synthesis reactions occur more steadily in nonpolar solvents, such as liquid methane (Benner *et al.,* 2004; Schulze-Makuch and Irwin, 2018a), and the low solubility of nitrogenous compounds in liquid methane offers possibilities for membrane formation (see Chapter 9.2.3.1).

Since methane is nonpolar, alternative biochemistry in liquid methane would likely be dependent on nonpolar interactions. These conditions could create challenges for an effective information storage system. McKay (2016) suggested that a binary-code information system based on hydrogen bonding between oxygen- and nitrogen-containing molecules could be feasible. Although the latter suffers from the solubility issues mentioned above, this type of bonding could act as an analog to nucleotide base pairs in terrestrial DNA. Furthermore, polymers that are electrochemically stable and can store charge throughout their entire volume could also function as the primary information molecule in a cold methane biochemistry due to their ability to exist in different redox states (Abdulla and Abbo, 2012; McKay, 2016).

9.2.2.5. Supercritical solvents. Alternative solvents that are in supercritical phase in a variety of planetary conditions can also serve as a medium for exotic life. Discussion of these solvents can be found in the supplementary information.

9.2.2.6. Solid- or gas-phase life. Certain aspects of solid-state systems exhibit lifelike properties. Crystals are nonliving structures that nonetheless replicate their structural patterns during growth. Environmental information can be preserved through defect formation (analogous to mutation) and propagation (Cairns-Smith and Hartman, 1986), potentially serving as an alternative, solid-state (*i.e.,* nonpolymeric) information system. While there is no comprehensive definition for life (see Chapter 2.4), it does require interactions among molecules. The low opportunity for interaction between separated regions in the solid phase is the main limitation to the idea of solid-phase life.

Similarly, gas molecules would collide infrequently in the absence of an enclosed space, making molecular interactions challenging. Gas-phase metabolic processes that are contained in compartments, for example surrounded by liquids or solids (*e.g.,* bubbles, vesicles, or vacuoles; Sagan and Salpeter, 1976; Pfeifer, 2006), would decrease the amount of energy available to keep the organism alive. Granular gases could provide another solution to the co-localization problem.

More information about granular gases and the possibility of some common terran biomolecules existing in the gas phase is discussed further in the supplementary information.

9.2.3. Alternative compartments: How important is the cell membrane?

The presence of a semipermeable barrier between the interior and exterior environment provides biochemical networks with a controlled internal chemical environment that can operate independently from the external environment, and it is fundamental to terran life (see Chapter 2.2.1). Terran cell membranes are composed of polar phospholipids (see Chapter 2.2.3.3), but these lipid bilayer membranes are not a viable construction outside of liquid water (or potentially other polar solvent-based environments). Assuming that phospholipid bilayers are a requirement for encapsulation would dramatically restrict our search for life. This section explores alternative means in which exotic life could partition from its environment.

9.2.3.1. Alternative cellular membranes. Known examples of organisms on Earth make use of nonlipid membranes temporarily. For example, when the lipid membrane of the algae *Bryopsis plumosa* is disrupted, a semipermeable, polysaccharide-based gelatinous envelope forms that allows selective materials transportation until the lipid membrane is rebuilt (Kim *et al.,* 2001). Models of alternative membranes that could form in other planetary environments, such as the icy moon Titan (see Chapter 9.3.2.1), include hypothetical membranes, termed azotozomes, composed of small organic nitrogen compounds (''azoto'' means nitrogen-containing). Like lipids, these azotozomes contain polar heads and nonpolar tails, but their nonpolar ends are oriented outward, making them compatible with a nonpolar solvent like methane. Although these membranes retain their flexibility in cryogenic temperatures, other work has questioned their thermodynamic stability and ability to self-assemble in nonpolar solvents (Sandström and Rahm, 2020). The search for alternative membranes is an active and ongoing area of research.

9.2.3.2. Non-membrane compartments. Exotic life may also exist in compartments not delineated by membranes. One type of proposed non-membrane compartment is mineral pores. Catalysis of chemical reactions or reaction cycles can be induced on mineral surfaces, and it has been suggested that porous structures could be used as alternative cellular compartments for early life (see Chapter 4.2.4.4). Mineral surfaces that contain networks of pores and cavities (*e.g.,* iron-sulfide precipitates, weathered potassium-feldspars, the spaces between mica sheets, and clays) provide relatively controlled environments (*i.e.,* micro-reactors) for chemical reactions (Russell, 2006; Branciamore *et al.,* 2009; Hansma, 2010). These networks are open to diffusion while being protected from the effects of a fully open system and from UV radiation. Additionally, thermal gradients can facilitate the accumulation of molecules in porous mineral systems, which can increase the chemical complexity such as is seen in porous hydrothermal systems (Branciamore *et al.,* 2009; Coveney *et al.,* 2012).

Droplets are another conceivable way of compartmentalizing without the need for membranes. Droplets can form as liquid suspensions in gas (aerosols) or in liquid (emulsions). Recent studies on droplets have uncovered complex behaviors previously thought to be exclusive to biological membranes, including autonomous division (Taylor *et al.,* 2017), cooperation, and predation (Points *et al.,* 2018). Although membraneless, coacervate droplets formed of oppositely charged polymers (*e.g.,* DNA and polypeptides) can spatially concentrate different molecules, facilitate important biochemical reactions, and have even been shown to be conducive to RNA catalysis (Aumiller *et al.,* 2016; Drobot *et al.,* 2018). Alternative organic polymers, such as polyesters, are also capable of developing into coacervates which could serve as compartments for exotic life (Jia *et al.,* 2019).

9.2.3.3. Non-compartmentalized life. Theories that propose life without any form of explicit compartmentalization are typically based on the ability of clay minerals to selectively catalyze the synthesis and adsorption of certain biologically relevant compounds (Cairns-Smith *et al.,* 1972; Rimola *et al.,* 2009). Defects on the mineral surface could promote a selective process that could eventually lead to microenvironments where complex chemical systems could evolve (Cairns-Smith and Hartman, 1986). However, this theory has not yet been experimentally proven. Co-localization of polymers could also lead to membraneless life. Walker *et al.* (2012) demonstrated that a simulated surface with relatively low diffusion rates for polymers and relatively high diffusion rates for monomers results in co-localization of polymers. This aggregate ''cell material'' is analogous to the temporary polysaccharide envelopes discussed above (see Chapter 9.2.3.1). Furthermore, these polymer clusters recycled the monomers they used to form polymers between the system's interior and exterior and showed growth in particular diffusion settings (Walker *et al.,* 2012). While it is not known whether membraneless life is possible, certain exotic life-forms could be membraneless for a portion, or perhaps even the entirety, of their life cycle.

9.2.4. Alternative energy sources

Chapter 6.2 outlined the two main energy sources for Earth-based life: solar and chemical. However, a much larger variety of energetic resources are available, and life could theoretically make use of alternative energy sources. This section will discuss various alternative exotic lifestyles that may utilize alternative energy sources that are found throughout the Solar System.

9.2.4.1. Thermal gradients. Thermal gradients are common in the universe, making them a good candidate energy source for life (Muller and Schulze-Makuch, 2006). Thermal gradients on Earth occur in hydrothermal vents, hot springs, interfaces (*e.g.,* water-air and water-ice), and convection cells (*e.g.,* mantle, ocean, and atmosphere), just to name a few examples. Thermal gradients are powered by solar radiation as well as geothermal energy—trapped heat from the planet's formation and from decaying radioisotopes (see Chapter 3.4.3.1). Beyond Earth, there are thermal gradients in the atmospheres of Venus, Jupiter, Saturn, Uranus, and Neptune. Radiogenic heating may also be present on Europa and Enceladus (see Chapter 9.3.2). Tidal influences from gravitational forces of other celestial bodies can generate friction on the surface or in the interior of a planetary body and lead to tidal heating. Impacts into the surfaces of icy moons such as Europa and Titan might also have been a major source of heat during their accretion (Sotin *et al.,* 2021) and provided transient energy sources after accretion (O'Brien *et al.,* 2005).

Researchers have speculated that thermotrophs, or organisms that use heat as a basic energy source (Muller and Schulze-Makuch, 2006), may use thermal gradients at hydrothermal vents to generate energy, by (i) taking advantage of the buoyancy of a warm water vacuole to float into cooler waters (Fig. 9.1a), (ii) having one side of an elongated organism attached to a hydrothermal vent while the other side moves in cooler water (Fig. 9.1b), or (iii) the uptake of cold water through a cavity and ejecting warm water, much like instances of water flow in barnacles and sponges (Fig. 9.1c) (see Schulze-Makuch and Irwin [2018a] for a more detailed description of these organisms and the use of thermal gradients as an energy source).

9.2.4.2 Mechanical energy. The movement of air or water could also be used to create and maintain chemical disequilibria (Bartlett and Wong, 2020). For example, a hypothetical mechanotrophic underwater organism (Fig. 9.2) attached to a static surface could use the flow of water to rotate an external appendage (such as a flagellum) connected to an internal rotation-induced, ATP-producing protein. The reversible nature of rotary motor proteins of ATP synthetase, used during flagellar motion, suggests such a possibility and is relatively well-studied (Yoshida *et al.,* 2001).

9.2.4.3. Gravitational energy. The idea of life using gravitational energy sources is explored further in the supplementary information.

9.2.4.4. Other radiation energy. Radiotrophy uses radiation as an energy source by converting ionizing radiation (typically gamma radiation) into chemical energy using pigments (*e.g.,* melanin) or similar molecules. Currently, this type of metabolic process has only been proposed in melanized fungi living near Chernobyl (Dadachova *et al.,*

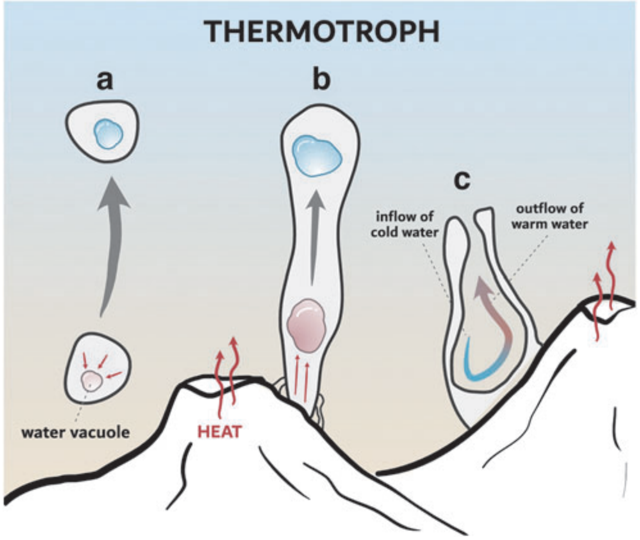

FIG. 9.1. Diagram representing hypothetical thermotrophs with a variety of lifestyles that take advantage of thermal gradients near hydrothermal vents. (**a**) Organism uses the buoyancy of warm water and moves to cooler areas using a water vacuole that has been heated by hydrothermal vents. (**b**) Organism attaches to a hydrothermal vent and moves intracellularly and metabolizes using the thermal gradient. (**c**) Organism uses the flow of cool water into a cavity, which is heated up by hydrothermal vents and subsequently ejected out of the cell, for energy. Adapted from Muller and Schulze-Makuch (2006).

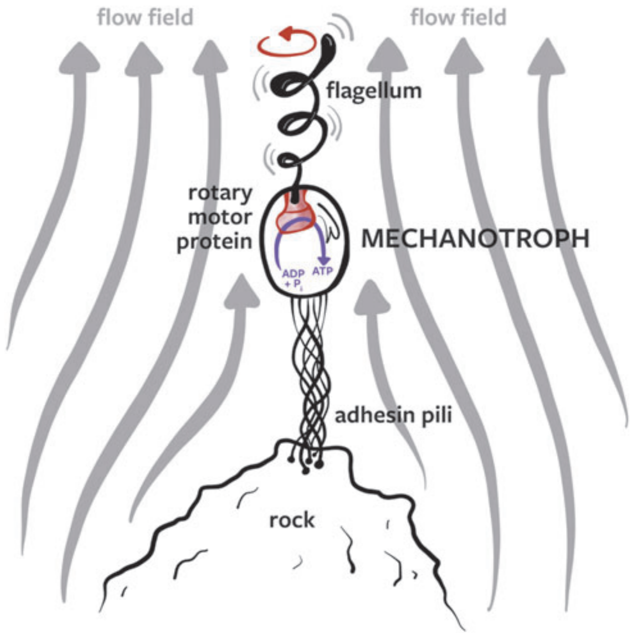

FIG. 9.2. Diagram representing a hypothetical mechanotroph, making use of the flow of surrounding solvent to turn a flagellum attached to a rotary motor protein. This movement is used to make a high-energy covalent bond (using the formation of adenosine triphosphate [ATP] from lower-energy adenosine diphosphate [ADP] and inorganic phosphate [Pi] as an example), thus providing energy for the organism. Reprinted with permission from Bartlett and Wong (2020).

2007), where fungal growth and spore germination are enhanced in the direction of radiation (see Chapter 6.3.5). If terran organisms can thrive in the presence of radiation, perhaps exotic organisms could use it as an energy source directly. Harnessing energy from radiation through the excitation of organic molecules (*e.g.,* pigments) would change the electronic properties of the pigments. For example, exotic life could harness energy through the direct or indirect utilization of these secondary electrons that could be the result of particle attenuation by a surface, such as an icy moon's ice shell (Stelmach *et al.,* 2018).

9.2.4.5. Osmotic gradients.

Environmental osmotic gradients might be a source of chemical energy required to sustain basic ecosystems (Schulze-Makuch and Irwin, 2002), specifically for Jupiter's moon Europa (see Chapter 9.3.2.2). Like hypothetical thermotrophic organisms (see Chapter 9.2.4.1), organisms in such an environment could, for example, move between areas of different salinity (in similar or different phases) or extend themselves between gradients to generate chemical energy (Fig. 9.3). The interface between the ice sheet and liquid water may also provide an environment with the necessary osmotic gradients. Organisms could exist that move through this interface, or live across this interface, with a tethered end and a free end. Osmotic gradients that are present within interfaces, including liquids and atmospheres, are thought to be common in the Solar System.

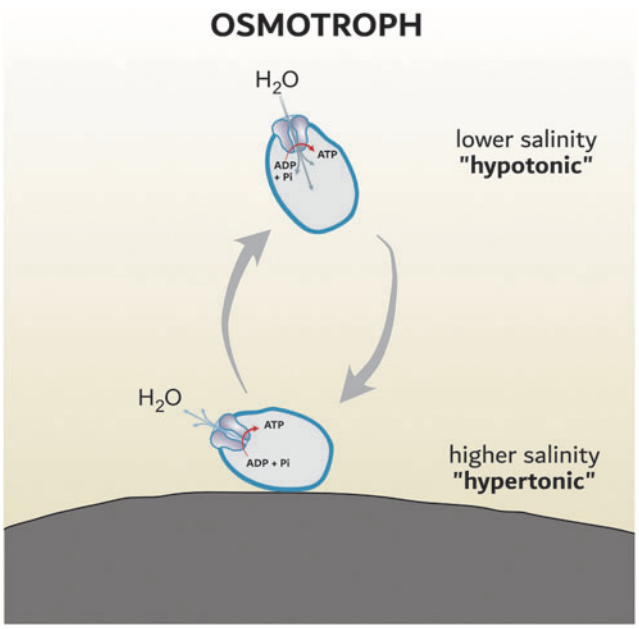

FIG. 9.3. Diagram representing hypothetical osmotrophs, taking advantage of an osmotic gradient. The organism makes use of the movement of water in and out the cell through a membrane complex that transduces the movement into a high-energy covalent bond that can be used by the cell (using the formation of adenosine triphosphate [ATP] from lower-energy adenosine diphosphate [ADP] and inorganic phosphate [Pi] as an example; adapted from Schulze-Makuch and Irwin, 2002).

9.2.5. Alternative information mechanisms

The central dogma of terran biology (see Chapter 2.2.3.1) describes how information in all living systems flows (with few exceptions) from DNA to RNA to protein. This section explores alternative genetic codes and alternative biopolymers that could be involved in exotic informational systems, and potential life that does not store information in polymers but rather propagates information through other means.

9.2.5.1. Alternative genetic codes. All life on Earth shares essentially the same universal genetic code (with minor variations), which determines how the 64 DNA triplet codons map to the 20 standard amino acids used by all living systems during translation. Nevertheless, questions remain. Why is there only one universal code used by life as we know it, and are there other options? Is our genetic code an inevitability resulting from processes that govern the emergence and complexification of life, or is it highly contingent on external conditions? Answering these questions would not only help us resolve some of the deepest mysteries related to the early evolution of life on Earth but also help assess the potential for life based on alternative genetic codes.

A strong indication that alternatives to terran life's genetic code are possible is that our own code has likely changed throughout evolutionary history together with the rest of biochemistry (Koonin and Novozhilov, 2009). Although the specifics are unknown, evidence indicates that early life was based on a primordial genetic code composed of just 10 of the canonical amino acids and possibly noncanonical amino acids that progressively evolved to give rise to the modern code (see Chapter 4.2.4.1). Alternative or expanded genetic codes have been engineered experimentally to include nonstandard amino acids for synthetic biology applications (Bain *et al.,* 1989; Noren *et al.,* 1989; Chin *et al.,* 2003), further demonstrating that life may not be confined to the known terran genetic code.

9.2.5.2. Alternative informational polymers. Genetic encoding and informational systems used by life could conceivably be based on alternative polymers to the canonical nucleic acids (RNA and DNA) and proteins. For example, meteorites and prebiotic synthesis experiments such as Miller-Urey spark-discharge syntheses contain a wide variety of amino acids that are not present in life's genetic code (see Fig. 3.9 and Chapter 3.3.3), and alternative sets of amino acids could potentially cover the space of necessary biochemical functions (Ilardo *et al.,* 2015). Similarly, noncanonical nucleobases have been discovered in meteorites, opening the possibility of using alternative nucleobases in DNA or DNA-like structures (Geyer *et al.,* 2003; Yang *et al.,* 2006). Another newly explored genetic system is the so-called Hachimoji, an expanded informational system built with eight building blocks instead of four (Hoshika *et al.,* 2019). This system can be transcribed into Hachimoji RNA and has been shown to be thermodynamically stable, with greater information density than the canonical four-base DNA. Thus, the ubiquitous presence of DNA as an informational polymer in terran biology may be due not to its functional superiority but simply to chance, suggesting that life elsewhere may not be restricted to such a system.

Informational polymers with a different backbone than the ones used in DNA and RNA have also been proposed. Polymers containing altered sugar backbones with alternative nucleobases (*e.g.,* peptide nucleic acids) are termed xeno-nucleic acids (XNAs) (Pitsch *et al.,* 1995) and could have predated RNA/DNA while being compatible with a later genetic takeover by endogenous nucleic acids (Eschenmoser, 1997). Some researchers have even suggested that the presence of a repeating backbone charge is a requirement for a functional genetic biopolymer to be capable of Darwinian evolution. Thus, genetics in exotic life-forms could also be based on polyelectrolytes (Benner, 2017) or even polymers with an uncharged backbone that could also be capable of directed evolution (Arangundy-Franklin *et al.,* 2019).

Other polymeric structures (*e.g.,* polypeptides) can exhibit properties that could make them candidates for the basis of an informational system. This includes the ability for peptides to replicate through a form of nonlinear autocatalysis (*i.e.,* hypercycles), where the synthesis of one peptide catalyzes the synthesis of another, which ultimately contributes to its own production (Lee *et al.,* 1997). Certain small peptides have also demonstrated self-replicating properties in a sequence-specific manner (Lee *et al.,* 1996). However, limitations to peptides as sequence-based informational polymers include their formation of secondary and tertiary structures and their propensity to cluster together.

9.2.5.3. Alternatives to polymer-based genetics. Compositional, or analog, information is harbored in the components of a system, whether they are molecules in a cell or chemical species in a metabolic network (Segré and Lancet, 2000). In contrast to the kind of ''digital'' information encoded in linear biopolymers, compositional information does not require the presence of complex structures to be constructed and maintained and may offer an alternative to polymer-based genetics.

In models of analog inheritance, groups of molecules store heritable differences in their composition (Shenhav *et al.,* 2003). Computational approaches show that different groups of molecules could exist in multiple states that can conserve their compositional integrity as they grow and split through the subsequent formation of complex autocatalytic feedback loops that resemble metabolic pathways (Kun *et al.,* 2008; Xavier *et al.,* 2020). These molecular assemblies can also undergo changes that lead to a transition from one compositional state to another, for example when single molecules with advantageous capabilities are randomly added to the assembly (Segré *et al.,* 2000). This work suggests that the inheritance of compositional information can arise in groups of molecules in the complete absence of linear encoding and digital biopolymers.

Similarly, surface-limited metabolism models (SLiMes) suggest that autocatalytic chemical systems might spontaneously form on mineral surfaces and display key life processes such as the ability to self-propagate and evolve (Wächtershäuser, 1988; Baum and Vetsigian, 2017; Vincent *et al.,* 2019). In these models, SLiMes are chemical reaction networks composed of multiple autocatalytic cores. The initiation of a new core by a rare reaction can function like a mutation, moving a localized SLiMe (*i.e.,* one that is absorbed on a mineral surface) to a new state characterized by a different chemical composition. Selection among competing systems with distinct compositions can allow for adaptive evolution without complex polymer-based genetics. These alternatives to traditional genetics provide tantalizing models of potential living systems that may look very different from life as we know it and challenge the notion that life requires polymers.

9.3. Where to Look for Exotic Life

There are several promising locations to search for exotic life in the Solar System. Some icy moons around the gas giant planets have subsurface liquid water oceans and have been proposed as important astrobiological targets for terran-like life (see Chapter 7.2). However, these potentially habitable worlds, and even gas giants themselves, may be more likely to host exotic life due to their different evolutionary histories and different geochemical environments (*e.g.,* lack of abundant solar energy). This section discusses the potential energy sources and chemical composition of several Solar System bodies that make them prime candidates to search for exotic life.

9.3.1. Terrestrial planets

9.3.1.1. Shadow biosphere(s) on Earth. The search for exotic life is not necessarily an interplanetary task. Indeed, some exotic life-forms have been found on Earth in areas that were previously thought uninhabitable (*e.g.,* radiotrophic fungi, see Chapter 6.3.5.3, or microorganisms living in spacecraft assembly clean rooms, see Chapter 10.3.1). One theory posits that Earth has a shadow biosphere whose inhabitants are yet undetected (Davies *et al.,* 2009). This theory stems from the idea that there were potentially multiple origins of life on Earth (or a divergence shortly after the origin of life), resulting in distinct biochemistries and concomitant biospheres that could be ecologically separated or integrated. Evolutionary divergence between these biospheres would create lineages of life that have either gone extinct or persist but remain undetected (Bartlett and Wong, 2020). Examples of these hypothesized life-forms include ones with RNA-based information processing, ones with differing chirality, ones using different sets of amino acids and/or nucleotides, or even life built on a different elemental set (Davies *et al.,* 2009).

9.3.1.2. Mars. As discussed in Chapter 7.2.1, Mars is a popular candidate for the search for life as we know it. In comparison with other planetary bodies, Mars has, in general, abundant energy sources. One of these sources is serpentinization that could produce high energy yielding chemical sources (*e.g.,* CH_4 and H_2; see Chapter 7.2.1.1), similarly to those proposed for the origin of life on Earth (see Chapter 4.2.3.1). Despite some similarities to terran environments and energy sources, Mars' different evolutionary history also makes it a candidate for hosting exotic life. In cases like Mars, where there are some of the necessary ingredients for terran life to develop (*e.g.,* presence of water), there is a need to be more open to the possibility of exotic forms of life having developed there and to change our approach to the search for extraterrestrial life accordingly (Johnson *et al.,* 2018; Carrier *et al.,* 2020; Chou *et al.,* 2021a). For example, Houtkooper and Schulze-Makuch (2007) hypothesized martian organisms utilizing a mixture of water and hydrogen peroxide as their intracellular solvents, which would lower the freezing point of the solvent and thereby extend the habitable regions to sub-arctic regions of Mars.

9.3.1.3. Venus. Venus is currently deemed inhospitable and probably uninhabitable to life as we know it due to its high surface temperatures and corrosive environment (see Chapter 7.2.2). However, scientists have long speculated on the possibility of life existing in the lower atmosphere of the planet where the cloud layers have atmospheric pressures like Earth's (Morowitz and Sagan, 1967; Grinspoon, 1997; Kotsyurbenko *et al.,* 2021). Although intense solar radiation potentially limits the habitability of these regions for Earth-like life-forms, radiation-tolerant microbes living in Venus's atmosphere have been imagined that use elemental sulfur as a protective coating against radiation (Schulze-Makuch *et al.,* 2004). In addition, the lower atmosphere of Venus is thick and liquid-like, facilitating chemical exchange between the cloud layer and the surface (Krasnopolsky, 2015). These conditions might allow for a dynamic environment and sustained thermodynamic disequilibria that life can take advantage of (Schulze-Makuch and Irwin, 2018a). More recently, Seager *et al.* (2021) proposed a spore-forming life cycle for hypothetical venusian microbes, where the organism can grow and reproduce for a time while drifting upward within cloud droplets, then dries out into a spore when the droplet becomes large enough to fall. Spores would then collect in lower haze layers (33–48 km), and turbulent phenomena would periodically lift them upward into the clouds to begin the life cycle again.

Life on Venus could also be based on different biochemistry than terran life by taking advantage of abundant alternative solvents in its atmosphere. For example, Benner *et al.* (2004) speculated that sulfuric acid, which may be as concentrated as 98% in certain altitudes (Krasnopolsky, 2015), could serve as an alternative solvent for exotic life (see Chapter 9.2.2.2). Sulfuric acid could exist in the form of aerosol droplets that may persist for several months in Venus's atmosphere as opposed to several days for cloud particles on Earth (Grinspoon, 1997). In addition, the high pressure and temperatures of the venusian surface (see Chapter 7.2.2) cause high levels of CO_2 to be supercritical and may provide a potential alternative solvent that could support exotic life (Matsuda, 2013; Schulze-Makuch and Irwin, 2018c).

9.3.2. Icy moons

9.3.2.1. Titan. Titan, the largest moon of Saturn, is an intriguing astrobiological target for the search for exotic life (for more information on Titan and its habitability for life as we know it, see Chapter 7.2.5). Like Earth, Titan's surface is speckled with rivers and lakes and clouds that periodically release flurries or generate storms. Yet these dynamic liquids are nothing like those on Earth. They are composed of hydrocarbons. The surface temperature on Titan is so cold (about -179°C) that methane is the predominant liquid. Photochemical reactions between methane and nitrogen in the atmosphere create numerous organic molecules that drift down to the surface, mixing with lakes and settling atop shifting hydrocarbon dunes (Raulin, 2008). Because light intensity on Titan is low due to a hazy atmosphere, it is possible that potential life-forms there have evolved to rely on non-solar energy sources such as tidal forces, osmotic gradients, or even thermal gradients (see Chapter 9.2.4).

A new framework is needed to imagine what life could be like on such a different world. For example, titanian life could be based on methane liquids (see Chapter 9.2.2.4). Life there would also need to be adapted to low temperature conditions. While the nonpolar nature of methane eliminates the use of lipids as membrane materials, Stevenson *et al.* (2015) proposed an alternative membrane composition made up of small organic nitrogen compounds that could fill a similar role as lipid membranes play in Earth life (see Chapter 9.2.3.1). Several sources of chemical energy are available at Titan to fuel metabolic activities, including the hydrogenation of photochemically produced acetylene into methane and the production of cyanide and cyanamide compounds from nitrogen and methyl radicals (Schulze-Makuch and Grinspoon, 2005). NASA's upcoming Dragonfly mission will explore Titan's surface and scout for signs of life.

A subsurface liquid ocean may also exist beneath the surface of Titan (Bills and Nimmo, 2011; Iess *et al.,* 2012) and could be suitable for the development of life (Fortes, 2000). Life in this ocean would have to be based on alternative biochemistry, as the cryogenic conditions and high pH of the putative ammonia-rich liquid would destabilize most terran biomolecules (see Chapter 9.2.2.1). The weathering of silicate materials in Titan's core by this subsurface ocean could also provide chemical energy to putative organisms living in Titan's interior (Fortes, 2000).

9.3.2.2. Europa.

As is the case with other icy moons, sunlight cannot penetrate Europa's icy shell, and any life-forms existing in its subsurface oceans would have to rely on alternative sources of energy. Radiogenic heating and tidal forces might induce high-temperature hydrothermal fluids to escape from Europa's silicate mantle into the cold-water ocean. These energy sources might also drive oceanic convection and the transport of nutrients and other molecules (Zolotov and Shock, 2003; Barr and Showman, 2009). Research has shown that geothermal radiation from hydrothermal vents on Earth could be used to support certain phototrophic microorganisms (Beatty *et al.,* 2005). Furthermore, these hydrothermal fluids coupled to a rocky interior could lead to serpentinization reactions and to significant chemical fluxes of hydrogen (Vance *et al.,* 2016), which could be used as energy by organisms. Osmotic gradients at the ice-water interface or at the bottom of the ocean of Europa, or thermal gradients and mechanical energy produced by radiogenic heating and oceanic convection, could also produce energy necessary to support putative forms of exotic life (see Chapter 9.2.4). Chemical disequilibria in the oceans of Europa have also been theorized to provide a source of chemical energy for potential oceanic life that may make use of oxidized species, which include CO_2 and SO_4^{2-}, and reduced species, which include H_2 and Fe^{2+}, among others (Schulze-Makuch and Irwin, 2002).

9.3.2.3. Enceladus and other icy moons.

On Enceladus, the power output from its cryo-volcanically active south polar region is roughly 10 times larger than what would be expected due to internal heating alone (Spencer *et al.,* 2006). This output, combined with extensive tectonic activity and high-activity plumes, points toward extensive additional heating due to tidal interactions with Saturn (Schubert *et al.,* 2007). Furthermore, silicates detected in Enceladus' plumes suggest that the material was propagated up from the moon's interior through the potential ocean layer via an internal heat source (Parkinson *et al.,* 2008). In addition, H_2 was also present in abundance in these plumes as detected by the Cassini mission (Waite *et al.,* 2017). These detections may point toward serpentinization reactions occurring. Similar serpentinization processes might be occurring in other icy moons, such as the jovian moons Ganymede and Callisto, due to the presence of water-rock interfaces and tidal and radiogenic forces (Vance *et al.,* 2007). An internal heat source and tidal friction would generate thermal gradients within these oceans that, alongside assumed osmotic gradients (Matson *et al.,* 2007; Parkinson *et al.,* 2008), may be able to support metabolic processes.

9.3.2.4. Other planetary objects.

The potential for life as we don't know it on planetary objects such as gas giants and rogue planets is explored in the supplementary information.

9.4. How to Look for Exotic Life: Agnostic Biosignatures

As discussed in Chapter 8, most established strategies for biosignature detections are based on the identification of well-established features of terran life (*e.g.,* the presence of certain molecules, isotopic ratios, enantiomeric ratios and compositions, and morphological patterns). However, the possible existence of exotic life may require that descriptions of life, and thus biosignatures, expand beyond Earth-centric perspectives. This is particularly important considering upcoming missions to ocean worlds that are isolated from exchange with terrestrial material and thus may contain life that does not share a common ancestry to ours. It is critical to abstract our conception of life and search for signatures of life in general, independent of its molecular basis (Johnson *et al.,* 2018; Cleland, 2019; Carrier *et al.,* 2020; Chou *et al.,* 2021a). Signatures of life that do not presuppose a particular molecular framework are referred to as ''agnostic biosignatures'' (Fig. 9.4).

Research in agnostic biosignatures seeks to identify unfamiliar features and alternative chemistries and biochemistries by using existing instrumentation in more inclusive ways, pursuing new leads, and analyzing data with probabilistic approaches. Some attributes of living things that can help distinguish them from inanimate matter are the ability to self-replicate, make use of diverse energy sources, and modify the surrounding environment to survive. Proposed agnostic biosignatures rely on such features of life and their natural consequences; they rely on ''what life does'' rather than ''what life looks like.''

Agnostic biosignatures, much like biosignatures based on terran life, need to be analyzed in the context of the environment in which they are (potentially) detected to ensure that they are unlikely to have arisen abiotically. It is important to study such proposed biosignatures using probabilistic models and considering results from several types of measurements in combination to decrease the likelihood of either a false positive or false negative signal (see Chapter 8.6).

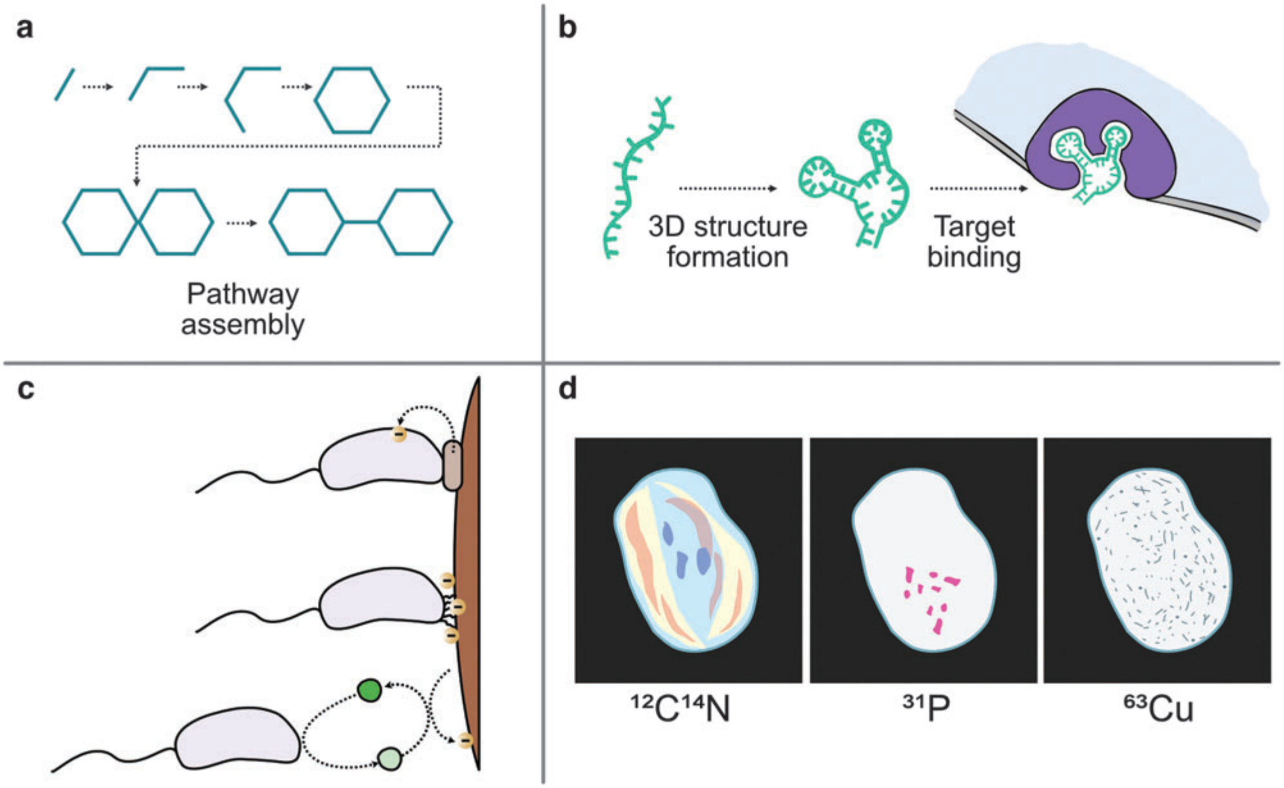

FIG. 9.4. Examples of proposed agnostic biosignatures: (**a**) molecular complexity; (**b**) fingerprinting surface complexity; (**c**) energy and metabolism agnostic biosignature; (**d**) structural, morphological, physical agnostic biosignature. Panel (b) is adapted from Johnson *et al.* (2018).

9.4.1. Chemical agnostic biosignatures

It can seem counterintuitive to talk about chemical agnostic biosignatures when we previously mentioned that, by definition, we are not presupposing any specific chemistry in unknown life-forms. However, chemical agnostic biosignatures refers to patterns in compounds (such as molecular complexity) or patterns within groups of compounds and samples. Astrobiologists agree that life as we know it tends to promote and express complex chemistry (Emmeche, 1997; Ruiz-Mirazo *et al.,* 2004). If a highly complex chemical compound (similar in complexity to an enzyme or DNA, for example) is found in a sample in significant abundance, it could be considered a potential product of biology (Fig. 9.4a; Marshall *et al.,* 2017). Similarly, a distinct distribution of compounds or chemical disequilibrium that is unlikely to occur abiotically could be a sign of a focused metabolism and thus could be a sign of life (McKay, 2004; Dorn *et al.,* 2011). This signature would not need to be specific to any metabolism and its resulting byproducts. For these signatures to be robust, the probability that a molecule or a distribution of molecules was produced abiotically or not must be established, and they must be analyzed within the context of the surrounding environment.

Another line of reasoning concerns the ubiquitous use of polymers in terran life. Polymers can explore a large functional chemical space (*i.e.,* many configurations can perform many different functions) due to their intrinsic modular structure (different combination of monomers leading to diverse structures). It is likely that these efficient functional entities have been selected into central roles in living organisms on Earth. This modularity also allows for the safeguard of information that can easily be copied and propagated. Researchers have suggested that polymers could have been harnessed by other possible biologies (Chapter 9.2.5.2), and polymeric chemical structures should be considered when searching for life (Benner and Hutter, 2002).

Other proposed chemical agnostic biosignatures are excesses in certain elements, isotopes, enantiomers, or even an overabundance of chiral molecules in general, when compared to the background environment (Glavin *et al.,* 2020). These types of chemical signatures can be searched for using current flight-ready technologies such as mass spectrometry and tandem mass spectrometry (see Chapter 8.2.2.1). Data collected by these instruments could help identify patterns linked to the presence of polymers, the complexity of a compound, or the distribution of compounds within a sample (Chou *et al.,* 2021b).

A recently proposed application of a common nucleic acid sequencing technology, referred to as the ''fingerprinting surface complexity'' method (Fig. 9.4b; Johnson *et al.,* 2018), utilizes oligonucleotides' ability to form a wide range of structures that have unique binding specificities and thus can bind selectively to different compounds (Stoltenburg *et al.,* 2007; Cleaves *et al.,* 2011; Sun and Zu, 2015). The resulting binding affinities can be explored through well-documented amplification and sequencing methods to inform us about the diversity of binding sites, giving us some insight into the heterogeneity of the sample that could reflect the potential biogenicity of a sample.

9.4.2. Structural, morphological, and physical agnostic biosignatures

When it comes to the morphological aspects of exotic life, there is reason to believe it might also take the basic form of a structurally separated or encapsulated structure distinct from its environment (see Chapter 9.2.3). The idea that ''molecules that stay together evolve together'' suggests that co-localization is important and might be essential to evolution (Szostak *et al.,* 2001; Orgel, 2004). Well established microscopy techniques can be used to look for structural differences that might be indicative of localized structures stemming from biology (organisms, groups of organisms or even ecosystems, see Chapter 8.1.2). Such structures might look similar even if composed of different biochemistry (Fig. 9.4d). Another proposed agnostic feature of life accessible by imaging techniques is nonrandom motility, wherein organisms move to search their environment for possible food and energy sources.

9.4.3. Energy and metabolism agnostic biosignatures

Metabolism is considered one of the most distinctive features of life as we know it (Neveu *et al.,* 2018). Ways to approach the detection of metabolisms discussed here include evidence of consumption and/or waste production, evidence of electron exchange, and evidence of specific chemical network structures.

Consumption-release experiments have been proposed (and even attempted in the Viking experiments; see Chapter 8.2.5.1 in the supplementary material for Chapter 8), as has the search for disequilibrium redox reactions distinct from abiotic processes. These kinds of agnostic biosignatures would not presuppose certain possible food sources or waste products based on terran biochemistry but rather would extrapolate from our understanding of the theoretical metabolisms possible in certain conditions and design experiments based on those theories. Evidence of energy transfer that could be fueling metabolic processes is another agnostic biosignature that could be linked to active metabolism (Fig. 9.4c) and could be measured using electrochemical probes (see Chapter 8.2.2.4). Finally, analysis of statistical patterns in chemical reaction networks of biological systems (Wuchty *et al.,* 2006) and in the atmosphere of Earth appear to be scale-invariant (Solé and Munteanu, 2004; Kim *et al.,* 2019). If some network features (*e.g.,* scale invariance) are a universal feature of life, then analyzing network topologies may present a method of differentiating living systems from nonliving systems, independent of the underlying biochemistry or the specifics of the potential metabolism.

9.5. Conclusion

Discussions about the existence of life elsewhere in the universe often stem from assumptions based on terran biology. Consequently, research into the origins of life, habitability, and life detection beyond Earth are biased toward life as we know it. Researchers might be missing insights into life in general or missing opportunities to discover unfamiliar or exotic forms of life (even here on Earth). The research described in this chapter attempts to broaden our perspectives on what life is, what it can be made of, where it could live, and how we could detect it. We acknowledge that it is difficult to truly be free of our terran bias, even when collating information about life as we don't know it. There is still much exciting work to be done to help increase our knowledge in these areas, helping us get closer to understanding what life is. Nevertheless, it is important to keep in mind where our assumptions come from and attempt to alleviate our terran bias by asking ourselves this vital question: how different could life be?

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Author Contributions

N. Grefenstette co-managed the team, co-edited and co-developed the structure of the chapter, and co-authored the chapter introduction, section introductions, and conclusions. N. Grefenstette co-authored the how the origin of life on Earth can inform us about life as we don't know it section, the how different life could be section, other planetary objects in where to look for exotic life section, and co-authored the how to look for life: agnostic biosignatures section.

L. Chou co-managed the team, co-edited and co-developed the structure of the chapter, and co-authored the chapter introduction, section introductions, and conclusions. L. Chou authored the Mars, Venus, Europa, and Enceladus sections and co-authored the how to look for life: agnostic biosignatures section.

S. Colón-Santos authored the metal oxides-based life, the formamide section, and co-authored the non-membrane compartments section.

T. Fisher authored the methane and supercritical CO_2 sections and contributed to the agnostic biosignatures section.

V. Mierzejewski authored the silicon-based and co-authored the boron-based life section.

C. Nural authored the gas phase life section, co-authored the non-membrane compartments section, and contributed to the alternative membranes section.

P. Sinhadc co-authored the how the origin of life on Earth can inform us about life as we don't know it section and the alternative genetic code section.

M. Vidaurri authored the alternative energy source section, including thermal, mechanical, gravitational, nuclear decay, osmotic gradient, tidal energy, radiogenic heating, and serpentinization section, as well as the shadow biosphere section.

L. Vincent authored the sulfur-based life, ammonia, sulfuric acid, alternative informational polymers, and alternatives to polymer-based genetics section, and co-authored the boron-based life and alternative genetic code section.

M.M. Weng authored the alternative membranes section and the Titan section and co-authored the non-membrane compartments section.

Supplementary Material

Supplementary Information
Supplementary Figure 9.S1

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Abbreviations Used

iCHELLs = inorganic chemical cells
POMs = polyoxometalates
SLiMes = surface-limited metabolism models