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► To cite this version:

Lorenzo Carré, Giuseppe Zaccai, Xavier Delfosse, Éric Girard, Bruno Franzetti. Relevance of earth-bound extremophiles in the search for extra-terrestrial life. *Astrobiology*, In press, 22 (3), pp.322-367. 10.1089/ast.2021.0033 . hal-03400442

HAL Id: hal-03400442

<https://hal.science/hal-03400442>

Submitted on 25 Oct 2021

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Astrobiology

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Journal:	<i>Astrobiology</i>
Manuscript ID	AST-2021-0033
Manuscript Type:	Reviews
Date Submitted by the Author:	08-Mar-2021
Complete List of Authors:	Carré, Lorenzo; Institut de Biologie Structurale Zaccai, Giuseppe; Institut de Biologie Structurale; Institut Laue-Langevin Delfosse, Xavier; Laboratoire d'Astrophysique de Grenoble Girard, Eric; Institut de Biologie Structurale Franzetti, Bruno; Institut de Biologie Structurale,
Keyword:	Habitability, Thermophiles, Halophiles, Psychrophiles, Piezophile
Manuscript Keywords (Search Terms):	Macromolecules, Biophysics, Biochemistry, Extreme conditions

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Relevance of earth-bound extremophiles in the search for extra- terrestrial life

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Extremophile biochemistry for exobiology

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Keywords

Astrobiology – Extremophiles biochemistry and biophysics – Extraterrestrial environments –

High pressure – Extreme temperatures – Hypersalinity

Abstract

The recent discovery of extrasolar earth-like planets orbiting in their habitable zone of their system, and the latest clues of presence of liquid water in subsurface of Mars and in subglacial ocean of Jupiter and Saturn moons, have reopened debates about habitability and limits of life. While liquid water, widely accepted as an absolute requirement for terrestrial life, may be present in other bodies of the solar system or elsewhere, physical and chemical conditions, such as temperature, pressure and salinity may limit this habitability. Nonetheless, life is observed in various extreme terrestrial environments which had been previously thought too harsh. Extremophilic microorganisms found in these environments are adapted to thrive in permanently extreme ranges of physical and chemical conditions. Their biochemistry and biophysics are currently studied actively, in an exploration of the limits for life at a molecular scale. In the context of habitability, these approaches may guide the search of extraterrestrial life. In this review, promising environments for life in the Solar System are discussed in terms of habitability by terrestrial-like extremophiles and the adaptive biophysics and biochemistry of extremophiles growing, respectively, in extreme temperature, pressure or salinity, are reviewed in some detail.

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Introduction

The existence of extraterrestrial worlds and life has long been debated by philosophers and thinkers (Gress and Mirault, 2016). The Copernican Revolution in particular has dramatically strengthened the idea that Earth is not the center of Universe and that similar world may host life elsewhere (Peretti, 2019; von Hegner, 2019b). Nonetheless, the question of extraterrestrial worlds and life has been addressed long before, for example during the occidental medieval period by theologians or philosophers such as Nicolas de Cues, Thomas Bradwardine or Jean Buridan. Scientific questions about extraterrestrial life are now at the center of the new field of astrobiology.

This thematic is undergoing a revolution by recently becoming a genuine scientific topic – with objects to be studied – thanks to major advances in several areas describing habitability of different bodies outside the Earth. The presence of liquid water is generally the first criterion (but not the only one) to define the habitability of extraterrestrial worlds. There is now irrefutable evidence that water flowed on the surface of Mars during its early phases (Wordsworth, 2016). The icy moons of the giant planets are also of considerable interest for the search of life since the presence of a water ocean under the icy crust is inferred for Ganymede, Europa, Enceladus and Titan (Kivelson *et al.*, 2000; Rambaux *et al.*, 2011; Thomas *et al.*, 2016). Beyond the Solar System, the last quarter of a century has seen the discovery of first extrasolar planets (Mayor and Queloz, 1995), followed a few years later by the discovery of telluric exoplanets in habitable zones around their stars (Delfosse *et al.*, 2013; Bonfils, Curto, *et al.*, 2013; Anglada-Escudé *et al.*, 2016). If such planets of satellites can host liquid water on the surface or below the surface, the conditions of pressures, temperatures,

salinities, pH, etc can however be extreme for a terrestrial type of life, and astrobiology is interested on eventual life forms in very diverse environments.

Astrobiology could be defined as “the study of the origin, evolution, distribution (and future) of life in the universe: extraterrestrial life and life on Earth” (Cottin, 2019). The term was first proposed in 1953 by the astronomer Gavriil Tikhov (Tikhov, 1953) and the field was first theorized by the biologist Joshua Lederberg in 1960 under the synonym term of exobiology (Lederberg, 1960). In fact, to date there is only one kind of life known: terrestrial life. Every cell on Earth has descended from a common ancestor and shares the same basic chemistry and biological processes. The discovery of a new kind of life, independent from the terrestrial life, would be of huge scientific interest.

Still, a general definition of life is required if we were to search for extraterrestrial one (von Hegner, 2019a). Many definitions have been proposed based on thermodynamics, chemical composition or evolution but as there is only one example of life to date, all definitions will be geocentric. It has been proposed that terrestrial life could be defined as a “genome-containing, self-sustaining chemical dissipative system that maintains its localized level of organization at the expense of producing entropy in the environment; which has developed its numerous characteristics through pluripotential Darwinian evolution” (von Hegner, 2019a).

Another way to understand life in regards to astrobiology is through its limits (Takai, 2019). Biological processes are based on the chemical reactions between the molecules constituting organisms: biomolecules. Physical and chemical properties of biomolecules determine the range of conditions compatible with their functions and thus the limits of biological activity. As a consequence, life has long been seen as a fragile thing, needing liquid

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water abundancy, warm temperatures, moderate salinity, no ionizing irradiation, etc (von Hegner, 2020).

In this context, the discovery of extremophiles has pushed farther away the limits of life. Many natural terrestrial environments have first appeared to be too harsh and incompatible with persistent life: hyperacidic volcanic hot springs, hyperbaric deep abysses, subzero polar environments or saturated salt lakes. Nonetheless, organisms permanently living in these extreme environments and adapted to live under their extreme conditions, have been discovered. While presence of microscopic infusory-like life in hypersaline lake has been hypothesized by Darwin during its journey on the Beagle (Darwin, 1839), the term of extremophiles was first coined in 1974 (Macelroy, 1974). By opposition, non-extremophilic life can be defined as mesophilic (living between extremes). As life could be found in new unsuspected environments, it has also appeared that classical definitions of life and habitability are generally mesocentric and even the definition of an extreme condition, environment or extremophile may present the mesocentric bias.

As a consequence, there is an opposition between two points of view, two ways to understand terrestrial life with consequences on the apparent probability of finding extraterrestrial life: 1) Life is fragile, needs precise conditions to appear and to develop and is thus uncommon in the Universe or 2) Life can adapt to many environments and large panel of physical and chemical conditions and could be more common in the Universe, widening the criterions of habitability. It should however be noted that the existence of extremophiles does not infer that life can appear in extreme environments.

In this review, a description of extraterrestrial environments promising for the search of life and a description of their extreme conditions will be provided. Then an overview of the

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3 impact and relevancy of extremophilic life in astrobiology will be discussed. Focusing on
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5 structural and dynamical scale, basis of biochemistry will be given in order to understand how
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7 biomolecules and biological processes are altered by extreme conditions. Molecular traits
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9 found in extremophiles allowing life under one or multiple extreme conditions will then be
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Where to search for life? Promising extraterrestrial environments

The search for extraterrestrial life, current or traces of past biological activity, is tightly related to the search for habitable environments (Javaux and Dehant, 2010). Because there is, so far, only one known kind of life yet, our notion of habitability is constrained by our understanding of Terran life, its origins, its evolution and its limits (O’Malley-James and Lutz, 2013; Zaccai, 2020). Even if not sufficient, liquid water is strictly essential for life as we know it (Jones and Lineweaver, 2010). The search for extraterrestrial liquid aqueous environments is thus fundamental.

In this context, the discovery of exoplanets within the so-called habitable zone associated with their star is promising and highly exciting (Gillon *et al.*, 2017). To be considered as potentially habitable, planets must be within the proper range of orbital distances to allow surface temperatures such that liquid water can be stable on their surface (Kasting *et al.*, 1993; Selsis *et al.*, 2007). This constraint also imposes limits on the planet mass and atmosphere conditions to be within the atmospheric surface pressure range allowing water to be liquid. The greenhouse effect plays a crucial role for the surface temperature, and is estimated in using climate models adapted to the characteristics of the central star. These models are becoming increasingly sophisticated from global one-dimensional models to a new generation of 3D Global Climate Model. But they cannot take into account the particularities of each planet, or even the composition of its bulk and its atmosphere which are unknown today. An important breakthrough will be made in the future, since some of these worlds in habitable

zones will become amenable to characterization of their atmosphere through transit spectroscopy either with James Web Spatial Telescope (JWST) (Doyon *et al.*, 2014; Beichman *et al.*, 2014) or by combining high-dispersion spectroscopy with high contrast imaging with Extremely Large Telescope (ELT) (Snellen *et al.*, 2015), and that a highly ambitious space missions such as the Large UV/Optical/Infrared Surveyor (LUVOIR), which could be in operation in the middle of the century, will have as its main objective the characterization of atmospheres of Earth-like planets around solar type stars. But today, if we know that telluric planets in habitable zones are very common (Bonfils, Delfosse, *et al.*, 2013; Dressing and Charbonneau, 2015; Bryson *et al.*, 2020), their environment is globally unknown to us and we can only imagine their diversity. So our review will focus on the better-known characteristic of the bodies of the Solar System.

Earth is the only planet in the Solar where solid, liquid and gaseous water co-exist on the surface. A co-existence that is the basis of the cycles that create the climatic conditions that shape our environment. It has been shown, however, that transient liquid water is possible on the surface of Mars (Möhlmann and Thomsen, 2011; Chevrier and Rivera-Valentin, 2012; Martín-Torres *et al.*, 2015). Subsurface Martian environments appear to be more habitable with persistent aqueous environments (Clifford *et al.*, 2010; Jones *et al.*, 2011; Orosei *et al.*, 2018), raising hope for the existence of a deep Martian biosphere, analogous to the deep biosphere of Earth's crust (Richard A. Kerr, 1997; Itävaara *et al.*, 2016) or deep subglacial Antarctic habitats (Michalski *et al.*, 2013; Mikucki *et al.*, 2015).

Farther away from Earth, liquid water is abundant under the surface of other bodies of the Solar System. Multiple evidence shows that several icy moons of giant planets possess liquid water oceans beneath a thick crust of ice. Among them, Enceladus, a moon of Saturn,

and Europa, a moon of Jupiter, are of particular interest for astrobiology and the search for life (Gaidos *et al.*, 1999; Jebbar *et al.*, 2020a)

Mars brines and depths

Several current geological aspects have given birth to the hypothesis of a water ocean covering large portions of Mars surface approximately 3-4 billion years ago (Brandenburg, 1987; Chapman, 2003; Di Achille and Hynek, 2010). This hypothesis proposes that surface habitable aquatic environments may have existed on Mars (Grotzinger *et al.*, 2014). Moreover, geochemical traces of past hydrothermal activity on Martian surface lead to the hypothesis that environments similar to acidic terrestrial Laguna Caliente lake (Hynek *et al.*, 2018) or El Tatio geothermal field (Barbieri and Cavalazzi, 2014), which are both habitable and colonized by extremophiles, may also have existed. The permanence of this ancient ocean remains an open question, climate models of Mars having difficulties in reconciling its potential existence in these period with the surface temperature estimated to be too cold, resulting in most surface water having to be trapped in ice (Head *et al.*, 2018; Palumbo and Head, 2018; Turbet and Forget, 2019). This enigma might be solved if the atmosphere of ancient Mars was dominated by H₂ and CO₂. Recent 1D numerical radiative-convective climate calculations suggest that this is a viable solution, and may be the only one, to warm the surface of Mars above the melting point of water (Turbet *et al.*, 2020) but this remains to be confirmed by 3D Global Climate Models computations.

However, even if the Martian surface was habitable in the past, it is now considered to be too extreme for life to develop and persist (McCollom, 2006). Major barriers are extreme aridity, cold temperature, abundance of perchlorates which strong oxidative power would impair most biomolecules (Quinn *et al.*, 2013; Wadsworth and Cockell, 2017) and intense UV irradiation which degrades organic matter too. It is also expected that iron, which is abundant in Martian soils, may catalyzes this degradation process (Stalport *et al.*, 2019). The main place where to look for life on the Red Planet may, therefore, be below the surface. In particular, a liquid water body has been recently discovered under the southern ice cap (Orosei *et al.*, 2018).

In the hope of finding active current biological activity or traces of past life, future exploration missions such as ESA ExoMars or NASA Mars 2020 will carry equipment such as rovers designed for studying subsurface environments with a special emphasis on biomarkers (Simoneit *et al.*, 1996; Vago *et al.*, 2015; Williford *et al.*, 2018). Indeed, the Martian subsurface is expected to be less harsh than the surface, shielded from sublimation and UV irradiation and where persistent liquid water within brines could be present and stable (Martínez and Renno, 2013).

The possibility of a present or past deep Martian biosphere in subsurface waters, based on chemosynthesis and analogous to terrestrial subsurface ecosystems, has been largely hypothesized (Boston *et al.*, 1992; Michalski *et al.*, 2013; Grotzinger *et al.*, 2014; Mikucki *et al.*, 2015). However, putative Martian subsurface organisms would have to cope with desiccation-rehydration cycles and fluctuating salinity, constraints that would select adaptations to cope with high salinity (Reid *et al.*, 2006; Kendrick and Kral, 2006; Vauclaire *et al.*, 2020). In particular, Martian brines may reach molar concentrations of various salt ions,

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3 similarly to the salinities of terrestrial hypersaline lakes such as Great Salt Lake or Dead Sea
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5 (Fox-Powell *et al.*, 2016).
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8 Temperatures are expected to be higher at greater depths under the Martian surface
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10 due to radiogenic heating allowing liquid water to exist (Clifford, 1993; Clifford *et al.*, 2010) as
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12 deep as 310km, at 427°C and a pressure of 4GPa (Jones *et al.*, 2011). By comparison,
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14 subsurface liquid water is only possible on Earth in the first 5km deep in the crust, (Jebbar *et*
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16 *al.*, 2020a) under a maximal pressure of 150MPa. Yet deep biosphere is mostly occupied by
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18 microorganisms in the first 1-2km (Magnabosco *et al.*, 2018) at pressure values around
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20 100MPa. Putative subsurface Martian life at warm temperatures would thus have to cope with
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22 higher pressure than its terrestrial counterpart.
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27 These present-days putative habitable Martian environments are represented in
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29 [Figure 1-A](#).
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38 Subglacial oceans of giant planets icy moons

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44 Earth is the only planetary body in the Solar System with present persistent surface
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46 liquid water. However, if subsurface liquid water is also taken into account then the blue
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48 planet appears to be rather dry compared to the icy moons of giant planets (Mosher and
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50 Cheng, 2018). Indeed, several of these moons may possess, under their ice surface layer, huge
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52 liquid water masses that could be called exooceans (Taubner *et al.*, 2020), with liquid water
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54 abundance relative to the total mass of the body exceeding Earth's. Furthermore, Europa,
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Callisto, Titan and Ganymede, subglacial liquid water volumes exceed Earth's total liquid water volume.

Europa and Enceladus, in particular, have oceans which are of particular interest for astrobiology because they would be directly in contact with rock allowing geochemical processes such as enrichment of water with various organic and inorganic molecules. On Earth, these interactions between liquid water and rock represent a source of energy and for existing microorganisms and may have been critical for the origin of life (Hinman, 2013). Hence, they are major targets for exploration and study by future space missions (Pappalardo, 2012; Cable *et al.*, 2016).

The subglacial ocean on Europa

First evidence of a liquid water ocean beneath the ice on Europa came from the Galileo spacecraft imaging in 1996 of the Jovian moon surface showing a geological young surface and evidence of hydrogeological activity (Kerr, 1996). Further analysis supported existence of a subsurface ocean of liquid water (Carr *et al.*, 1998; Pappalardo *et al.*, 1999), whose origin is the tidal effect of Jupiter's gravity resulting in a massive heating of its satellite (Greenberg, 2009).

Beneath the ice, the ocean would be global, up to 100-200km deep, depending on the model used (Anderson *et al.*, 1997, 1998; Pappalardo *et al.*, 1999; Spohn and Schubert, 2003; Marion *et al.*, 2003; Thomas *et al.*, 2016). Its volume would exceed that of all terrestrial oceans volume (Chyba and Phillips, 2001). Models depict an ice shell 10km thick (Park *et al.*, 2015)

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with local variations in thickness (Thomas *et al.*, 2016). The layer of ice acts as a barrier against cosmic rays, thus offering protection to hypothetical current oceanic life or traces of past life. Europa may also be the only Solar System body other than Earth exhibiting plate tectonics and subduction (Kattenhorn and Prockter, 2014). Subduction in the ice shell of Europa is a particularly interesting feature as it could bring molecules from the surface down the liquid water ocean, molecules such as oxidants produced by photochemistry (Pasek and Greenberg, 2012) or prebiotic molecules delivered by meteorites and comets (Pierazzo and Chyba, 2002; Kimura and Kitadai, 2015).

What makes Europa even more interesting for astrobiology is the putative existence of hydrothermal processes at the bottom of the ocean. Modeling of Europa’s geology (Lowell and DuBose, 2005) and ocean’s chemical composition (Zolotov, 2003) argue in favor of abyssal hydrothermalism. By comparison with Earth’s black smokers ecology, hydrothermal vents on Europa would provide an habitable environment with energy source, chemical and physical gradients, making Europa’s abysses strong candidates for habitable environments with extraterrestrial life (Gaidos *et al.*, 1999; Chyba and Hand, 2001; Pierazzo and Chyba, 2002; Zolotov, 2003; Marion *et al.*, 2003).

The chemical composition of Europa’s subglacial ocean has also been modeled. It is expected to be acidic because of the intake of oxidants produced on the surface by photochemistry (Pasek and Greenberg, 2012). Nonetheless, pH could vary close to the putative hydrothermal vents, since hydrothermal fluids which are expected to be alkaline similarly to Earth’s (Zolotov, 2003). Preliminary studies have implied that salinity would be close to saturation (Hand and Chyba, 2007), similar to terrestrial hypersaline lakes, and dominated by $MgSO_4$ (Kargel *et al.*, 2000; Fanale *et al.*, 2001; Zolotov and Shock, 2001; McKinnon and Zolensky, 2003). However, $MgSO_4$ concentration is supposed to decrease

significantly with depth and pressure (Schmidt and Manning, 2017). Other works suggest instead that NaCl would be the dominant salt (Valenti *et al.*, 2012), recently supported by Hubble Space Telescope detection of sodium chloride on Europa's surface which may originate from under the ice layer (Trumbo *et al.*, 2019).

The subglacial ocean on Enceladus

Enceladus is much smaller than Europa. It is also heated by the tidal effect of Saturn (Tyler, 2009). First evidence of liquid water beneath the ice came thanks to Cassini-Huygens mission which flew over the moon in 2005 and 2007. It detected various hydrogeological activities at the south pole of the moon: cryovolcanism (Porco *et al.*, 2006), surface thermal emissions (Spencer *et al.*, 2006) and a massive water vapor plume (Hansen *et al.*, 2006). All these elements argue in favor of a local liquid water ocean beneath the south pole of Enceladus.

Other evidence includes gravimetric anomalies (Iess *et al.*, 2014) and chemical analysis of the plume, which led to sampling the composition of the subglacial ocean. Many chemical species have been thus identified: the dominant species of the plume is water, CO₂ and CH₄ are present in significant amounts and small amounts of NH₃, HCN, propane and acetylene were also detected (Waite, 2006). Further analysis also showed presence of benzene, methanol, various alkenes and alkanes (Waite *et al.*, 2009) and even larger organic molecules with masses above 200Da in ice grains in the plume closer to the icy surface (Postberg *et al.*, 2018).

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The presence of organic molecules has been interpreted as proof that hot aqueous chemistry (Matson *et al.*, 2007) and hot rock-water interactions (Sekine *et al.*, 2015) are currently active in the Enceladus subglacial ocean. Accumulation of evidence has strengthened the hypothesis of the existence of hydrothermal vents at the bottom of the Enceladus subglacial ocean (Parkinson *et al.*, 2008; Tobie, 2015; Sekine *et al.*, 2015; Waite *et al.*, 2017), making this ocean an even stronger candidate for a habitable extra-terrestrial environment.

Enceladus’s subglacial ocean is estimated to be about 10km deep (Tyler, 2009), well protected by the ice sheet from cosmic rays (less *et al.*, 2014). Chemical models of the subglacial ocean based on the study of the plume have been proposed. Enceladus present-day subglacial ocean would be a solution of NaCl and carbonate (Zolotov, 2007), with some KCl, MgCl₂ (Brown and Hand, 2013) with a strongly alkaline pH of approximatively 11-12, as determined by models of carbonate speciation (Glein *et al.*, 2015). This would make Enceladus’s ocean similar to the terrestrial soda lakes.

Other icy moons

Titan, a moon of Saturn, is considered by many as an icy body of huge astrobiological interest. Several attempts have been made to conceptualize putative life or biomes on Titan (Fortes, 2000; McKay and Smith, 2005). A subglacial ocean hundreds kilometers-deep has been hypothesized for Titan (Grasset and Sotin, 1996); it would be extremely cold, however, and composed of water and ammonia (Grindrod *et al.*, 2008; Baland *et al.*, 2011). In spite of

the fact that various bacteria have been shown to survive in highly concentrated ammonia solutions (Kelly *et al.*, 2012), these exotic conditions are largely unknown to natural terrestrial life. If there were life in such subglacial ocean, it is likely that it would be based on a radically different chemistry than on Earth. However, the existence of hydrothermal vents, as possible sites for prebiotic chemistry, at the bottom of the subglacial ocean, has also been hypothesized (Lunine and Rizk, 2007). The surface of Titan is more promising with regard to prebiotic chemistry because of its complex carbon hydrogeology (Raulin *et al.*, 2012).

Ganymede, a satellite of Jupiter, is the biggest moon in the Solar System and may have the largest subglacial ocean (McCord *et al.*, 2001). However, it is also expected that a thick high-pressure tetragonal ice layer would separate liquid water and rock (Vance *et al.*, 2014), limiting chemical exchanges.

Other icy moons with putative subglacial oceans include Dione, satellite of Saturn (Beuthe *et al.*, 2017), Mimas, also of Saturn (Rhoden *et al.*, 2017) and Triton, satellite of Neptune (Ruiz, 2003; Gaeman *et al.*, 2012).

Icy moons environmental conditions

It should be kept in mind that temperature, salinity, pressure, pH and chemical composition may vary within sub-glacial oceans, leading to multiple types of environment of various habitability: hydrothermal abyssal fields, seafloors, hypersaline brines and the top of the oceans close to the ice (Gaidos *et al.*, 1999; Chyba and Phillips, 2001; Marion *et al.*, 2003). These putative habitable icy moons environments are represented in [Figure 1-B](#).

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While black smokers have been proposed as models for icy moons abyssal environments, deep hypersaline anoxic basins (DHABs) have been proposed as models for subglacial general waters (Antunes *et al.*, 2020). In fact, DHABs like the Mediterranean Discovery, Urania and Kryos basins or Red Sea brine lakes could share some conditions with icy moons oceans such as anoxia, absence of light, high salinity, high pressure and warm/low temperatures.

Subglacial bodies of liquid water are also present on Earth such as the Antarctic lake Vostok. As they low-temperature environments isolated by ice from the outside, they have also been proposed as models for icy moons subglacial oceans (Petit *et al.*, 2005; Cockell *et al.*, 2011).

Laboratory experiments from many scientific fields designed for simulating icy moons conditions have recently be reviewed (Taubner *et al.*, 2020).

Extremophiles and astrobiology

While the previous Martian and icy moons environments appear rather extreme compared to classical biological standards, they share multiple features with extreme terrestrial environments. In this part, extremophilic microorganisms, which have successfully colonized most of these environments, will be presented in the context of astrobiology and extraterrestrial habitability.

Extremophiles are considered as key model organisms for astrobiology by many (Seckbach, 2000; Cavicchioli, 2002; Reid *et al.*, 2006; Marion and Schulze-Makuch, 2007;

Pikuta, 2007; Stojanovic *et al.*, 2008; Canganella and Wiegel, 2011; Merino *et al.*, 2019; DasSarma *et al.*, 2020; Jebbar *et al.*, 2020a). Their ability to grow at temperatures above 100°C or under 0°C, at pH under 1 or above 10, at nearly saturating salinity, or at pressure over 100MPa are particularly impressive from our mesophilic anthropocentric point of view. Taken as a whole, extremophiles encompass all kinds of environments inhabited by present-days terrestrial life and show that, as long as there is water, most terrestrial environments can be inhabited and actually are. Existence and diversity of extremophiles argue against the long-lived belief that life is intrinsically fragile (von Hegner, 2020). In contrast, the idea that life can work in a various and large range of physical and chemical conditions opens the spectrum of potentially habitable terrestrial and extraterrestrial environments. On the other hand, a precise understanding of life at the extremes may also help to tell which extraterrestrial environments would be too extreme, even for the billion's years-old evolved terrestrial life. However, as discussed later, feasibility of abiogenesis in extreme environments is still largely debated. Therefore, it should be stressed that environments putatively habitable by extremophiles should not be considered necessarily favorable for life to appear.

In general, it is expected that extraterrestrial environments such as Martian depths and icy moons oceans are more extreme than terrestrial counterparts. Therefore, putative life in these environments would have like terrestrial extremophiles to face extreme chemical and physical conditions. As extreme conditions effects on molecular systems are universal, it would also be expected that putative extraterrestrial life would use strategies similar to terrestrial extremophiles to cope with their environment.

Extremophiles are present in three domains of cellular life, which all share the last universal common ancestor (LUCA): *bacteria*, *archaea* and *eukarya*. While some

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extremophiles, mainly among fungi, protists and green algae, are indeed eukaryotic (with a nucleus), prokaryotes (without nucleus) usually dominates in extreme environments. Macroscopic, which are mostly eukaryotes, are scarce in extreme environments and microorganisms only can permanently thrive and grow in extreme environments by adapting their whole biomolecules to the extreme conditions. Moreover, extremophilic prokaryotes may belong to extensive phylae with extremophiles only, showing a long-evolved adaptation to extreme conditions.

Among them, archaea are dominant in extremely hot, acid and hypersaline environments while bacteria are dominant in cold and alkaline environments. Depending on the ambient temperature, deep-sea organisms are typically represented by psychrophilic bacteria or thermophilic archaea. Extremophiles found in marine or continental subsurface rocks are generally bacteria. Some extremophiles and their representative natural environments are represented in [Table 1](#).

Like any other cellular organism, extremophiles may be infected by viruses. In fact, virions, the extracellular dispersion form of viruses, have been found in black smokers (Geslin *et al.*, 2003), acidic hot springs (Prangishvili and Garrett, 2005), hypersaline lakes (Sime- Ngando *et al.*, 2011) and cold polar environments (Wells and Deming, 2006). As they infect cells inhabiting extreme environments, viruses of extremophiles also possess biomolecules adapted to these extreme conditions and should be considered as part of extremophilic biodiversity. While the status of viruses, alive or not, is still debated (Koonin and Starokadomskyy, 2016; van Regenmortel, 2016), virions are the most abundant biological objects on earth and their biomass may exceed total biomass of protists (Suttle, 2007). Due

to their uncontested contribution to biogeochemical cycles and evolutions of cells, viruses should be considered as a matter of interest for astrobiology (Berliner *et al.*, 2018).

Experimental astrobiology: using extremophiles to test habitability

Besides theoretical considerations, experimental studies have been used to assess compatibility between extremophiles physiology and extraterrestrial conditions expected to be encountered in icy moons subglacial oceans or Martian brines and subsurface.

As no sunlight is available in icy moons oceans, photosynthesis is expected to be absent from putative subglacial oceanic life (Gaidos *et al.*, 1999; Chyba and Hand, 2001) and ecosystems would be rather based on chemosynthesis. The same is expected for putative subsurface life on Mars (Boston *et al.*, 1992). Without photosynthesis-driven dioxygen production, these environments would be anoxic. It should be noted that primitive Earth atmosphere and waters were anoxic until about 2.4 billion years, yet allowing life to appear. Anoxia should not be considered as an extreme condition for biological systems or a barrier for life *per se*. On the contrary, since the oxygenation of atmosphere and waters by photosynthetic microorganisms, aerobic microorganisms have to cope with huge oxidative stress through various long-evolved molecular systems.

In terrestrial anaerobic environments, methan-producing microbes (methanogens) are key organisms to understand such biotops. Methanogens are widespread microorganisms from the *Archaea* domain encountered in both extreme and moderate environments. They

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are either autotrophs (producing their own organic matter) or heterotrophs (needing organic matter from the environment). In all cases, CH₄ is a byproduct of their growth, which can be then metabolized by methanotrophs (bacteria and archaea). Hence, methanogens using CO₂ as the carbon source and H₂ as the reducing agent (Lyu *et al.*, 2018), and extremophilic ones in particular, are recurring model organisms in astrobiology which are expected to be able to thrive in extraterrestrial environments (Reid *et al.*, 2006; Kendrick and Kral, 2006; McKay *et al.*, 2008; Taubner *et al.*, 2015). Moreover, as most methane on Earth is of biological origin (Liu and Whitman, 2008) it could be considered to some extent as a biomarker (Jebbar *et al.*, 2020b). This must however be balanced by the fact that existence of CH₄ could be a purely abiotic phenomenon in a case of an H-rich atmosphere. CH₄ being a natural reservoir of carbon (Woitke *et al.*, 2020). The biomarker status of this molecule depends therefore on the type of planetary atmosphere.

For the CO₂-rich Martian atmosphere, calculations indicate that the presence of methane is not expected if the atmosphere is at thermodynamic equilibrium (Levine *et al.*, 2010). If emitted, it cannot survive long, with a relatively short lifetime of about 300years. Its detection, specially within local releases (Formisano *et al.*, 2004; Mumma *et al.*, 2009; Webster *et al.*, 2015, 2018), therefore argues for a very young source. Martian methane origin remains an open question, the debate not being settled between geochemical/geological processes or microbial life (Lyons *et al.*, 2005; Yung *et al.*, 2018) questioning the existence of methanogens viability on Mars. Experimental works have shown that extremophilic methanogens could withstand many Martian surface or subsurface extreme conditions: thermophilic *Methanothermobacter wolfeii* survived long Martian-like desiccation (Kendrick and Kral, 2006) and low pressure (Mickol and Kral, 2017), psychrophilic *Methanobacterium articum* could tolerate perchlorate and even use it as a substrate (Shcherbakova *et al.*, 2015),

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3 Siberian permafrost methanogens survived simulated Martian thermal daily variation
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5 (Morozova *et al.*, 2007), long freezing, high salinity and starvation (Morozova and Wagner,
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7 2007) and psychrophilic *Methanosarcina soligelidi* and *Methanococcoides burtonii* could
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9 manage methanogenesis at Martian subsurface pressure and/or at temperatures down to -
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11 5°C (Reid *et al.*, 2006; Schirmack *et al.*, 2014).
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15 Since H₂, CO₂ and CH₄ have been detected in Enceladus's plume (Waite, 2006; Waite
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17 *et al.*, 2009, 2017), it has been proposed that methanogens could be thriving in Enceladus
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19 subglacial ocean. Based on this assumption, recent experimental study have shown that
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21 growth of the thermophilic methanogen *Methanothermococcus okinawensis* is possible under
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23 extrapolated Enceladus hydrothermal vents conditions (Taubner *et al.*, 2018).
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31 Extremophiles from hypersaline environments have also been considered in simulated
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33 extraterrestrial conditions. As discussed above, many extraterrestrial environments such as
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35 brines of Mars, Enceladus and Europa, may be hypersaline, in which halophilic and
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37 halotolerant microorganisms, for which terrestrial counterparts represent good models,
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39 thrive (DasSarma, 2006; Reid *et al.*, 2006; Sundarasami *et al.*, 2019). Moreover, intracellular
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41 accumulation of salts, particularly manganese salts (Webb *et al.*, 2013), in halophilic cells
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43 provides protection against ionizing radiation (Kish *et al.*, 2009), which is prevalent in some
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45 extraterrestrial environments.
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50 Experimental studies showed that some halophilic archaea can tolerate high concentrations
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52 of perchlorates similar to what would be faced in Martian brines (Oren *et al.*, 2014; Matsubara
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54 *et al.*, 2017; Laye and DasSarma, 2018), Martial-like UV irradiation (Fendrihan *et al.*, 2009),
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56 desiccation-rehydration cycles (Vauclare *et al.*, 2020), long-term desiccation and freezing
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cycles (Mancinelli *et al.*, 2004) or could grow at Martian subsurface temperature (Reid *et al.*, 2006; Laye and DasSarma, 2018).

Extremophiles and abiogenesis

Existence of extraterrestrial conditions compatible with terrestrial life is not a sufficient reason to believe extraterrestrial life has actually originated. In fact, extremophilic life is a complex lifestyle achieved with many mechanisms: large molecular assemblies, efflux/influx systems, molecular glues and rivets, channeling, etc. Evolution has driven life for at least 3,5 billion years, leaving time to evolve new mechanisms allowing adaptation to new environments, but discourses on origin of life, on abiogenesis, are only speculative.

In silico predictions of LUCA’s genome offered several arguments in favour of a thermophilic origin of modern cells: G+C-rich content (Di giulio, 2000), which is largely associated with modern-days thermophily as GC basepair is less susceptible than AT to thermodenaturation, predicted proteins with amino acid sequences similar to modern-days thermophilic homologs (Giulio, 2003) and the possession of a gene coding reverse gyrase enzyme, a hallmark trait of hyperthermophiles (Catchpole and Forterre, 2019) lead to the model where the last ancestor of all cells have emerged from inorganic natural compartmentation in hydrothermal vents. (Koonin and Martin, 2005). Others have proposed that even first biomolecules have originated in rich hydrothermal prebiotic chemistry (Martin

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3 *et al.*, 2008) and land hydrothermal fields have also been proposed as the place for precellular
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5 evolution (Mulkidjanian *et al.*, 2012).
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8 High pressure encountered in hydrothermal vents environments could have also
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10 facilitated some prebiotic reactions, notably by countering destabilizing effect of high
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12 temperature. It has thus been proposed that LUCA, the last ancestor of all cellular organisms,
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14 was pressure-adapted (Daniel *et al.*, 2006) or that high pressure in hydrothermal vents
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16 environments facilitated synthesis of prebiotic molecules (Hazen *et al.*, 2002; Daniel *et al.*,
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22 All these elements place abyssal hydrothermal vents at the center of abiogenesis. Such
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24 claims may be supported by the discovery of putative 3.8-4.3Ga old microorganism fossils in
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26 hydrothermal vents precipitates (Dodd *et al.*, 2017), in spite of the fact that such evidence of
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28 life older than 2.7Ga is controversial (Gargaud *et al.*, 2012).
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34 However, others have pointed out that a thermophilic origin of life is unlikely (Miller
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36 and Lazcano, 1995; Levy and Miller, 1998; Galtier *et al.*, 1999; Bada and Lazcano, 2002; Islas
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38 *et al.*, 2003). In general, some have argued that in the absence of complex long-evolved
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40 adaptations, extreme conditions would have been too harsh for first cells or first
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42 biomolecules, hence excluding the relevance of extremophiles for LUCA lifestyle, RNA world
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44 or first prebiotic reactions (Cleaves and Chalmers, 2004; Islas *et al.*, 2007).
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49 Hence, existence of extreme extraterrestrial environments, such as subglacial
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51 hydrothermal vents of Enceladus or Europa, which could be habitable for terrestrial
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53 extremophiles does not imply that life actually emerged and adapted there. Extraterrestrial
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55 life may not be necessarily extremophilic (von Hegner, 2020).
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Beyond the cell: extremophilic biochemistry in extraterrestrial conditions

As the cellular level appears to be too complex and evolution-dependent, extraterrestrial habitability should also be tested at smaller scales. Before the potential existence of cells, with long-evolved mechanisms to cope with extreme conditions, prebiotic systems have to emerge from prebiotic chemistry. Even if the question of terrestrial abiogenesis is still open, extensive work has been done about extraterrestrial prebiotic chemistry (Cleaves, 2014). In particular, several experimental setups for simulating prebiotic chemistry in icy moons putative hydrothermal vents conditions have been proposed (Barge and White, 2017; Taubner *et al.*, 2020). Other extraterrestrial environments relevant to prebiotic chemistry have been experimentally simulated. In interstellar ice, prebiotic molecules such as amino acids have been found to be abiotically synthesized, a process which have been simulated in laboratory conditions (Caro and Dartois, 2013). Amino acids and nucleotides have also been for example synthesized in simulated Titan atmosphere (Hörst *et al.*, 2012).

However, the aim of this review is to focus on the scale between cell physiology and prebiotic chemistry: biochemistry. The study of extremophiles encompasses biochemistry, cell biology, microbiology and ecology. Nonetheless, experimental astrobiological studies using extremophilic systems are largely limited to the cell scale. Little work has been done with biomolecules from extremophiles such as proteins and lipids in planetary conditions.

By contrast, multiple experimental studies have pushed terrestrial biochemistry in conditions far beyond their normal context and their expected physical and chemical limits. An example of such work include enzymatic catalysis in organic solvents (Dordick, 1989; Klivanov, 1989) or ionic liquids (Kragl *et al.*, 2002). In this context, enzymes from extremophiles have shown many industrial applications (Horikoshi, 1999; Vieille and Zeikus, 2001; Demirjian *et al.*, 2001; Dumorne; *et al.*, 2017). To some degree, these studies push back the limits of life, understood as fundamental biochemical processes. However, these works are generally made outside of the context of astrobiology.

In this review we would like to defend the position that experimental studies using biomolecules such as enzymes from extremophilic terrestrial organisms (extremozymes) are of particular interest for astrobiology. In fact, studying viability of terrestrial extremophiles in simulated extraterrestrial environments mainly gives information about one precise microorganism capacity. In fact, survivability of halophiles in simulated Martian conditions, for example, has been proven to be dependent on the strain used (Peeters *et al.*, 2010). Indeed, growth, metabolism and cell division are complex biological phenomenon requiring multiple enzymes, coordinated processes and are thus largely interdependent. In contrast, given precise fundamental biochemical processes such as assembly of a macromolecular edifices, protein synthesis, DNA replication or metabolite production appear simpler to study and to extrapolate. Even if putative extraterrestrial life is based on the same chemistry as terrestrial's, large-scale cell processes may significantly differ. Smaller-scale fundamental biochemistries however could share more similarities.

Therefore, studies of biomolecules from extremophiles in simulated planetary conditions may offer insights about limits of given biological processes in extraterrestrial environments. Moreover, in the context of abiogenesis or early life, in absence of most of the

long-evolved cellular tools modern cells possess, studying fundamental biological processes may give insights on the feasibility of biochemistry in extraterrestrial environments.

Indeed, some extraterrestrial environments are more extreme than their terrestrial commonly accepted analogs. Martian surface is not only extremely dry and UV-irradiated like Atacama Desert but also face generally extremely low temperatures and perchlorates abundancy. For icy moons, it is often believed that putative hydrothermal vents would be analogous to terrestrials. However, depth and pressure significantly differ. Deepest hydrothermal vent on Earth lies at approximatively 5km under the sea level with *in situ* pressure of 50MPa (Connelly *et al.*, 2012). In contrast, some icy moons subglacial oceans are, according to several models, hundreds kilometers deep (Schmidt and Manning, 2017). Europa subglacial ocean for example, would be 100-200km deep (Anderson *et al.*, 1997; Pappalardo *et al.*, 1999; Spohn and Schubert, 2003; Marion *et al.*, 2003; Thomas *et al.*, 2016). Taking account of the 10km thick ice crust (Park *et al.*, 2015), the 100-200km deep ocean and gravity field of the moon, the hydrostatic pressure at the bottom of the water, where hydrothermal vents would be located, is expected to be approximatively 130-260MPa (Naganuma and Uematsu, 1998), which would correspond on Earth to a theoretical oceanic depth of 13-26km under the sea. There is no known terrestrial hydrothermal vent at such pressure and the highest pressure at which life has been found is approximatively 108MPa at Mariana Trench.

High pressure processing has been used since 1914 (Hite *et al.*, 1914) to sterilize food and typically 200-300MPa treatment kills most non-piezophilic bacteria whereas 400-700MPa treatment kills most spores (Huang *et al.*, 2014). The first known obligate piezophile microorganism is the black smoker hyperthermophile *Pyrococcus yayanosii* HC1 which has an optimal growth pressure of 58MPa and is able to grow at pressures as high as 120MPa (Zeng

et al., 2009; Birrien et al., 2011). However, piezoresistance has been engineered in non-piezophilic bacteria: selection of pressure-resistant mutants allowed, for example, significant survival of *Escherichia coli* MG1655 after exposure to 20GPa (Vanlint et al., 2011) but with no sign of active growth. In similar experiments, some active enzymatic activity has also been reported at pressures as high as 14GPa (Sharma, 2002; Yayanos, 2002). However, these results are controversial.

Yet, little is known about how given biochemical processes, independently of the cellular context, behave in pressures similar to what would be face in Europa's depths. Biomolecules from terrestrial abyssal extremophiles may thus provide insights on how these processes would work under such pressures. More generally, extremophilic molecular adaptations may help understanding how fundamental biological processes could or not work under extraterrestrial conditions.

Extreme conditions overview

The scientific field of extremophiles includes both *stricto sensu* extremophilic systems (needing an extreme condition to work properly) and systems which are just resistant to conditions which would impair most biological processes. It also includes adaptation based on the exclusion of extreme condition out of the cell interior when possible and adaptation of all biomolecules within the cell. Strategies based on special long-term resistance form (such as spores) with reduced metabolism and paused growth are sometime included in this field.

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Extreme conditions may either be of physical or chemical origin. While chemical extreme conditions are diverse, including high abundancies of various compounds, physical extreme conditions are limited to fewer situations. Among them, high or low temperature and pressure are particularly challenging for life at the molecular scale. Indeed, microorganisms, which are dominant in extreme environments, cannot form barriers against temperature or pressure change nor they can maintain internal temperature or pressure different from the environment. Hence, adaptation of all biomolecules is necessary in order to grow under these conditions.

Biological membranes, which separate interior the cell from the external environment, are permeable to water and not fully impermeable to small solutes. Thus, in hypersaline environments, osmotic pressure favors loss of intracellular water and increase of intracellular salinity. The latter makes high salinity a chemical extreme condition different from desiccation and affecting the internal environment, needing adaptation of all biomolecules.

As described latter, structure and dynamics traits of proteins are the keys for life at high (HT) or low temperature (LT), high salinity (HS) and high pressure (HP). Because of these fundamental structural changes, growth or survivability of extremophiles may be reduced or impossible under mesophilic conditions, making them “true” extremophiles.

Effects of these conditions on biomolecules and molecular traits found in extremophiles to cope with them will be detailed in the next chapters of this review. Terrestrial environments displaying one or multiple extreme conditions among these are represented in [Figure 2](#).

Other extreme conditions can be handled by organisms at a cellular scale and without adapting general structure of their biomolecules. In the context of astrobiology, adaptations at the scale of the cell may appear to be less relevant than adaptations at the scale of the

structure and dynamics of biomolecules. Nonetheless, these other extreme conditions will be briefly reviewed in this chapter.

A general overview of extreme conditions, extremophily types, associated terrestrial environments and extraterrestrial environments putatively displaying these conditions is presented in [Table 1](#).

Extreme pH

Acidophiles and alkaliphiles are extremophilic organisms needing respectively low (< 3) or high pH (> 9) to grow. Acidic environments on Earth where acidophiles thrive include volcanic hot springs, solfataric fields, acid mine drainage, bioreactors and coal spoils. Alkaline environments where alkaliphiles thrive include alkaline hot springs, white smokers, soda lakes and sewage waters.

Several attempts to compare acidic terrestrial environments to extraterrestrial ones have been made. In particular, the acidic metal-rich waters and sediments of Río Tinto have been compared with ancient aqueous acidic sulfate-rich martian fields (Clifford 1993; Clifford *et al.* 2010). Enceladus ocean is expected to be alkaline (Glein *et al.*, 2015) but pH of extraterrestrial environments is still largely debated.

For terrestrial life, extreme pH is not a condition that cells can afford to totally let in. Because metabolism lies in generating ATP using pH gradients across the cell membrane, equilibration of pH between cytoplasm and external acidic or alkaline environment has to be avoided by cells (Krulwich and Guffanti, 1983; Krulwich, 1995). Therefore, instead of adapting

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proteins and the rest of cellular content to $\text{pH} < 3$, intracellular pH in acidophiles is generally maintained above 6 (Baker-Austin and Dopson, 2007; Krulwich *et al.*, 2011). The same goes for alkaliphiles which keep an intracellular pH lower than the environment pH (Krulwich *et al.*, 2011). For example, the model extremely alkaliphilic bacteria *Bacillus pseudofirmus* OF4 grows at pH 10.5 but keeps a cytoplasmic pH of 8.3 (Sturr *et al.*, 1994) and the most extreme acidophile organism *Picrophilus torridus* which can grow around pH 0 has an intracellular pH of 4.6 (Fütterer *et al.*, 2004).

Even if terrestrial acidophiles and alkaliphiles possess specific molecular traits, such as charged cell wall, high membrane impermeability and powerful proton pumps (Krulwich *et al.*, 2011; Kulkarni *et al.*, 2019), extreme pH is an extreme condition that is largely kept out of the cell, thus not needing an overall large adaptation of proteins.

Desiccation

Today, Mars lacks stable surface liquid water. Some terrestrial deserts share several conditions with Martian surface. In particular, the Atacama desert is considered as one of the best model environment for present-Mars surface (Dose *et al.*, 2001) as it displays extreme low abundance of liquid water, extensive UV irradiation and Mars-like soils (Navarro-González *et al.*, 2003; Fletcher *et al.*, 2012).

However, extremely arid terrestrial environments such as Atacama Desert or Antarctic surface are among the least inhabited places on Earth. If extraterrestrial life shares liquid water-dependency with Terran life, then it should be considered that current active life would be harder to find in arid extraterrestrial environments.

Indeed, most molecular processes in cell require water as a solvent to work. Under desiccation, reactive oxygen species accumulate, damaging all biomolecules, protein and nucleic acids undergo direct damage, metabolism is hindered and membrane impermeability and transport are disrupted (Lebre *et al.*, 2017). Moreover, water activity (a_w) decreases, meaning that less water molecules are available for solvation or catalysis.

In this state, life processes are significantly reduced until water returns. Molecular traits of xerotolerant organisms include accumulation of compatible solutes which replace water, stabilize membranes and lower oxidative damage and water retention. Under extended desiccation, xerotolerant organisms undergo reversible cellular states such as dormancy, sporulation and anhydrobiosis (Crowe *et al.*, 1992). In such state, metabolism and most biological processes are inhibited or stopped until water is abundant again. Therefore, xerotolerant organisms may not be described as truly xerophilic.

Ionizing radiation

Highly irradiated environments are uncommon on Earth and most are of anthropic origin. However, other putatively habitable extraterrestrial environments may be more irradiated than terrestrial surface. Ionizing radiation, which is either caused by α or β particles or by γ or X photons, alters all kinds of biomolecules, mainly by producing radicals. While radicals lead to both DNA damage, leading to accumulation of mutations and loss of genetic information, and membrane damage, leading to the loss of cell integrity, protein damage,

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caused by radical-induced carbonylation (Suzuki *et al.*, 2010), is the most important and lethal effect for microorganisms of ionizing radiation (Daly, 2009).

Radioresistant organisms such as the bacteria *Deinococcus radiodurans* (Anderson *et al.*, 1956) and the archaea *Thermococcus gammatolerans* (Jolivet, 2003) can withstand high doses of ionizing radiation. Proteins (Krisko and Radman, 2010) and DNA (Hutchinson, 1985) are directly damaged by ionizing radiation. Several molecular traits have been related to radioresistance, particularly in the model organism *D. radiodurans*: proteome protection, cytoplasmic accumulation of Mn²⁺ and extensive DNA repair (Krisko and Radman, 2013). Several strategies providing radioresistance, such as Mn²⁺ accumulation, are shared with several archaea (Kish *et al.*, 2009; Webb *et al.*, 2013). However, growth of radioresistant organisms is still inhibited by ionizing radiation: they are not radiophilic. Nonetheless, as some extraterrestrial environments are expected to be highly irradiated, they are still considered by some as an interesting model for astrobiology (Pikuta, 2007; Canganella and Wiegel, 2011; Merino *et al.*, 2019).

However, it has to be mentioned that some organisms can actually benefit from sources of ionizing radiation. Melanized fungi found in remains of Chernobyl Atomic Energy Station have shown radiotrophic properties: they tend to grow toward sources of ionizing radiation (Zhdanova *et al.*, 2004) and to produce more biomass as they are irradiated (Dadachova and Casadevall, 2008). It has been shown that melanine enhances radioresistance and radiotrophy (Dadachova, Bryan, Huang, *et al.*, 2007; Dadachova, Bryan, Howell, *et al.*, 2007) and that it could acts as a transductor of energy to the benefit of the cell (Dadachova and Casadevall, 2008). Another example of an organism benefiting of radioactivity is the bacteria *Candidatus Desulforudis audaxviator* isolated from deep continental environments. This organisms has been found to live in mono-specie colonies and to use radiolytically

produced molecules as a source of energy and nutrients making this ecosystem independent from surface photosynthesis (Lin *et al.*, 2005, 2006; Chivian *et al.*, 2008). Even if these two examples of organisms taking advantage of ionizing sources are less radioresistant than model *D. radiodurans* and *T. gamatolerans*, they still provide examples of how life could adapt in heavily irradiated extraterrestrial environments (Atri, 2016; Stelmach *et al.*, 2018).

Organic solvents

Organic solvents are highly toxic to biological systems, in particular for membranes and protein structures which rely on hydrophobic interactions in a polar medium (Sikkema *et al.*, 1995). Solvent tolerant organisms such as the bacteria *Staphylococcus haemolyticus* can resume growth after long exposure to 100% toluene, benzene, and p-xylene (Nielsen *et al.*, 2005). In spite of the fact that resistance to organic solvents can be achieved by bacteria and archaea by various molecular traits mainly based on the exclusion of solvent molecules out of the cell (Ramos *et al.*, 1997, 2002; Takuichi *et al.*, 1997; Sardesai and Bhosle, 2002; Usami *et al.*, 2005), to our knowledge no organism requires organic solvents to survive: there is no solvophilic organism.

Heavy metals

Heavy metal-rich environments are uncommon and mainly of anthropic or occasional geologic origin (Nies, 2000; Krami *et al.*, 2013). Therefore, most are geologically extremely recent as they follow industrial revolution. Natural metal-rich environments include geothermal fields, hydrothermal vents and ultramafic soils (Mergeay, 2006). In these environments, monoatomic ions of heavy metals such as Cu, Zn, Hg, Cd, As, Ni, Pb, Cr, Co, V, Ag, Au and Pt and several metal oxides can be highly abundant and toxic to eventual life. Their toxicity comes mainly from the ability to produce reactive oxygen species (ROS) (Prasad and Hagemeyer, 1999; Pinto *et al.*, 2003; Shahid *et al.*, 2014), their chaotropicity and their ability to replace other ions involved in enzymatic activity (Assche and Clijsters, 1990). For example, many metalloenzymes physiologically possess Ni^{2+} in their catalytic center whose replacement by Zn^{2+} makes them inactive (Boer *et al.*, 2014). Moreover, heavy metals disrupt salt and disulfide bridges of proteins favoring unfolded state (Tamás *et al.*, 2014). Their toxicity is typically ranked in the following order: $\text{Zn}^{2+} < \text{Ag}^+ < \text{Ni}^{2+} \ll \text{Cu}^{2+} < \text{Hg}^{2+} \ll \text{UO}_2^{2-} < \text{Co}^{2+} < \text{CrO}_4^{2-} < \text{Au}^{3+} < \text{Cd}^{2+} < \text{Mn}^{2+} \lll \text{Pb}^{2+}$ (Nies, 2000).

Metalloresistance is mainly achieved by energy dependent efflux of toxic ions (Silver and Phung, 1996) and by chelating agents (Daniels *et al.*, 1998; Cobbett, 2000). Other sequestration mechanisms in bacteria (Kothe *et al.*, 2010) include biomineralization (Bäuerlein, 2003) and cell wall adsorption (Merroun *et al.*, 2005). Periplasmic sensors are also of particular importance (Trepreau *et al.*, 2014; Nies *et al.*, 2017). Interestingly, metalloresistance is common in acidophilic archaea and bacteria whose sulfur metabolism may increase extracellular metal concentration (Dopson *et al.*, 2003, 2014). Nonetheless, heavy metal concentration is also an extreme condition that can be, to a certain extent, kept out of the cytoplasm and not requiring an adaptation of all proteins.

It should however be noted that, in some anoxic environments, many microorganisms, such as the bacteria *Geobacter metallireducens*, can achieve anaerobic respiration using metals, metalloids or radionuclides (Lovley *et al.*, 1993). These microorganisms can use extracellular Fe(III), Mn(IV), Co(III), Cr(VI) or U(VI) as electron acceptors which they respectively reduce into Fe(II), Mn(II), Co(II), Cr(III) and U(IV) (Gralnick and Newman, 2007; Richter *et al.*, 2012).

Nutrient scarcity

Oligotrophic environments are characterized by low nutrient and energy availability and low biomass. Oligotrophs are slow-growing microorganisms that can persist for long under oligotrophic conditions (Hoehler and Jørgensen, 2013; LaRowe and Amend, 2015) but still they require nutrients and energy sources to grow. To our knowledge, no molecular traits of biomolecules enabling oligotrophy is known.

However, organisms adapted to low-energy environments have been considered by some as interesting models for extraterrestrial life since several extraterrestrial environments such as Martian surface appear to be low-energy (Jones *et al.*, 2018).

Other extreme conditions

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Some other extreme chemical and physical conditions and their related adapted organisms have fallen into the scientific field of extremophiles: oxygen tension, hypobaric pressure (Schwendner and Schuerger, 2020), vacuum (Stojanovic *et al.*, 2008) and even chaotropicity (Williams and Hallsworth, 2009; Oren, 2013; Zajc *et al.*, 2014). However, most of the organisms associated with these extreme conditions are not considered as classical extremophiles and do not possess clear molecular traits enabling adaptation.

Biochemistry and Biophysics handbook: terrestrial biomolecules and extreme conditions

Terrestrial biological systems (cells and viruses) are made of various biomolecules: peptides and proteins, nucleic acids (DNA and RNA), lipids, sugars and various small-molecular-weight molecules such as metabolites, osmolytes or messenger molecules. However, as intracellular water makes most of the living cell mass (up to 70-80% for most cell) it is often considered as a biomolecule itself.

In the next part of this review, biophysical and biochemical properties of major biomolecules will be briefly summarized under the structure-dynamics-function relationship paradigm. Effects of extreme conditions considered by this review (low temperature, high temperature, high salinity and high pressure) on these biomolecules will then be described. As adaptation to extreme temperature, salinity or pressure relies on macromolecular structural and dynamics changes (Panja *et al.*, 2020), it is essential to understand these properties of biomolecules and how they are altered by extreme conditions.

Structure-dynamics-function relationship in biomolecules

Solution thermodynamics

Biology takes place in aqueous solution and interactions with the solvent environment play an essential role in the folding and stabilization of proteins and nucleic acids and organization of lipids to form membranes. In fact, the discovery of biological macromolecules in the late 19th century and their initial characterization in the 20th is tightly interwoven with the development of solution thermodynamics. Useful definitions:

The change in *enthalpy*, ΔH , during a chemical reaction is the heat absorbed or released in the breaking and formation of bonds. The heat energy invested by a system in order to sample all the configurations available to it, at a given temperature, is equal to its *entropy*, S , multiplied by the temperature (in Kelvin units). *Gibbs free energy*, G , represents the part of the energy in a system that is not used to populate the different entropy configurations (thermal agitation disorder) and which can be transformed into useful work, hence its name. At constant pressure and temperature, the free energy released by a reaction is given by:

$$\Delta G = \Delta H - T \Delta S \tag{1}$$

A system that exchanges energy with its surroundings evolves in order to maximize its free energy. The spontaneous sense of a reversible reaction is in the direction for which $\Delta G < 0$. When two states of a system are at equilibrium, their free energy difference is zero, $\Delta G = 0$. In the case of a chemical equilibrium (e.g. between the folded and unfolded state of a protein), free energy (and consequently enthalpy and entropy) are calculated from the measured equilibrium concentrations of the two states.

Water: a structured solvent and interactant

Water is not only the most abundant biomolecule but is also occupies close to three quarters of the volume in most organisms. Water molecules interact with all biomolecules, which they solvate and act as a lubricant in the crowded intracellular environment, as well as participating directly in chemical reactions. Water in the liquid state is highly dynamic. Neighboring molecules exchange partners through hydrogen bonds on the picosecond timescale (Lynden-Bell *et al.*, 2010). This turns water into a vital entropy sink for biochemical reactions in aqueous solution, as well as for the hydration effects that trigger functional dynamics in macromolecules. Apolar solutes, which cannot form hydrogen bonds with water, interfere unfavorably with its dynamic leading, for example, to the low solubility of oil in water. This is called the hydrophobic effect. The free energy change (eq. 1) upon dissolution reflects the balance between enthalpy and entropy changes in both solvent and solute. Interestingly, this leads to a minimum in solubility at a given temperature (strongest hydrophobic effect) with increased solubility at lower as well as high temperatures. Because of the crowded environment, the properties of intracellular water did not escape controversy (Ball, 2008), until clarified experimentally by neutron scattering and NMR experiments (Jasnin *et al.*, 2010). In fact, despite the extreme concentration and crowding of the cell, macromolecules remain soluble (Costenaro *et al.*, 2002), and about 90% of the water flows freely, maintaining the thermodynamic properties of liquid water, while about 10% is dynamically confined in macromolecular solvation shells (Jasnin *et al.*, 2010). Water dynamics is characterized by various movements at diverse timescales, see Figure 3.

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Most importantly, water determines both folding and catalytic functions of proteins and nucleic acids (Bellissent-Funel *et al.*, 2016) as will be discussed further below.

Proteins

Proteins are the most diverse of all macromolecules, varying in length, size, shape and physical and chemical properties. Their functions are as diverse with, for example, collagen structuring the extracellular matrix, muscle and flagella converting force into motion, membrane proteins acting as signal receptors and, of course, enzymes that are fundamental for biological systems as they enable catalysis at lower substrate concentrations and with higher specificity than abiotic catalysis. Depending on the method, 1000-2300 different proteins can be detected in cells of *E. coli*, one of the most studied organisms, (Han and Lee, 2006; Ishihama *et al.*, 2008; Soufi *et al.*, 2015) whose genome typically encodes up to 4300 putative protein sequences (Serres *et al.*, 2001; Hu *et al.*, 2009).

Proteins are synthesized by large intracellular macromolecular assemblies called ribosomes composed of proteins and catalytic RNA (rRNA), which decodes the information of messenger RNA (mRNA) in the process of translation. mRNA has itself been transcribed from genomic DNA.

Proteins are structured (folded) and dynamic polymers whose chemical name is polypeptide chains. The combination of these two properties is fundamental to their physical and chemical properties and their biological function as it allows possibility to build active sites, interactions, stability, specificity, etc. Loss of protein dynamics or structure generally

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3 results in a loss of function. Even intrinsically disordered proteins (IDPs) or IDP domains in
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5 proteins, with little to no persistent three-dimensional structure, obey this structure-
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7 dynamics-function relationship. Indeed, their apparent lack of structure is fundamental to
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9 their functions, they can adopt local structures upon binding (Kragelj *et al.*, 2013), they
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11 complement structured proteins (Oldfield and Dunker, 2014) and are fundamental in many
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13 cellular processes (Wright and Dyson, 2015).
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18 Of particular relevance for effects of extreme conditions and adaptation to extreme
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20 environments is the fact that protein structure and dynamics rely heavily on interactions with
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22 solvents. Consider the free energy difference (eq. 1) between the folded and unfolded state
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24 of polypeptide chain. The ΔH , ΔS display a complex dependence on temperature: ΔH , because
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26 of bonding within the chain as well as between the chain and solvent; ΔS , because of chain
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28 disorder as well as solvent disorder (the hydrophobic effect). This leads to the native state
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30 being stabilized in a narrow temperature range (close to 37°C for mesophilic proteins) falling
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32 off at temperatures above (thermal unfolding) and below (cold denaturation). The maximum
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34 stabilisation free energy is interestingly small. At about 50 kJ/mol it corresponds, for example,
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36 to the enthalpy gained by breaking two or three H-bonds in the protein interior, when
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38 hundreds are involved in internal protein and protein solvent interactions.
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48 The structure of proteins is in general understood as the combination of four structural
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50 levels, to which a fifth, quinary structure, has however been added for interactions between
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52 different proteins within cells (McConkey, 1982; Cohen and Pielak, 2017).
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55 The primary structure is the amino acid sequence. While protein is the functional
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57 molecule, its chemical composition is a polypeptide linear polymer made up of amino acids
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59 (AA) linked by peptide bonds. They share a basic structure with a central α carbon bonded to
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a hydrogen, an amino group, a carboxyl group and a side chain. All AA found in proteins (proteinogenic AA) are L-stereoisomers. The side chain determines the nature of the AA residue in chain. There are twenty AA in the genetic code. However, two other AA that are not in the universal genetic code can also be found in some proteins: selenocysteine in all three domains of life and pyrrolysine in methanogenic archaea and some bacteria (Rother and Krzycki, 2010). Amino acids can be classified according to their characteristics (small/large, polar/non-polar, charged/uncharged, acid/basic, aromatic or not, etc.) or to their properties (rigid/flexible, proton donor/acceptor, metal-binding, DNA and RNA binding, Mg^{2+} and Ca^{2+} -binding, etc.). AA residue names are generally written with one-letter or three-letters abbreviations. Primary structures of proteins can be determined by translating open reading frames in genomic DNA.

Secondary structure corresponds to local, defined structural organization in segments of a protein. The α -helix and β -sheet are the most common secondary structures, but β -turns (Hutchinson and Thornton, 1994) and Ω -loops (Leszczynski and Rose, 1986; Fetrow, 1995) are also frequent. Tight turns and flexible loops are usually found between helices and sheets. Secondary structures result from hydrogen bonds between amino and carboxyl groups of the same or different AA backbones, thus not directly involving side chains. However, some AA or AA sequences are known to favor or to block secondary structure formations. For example, Met, Ala, Leu, Glu and Lys are common in helices whereas Pro and Gly block helices formation and are common in turns. Secondary structure can often be predicted *in silico* with primary structure as the sole information.

Tertiary structure is the general three-dimensional architecture defined by the spatial coordinates of each atom of the protein. At this level, prediction by informatics remains really challenging unless through modelling based on experimental data from a homologous

structure. The experimental determination of 3D-structures is made by X-ray crystallography (XRC), nuclear magnetic resonance (NMR) or cryo-electronic microscopy (cryo-EM). Tertiary structure involves interactions between side chains: hydrophobic interactions, disulfide bridges, hydrogen bonds, hydrophilic interactions and ionic bonds. The process under which linear AA polymers acquire their tertiary structure is called folding. Folding determines protein properties: affinity with other biomolecules, thermostability, catalytic activity and specificity, dynamics, etc.

Most proteins assemble within homomultimers or heteromultimers (Lynch, 2013). Quaternary structure is the transitory or stable resulting macromolecular structure made by association of proteins with other proteins, nucleic acids or other cofactors. Quaternary structure not only relies on subunits' 3D-shapes but also on the contact surfaces. Interactions between subunits are mainly based on hydrophobic interactions but can also be stabilized by electrostatic, hydrogen and occasionally disulfide bonds.

Another level of complexity and variation also lies in post-translational modifications. Following translation on the ribosome during which they are synthesized, many proteins undergo covalent chemical modification of carboxy/amino termini or of side chains. These modifications, which are generally catalyzed by enzymes, are various and include glycosylation, phosphorylation or addition of aliphatic chains. Although these modifications are in general not critical for protein structure, they are nonetheless fundamental to many biochemical and cellular processes.

It should also be kept in mind that the state of the proteome, i.e. the set of all proteins produced by a cell, is tightly regulated. Not only do cells regulate protein synthesis, folding and post-translational modification but also their repair and degradation are fundamental.

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Protein degradation in particular is achieved by central macromolecular assemblies called proteasomes and allows the essential regulation of protein quantity and quality control (Sontag *et al.*, 2017).

In spite of the fact that countless biologically relevant information has been obtained with more than 160000 solved protein 3D-structures, classical structural biology approaches tend to give time-average representations. However, it is now clear that proteins are dynamical objects. Techniques such as NMR, THz and neutron spectroscopy and neutrons/X-rays scattering have unraveled various kinds of intramolecular movements within proteins with different timescales (Xu and Havenith, 2015), see [Figure 3](#). On one hand, flexibility of proteins is particularly critical for enzymes: substrate selection, entry, binding and catalysis is permitted by both local and general protein intramolecular movements (Zhao, 2017; Sen *et al.*, 2017; Rout *et al.*, 2018; Fürst *et al.*, 2019). On the other hand, stiffer regions within structures have been shown to be relevant for proton or electron transfer (Réat *et al.*, 1998; Sacquin-Mora *et al.*, 2007).

Nucleic acids

Nucleic acids are the biomolecules that harbor the genetic information, which can be transferred horizontally from organism to organism, or vertically from generation to generation. They chemically correspond to linear polymers of nucleotides linked by phosphodiester bonds. Nucleotides are made of a sugar (ribose for RNA and deoxyribose for DNA) associated with a nitrogenous base which can be a purine (adenine, guanine) or a

pyrimidine (cytosine, thymine for DNA or uracil for RNA). The base defines the nucleotide leading to the one-letter abbreviation: A, C, G, T (or U).

DNA is synthesized *in cellulo* on a DNA template by enzymes called DNA-polymerases, during the process of semi-conservative replication. However, in cells infected by retroviruses, DNA can also be synthesized from an RNA template by retro-transcriptase enzymes. In contrast, RNAs are synthesized from a DNA template during transcription in the cell but can also be replicated from an RNA template in some RNA-virus-infected cells.

In Watson and Crick base pairing, bases interact with each other as A-T, A-U, G-C. It can either lead to the interaction between two separate strands or to the folding of a single strand. This pairing also leads to the right-handed helical structure of double-stranded nucleic acids. The DNA double-helix is usually in a right-handed form called B-DNA. However, as a function of the environment, two other helical structures can be found: the A-form, with increased diameter, and the left-handed Z-form (Henderson and Krude, 2004).

The nucleotide sequence constitutes the primary structure of a nucleic acid, secondary structure results from base pairing and tertiary structure from the resulting three-dimensional folding. Nucleic acids can also be chemically modified by enzymes. DNA can be methylated while RNA can undergo many different post-transcriptional modifications (Nachtergaele and He, 2017). DNA is also generally topologically negatively supercoiled *in cellulo*.

Depending mainly on sequence and length, all double-stranded nucleic acids possess a melting temperature (T_m) above which half of the molecules will be turned into single-stranded form. However, nucleic acid stability and folding is also a function of salt concentration as they interact with ions, mainly cations (Von Hippel and Schleich, 1969). tRNA is for example, has been shown to be stabilized within salt crystals (Tehei *et al.*, 2002).

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While DNA mostly serves as storage of genetic information, RNA molecules may possess many functions.

Among them, ribozymes possess catalytic activity which, like protein enzymes, is dependent on structure and dynamics (Doherty and Doudna, 2001). Ribosomal RNAs (rRNAs) are essential and universal ribozymes found in all cells. rRNAs assemble with proteins in a macromolecular assembly called the ribosome. Ribosome decodes genetic information of mRNA into protein sequence which they synthesize (Cech, 2000). This process of translation also requires another type of RNA, transfer RNA (tRNA), which recognizes a three nucleotides sequence (codon) on mRNA, and brings, to the ribosome, the corresponding amino acid (according to the genetic code) to be inserted in the growing polypeptide.

Damage to nucleic acids can be spontaneous or induced by chemicals or physical conditions. These damages can lead to mutations and change or loss of the genetic information. While most mutations are generally repaired by enzymes, they still permit phenotypic variation, adaptation and natural selection and, thus, contribute to Darwinian evolution.

Lipids

Lipids are amphiphilic molecules with polar heads and hydrophobic tails. In aqueous solution, they spontaneously form diverse structures of which the bilayer is the most relevant for membrane formation. Note that in certain archaea, the lipid molecules in the outer and

inner leaflets are fused by the tails effectively turning the bilayer into a monolayer. The permeability barrier in biological cellular membranes is the bilayer made up of phospholipids and glycolipids and other hydrophobic molecules such as sterols into which transmembrane proteins are inserted. Membranes segregate cell components from extracellular environments and allow compartmentation. Control of diffusion and active transport across the membrane enables the possibility for cellular homeostasis and chemical gradients which can be used to produce energy.

Lipid bilayers take up different thermodynamic phases as a function of temperature and pressure, nature and length of hydrophobic tails, positions of unsaturated bonds in the tails, cyclization etc. Typically, the physiological state of a biological membranes is relatively fluid while not too disordered, allowing balance between fluidity and rigidity (see [Figure 4](#)) to favor membrane protein dynamics and function.

Furthermore, protein-lipid interactions modulate many membrane functions such as enzymatic activity and microdomain structure (Tien and Ottova-Leitmannova, 2000; Richens *et al.*, 2015).

Effects of extreme conditions on terrestrial biomolecules

Temperature, salinity and pressure conditions deeply affect structural and dynamical properties of proteins. Even if some general alterations can be recognized, effects of these conditions depend on the biomolecule. In particular, it should be kept in mind that, depending

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on their folding, all proteins react differently to these conditions. Therefore, every protein, even within one organism, possesses its own temperature, salinity and pressure limits.

Effects of low temperature on biomolecules

About 75% of the terrestrial biosphere can be considered as cold ($T < 5^{\circ}\text{C}$) with many environments that are permanently cold such as deep ocean and polar habitats (Cavicchioli, 2006).

Temperature is a general physical parameter governing all biomolecules behavior. The main result of lowering temperature is dynamical, decreasing the motion of atoms in water and biomolecules. As a consequence, processes requiring flexibility such as catalysis can be inhibited at low temperature, unless adaptation has led to weaker intramolecular bonding to allow the flexibility (Tehei *et al.*, 2004). Moreover, as motion is reduced, diffusion is also slowed down at low temperatures, limiting substrate and cofactors availability. According to Arrhenius law, a decrease of 20°C for example lowers reaction rates constants by four orders of magnitude (Russell *et al.*, 1990).

In addition to this effect, as a result of a decreased motion of atoms, proteins and all other biomolecules, loose flexibility at cold temperatures. As flexibility is critical for substrate acceptance and chemical transformation, cold temperature disrupts catalytic potential of enzymes.

Upon further temperature decrease, many observations tend to show that proteins undergo a process called cold denaturation leading to unfolding (see above, section Structure-Dynamics-Function in Biomolecules). In fact, through the hydrophobic effect, hydration of non-polar residues by water promoting unfolding is favored at low temperatures (Tsai *et al.*, 2002; Yoshidome and Kinoshita, 2012). In particular, formation of ice-water interfaces promotes this unfolding (Arsiccio *et al.*, 2020). However, some have argued that, instead, contribution of hydrophilic groups would be more important (Ben-Naim, 2013). Nonetheless, folding of proteins is impaired or slowed down by low temperatures. This can lead to the disassembly of large molecular edifices such as viral capsids (Da Poian *et al.*, 1995). At low temperatures, ribosome function is also reduced, impairing or slowing down even more protein folding.

In contrast to cold-denaturation of proteins, folding of nucleic acids is enhanced at low temperatures, which stabilize secondary structures, including non-native folding (D'Amico *et al.*, 2006). mRNAs in particular are considered more cold-sensitive than proteins (Narberhaus, 2002). However, increased stability of structures, like DNA hairpins for example, impairs processes like replication, transcription or translation as the polymerases or ribosomes are blocked by these structures (Inouye and Phadtare, 2008).

Processes involving DNA are also impaired by the increase of negative supercoiling induced by cold temperature (Wang and Syvanen, 1992; Mizushima *et al.*, 1997).

Reduced flexibility of biomolecules also affects membrane structure and functions. Lowering temperature promotes gel phase transitions in membranes, increasing compacity of lipid layer, rigidity and reducing permeability and lipid motion (Jebbar *et al.*, 2015, 2020a), see

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Figure 4. This impairs processes such as secretion and transport. Formation of ice crystals can even perforate cellular membranes (Muldrew and McGann, 1990; Parrilli *et al.*, 2011).

Water properties dramatically change with lowered temperature. Viscosity is increased and water activity (a_w) is decreased, meaning that there is less water available for catalysis, solvation and interaction with biomolecules.

From a general point of view, low temperature causes osmotic stress. When ice forms in the extracellular environment, osmotic pressure of cytoplasmic water is higher than extracellular ice leading to the efflux of water out of the cell and thus to a dramatic increase of many cytoplasmic compounds concentrations (Russell *et al.*, 1990). Concentration of the extracellular environment may make the environment more extreme (Banciu and Sorokin, 2013). However, due its composition, intracellular water freezes at temperatures under 0°C. In *S. cerevisiae*, for example, cytosol vitrification typically happens at -17°C (Fonseca *et al.*, 2016). At this point, intracellular ice formation concentrates even more the cytoplasm, altering pH, salinity, ionic strength, and a_w (Russell *et al.*, 1990).

Another general effect of low temperature on water is an increased solubilization of gas such as oxygen (D’Amico *et al.*, 2006), leading to an enhanced production of ROS species and thus to an oxidative stress (Abele and Puntarulo, 2004; Vinagre *et al.*, 2012).

Effects of high temperature on biomolecules

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3 In general, increased temperature means increased molecular movements and overall
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5 increased flexibility (Daniel and Cowan, 2000). A first consequence is that temperature acts as
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7 a catalyzer of most reactions, like the spontaneous degradation of many small molecules in
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9 biological systems. Unstable metabolites like ATP, NAD and acetyl phosphate, for example,
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11 have a short half-life at 95°C (Daniel and Cowan, 2000).
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18 For protein heat damage, it is possible to distinguish irreversible (degradation) and
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20 reversible (denaturation) damage (Daniel *et al.*, 1996).
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23 Protein flexibility enhancement at high temperature can also lead to a decrease of
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25 stability. As discussed before in this review, protein folding relies on various intramolecular
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27 interactions such as hydrophobic contacts, for example. As at high temperature atomic
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29 motions are increased, these bonds loose some strength and exposure of hydrophobic regions
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31 is enhanced, favoring unfolding (Das and Surewicz, 1995). This local denaturation is to some
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33 extent reversible as some protein can spontaneously fold back upon temperature decrease
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35 (Daniel *et al.*, 1996). Exposure of hydrophobic regions may, however, promote, interaction
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37 between unfolded proteins and hence to irreversible aggregation.
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42 In short, high temperature promotes unfolding of proteins which can be reversible.
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47 Several irreversible chemical modifications of amino acids composing proteins are
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49 enhanced by high temperatures: deamidation of Asn and Gln residues, succinimide formation
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51 at Glu and Asp and oxidation of His, Met, Cys, Trp and Tyr (Ahern and Klivanov, 1985; Zale and
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53 Klivanov, 1986; Daniel *et al.*, 1996; Daniel and Cowan, 2000). These irreversible degradations
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55 not only impair folding but can also lead to cleavage of peptide bond, leading to the loss of
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57 structure and function of proteins.
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Nucleic acids are also altered by high temperature in both reversible and irreversible ways. As temperature increases, subtle alterations of DNA double helix occur: the winding angle expands which lengthens the repeat and reduces the twist in the DNA molecule. A further effect of temperature is the reversible denaturation of the double helix by unbinding of base pairs. When turned into single stranded form, DNA is more sensitive to chemical irreversible damage: hydrolysis which leads to purine deamination and formation of abasic sites, oxidation leading to the formation of hydroxy-guanine or even to a rupture of the helix, saturation of double bonds in nucleobases and non-enzymatic methylation (Lindhal, 1993; Daniel and Cowan, 2000). All these damages impair normal Watson-Crick pairing of nucleobases and can lead to the accumulation of deleterious mutations if not repaired by the cell.

RNA is not only be altered by the previous effects but its differences with DNA make it even more thermosensitive. Because the sugar moiety in RNA is ribose, which possesses an additional hydroxyl group, it is sensitive to thermally-enhanced hydrolysis leading to the cleavage of the backbone (Voet and Voet, 2016). Moreover, as RNA incorporates uracil, which lacks a methyl group, instead of thymine, base stacking is less stable than in DNA. Both effects equally diminish thermostability of RNA in comparison to DNA (Wang and Kool, 1995), dramatically shortening its half-life at high temperatures.

Membrane properties are also directly temperature-dependent. Increasing temperature promotes fluid phase transitions in the lipid components, increasing disorder, fluidity, permeability and lipid motion (Jebbar *et al.*, 2015, 2020a), see [Figure 4](#). Increased molecular agitation of hydrophobic tails means less interactions between them and increased permeability of sodium and protons, particularly in non-thermophile membranes (Driessen *et*

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3 *al.*, 1996; Daniel and Cowan, 2000). As a result, non-thermophilic organisms face problems in
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5 maintaining homeostasis in high temperature environments. Transmembrane gradients are
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7 hence impaired which can turn into bioenergetic failure.
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13 At high temperature, water solubilization of gases is decreased, water molecules are
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15 more disordered and interact less with charges on biomolecules, impairing protein solvation
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25 Effects of high salinity on biomolecules

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31 As for other biochemical parameters, cells actively maintain their cytoplasm at a given
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33 salinity in composition and concentration that is different from that in the extracellular
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35 environment. In particular, in all cells, while the Na^+ ion is dominant outside, the K^+ is
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37 dominant within the cell. This is true in human blood, for example, in which an injection of
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39 physiological 0.9% NaCl into the serum will contribute to treat a dehydrated patient, while an
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41 equivalent concentration of KCl would kill the same patient, by overwhelming the membrane
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43 pumps acting on ion exchange. In hypersaline environments such as salt lakes and marshes,
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45 rock salts, brine ice-inclusions and sea brines (Kanekar *et al.*, 2012), the outside osmolarity
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47 poses a further challenge, counterbalanced in the cytoplasm by neutral molecules like glycerol
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49 or ectoine, or, in certain archaea and bacteria, by KCl. While the dominant salt in marine
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51 hypersaline environments is NaCl, other natural and artificial environments can be KCl, MgCl_2 ,
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53 MgSO_4 or CaCl_2 -rich (Schneegurt, 2012; Oren, 2013; Jebbar *et al.*, 2020b).
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Salinity-induced toxicity results from two distinct effects: non-specific osmotic stress and ion-specific toxicity (Serrano, 1996). Depending on the organism, one can be more important than the other. For example osmotic stress is more toxic in *E. coli* while sodium-specific toxicity primes in *S. cerevisiae* (Serrano and Gaxiola, 1994).

Specific toxicity of ions comes from their excessive interaction with proteins. While proteins require ions interacting with their charged or polar uncharged residues, excessive ions abundance can impair protein integrity or interactions, for example, through charge screening.

A change of cytoplasmic ion composition may hence alter these interactions: some ions with other steric properties will quench previous ones or new destabilizing ionic interactions will appear (Larsen, 2011; Okur *et al.*, 2017).

At molar concentrations, ions can have chaotropic effects, decreasing surface tension and increasing hydrophobic solubility. Chaotropic ions may either be cations or anions depending on their hydration and their effect can be countered by kosmotropic ions with opposite effects, see [Table 2](#).

Chaotropic ions (either strongly hydrated cations or weakly hydrated anions) interact with protein backbone (see [Figure 5](#)) and thus promote exposure of backbone to solvent, leading to unfolding (Kumar and Venkatesu, 2014; Mazzini and Craig, 2017). Chaotropic divalent cations Mg^{2+} or Ca^{2+} also interact with side-chains of negatively charged amino acids, quenching their eventual physiological interactions involved in structure or function (Okur *et al.*, 2017). These ions are intracellularly accumulated in athalassohaline environments (Oren, 2013), which can be defined as aquatic environments with ionic proportions different from seawater.

Chaotropic anions (weakly hydrated) however interact less with side-chains of positively charged residues than kosmotropic anions (strongly hydrated) (Okur *et al.*, 2017) and hence represent a lesser threat to proteins.

It has been proposed that chaotropic ions also alter general structure and properties of water (Marcus, 2009) but this idea has been debated (Ball and Hallsworth, 2015).

In general, high concentrations of salt ions directly impair interactions between all charged biomolecules: proteins, DNA, RNA, lipid heads, metabolites, etc (Eisenberg, 1995). However, each protein reacts differently depending on its folding and its charge.

Osmotic stress comes from the binding of water by salt ions, lowering water activity and causing hydric stress. In a hypersaline environment, water molecules are more bound to salt ions, hence less diffusive and less available for catalysis, solvation and interaction with biomolecules.

Effects of high pressure on biomolecules

High hydrostatic pressure ($P > 10\text{MPa}$) concerns mainly two environments: deep waters (88% of the total oceanic volume has a depth $> 1\text{ km}$) and continental and marine subsurface biosphere, which contains a significant proportion of total earth biomass (McMahon and Parnell, 2014). Hence, high pressure, like cold temperature, is a common extreme condition that terrestrial life faces (Jebbar *et al.*, 2015).

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Le Chatelier’s principle governs systems under pressure changes: equilibriums of non-isovolumetric reactions will react to pressure increases by shifting towards a decrease of volume. Therefore, reactions leading to net increase of charges, such as production of ions, are favored by pressure as charges can condensate water molecules, which will occupy less volume (Gross and Jaenicke, 1994; Fumiyoshi *et al.*, 1999). Another consequence is a favored overall compaction of biomolecules, impairing flexibility and dynamics.

The first effect of pressure on proteins is the dissociation of large molecular assemblies such as ribosomes (Gross *et al.*, 1993) or DNA-protein complexes (Kawano *et al.*, 2005). In fact, quaternary structure often relies on charged residues, which can, upon dissociation, condense water molecules. Most multimeric assemblies dissociate at 200MPa (Aertsen *et al.*, 2009).

Tertiary structure can also be altered and even lost under pressure. Pressure-induced protein unfolding has long been known (Bridgman, 1914), however, unlike thermal denaturation, mechanisms behind this process are still unclear. As pressure tends to enhance compaction of biomolecules, proteins with internal cavities are particularly affected (Girard *et al.*, 2010; Matthews, 2012; Roche *et al.*, 2012). As a general rule: if a protein occupies more space when folded, which is not the case of all of them, then pressure will favor its unfolding (Jebbar *et al.*, 2020a). Another effect favoring unfolding of proteins is that exposure of hydrophobic residue to solvent enables Van Der Waals interactions with water molecules hence condensing them (Gross *et al.*, 1993). Most proteins unfold at 400MPa (Aertsen *et al.*, 2009).

Extensive research has been done about effects of high pressure on DNA-stabilization. As for other macromolecules, DNA is compacted under pressurization. Preliminary work

showed that double-stranded DNA solutions can undergo pressure up to 1GPa without being denatured (Hedén *et al.*, 1964). Effect of pressure on DNA stability can be assessed by measuring changes of T_m which measures the temperature above which half of a given DNA undergoes denaturation. With the exception of some short simple synthetic DNA double-strands which are destabilized by high pressure (Hughes and Steiner, 1966; Gunter and Gunter, 1972), a general rule is that nucleic acids are stabilized under high pressure (Macgregor, 1998), the longer the more stable (Macgregor, 1996). More precisely, stabilization of nucleic acid duplexes is a function of their T_m at P_{atm} (Dubins *et al.*, 2001; Rayan and Macgregor, 2005). Crystallographic studies have shown that DNA can act as a molecular spring, being reversibly compacted under high pressure (Girard *et al.*, 2007).

However, since the DNA double-helix needs to be opened for several biological processes, high pressure-induced stabilization of double-stranded nucleic acids impairs fundamental biological processes such as replication, transcription and translation (Macgregor, 2002).

As with temperature, membrane rigidity and permeability are pressure-dependent (Hazel and Eugene Williams, 1990). Increasing pressure promotes gel phase transition of membranes, increasing compacity of lipid layer, rigidity and reducing permeability and lipid motion (Jebbar *et al.*, 2015, 2020a), see [Figure 4](#). As effect of pressure on proteins and nucleic acids appear to be moderated in comparison to other extreme conditions, membrane instead is considered to be the most pressure-sensitive biomolecule (Oger and Jebbar, 2010).

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Cytosolic water is also deeply affected by pressure. Condensation of water molecules being favored, water tends to lose its structure (Gross and Jaenicke, 1994), especially in the crowded cytoplasmic environment.

According to Henry-Dalton law, gas solubility in water is increased at high pressure (Gross and Jaenicke, 1994). Dissolution of CO₂ into water in particular releases protons which leads to acidification of cytosol (Abe and Horikoshi, 1998). This phenomenon is increased by the pressure-enhanced deprotonation of phosphate groups (Fumiyoshi *et al.*, 1999) and membrane rigidification which inhibits H⁺ export out of the cell (Abe and Horikoshi, 1995).

Interestingly, in *E. coli*, pressure stress induces synthesis of proteins that are involved in both cold-shock and heat-shock response (Welch *et al.*, 1993). In fact, pressure acts similarly to both low and high temperature as it increases stability of membrane and DNA and destabilizes proteins (Jebbar *et al.*, 2015).

Molecular traits allowing life at extreme temperatures, salinity and pressure

Psychrophilic molecular traits

The concept of psychrophily has been debated (Cavicchioli, 2015) to include both organisms requiring a low temperature for growth and organisms tolerant to coldness but growing faster at warmer temperature. A practical general definition would be that psychrophiles are organisms found in permanently cold environments.

Psychrophilic biomolecules are generally characterized by an overall increased flexibility and a lower thermostability. While in hot environments macromolecules “just” need molecular traits to increase their thermostability, in a cool environment (still above cold denaturation), macromolecules are stabilized by weaker forces that still allow functional dynamics as measured in a psychrophilic proteome (Tehei *et al.*, 2004). In contrast, the available energy for catalysis is so scarce that psychrophilic enzymes face the problem of maintaining an activity. However, no universal protein structural signature has been found in all psychrophilic proteins. Instead, each system uses different tools for increasing flexibility and rising catalytic efficiency (Smalås *et al.*, 2000; Bowman and Deming, 2014). As a consequence, these traits uniformly reduce thermal stability of psychrophilic proteins (Feller and Gerday, 2003). It should be noted that psychrophilic enzymes do not work optimally at low temperatures but instead maintain activity at lower temperatures than mesophilic and

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thermophilic counterparts. In contrast to mesophilic and thermophilic enzymes, which start to loose activity as they unfold, psychrophilic enzymes loose activity as temperature increases before unfolding (D’Amico *et al.*, 2003; Feller, 2013). [Figure 6](#) illustrates stability and activity dependence of a psychrophilic, mesophilic and thermophilic enzyme, respectively.

Increased glycine/proline residues ratios appears to be common in psychrophilic proteins (Zheng *et al.*, 2016). Gly residue (which side chain is replaced by an hydrogen) has an increased rotational freedom compared to other residues, its abundancy in psychrophilic proteins may hence provide local flexibility in protein chain. Surface Gly residues can be found far away from the active site (Fields and Somero, 1998), conferring local flexibility of domains increasing general wiggling capacities of the enzyme (Deniz, 2018). Gly abundancy can however also increase in binding pockets (Hashim *et al.*, 2018) increasing flexibility around the active site which improves turnover number of the enzyme at the expense of its affinity for its substrate (Fields and Somero, 1998; Georlette *et al.*, 2000). In general, catalytic potential in the active site is achieved by optimized electrostatics (Smalås *et al.*, 2000).

In contrast, the Pro residue, which has less movement freedom and bares rigidifying properties, is scarce in psychrophilic enzymes (Zheng *et al.*, 2016), particularly in protein loops (Hashim *et al.*, 2018), which are longer and more flexible (Wallon, Lovett, *et al.*, 1997).

As revealed by several X-ray crystal structures, flexibility of psychrophilic enzymes is largely achieved by a weakening of all intramolecular interactions (Gerday *et al.*, 1997; Casanueva *et al.*, 2010) as suggested by proteome molecular dynamics (Tehei *et al.*, 2004). Surface salt bridges involved in tertiary or quaternary structure are less frequent (Wallon, Lovett, *et al.*, 1997; Riise *et al.*, 2007; Michaux *et al.*, 2008; Bauvois *et al.*, 2008; Parvizpour *et al.*, 2017) and surface charged residues, even if they are often less abundant (Aghajari *et al.*,

1998), are generally unpaired (Arnórsdóttir *et al.*, 2005; Almog *et al.*, 2008; Pedersen *et al.*, 2009), hence facilitating binding of water and stability of solvation shell. Hydrogen bonds too are less abundant (Leiros *et al.*, 2007; Michaux *et al.*, 2008; Altermark *et al.*, 2008; Xie *et al.*, 2009). In addition to lowering overall rigidity, reduced hydrophobic interactions in psychrophilic enzymes (Arnórsdóttir *et al.*, 2005; Huston *et al.*, 2008), especially those implying aromatic residues (Wallon, Lovett, *et al.*, 1997), also counters hydrophobic effect caused by low temperature.

Psychrophilic protein cores are also made less rigid and compact by reducing their hydrophobicity (Hashim *et al.*, 2018), decreasing the number of Arg and Pro residues (Aghajari *et al.*, 1998) or by introducing salt bridges across it, which facilitate solvent penetration and destabilize the core (Parvizpour *et al.*, 2017). In fact, exposure to solvent increases entropy and thus participate to the gain of flexibility in psychrophilic proteins (Wallon, Lovett, *et al.*, 1997; Arnórsdóttir *et al.*, 2005; Thorvaldsen *et al.*, 2007; Bauvois *et al.*, 2008; Zheng *et al.*, 2016).

Structural traits in cold-adapted proteins are represented in [Figure 7](#).

Some studies have found other AA biases associated with psychrophily such as more Ser and Leu and less Arg, Glu, Thr and acidic residues (Médigue, 2005; D'Amico *et al.*, 2006; Casanueva *et al.*, 2010; Raymond-Bouchard *et al.*, 2018). However, relations between these sequence biases and structural modifications are less common than the increased Gly/Pro ratio.

Psychrophiles also produce a specific kind of protein to cope with subzero temperatures: antifreeze proteins (AFP). AFP are highly soluble and specifically bind ice nuclei with their hydrophobic residue by strong Van der Waals interactions (Howard *et al.*, 2011,

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2014), limiting crystallization of the cytoplasm (Scotter *et al.*, 2006; Venketesh and Dayananda, 2008).

Since nucleic acid secondary structures are more stable at low temperatures, many processes involving their opening, such as transcription for DNA and ribosome biogenesis for RNA, are impaired. This could be managed in psychrophiles by overexpression of helicases (Lim *et al.*, 2000), which are enzymes able to open nucleic acid double helix in either double stranded or folded molecules.

Increased flexibility in psychrophiles is also met at the membrane level. In contrast to proteins, psychrophilic lipids are not more flexible but possess more steric constraints reducing the packing of hydrophobic queues and thus leading to a less rigid membrane. Incorporation of unsaturation in hydrophobic tails of lipids is a common feature as it induces bends in the tails (Dexter and Cooke, 1984; Okuyama *et al.*, 1993; Jebbar *et al.*, 2020a). Other trait perturbing compacity and increasing fluidity include methyl-branched lipids (Russell, 1997) and shorter acyl chains (Anesi *et al.*, 2016; Rafiq *et al.*, 2019). Some of these traits are summarized in Figure 8. Membrane fluidity can also be modulated by insertion of hydrophobic carotenoid pigments (Jagannadham *et al.*, 2000; Fong *et al.*, 2001a). While polar carotenoid insertion rigidifies membranes, non-polar carotenoids fluidify them (Subczynski *et al.*, 1992).

Psychrophilic cytoplasmic content is characterized by an abundance of small molecules. Like organisms coping with high salinity or desiccation, psychrophilic organisms counteract reduced water availability by enriching their cytoplasm with several classes of compatible solutes. Cryoprotectant molecules such as trehalose and exopolysaccharides (EPS)

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3 lower water freezing point (D'Amico *et al.*, 2006), bind water molecules limiting their effusion
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5 out of the cell and may prevent protein denaturation and aggregation (Phadtare, 2004);
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7 interestingly, neutron spectrometry has shown that trehalose stiffens dynamics and reduces
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9 flexibility in proteins (Cordone *et al.*, 1999), an unfavorable property for psychrophiles.
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11 Trehalose also protects membranes during cold-induced dehydration (Goodrich *et al.*, 1988).
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13 Polyols, such as glycerol and mannitol, are also common molecules produced by psychrophiles
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15 which act as buffering agents for proteins (Jennings, 1984).
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20 It has also been shown that chaotropic agents are preferentially accumulated by
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22 psychrophiles and that adding such agents in their medium enhances their ability to grow at
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24 low temperatures (Chin *et al.*, 2010).
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33 Thermophilic molecular traits

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39 Thermophiles and hyperthermophiles are organisms defined by optimal growth
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41 temperatures above 60 and above 80 °C, respectively. Hyperthermophiles are only
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43 represented in bacteria and archaea. The most hyperthermophilic microorganisms are
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45 archaea.
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48 At a given temperature, while psychrophilic biomolecules possess increased flexibility
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50 and catalytic potential, thermophilic biomolecules are generally characterized by increased
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52 rigidity, expressed in higher resilience or effective force constants maintaining
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54 macromolecular structure, which would contribute to their stability at high temperatures
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56 (Tehei *et al.*, 2004). As a consequence, these traits make catalysis with thermophilic enzymes
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extremely inefficient at moderate or low temperatures. Despite their exceptional thermostability, most thermophilic enzymes *in vitro* start to unfold at 80°C. As, according to the Arrhenius law, reaction speeds keep increasing with temperature, stability of enzymes is the only upper limit for catalysis in hyperthermophiles. Optimal temperature for activity is actually reached at a temperature so high that it unfolds most molecules, see [Figure 6](#).

Thermophilic proteins in particular are more stable and more resilient (Tehei and Zaccai, 2007; Zaccai, 2013). In fact, resilience has been pointed out as a better term than rigidity for explaining thermostability (Aguilar *et al.*, 1997). Thermophilic enzymes usually display significantly reduced catalytic activity at room temperatures, possess higher optimal temperatures, exceptional thermostability (Vieille *et al.*, 1996; Vieille and Zeikus, 2001) and some, such as amylopullulanases from the archaea *Pyrococcus furiosus* and *Thermococcus littoralis*, display activity at temperatures as high as 110-125°C (Brown and Kelly, 1993). As the environment provides much of the energy, catalysis is not a challenge for thermophilic enzymes.

In contrast, thermal denaturation being the challenge, thermophilic proteins have evolved multiple ways to achieve thermostability. At first sight, thermophilic, mesophilic and psychrophilic proteins do not display much difference (Vieille and Zeikus, 2001) with sequence identity of 40-85% (Davies *et al.*, 1993), superimposable tridimensional structures (Gianese *et al.*, 2002) and identical catalytic mechanisms (Zwickl *et al.*, 1990; Bauer *et al.*, 1998). In fact, thermophilic properties are achieved by a small number of specific mutations which obey to no clearly understood yet rule (Han *et al.*, 2019) but tend to improve compaction (decreasing surface/volume ratio), resilience and enhance hydrophobic interactions within the protein core (Haney *et al.*, 1999; Feller, 2010).

Comparative studies of protein dynamics and primary, secondary, tertiary and quaternary structures have given some insights about the ways to achieve thermostability (Irimia *et al.*, 2004; Koutsopoulos *et al.*, 2005a, 2005b; Karlström *et al.*, 2006). It appears that the most recurring molecular traits in thermophilic proteins are salt bridges and ion-pair networks lowering exposure of the protein to the solvent (Goldman, 1995; Yip *et al.*, 1995; Kumar *et al.*, 2000). Indeed, as at high temperatures water molecules interact less with charged residues, these residues can form stabilizing interactions (Elcock, 1998).

For thermophilic multimeric assemblies, increased hydrophobicity of subunit interfaces (Wallon, Kryger, *et al.*, 1997; Hatanaka *et al.*, 1997) and introduction of intermolecular disulfide bridges (Dick *et al.*, 2016) favor stability of the quaternary structure. Within globular proteins, interactions between aromatic residues (Phe, Trp, Tyr) have also been proposed as a way to achieve thermostability (Karlström *et al.*, 2006). Probably as a way to protect them from oxidation which is enhanced at high temperature, Cys residues involved in disulfide bridges are often buried within the protein (Choi *et al.*, 1999; Kumar and Nussinov, 2001). As Gly and Pro residues respectively induce flexibility or rigidity in protein backbone, many thermophilic proteins display decreased Gly/Pro ratio (Vieille and Zeikus, 2001). However Gly are more abundant in alpha helices where they increase stability (Petski, 2001). Flexible surface loops can also be shortened or deleted in thermophilic proteins (Thompson and Eisenberg, 1999; Yamagata *et al.*, 2001; Tanaka *et al.*, 2004), hence decreasing surface/volume ratio (Petski, 2001).

Figure 7 summarizes some major interactions and structural features enabling thermostability in thermophilic proteins.

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In contrast to the negatively supercoiled DNA in mesophiles, all hyperthermophiles have the reverse gyrase enzyme that positively supercoils DNA *in vitro* (de La Tour *et al.*, 1990). This enzyme is specific to thermophiles, possessed by all both bacterial and archaeal hyperthermophiles and is absent from mesophiles (Catchpole and Forterre, 2019). However, the importance of this enzyme for hyperthermophilic life has been long debated (Forterre, 2002; Atomi *et al.*, 2004; Heine and Chandra, 2009). Nonetheless, it is now clear that growth above 90°C requires reverse gyrase (Lipscomb *et al.*, 2017). The *in vivo* function of reverse gyrase is yet still unclear. Reverse gyrase may prevent thermal denaturation (Ogawa *et al.*, 2015), maintain constant helical repeat in the DNA molecule (Forterre *et al.*, 1996), assure DNA recombination (Jamroze *et al.*, 2014) or repair thermally-depurinated bases (Kampmann and Stock, 2004).

Other major traits found in some thermophiles for DNA protection include: compaction of DNA by cationic histone-like proteins (Green *et al.*, 1983; Grayling *et al.*, 1996), stabilization by high intracellular ions concentrations (Hensel and König, 1988) and by polyamines such as putrescine, spermidine, norspermidine, thermospermine and spermine (Friedman and Oshima, 1989).

We could also mention the case of the virion of SIRV2, a virus infecting the hyperthermophilic archaeon *Sulfolobus islandicus*. In this virion, tight polar contacts between viral proteins and viral DNA force him to adopt A-form instead of canonical B-form, limiting contact with solvent and hence protecting it (DiMaio *et al.*, 2015). This way to protect DNA in absence of active repair system is also found in some bacterial spores which are particularly resistant to heat (Setlow, 1992).

Some post-transcriptional chemical modifications of RNA have also been found to enhance thermostability, such as methylation of rRNA (Noon *et al.*, 1998) and tRNA (Edmonds *et al.*, 1991; Kowalak *et al.*, 1994).

Similarly to their proteins, lipids of thermophiles and hyperthermophiles are more rigid and stable than their mesophilic counterparts. Lipids of hyperthermophilic archaea possess several features increasing membrane thermostability. Firstly, instead of an ester bond, polar head and hydrophobic tails are bound by an ether bond, which is less susceptible to hydrolysis. While most lipids in mesophiles possess one head for two hydrophobic tails, hyperthermophilic archaea have lipids with two polar heads, one at either end of the molecule, which can either occupy the same side or opposite sides of the membrane (De Rosa and Gambacorta, 1988). Such lipids form a monolayer in the membrane, like a bilayer of molecules whose tail ends are fused together, which is a structure believed to be particularly rigid. Other determinants of thermostability in lipids are hydrophobic tails of increased length and branching isoprenoid cyclization and crosslinking of isoprenoid chains (Gliozzi *et al.*, 1983; Tolner *et al.*, 1997). These traits increase interactions between lipids and other membrane components, enhancing overall rigidity, stability and impermeability of hyperthermophilic membrane (Gambacorta *et al.*, 1995).

Recently, it has been shown that insertion of squalane, an apolar molecule found in membranes of hydrothermal vent hyperthermophilic archaea, may contribute to membrane thermostability by inducing non-lamellar phase (Salvador-Castell *et al.*, 2020).

In spite of the fact that these features are mostly archaeal traits, some hyperthermophilic bacteria also possess lipids with one of more of these properties (Koga, 2012; Vinçon-Laugier *et al.*, 2017).

See [Figure 8](#) for a summary of main thermophilic lipid traits (Jebbar *et al.*, 2015, 2020a).

As with DNA protection, hyperthermophiles also produce various small soluble organic molecules which improve stability or refolding of proteins (Lamosa *et al.*, 2000; Faria *et al.*, 2004; Empadinhas and Costa, 2006; Mukaiyama *et al.*, 2008). These thermoprotectants are typically molecules derived from sugar, amino acid or energetic metabolism with kosmotropic properties (Faria *et al.*, 2004; Schiefner *et al.*, 2004).

Halophilic molecular traits

Organisms able to grow at salinities above sea average are generally considered halotolerant. True halophiles are organisms with an absolute requirement for high salt concentrations in order to grow. Two strategies for salt adaptation are generally observed, depending on which osmolyte counterbalances high extracellular salt: salt-in-cytoplasm and organic-osmolyte cytoplasm (Jebbar *et al.*, 2020a). While the latter enables to some extent exclusion of salinity out of the cytoplasm, the fact that the most extreme halophiles all display salt-in strategy leads to the idea that organic-osmolyte strategy may not be sufficient in extremely saline environments. The salt-in-cytoplasm strategy has been proposed in halophilic protozoa (Harding, 2018) and allows extreme halophilic archaea such as *Halobacterium salinarum* to grow in salt-saturated environments (Ventosa and Oren, 1996). Moreover, the salt-in strategy implies adaptation of the whole proteome to the extreme

condition. Halophiles may also use a combination of both strategies to cope with their environment.

Halophiles counter the outside osmolarity of Na^+ by accumulating K^+ in the cytoplasm (Ginzburg and Ginzburg, 1976). Intracellular concentration of K^+ typically reaches 2-5.5M in extremely halophilic archaea while Na^+ concentration is generally maintained under 2M (Oren, 2002).

In order to function in such a hypersaline cytoplasm, halophilic proteins possess several molecular traits allowing stability, activity and dynamics under these conditions. It was initially believed that the solvation shell in these proteins excluded salt, until a battery of complementary biochemical and biophysical methods established the opposite: Na^+ , K^+ , Cl^- ions are, in fact, recruited into the solvation shell and into the structure, and are essential for stabilization and dynamics (Bonneté *et al.*, 1993). Remarkably, even if halophilic malate dehydrogenase, the model studied, is from a salt-in-cytoplasm archaeon, it can also be stabilized similarly to mesophilic proteins by high concentrations of kosmotropic salts (Bonneté *et al.*, 1994).

Compared to mesophilic homologs, halophilic proteins are enriched in acidic residues (Asp and Glu). Surfaces of halophilic proteins in particular present more acidic patches (Dym *et al.*, 1995) and clusters of salt bridges, especially in interfaces between subunits of multimeric proteins (Richard *et al.*, 2000). This enrichment leads to highly negatively charged proteins (Kutnowski *et al.*, 2018; Niero *et al.*, 2020; Shirodkar *et al.*, 2020). In hypersaline solvents, such accumulation of charges has been proven to stabilize proteins. The reason lies in both increased ion-binding and solvation shell-structuring properties. In facts, more surface negatively charged residues can bind more solvent cations (Ebel *et al.*, 2002). On the other

hand, anions appear to be strongly bind by fewer sites (Ebel *et al.*, 2002). This ion-rich shell may limit water condensation on protein surface which would lead to excessive interaction between proteins and, hence, to their aggregation (Costenaro *et al.*, 2002). Nonetheless, as shown by neutron diffraction experiments, aspartic acid is also particularly efficient for tightly binding water molecules in hypersaline solutions (Lenton *et al.*, 2016). Thus, acidic surfaces of halophilic proteins strongly bind water (Frolov *et al.*, 1996) into a dense, highly structured solvation shell with multiple layers (Britton *et al.*, 2006) and disruption of pentagonal water networks (Talon *et al.*, 2014). In comparison, positively charged and uncharged protein surfaces appear to bind less tightly water molecules (Kim *et al.*, 2016). Models of interactions between salt, water and proteins have proposed that acidic surface composition may provide specific binding sites for water and salt ions on protein leading to a highly structured and salty solvation shell (Madern *et al.*, 2000). Because of this shell, surfaces are less accessible and overall interaction with bulk solvent is reduced, enhancing solubility and stability in low water activity environment (Tadeo *et al.*, 2009).

In contrast, halophilic proteins tend to have fewer Lys residues, slightly more small hydrophobic residues such as Gly, Ala and Val and less haliphatic residues like Phe (Madern *et al.*, 1995, 2000). Computational analysis of halophilic sequences also revealed a preference for small, polar and charged residue (Gln, His, Pro) and lower amounts of Asn, Cys, Ile, Met, Ser (Nath, 2016). As a consequence of these sequence bias, halophilic proteins tend to display increased flexibility (Paul *et al.*, 2008) and polarity and avoid positively charged residues and hydrophobicity (Nath, 2016), weakening hydrophobic contacts (Siglioccolo *et al.*, 2011) and leading to more polar and charged cores (Nayek *et al.*, 2014).

Since they have fewer Lys residues, halophilic proteins use more Arg residues in salt bridges which are crucial for stability in hypersaline environments (Madern *et al.*, 2000). On

average, halophilic proteins have two times more salt bridges than mesophilic homologs, both buried or exposed at surface, stabilized within salt bridges networks (Nayek *et al.*, 2014).

Major structural traits of halophilic proteins are represented in [Figure 7](#).

Interestingly, halophilic enzymes show resistance to organic solvent-induced unfolding (Niero *et al.*, 2020; Shirodkar *et al.*, 2020). Moreover, compared to their mesophilic homologs, halophilic enzymes tend to renature easier after heat-induced (Tokunaga *et al.*, 2004) or methanol-induced denaturation (Tokunaga *et al.*, 2017). This property is believed to originate from their high solubility countering the hydrophobic-based aggregation which normally makes denaturation irreversible (Tokunaga *et al.*, 2017).

Most of these traits make halophilic proteins highly unstable in low salinity environments: most unfold under 2-3M KCl (Eisenberg, 1995). Nonetheless some proteins found in halophiles show both properties of halophilic proteins, such as high solvation shell salt concentration, and non-halophilic proteins, such as the water-binding potential, making them resistant to both salinity-induced denaturation and to low-salinity denaturation (Coquelle *et al.*, 2010).

As halophilic proteins avoid positively charged residues, interaction with the negatively-charged DNA is achieved through cations tightly bound by negatively charged residues deep in the protein (Kutnowski *et al.*, 2018).

Membrane of halophiles possess various traits which may be helpful for living in hypersaline environments. In particular, to keep K^+ in their cytoplasm and to maintain Na^+ exclusion, membrane permeability is critical. Main adaptation is that halophilic lipids possess

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sulfate groups with negative charges shielding salt cations (Russell, 1989), limiting H⁺ and Na⁺ permeability and increasing stability (van de Vossenberg *et al.*, 1999).

Carotenoid insertion is observed in both halophilic archaea (Calegari-Santos *et al.*, 2016) and bacteria (Köcher and Müller, 2011). These hydrophobic pigments inserted in membrane act as membrane rivets, increasing its rigidity and impermeability (Lazrak *et al.*, 1988) and thus preventing cell lysis (Köcher and Müller, 2011). While polar carotenoid insertion rigidifies membranes, non-polar carotenoids fluidify them (Subczynski *et al.*, 1992).

Neutron scattering is particularly sensitive to intracellular water dynamics and as hence be used to study traits of water in halophilic cells (Jasnin *et al.*, 2010). Experiments on *Haloarcula marismortui*, an halophilic archaeaon from the Dead Sea, revealed a fraction of exceptionally slow water in the hypersaline cytoplasm, hypothetically organized by structured K⁺ ions (Tehei *et al.*, 2007; Frölich *et al.*, 2008).

The organic-osmolyte compatible solute strategy is characterized by the intracellular accumulation of small organic molecules such as betaine (Galinski and Trüper, 1982; Imhoff and Rodriguez-Valera, 1984; Nyyssola *et al.*, 2000) and ectoines (Ono *et al.*, 1998; Vargas *et al.*, 2006). Compatible solutes in halophiles are highly soluble molecules, polar but uncharged at intracellular pH, with kosmotropic properties that stabilize the solvation shell of proteins (Galinski, 1993; Zaccai *et al.*, 2016).

Piezophilic molecular traits

Growth of hyperthermophiles in black smokers at temperatures above the boiling point of water at surface pressures naturally requires high pressure to keep water liquid (Marteinsson *et al.*, 1999). However, many non-thermophilic deep sea bacteria (Kato *et al.*, 1998; Nogi *et al.*, 2007; Kusube *et al.*, 2017) demonstrated strict piezophily (impossibility to grow at atmospheric pressure) such as *Colwellia marinimaniae* sp. nov. which has been isolated in Mariana Trench, has a growth range of 80-140MPa and optimally grows at 120MPa (Kusube *et al.*, 2017). Obligate piezophiles have also been found in the archaeal domain (Zeng *et al.*, 2009; Birrien *et al.*, 2011). Remarkably, the most naturally pressure-tolerant archaea, *Thermococcus piezophilus* CDGST, which optimally grows at 50MPa and at pressure as high as 130MPa, can still grow at atmospheric pressure (Dalmasso *et al.*, 2016).

In deep-sea organisms, evidence of molecular adaptation to high pressure has been found that depends on the habitat: hot hydrothermal vents or cold deep-sea waters and sea-floor. Nonetheless, comparisons to closely related non-piezophilic thermophiles, mesophiles and psychrophiles relatives have permitted to underline several piezophilic specificities.

Neutron scattering experiments have been used to measure dynamical response of macromolecules in piezophilic and non piezophilic under high pressure (Zaccai, 2020). Contrary to what is expected from Le Chatelier's principle, piezophilic protein dynamics is enhanced and solvation shell dynamics decreased under high pressure (Martinez *et al.*, 2016), a behavior believed to be an adaptive strategy. In contrast, non-piezophilic proteins lose flexibility and undergo compaction under high pressure. Bulk water dynamics is equally affected in piezophile and non-piezophiles. Enhanced protein flexibility, which has also been

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observed for individual piezophilic proteins (Shrestha *et al.*, 2015), and a more compact and less dynamic solvation shell may hence be an adaptive strategy for piezophiles.

Enhanced flexibility compensates pressure-induced rigidification while compaction of the volume occupied by water molecules bound to proteins could stabilize them.

High pressure environments tend to constrain protein sequence evolution towards the reduction of side-chain volume (Gunbin *et al.*, 2009) and assembly of multimeric assemblies tend to display reduced volume changes in deep sea organisms (Morita, 2003). An explanation could be that folding or assembly processes which are less dependent on volume changes are less pressure-sensitive.

Sequence analysis revealed that sequence bias in piezophiles is actually dependent on the thermophilic/psychrophilic status. Nonetheless, in comparison to non-psychrophilic homologs, psychrophilic proteins tend to have more polar, hydrophilic and small amino acids (Di Giulio, 2005; Nath and Subbiah, 2016), counting for the reduction of side-chain volume. For non-psychrophilic piezophiles, hydrophobic, non-polar and aliphatic amino acids are also over-represented in comparison to non-psychrophilic relatives (Nath and Subbiah, 2016).

Loosely packed protein structures also seem to offer a way to decrease deleterious volumetric effects of high pressure (Nagae *et al.*, 2012). Indeed, when structure and activity rely on internal cavities, larger internal volumes in piezophilic proteins permit to maintain some internal cavities at abyssal pressures while, in comparison, non-piezophilic counterparts will lose too much internal volume. Major structural traits of pressure-adapted proteins are represented in [Figure 7](#).

As quaternary structure is primarily affected by high pressure, it has been found that multimeric assemblies, such as SSB DNA-binding proteins from piezophilic *Shewanella* strains

(Chilukuri *et al.*, 2002) and large TET peptidases assemblies from piezophilic *Pyrococcus horikoshii* (Rosenbaum *et al.*, 2012), are more resistant to pressure-induced dissociation.

Some nucleic acid piezophilic traits have been found. For example, an increased length of helices in 16s rRNA improves interaction with proteins and overall resistance to pressure-induced dissociation of ribosomes (Lauro *et al.*, 2007).

Piezophilic membrane traits have been described. The common feature of piezophilic membrane is increased fluidity (Siliakus *et al.*, 2017) which is achieved with molecular traits shared with psychrophiles: more unsaturated (DeLong and Yayanos, 1985; Hazel and Eugene Williams, 1990) and shorter (Bartlett and Bidle, 1999) hydrophobic tails. Branched lipids, which increase membrane fluidity, have also been found to be produced by deep sea microorganism as a response to high pressure (Scoma *et al.*, 2019). In abyssal hydrothermal environments however, high temperature may lead to a preference for typical thermophile lipids and to highly stable and rigid membranes.

Accumulation of compatible solutes in piezophiles may also help to cope with high pressure. β -hydroxybutyrate has been shown to be accumulated in the piezophilic bacterial response to high pressure (Martin *et al.*, 2002) and other small organic molecules may enhance protein stability under pressure in piezophilic cells (Jebbar *et al.*, 2015).

Interestingly, insertion of squalane, an apolar molecule found in hydrothermal vent archaea, in membranes not only enhances thermal stability but also resistance to pressure (Salvador-Castell *et al.*, 2020).

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It should be noted that high pressure triggers both cold-shock (Fujii *et al.*, 1999) and heat-shock responses (Sato *et al.*, 2015) in piezophilic *Shewanella* bacterial strains. Both pathways induce production of proteins or small molecules related to pressure-resistance.

Living with many extremes: Polyextremophily and limits of life

Terrestrial extreme environments often possess more than one extreme physical or chemical condition, see Figure 2. Inhabitants of these poly-extreme environments are thus called polyextremophiles. Abyssal black smokers thermophilic life also faces high pressure and high concentrations of toxic heavy metals which the hydrothermal fluid is enriched with (Seegerer *et al.*, 1993; Luther *et al.*, 2001; Damm, 2013) leading to the selection of metalloresistance (Vetriani *et al.*, 2005) whereas thermophiles in continental volcanic hot springs are exposed to high acidity and are thus also acidophilic (Seegerer *et al.*, 1993; Burton and Norris, 2000; Siering *et al.*, 2006; Seckbach *et al.*, 2013), soda lakes halophiles are also alkaliphilic (Jones *et al.*, 1998; Sorokin *et al.*, 2014), Río Tinto river microorganisms face acidic pH and high heavy metals concentrations (Amils *et al.*, 2014) and many brines, either abyssal, subglacial or on the surface, display combinations of high salinity and high pressure, chaotropicity, ultraviolet radiation, heavy metal abundancy, extreme pH and extreme temperatures (Hallsworth, 2019; Varrella *et al.*, 2020).

As it was described before, extraterrestrial environments can also have one or more extreme condition in regard of terrestrial life, see Figure 1. Mars subsurface hypersaline environments can be, depending on the depth, cold or hot and under pressure; icy moons

oceans can present, depending on the depth and the proximity with a hydrothermal vent, extreme temperatures, extreme pH and high pressure. Therefore, terrestrial environments where polyextremophilic thrive can be considered as good models for extraterrestrial environments (Capece *et al.*, 2013). However, some extreme conditions have synergic deleterious effects on biological systems and some natural polyextreme environments are well known for being the harshest for life, sometimes over the limits of life. If extremely dry environments are excluded from this concern, as liquid water presence is a *sine qua non* condition for terrestrial life processes, some natural aqueous environments are devoid of significant life and therefore considered as the most extreme for life.

Dallol volcanic hypersaline volcanic springs presents ponds with high temperature, saturated salinity, high abundance of iron and extreme acidity (Cavalazzi *et al.*, 2019; Kotopoulou *et al.*, 2019). In this unique environment, considered by some as a potential analog of ancient Mars (Gómez *et al.*, 2019), even if lipids biomarkers have been detected (Carrizo *et al.*, 2019) and some ultra-small archaea and bacteria may have been observed close to the most extreme ponds (Gómez *et al.*, 2019), two strict barriers for life have been identified: combination between extreme salinity and extreme acidity and combination between extreme salinity and extreme chaotropicity resulting from Mg^{2+}/Ca^{2+} abundancy (Belilla *et al.*, 2019).

Other examples of poly-extreme environment devoid of life are the Mg-rich deep hypersaline anoxic basins (DHAB). These deep sea brines, which can host deep-sea halophilic and piezotolerant microorganisms (van der Wielen, 2005), are considered by some as good models for icy moons subglacial oceans (Antunes *et al.*, 2020). In spite of the fact that some evidence of microbial activity have been found in Discovery Basin (van der Wielen, 2005), the nearly saturating concentrations of the chaotropic cation Mg^{2+} in this DHAB makes it

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3 exceptionally hostile to life (Hallsworth *et al.*, 2007). The same goes for Hephaestus DHAB
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5 where no life has been found yet (Cono *et al.*, 2019). At such low water activity values and
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7 high concentrations of Mg^{2+} , life appears to be only possible if kosmotropic ions are present
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9 to counteract toxicity of Mg^{2+} , like Na^+ in the Kryos DHAB (Yakimov *et al.*, 2015; Steinle *et al.*,
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15 In these two examples, chaotropicity induced by natural divalent cation abundance
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17 strongly limits life and may, similarly, represent a barrier for life in Martian brines (Fox-Powell
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19 *et al.*, 2016). This is supported by the fact that present-days increasing concentration of Mg^{2+}
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21 and Ca^{2+} in Dead Sea is progressively making it too hostile for halophiles (Oren, 2010).
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25 Although these natural terrestrial environments provide good insights on limits of
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27 terrestrial life with regard to water activity and chaotropicity, other poly-extreme conditions
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29 that could be present in extraterrestrial environments are still largely unknown. Some
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31 combinations of extreme conditions are indeed not associated with any known
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33 polyextremophile although the reasons for this absence are unclear. For some cases, synergic
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35 deleterious effect would be too much for biomolecules and molecular traits would be
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37 mutually incompatible. In other cases, natural environments with a given combination could
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39 just not exist. These combinations include psychroacidophily (combination of low-
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41 temperature and low pH adaptations), haloacidophily (high salinity and low pH) and
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43 piezoalkaliphily (high pressure and high pH) (Bowers *et al.*, 2009; Capece *et al.*, 2013; Harrison
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52 In the next part of this review, combinations between high/low temperature, high
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54 salinity and high pressure, will be discussed in regard to their effect on biomolecule and their
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56 eventual polyextremophiles.
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Thermohalophily

Hot hypersaline environments, like Atlantis II DHAB (Anschutz *et al.*, 1999) or Dallol hypersaline volcanic hot springs (Kotopoulou *et al.*, 2019), are uncommon on Earth and so are organisms adapted to both high temperature and high salinity. In the *archaeal* domain, only moderate thermophiles have shown extreme halophily (Mesbah and Wiegel, 2005; Harrison *et al.*, 2013). In general, combination of high temperature and high salinity is considered hard to handle by microorganisms (Chin *et al.*, 2010). One reason lies in the nature of the proteome: thermophilic proteins tend to have overall less charged proteins which are unstable in environments with high ionic concentrations such as the cytoplasm of halophiles (Zaccai, 2011). Moreover, halophilic protein traits include weakening of hydrophobic interactions (Sigliocco *et al.*, 2011) which are fundamental for thermostability (Haney *et al.*, 1999). Yet, halophilic proteins are more thermostable than non-halophilic counterparts and some protein traits could be shared between thermophiles and halophiles. Salt bridges for instance are both a way for thermostability (Tanaka *et al.*, 2004; Xia *et al.*, 2018) and haloresistance (Nayek *et al.*, 2014). It has indeed been discovered in a thermohalophilic enzyme an abundance of salt bridges but also of hydrogen bonds (Badiea *et al.*, 2019). It has also been shown that halophilic enzymes can be protected from thermal denaturation when trapped in dry salt crystals (Tehei *et al.*, 2002), showing that relation between high temperature and high salinity conditions may be complex.

It has been proposed that in deep sea hypersaline environments, salinity might increase the upper temperature limit of enzymes (Alcaide *et al.*, 2015). Moreover, as

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halotolerant (Kaye and Baross, 2000) and moderately halophilic (Kaye *et al.*, 2004) bacteria as well as halophilic archaea (Takai *et al.*, 2001; Ellis *et al.*, 2008) have been identified in continental or abyssal hydrothermal environments, it has also been proposed that hot hydrothermal chimneys may host hypersaline hot microenvironments allowing colonization by halophiles (Takai *et al.*, 2001).

To date, the most halophilic hyperthermophile is *Thermococcus waiotapuensis* *sp. nov.* which grows at temperatures as high as 90°C and salinity as high as 2.2M NaCl (González *et al.*, 1999) and the most thermophilic extreme halophile is *Natronolimnobius aegyptiacus* which grows at saturated concentration of Na⁺ and at temperature as high as 56°C (Bowers and Wiegel, 2011; Zhao *et al.*, 2018).

Thermopiezophily

Generally, pressure and temperature have opposite volumetric effects on macromolecular systems: while temperature increases flexibility and pushes objects to occupy more space, pressure increases rigidity and tends to reduce volumes of objects. One major effect of high pressure is the elevation of boiling temperature of water. In abyssal hydrothermal environments, pressure hence widens the range of liquid water conditions, opening new environments for a water-based life to develop. There is no known hyperthermophile with an optimal growth temperature above 100°C. However, the most thermophilic organism *Methanopyrus kandleri*, which optimally grows at 98°C (Kurr *et al.*, 1991), has been shown to grow at temperatures as high as 122°C (Takai *et al.*, 2008) which

can only be achieved at abyssal pressures. It has also been shown that for both abyssal archaea *M. kandleri* and abyssal bacteria *Marinitoga piezophile* optimal growth temperature is proportional to pressure (Miroshnichenko and Bonch-Osmolovskaya, 2006; Takai *et al.*, 2008).

Some individual enzymes can however be more efficient at temperatures above 100°C. Protease from *Methanococcus jannaschii* for example works optimally at 116°C and displays activity at temperatures as high as 130°C (Michels and Clark, 1997). Thermophilic and piezophilic molecular traits may be synergetic (Capece *et al.*, 2013), such as insertion of squalane in lipid bilayers which improves membrane stability under both high temperature and high pressure (Salvador-Castell *et al.*, 2020). Nonetheless, structural signatures of thermopiezophily have yet to be described.

The fact that high pressure induces heat-shock response in both piezophilic (Sato *et al.*, 2015) and pressure-sensitive microorganisms (Iwahashi *et al.*, 1991; Aertsen *et al.*, 2004) goes along with the idea that some thermophilic traits can also cope with high pressure. In particular, molecular systems coping with protein instability may be useful for both heat-induced and pressure-induced unfolding.

Psychrohalophily

Polar cold environments are often associated with high salinity. In spite of the fact that salt solubility in water decreases with temperature, freezing of water excludes salt from the ice thus concentrating the remaining liquid which becomes hypersaline. Moreover, high salinity lowers freezing point of water to the point that saturated NaCl brines are liquid at

temperatures as low as -21°C (Brady, 1992). The saltiest liquid water body on earth is also the coldest: Antarctic Don Juan pond remains liquid at temperatures as low as -50°C because of its high CaCl_2 concentration (Harris and Cartwright, 2013). As with water above 100°C requiring high pressure to remain liquid, subzero liquid water is possible at high salinity, opening the window for life. The most psychrophilic halophile is *Psychromonas ingrahamii* which grows at temperatures as low as -12°C at 3.3M NaCl (Auman *et al.*, 2006) and the most halophilic psychrophile is *Planococcus halocryophilus* Or1, which grows at salinities as high as 18% at -15°C (Mykytczuk *et al.*, 2013). While true psychrophiles found in hypersaline cold environment are bacteria, Antarctic hypersaline Deep Lake is dominated by cold-tolerant halophilic archaea (DeMaere *et al.*, 2013; Williams *et al.*, 2017).

At the biomolecular level, psychrophily and halophily appear to have some degree of synergy (Capece *et al.*, 2013). Intracellular accumulation of chaotropes such as Na^+ , Mg^{2+} or Ca^{2+} , which is easy in hypersaline environments, facilitates life at low temperature as they counter the cold-induced stabilization of proteins (Chin *et al.*, 2010). As both low temperature and salinity cause osmotic stress, compatibles solutes such as polyols may also be critical to psychrohalophily. Both salt-in-cytoplasm and compatible solute strategies for halophily thus enhance psychrophily.

At the membrane level, cold temperature rigidifies membranes and increases their impermeability, which serves resistance to high salinity. Moreover, carotenoids are observed in both halophiles and psychrophiles where they serve membrane fluidity control. However, depending on their nature, they have either rigidifying or fluidifying properties (Subczynski *et al.*, 1992). As psychrophilic bacteria increase production of carotenoids as response to increased salinity (Fong *et al.*, 2001b), they may represent a tool for adjusting membrane fluidity in cold hypersaline environments.

At less extreme values of cold and salinity, plants possess common pathways for resisting to drought, coldness and salinity (Xiong *et al.*, 2002; Zhu, 2002; Seki *et al.*, 2003).

Psychropiezophily

Deep ocean cold and pressurized waters represent most of the biosphere volume. While organisms of hydrothermal vents benefit from compensating effects of high pressure and high temperature on molecular flexibility, deep sea organism molecules are rigidified by both low temperature and high pressure (Capece *et al.*, 2013).

Remarkably, high pressure induces cold-shock response in both mesophilic (Welch *et al.*, 1993; Wemekamp-Kamphuis *et al.*, 2002) and piezophilic bacteria (Fujii *et al.*, 1999). This pathway is critical to psychropiezophily. In particular, it enables production of unsaturated lipids to counter cold-induced and pressure-induced crystallization (Kato, 2010). Cryoprotectant extracellular polysaccharides (EPS) production also appears to be induced by high pressure conditions in deep-sea bacteria (Marx *et al.*, 2009; Bælum *et al.*, 2012), however the exact function of EPS for pressure-tolerance is still unclear.

Halopiezophily

Most piezophiles have been isolated at low salinity and most halophiles at atmospheric pressure (Harrison *et al.*, 2013). Hypersaline deep environments are limited to DHABs.

However, nature of salt may significantly reduce the window for life. For example, high concentrations of MgCl_2 in Discovery and Hephaestus DHABs strongly limits life independently from pressure (Hallsworth *et al.*, 2007; Cono *et al.*, 2019; Antunes *et al.*, 2020).

Halophilic and halotolerant bacteria are commonly found in deep sea environments (Takami *et al.*, 1999; Lu *et al.*, 2001) where they generally face low temperature too.

Nonetheless, halophilic piezophilic and piezotolerant bacterial enzymes from 3-3.5km deep Medee, Bannock and Kryos DHABs have been described (Alcaide *et al.*, 2015). Interestingly, these enzymes displayed various combinations of traits observed in proteins of thermophiles (such as aromatic interactions or less glycine), halophiles (such as acidic surface) and pizeophiles or piezotolerant (such as less salt bridges, small amino acids) organisms (Alcaide *et al.*, 2015).

Some halophiles also seem to be rather piezotolerant. For example, the extreme halophilic archaea *Halobacterium salinarum* NRC-1 which is found in surface hypersaline environments has been shown to survive 400MPa exposure (Kish *et al.*, 2012) and moderate halophilic seawater bacteria *Micrococcus roseus* shows some resistance to 207MPa exposure (Tanaka *et al.*, 2001).

Adaptation to both high pressure and high salinity may use compatible solutes conferring both haloresistance and piezotolerance such as β -hydroxybutyrate (Martin *et al.*, 2002).

Extremophilic molecular traits as biomarkers

The search for extraterrestrial life is not limited to the search of environments compatible with life but can also be extended to the search of traces of a current or past biological activity. These traces are commonly called biomarkers or biosignatures. Biomarkers may either be remotely observed on or found *in situ* by space missions. However, reliability and detectability of a biomarker seems to be mutually incompatible. For example, CH₄ and dioxygen have been proposed as biomarkers and can be remotely detected in extraterrestrial atmospheres. As discussed before, CH₄ is mostly biogenic on Earth but can accumulate abiotically in other planetary contexts. As significative accumulation of dioxygen in terrestrial atmosphere was caused and is maintained by photosynthesis, it has been proposed that oxygen-related species could be used as biomarker (Mendillo *et al.*, 2018; Meadows *et al.*, 2018). However, like CH₄, oxygen gases may also be produced abiotically in significant amounts (Luger and Barnes, 2015; Narita *et al.*, 2015). Even simple organic molecules that constitute essential bricks of life have been proven to be easily produced in abiotic conditions. The famous Murchison meteorite for example contains for example various amino acids (Kvenvolden *et al.*, 1971; Cronin and Pizzarello, 1983), nucleobases (Martins *et al.*, 2008) and even sugar-like polyols (Cooper *et al.*, 2001) that could all be produced by abiotic or prebiotic chemistry. On the other hand, detection of macromolecules and polymers with several features like complexity, chirality or sequence, like proteins or nucleic acids, would constitute a strong evidence for biological activity but would be more difficult to detect in large amounts. Moreover, terrestrial complex biomolecules can be considered as extremely fragile in comparison to their constituents or the products of cell metabolism. Proteins and RNA for example are known to be difficult to handle in laboratory conditions, requiring various procedures to increase their stability. The same goes *in cellulo* as organisms have evolved

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3 numerous biological repairing or recycling processes, such as chaperones and proteasomes,
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5 to cope with this instability.
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8 In this context, biomolecules from certain extremophiles may offer the advantage of a
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10 greater stability, a point which as recently be reviewed (Jebbar *et al.*, 2020a). As mentioned
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12 before, lipids and proteins of thermophiles are exceptionally stable in numerous chemical and
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14 physical conditions, which would make them easier to detect in extraterrestrial environments.
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16 Moreover, many extremophiles accumulate large amounts of compatibles solutes and small
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18 organic molecules to cope with extreme conditions. With the proper geological and chemical
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20 setting, these molecules could be stable over long periods and hence represent interesting
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22 biomarkers.
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27 Another example of biomarker related to extremophiles is the case of pigments.
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29 Indeed, chlorophyll pigment produced by terrestrial photosynthetic organisms cause a
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31 distinctive increase of red light reflection a the planetary level which has remotely detected
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33 (Sagan *et al.*, 1993). Red edge on reflectance spectra of exoplanets and exomoons has hence
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35 been proposed as a surface biomarkers (Seager *et al.*, 2005; O'Malley-James and Kaltenecker,
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37 2019), and may be extended to other wavelengths, depending on the light received by the
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39 extraterrestrial body which may influence the type of pigments needed by putative organisms
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41 (Kiang, 2008; O'Malley-James *et al.*, 2012). However, in addition to light-harvesting, pigments
42
43 also serve photoprotection and can be accumulated in large amounts. This is especially true
44
45 for terrestrial halophiles which accumulate carotenes pigments, increasing their resistance to
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47 UVs, giving to hypersaline lakes a distinctive red color. As pigment-based radioprotection and
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49 putative radiotrophy has been observed in melanized fungi, pigments may also be
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51 accumulated by putative life in heavily irradiated extraterrestrial surface environments. It
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3 should nonetheless be kept in mind that remote detection of extraterrestrial pigments may
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5 be technically difficult or even impossible (Björn *et al.*, 2009).
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8 To conclude with, terrestrial extremophiles also present special metabolisms which
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10 could produce biomarkers. For example, in volcanic or hydrothermal environments, which
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12 present various combinations of extreme conditions (high temperature, low pH, high
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14 pressure, heavy metal abundance, etc), many organisms metabolize sulfur-containing
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16 compounds, leading to isotopic signatures that could be, as for carbon, used as a biomarker
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18 (Chela-Flores, 2019). While this type metabolism is not limited *per se* to extreme
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20 environments, it is still more abundant in acidic hot springs and deep sea hydrothermal vents.
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Conclusion

Several extraterrestrial environments in the Solar System and beyond may allow persistence of relatively warm liquid water bodies, raising hope for finding habitable environments, prebiotic chemical processes or even biological activity. Based on our current knowledge about limits of life and the Solar System, habitable extraterrestrial environments are likely to be found in subsurface Martian brines and the subglacial oceans of the Europa and Enceladus moons. Other habitable environments, such as surfaces of exoplanets, may be characterized in the future.

These extraterrestrial environments appear to have extreme physical and chemical conditions, hence presenting similarities with terrestrial extreme environments. Extremophilic microorganisms found in hydrothermal vents, deep sea, hypersaline lakes or polar environments have evolved several strategies to cope with the extreme conditions of their environment. Some of these strategies could be shared with putative extraterrestrial life facing similar extreme conditions. Indeed, extremophiles such halophiles or psychrophilic methanogens have been successfully cultivated in extraterrestrial conditions to show feasibility of life out of the earth. While some strategies of terrestrial extremophiles happen at the scale of the cell, adaptation of the nature and properties of their biomolecules is generally required. Smaller-scale systems, such as machineries and assemblies from extremophiles, could be used instead to check general behavior of biomolecules in extraterrestrial conditions.

Extreme conditions such as extreme pH, ionizing radiation, high concentrations of heavy metals or organic solvents are managed by terrestrial extremophiles with adaptations

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3 at the cellular level and require less to no molecular trait. In contrast, high and low
4
5 temperature, high salinity and high pressure are extreme conditions commonly faced by life
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7 on Earth through adaptations requiring specific traits in all biomolecules, in particular
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9 proteins. Extreme conditions impair normal structure and dynamics of biomolecules, altering
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11 properties such as flexibility, stability or activity. In particular, alteration of solvation shell,
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13 which is fundamental for structure and dynamics of biomolecules, is critical.
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17 Each type of biomolecule (protein, nucleic acids, lipids) from extremophiles displays its
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19 own set of molecular traits associated with extremophily. Individual proteins may present one
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21 or many of these traits allowing function under extreme conditions. Usually, no clear signature
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23 of extremophilic adaptation can be recognized, only trends. These adaptations tend to
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25 counter the effect of extreme conditions, for example through increased flexibility of
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27 psychrophilic proteins to counter rigidification induced by low temperatures or increased
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29 solubility of halophilic proteins to counter enhanced aggregation under hypersalinity. For
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31 proteins, relatively small changes of overall structure can be observed in crystal structures. In
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33 contrast, huge differences with mesophilic proteins can be observed at the dynamical level by
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35 techniques such as neutron scattering, spectroscopy or NMR. In particular, adaptation of the
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37 solvation shell, which should be understood as a part of the proteins, is fundamental to cope
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39 with extreme temperature, salinity or pressure.
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47 While most extremophilic systems are studied in regard to one extreme condition,
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49 terrestrial extreme environments actually generally display more than one such condition. The
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51 same goes for extraterrestrial environments which can also be called polyextreme. Some
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53 combinations of extreme conditions are however unknown on Earth and associated with no
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55 organism to date. Combinations of extreme conditions may either be compensating or
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57 synergistically deleterious for biological system. High pressure and high temperature present
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3 in abyssal hydrothermal vents for example have opposite effects on molecular flexibility
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6 whereas low temperature and high pressure in deep sea environments both increase rigidity
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8 of biomolecules. While some strategies may be shared between two extreme condition,
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10 molecular traits allowing life under polyextreme conditions are still poorly understood.
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13 Most data about extremophilic adaptation have been obtained through study of
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15 simple systems such as monomeric proteins. However, complex biological processes such as
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17 DNA replication, transcription, translation, protein degradation or metabolism largely rely on
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19 large molecular assemblies. Systems like multimeric enzymes, DNA/RNA polymerases,
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21 ribosomes or even viral capsids may represent an intermediary scale between simple proteins
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23 and complex cellules. Studying their stability and dynamics under extraterrestrial extreme
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25 conditions may bring interesting data about habitability of extraterrestrial environments and
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27 how life could manage extreme conditions out of Earth.
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35 Existence and diversity of extremophiles have opened the window of habitability,
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37 suggesting that life would be possible in extraterrestrial environments similar to extreme
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39 terrestrial ones. Yet, some open questions still remain.
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42 Can putative extremophilic biomarkers be find in extraterrestrial environments? The
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44 study of extremophiles has shown that they may produce biomarkers relevant for
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46 astrobiological concern. In particular, biomolecules in thermophiles and halophiles display
47
48 exceptional stability and resilience and thus could make interesting biomarkers.
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52 How fast can life adapt to extreme conditions? It has been showed here that few
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54 molecular traits allow single biomolecules such as proteins to work under extreme conditions.
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56 These modifications may appear simple but they lead to dramatical changes of dynamical
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properties. At the scale of the proteome however, numerous changes are required for organisms, leading to the need of a longer evolution.

Can macroscopic life appear in extreme environments? Most extremophiles are indeed microorganisms, mostly bacteria and archaea. Extreme environments may host macroscopic life, such as shrimps and tube worms around black smokers, but ancestors of these macroorganisms have originated in mesophilic environments. Furthermore, the hypothesis of abiogenesis taking place in extreme environments is largely debated.

Can macroscopic life appear without photosynthesis? Even if claims about life billion years ago are controversial, it is generally believed that multicellular and macroscopic life have appeared long after photosynthesis. Before photosynthesis, ecosystems must have relied on chemosynthesis, just like many modern extreme environments, such as great depths or extremely hot environments, which are largely devoid of photosynthetic organisms. Because metabolic yields are low compared to photosynthesis, it is not certain that macroscopic life could appear in ecosystems with chemosynthesis only. Hence, putative life in Martian subsurface or icy moon abyssal environments may be limited to microorganisms.

Where should one search for extraterrestrial life? In the absence of known extraterrestrial mesophilic environments, extreme extraterrestrial environments such as Martian depths or icy moons abysses may be the most habitable. As adaptation to extreme conditions limits the possibility for extremophiles to thrive under mesophilic conditions, environmental variations of physical and chemical conditions may be a limiting factor. Thus, stable extreme environments should be favored over those displaying variations. As pointed by many, the search for hydrothermal should also be a priority. Liquid water may however not be enough, making the search for salt-enriched liquid water environment a priority too. In fact, biochemistry relies on a solvent which is an ionic solution rather than pure water and

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many biological processes such as folding and metabolism require ions to work. In particular, hydrothermal processes not only favors organic matter enrichment but also accumulation of ions in water. In terrestrial life, solvent is not pure water but an ionic solution where charges enable biological processes such as metabolism. Interestingly, hydrothermalism favors both organic matter and mineral ions enrichment of water.

In this context, the search for rocky exoplanets with surface mesophilic conditions may unravel new habitable environments.

Acknowledgments

This work is supported by the French National Research Agency in the framework of the Investissements d'Avenir program (ANR-15-IDEX-02), through the funding of the "Origin of Life" project of the Univ. Grenoble-Alpes.

IBS acknowledges integration into the Interdisciplinary Research Institute of Grenoble (IRIG, CEA)

Author contributions

All proposed and contributed in their respective expertise (L.C., E.G. and B.F. microbiology, biochemistry and structural biology, G.Z. biophysics, X.D. planetology and astronomy) and participated in writing the manuscript. L.C. wrote the paper and made the figures, G.Z. and X.D. wrote and corrected the paper, E.G. corrected the paper and B.F. supervised the writing process.

Disclosure statement

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Figure 1: Extreme extraterrestrial environments

Martian (A) and icy moons (B) putative habitable extraterrestrial environments. Putative extreme conditions are showed, abbreviations are: HS (high salinity), LT (low temperature), HP (high pressure), HT (high temperature). Main extreme constitutive conditions are written in bold while secondary or facultative extreme conditions are indicated in parentheses. Environments may possess additional extreme conditions such as pH.

Figure 2: Terrestrial environments with permanent extreme conditions

On left, archetypal permanently extreme terrestrial environments and examples. On right, abbreviated extreme conditions among those considered by this review: HS (high salinity), LT (low temperature), HP (high pressure), HT (high temperature). Main extreme constitutive conditions are written in bold while secondary or facultative extreme conditions are indicated in parentheses. These environments may possess additional extreme conditions such as pH.

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Figure 3: Dynamical properties of proteins and solvent

Representative timescales of solvent and protein intermolecular and intramolecular processes. Timescale is logarithmic from 1fs to 1s. HB means hydrogen bond. Adapted from (Gáspári and Perczel 2010; Zwier and Chong 2010; Xu and Havenith 2015).

Figure 4: Phase transitions of lipid membranes

This figure represents simple lipid bilayers, not biological membranes. The functional state of lipids in biological membranes is close to a disordered phase, between L_{β} and L_{α} . Reversible phase transitions can be triggered by low temperature (LT), high pressure (HP) or high temperature (HT). Locally, some types of lipids are found in the ripple phase P_{β} which is an intermediary between the gel and fluid phases. Bilayer pictures taken from: <http://www.vwalter.fr/ressources/scheme/>
Adapted from (Jebbar et al. 2015).

Figure 5: Interactions between ions and proteins

The protein here is *Pyrococcus abyssi* DNA Polymerase B (green) interacting with DNA (orange). Electrostatic interactions between ions and proteins are represented by arrows whose thickness illustrates bond strength. Ions are colored depending on their charge and the thickness of their shell represents their hydration. Adapted from (Okur et al. 2017).

Figure 6: Theoretical stability/activity thermal curves of three homologous extremophilic enzymes

Activity (% of maximum activity measured for the protein) and stability (% of folded protein) theoretical curves for three psychrophilic (blue), mesophilic (green) and thermophilic (red) homologous enzymes are given. Activity is relative to each enzyme. At temperature allowing maximum activity, psychrophilic enzymes are still stable (A) while thermophilic enzymes are largely denatured (B). Adapted from (D'Amico et al. 2003).

Figure 7: Structural features of extremophilic proteins

Pictures on the left are graphical representations of protein sites. Residues involved with an interaction are colored. Adapted from (Alcaide et al. 2015; Pucci and Rooman 2017)

Figure 8: Molecular traits of lipids in extreme conditions

Adaptive membrane changes in archaea. Lipids may adopt multiple traits at the same time. Traits reducing membrane permeability may also protect against other extreme conditions such as low pH. Adapted from (Jebbar et al. 2015).

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Table 1: General overview of extremophily

Extreme condition and exclusion possibility	Range of extremophily	Maximum extreme reached by terrestrial life	Examples of terrestrial environment	Extraterrestrial environments	Remarks
True extremophily types described in this review					
High Temperature (Impossible to exclude)	Thermophiles grow at $T > 50^{\circ}\text{C}$ Hyperthermophiles grow at $T > 80^{\circ}\text{C}$	Growth reported at 122°C (<i>Methanopyrus kandleri</i>)	Marine hydrothermal vents, volcanic hot springs and lakes	Icy moons putative hydrothermal vents	Evidence of survival after exposure to 130°C (<i>Geogemma barossii</i> Strain 121)
Low Temperature (Impossible to exclude)	Psychrophile can grow at $T < 15^{\circ}\text{C}$	Growth at -18°C (<i>Rhodotulura glutinis</i>)	Polar ocean, deep sea, ice interiors, Antarctic surface	Mars surface and subsurface rocks and brines Icy moons subglacial oceans top, middle and bottom waters	Evidence of metabolism at -33°C (<i>Paenispinosarcina</i> sp. B5)
High Salinity (Partially possible to exclude)	Halophiles grow at salinity $> 8,8\%$ Hyperhalophiles grow at salinity $> 14,6\%$	35% NaCl (saturation) (<i>Halobacterium salinarum</i>)	Saline marshes and lakes, seawater brines, DHAB, salt rocks, solar salterns	Mars Brines Icy Moons Oceans	
High hydrostatic Pressure (Impossible to exclude)	Piezophiles grow at $P > 10\text{Mpa}$ Hyperpiezophiles grow at $P > 50\text{MPa}$	Growth at 130MPa (<i>Thermococcus piezophilus</i> CDGST)	Deep sea, DHAB, oceanic and continental subsurfaces	Mars deep brines and rocks Icy Moons abysses	Non-piezophilic bacteria have been engineered to survive exposure to 20GPa
Other true extremophily types					
Low pH (Partially possible to exclude)	Acidophiles grow at $\text{pH} < 5$ Hyperacidophiles grow at $\text{pH} < 3$	Confirmed growth at $\text{pH} 0,7$ but evidence of growth at negative pH (<i>Picrophilus torridus</i>)	Acid mine drainage, solfataric fields, acido-thermal hot springs, fumaroles, coal spoils	Martian surface, Europa subglacial ocean	Most acidic cytoplasm $\text{pH} 4.6$ (<i>Picrophilus torridus</i>)
High pH (Partially possible to exclude)	Alkaliphiles grow at $\text{pH} > 9$ Hyperalkaliphiles grow at $\text{pH} > 11$	Growth at $\text{pH} 13.2$ (strains of <i>Bacillus</i> and <i>Clostridium</i>)	Soda lakes, alkalithermal hot springs, hydrothermal systems, sewage water	Enceladus subglacial ocean	Most alkaline cytoplasm $\text{pH} 10.5$ (<i>Bacillus pseudofirmus</i> OF4)
“Pseudo-extremophily” types					
Low water activity (Partially possible to exclude)	Xerophiles can grow at $a_w < 0,9$	Growth at $a_w 0,605$ (<i>Xeromyces bisporus</i>)	Desert, brines	Martian surface, subsurface and brines Icy moons subglacial brines	No true xerophile known yet

Ionizing radiation (Impossible to exclude)	Radioresistant organisms survive exposure to 500Gy	Survival after exposure to 30kGy (<i>Thermococcus gammatolerans</i>)	Anthropically contaminated environments, Guyamas basin hydrothermal vent	Martian surface	Less radioresistant but radiotrophic fungi are known
Chaotropes abundance (Possible to exclude)	Chaotolerant organisms tolerate high concentration of MgCl ₂ or CaCl ₂ without kosmotropic counterion	Growth at 2.1M MgCl ₂ (<i>Aspergillus proliferans</i>) or 2.0M CaCl ₂ (<i>Aspergillus sydowii</i>)	DHABs, athalassohaline lakes	Europa subglacial ocean, Martian surface	No true chaophile known yet
Heavy metal abundance (Possible to exclude)	Metalloresistant organisms can grow at millimolar concentrations of heavy metal ions	Growth at 20mM Zn ²⁺ , 20mM Co ²⁺ or 5mM Cd ²⁺ (<i>Ralstonia</i> sp. CHR4) > 48h survival on pure copper surface (<i>Kocuria palustris</i> and <i>Brachybacterium conglomeratum</i>)	Contaminated soils, Río Tinto,	Ancient Martian surface	No true metallophile known yet
Organic solvents abundance (Partially possible to exclude)	Solvoresistant organisms can grow at organic solvents concentration > 1% or survive at higher concentrations	Surviving 100% toluene, benzene and <i>p</i> -xylene (<i>Staphylococcus haemolyticus</i>)	Contaminated environments, petroleum seeps	Titan atmosphere and surface	No true solvophile known yet
Nutrients scarcity (NA)	Oligotrophs grow with extremely low nutrient abundance at low rates, growth often inhibited by high nutrient mixtures	<i>Pelagibacter ubique</i>	Ocean waters, deserts, oligotrophic soils	Martian surface and subsurface? Icy moon waters?	

Table 2: Chaotropic versus kosmotropic properties of ions

Ions irrelevant to biological contexts or absent from natural environments have been excluded from this table. Based on the Hofmeister series (Zhang and Cremer 2006).

Properties	← Kosmotropic = Salting out ← Stabilize proteins ← Decrease hydrophobic solubility ← Increase surface tension	Chaotropic = Salting in → Promote denaturation → Increase hydrophobic solubility → Decrease surface tension →
Anions	$\text{CO}_3^{2-} > \text{SO}_4^{2-} > \text{HPO}_4^{2-} > \text{CH}_3\text{COO}^- > \text{Cl}^- > \text{NO}_3^- > \text{ClO}_3^- > \text{ClO}_4^- > \text{SCN}^-$	
	← Strongly hydrated	Weakly hydrated →
Cations	$\text{NH}_4^+ > \text{K}^+ > \text{Na}^+ > \text{H}^+ > \text{Mg}^{2+} > \text{Ca}^{2+} > \text{Al}^{3+}$	
	← Weakly hydrated cations	Strongly hydrated cations →

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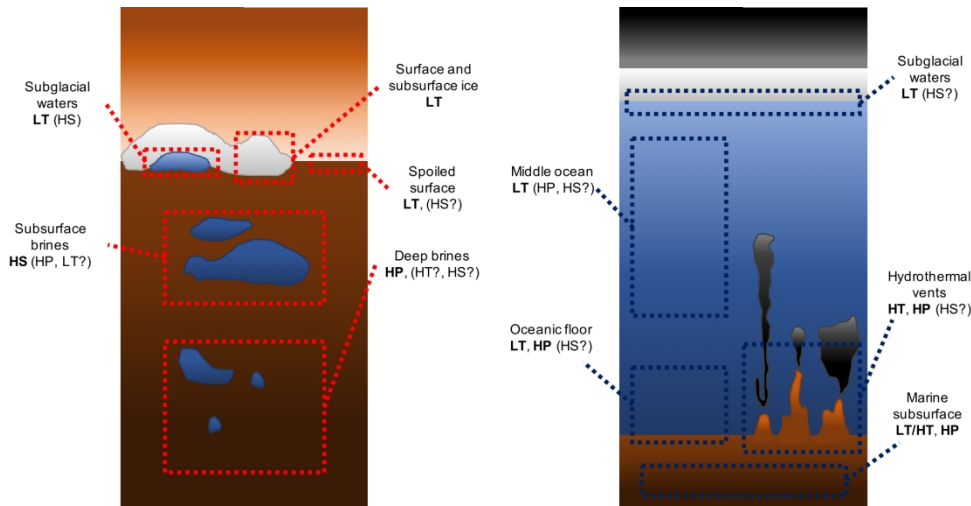


Figure 1: Extreme extraterrestrial environments

Martian (A) and icy moons (B) putative habitable extraterrestrial environments. Putative extreme conditions are showed, abbreviations are: HS (high salinity), LT (low temperature), HP (high pressure), HT (high temperature). Main extreme constitutive conditions are written in bold while secondary of facultative extreme conditions are indicated in parentheses. Environments may possess additional extreme conditions such as pH.

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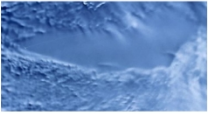

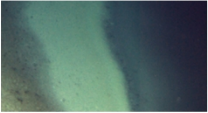

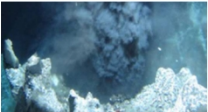




Subzero brines Don Juan Pond		LT HS
Subglacial lakes Lake Vostok, lake Willhans, lake Ellsworth		LT (HP, HS)
Sea ice, permafrost, polar regions		LT
DHAB, cold seeps, hypersaline sdiments Discovery basin, Kryos basin		HS (HP, LT)
Deep ocean, trenches Marianna trench		HP (LT, HS)
Hydrothermal vents Pito seamount, Lost City hydrothermal field		HT (HP)
Marine and continental subsurface		HP (LT/HT)
Volcanic hot springs, hydrothermal fields Yellowstone hot springs field, Solfataric fields		HT
Hypersaline hot springs Dallol volcano		HT HS
Hypersaline lakes and sediments Dead sea, lake Natron		HS (HT)

Figure 2: Terrestrial environments with permanent extreme conditions

On left, archetypal permanently extreme terrestrial environments and examples.
On right, abbreviated extreme conditions among those considered by this review: HS (high salinity), LT (low temperature), HP (high pressure), HT (high temperature). Main extreme constitutive conditions are written in bold while secondary or facultative extreme conditions are indicated in parentheses. These environments may possess additional extreme conditions such as pH.

Image credits from top to down: Goddard Space Flight Center NASA (x2), NASA/Dick Ewers, Virginia Edgcomb WHOI/NSF/ROV Jason/©WHOI, Jeremy Bishop on Unsplash, NOAA, James St John on Flickr, Lukas Kloeppel on Pexels, Pierre c 38 on Flickr, Ralf Steinberger on Flickr (all modified).

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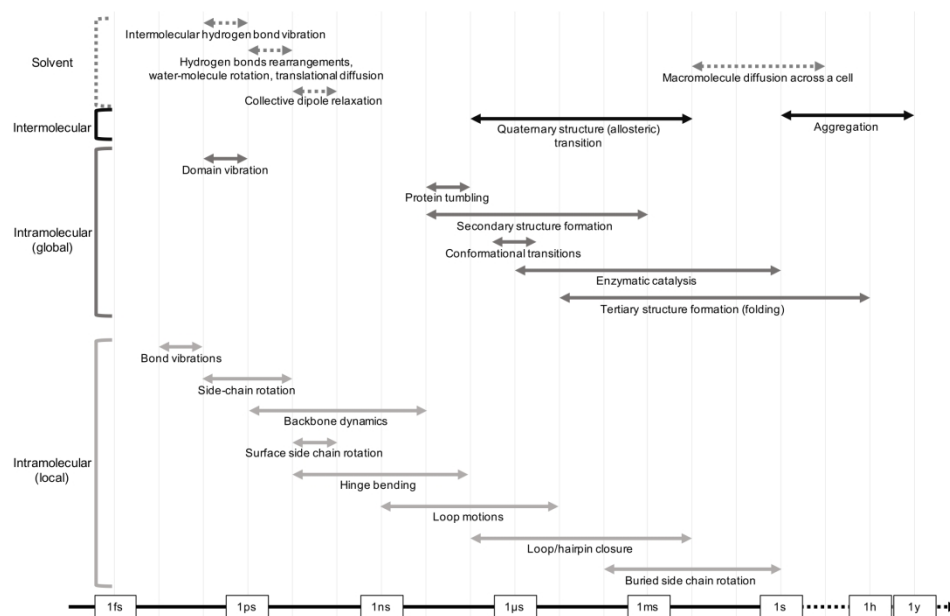


Figure 3: Dynamical properties of proteins and solvent

Representative timescales of solvent and protein intermolecular and intramolecular processes. Timescale is logarithmic from 1fs to 1s. HB means hydrogen bond. Adapted from (Gáspári and Perczel 2010; Zwier and Chong 2010; Xu and Havenith 2015).

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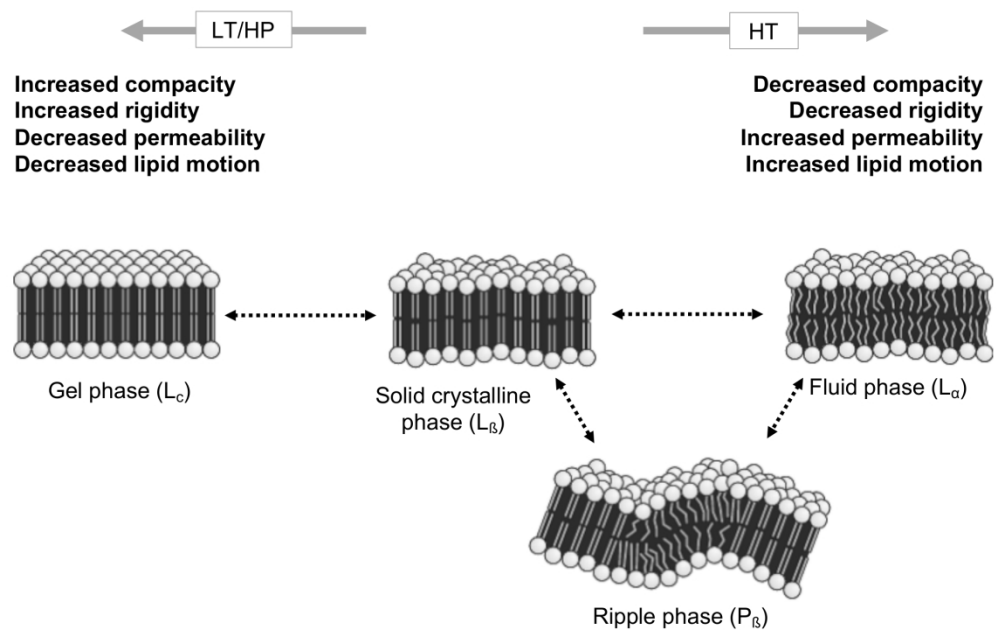


Figure 4: Phase transitions of lipid membranes

This figure represents simple lipid bilayers, not biological membranes. The functional state of lipids in biological membranes is close to a disordered phase, between L_β and L_α . Reversible phase transitions can be triggered by low temperature (LT), high pressure (HP) or high temperature (HT). Locally, some types of lipids are found in the ripple phase P_β which is an intermediary between the gel and fluid phases. Bilayer pictures taken from: <http://www.vwalter.fr/ressources/scheme/>
Adapted from (Jebbar et al. 2015).

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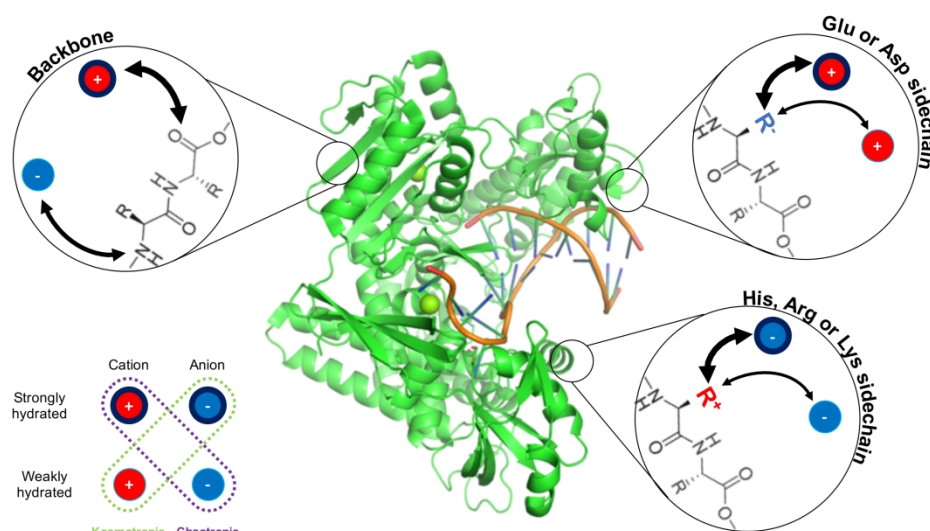


Figure 5: Interactions between ions and proteins

The protein here is *Pyrococcus abyssi* DNA Polymerase B (green) interacting with DNA (orange). Electrostatic interactions between ions and proteins are represented by arrows whose thickness illustrates bond strength. Ions are colored depending on their charge and the thickness of their shell represents their hydration. Adapted from (Okur et al. 2017).

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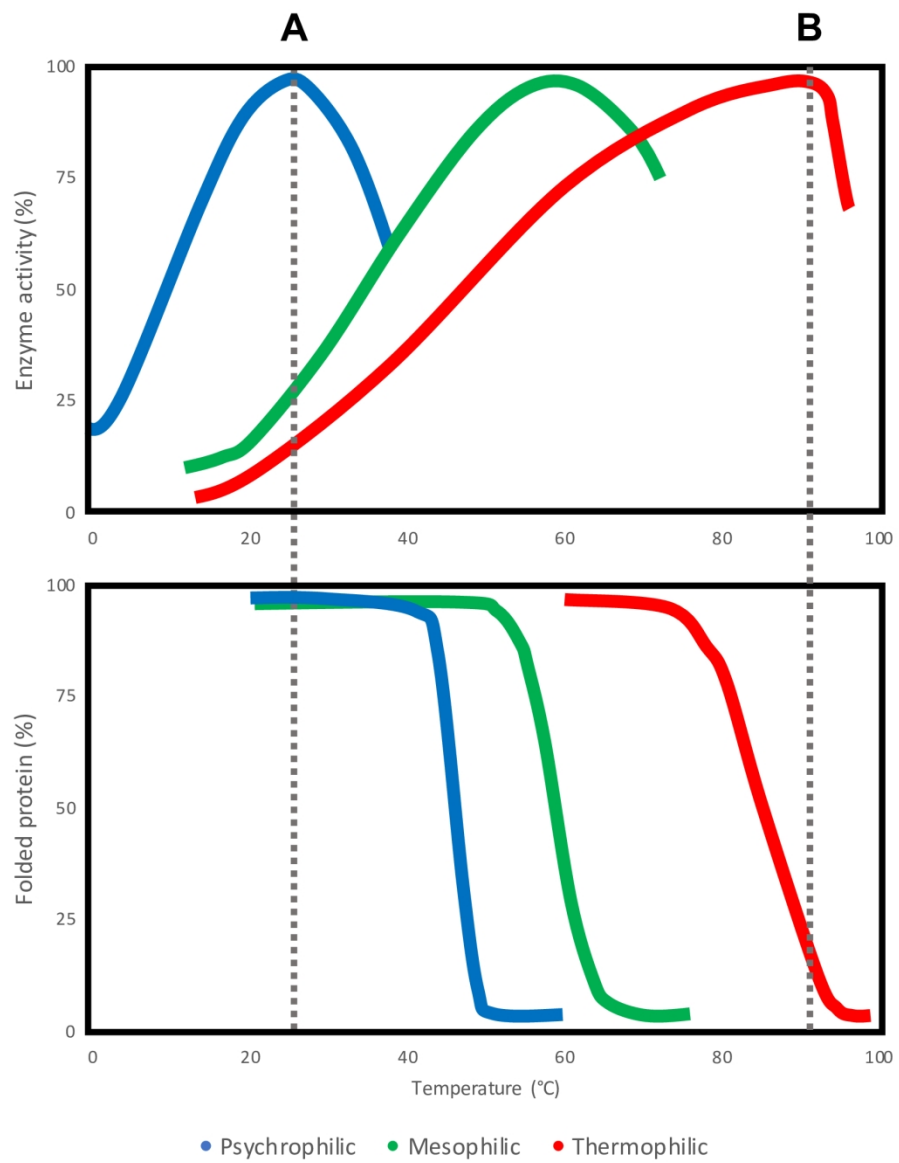


Figure 6: Theoretical stability/activity thermal curves of three homologous extremophilic enzymes

Activity (% of maximum activity measured for the protein) and stability (% of folded protein) theoretical curves for three psychrophilic (blue), mesophilic (green) and thermophilic (red) homologous enzymes are given. Activity is relative to each enzyme. At temperature allowing maximum activity, psychrophilic enzymes are still stable (A) while thermophilic enzymes are largely denatured (B). Adapted from (D’Amico et al. 2003).

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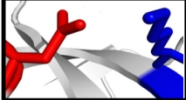
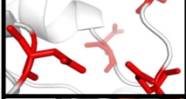
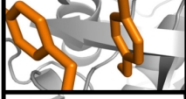


Interactions and involved residues	Thermophilic protein	Psychrophilic protein	Halophilic protein	Piezophilic protein
 Hydrophobic effect Trp, Tyr, Phe, Leu, Ile, Val, Ala	Overall increased hydrophobicity, surface less hydrophobic	Less hydrophobic interactions	Overall decreased hydrophobicity	?
 Salt bridges Glu or Asp + Arg or Lys (or occasionally His, Tyr or Ser)	More abundant, form ion-pair networks	Less abundant in surface, more abundant in the core	More abundant, more Arg-based	Less abundant
 Hydrogen bonds Ser, Thr, Tyr, Lys, Arg, Trp, His and backbone + His, Trp, backbone	Form surface networks	Less abundant	?	?
 π-π interaction Trp, Tyr, Phe	More abundant	Less abundant	Less abundant	?
 π-cation interaction Trp, Tyr, Phe + Arg, His, Lys	More Arg-based	Less abundant	Less abundant	?
 Disulfide bridge Cys	More abundant in flexible regions, buried	?	?	?

Figure 7: Structural features of extremophilic proteins

Pictures on the left are graphical representations of protein sites. Residues involved with an interaction are colored. Adapted from (Alcaide et al. 2015; Pucci and Rooman 2017)

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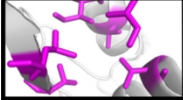

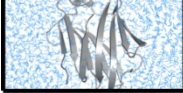
Other features		Thermophilic protein	Psychrophilic protein	Halophilic protein	Piezophilic protein
	Loops	Shorter or deleted, more Pro	Longer, more flexible, less Pro	?	?
	Core	More hydrophobic, less cavities	Less dense, less rigid more cavities, less Arg and Pro	Denser, smaller residues, less hydrophobic,	Denser, smaller residues
	Dynamics	Increased rigidity/resilience	Increased flexibility	Increased flexibility	Increased flexibility
	Hydration shell	Larger, increased interaction between solvent and residues	Water bounded by unpaired charged residues	Saltier, more structured, water tightly bound	Denser, less dynamic

Figure 7: Structural features of extremophilic proteins

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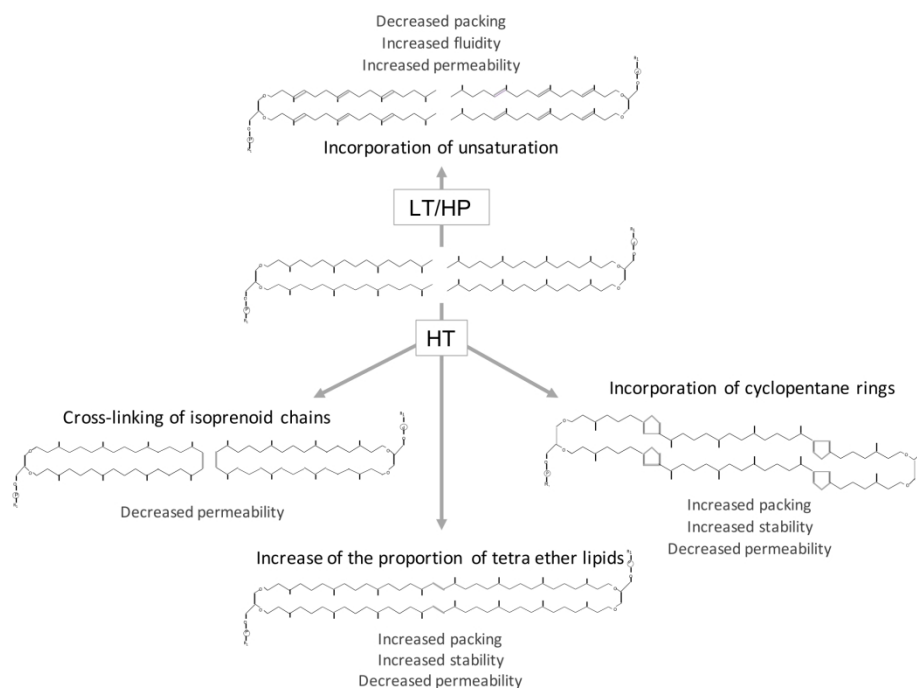


Figure 8: Molecular traits of lipids in extreme conditions

Adaptive membrane changes in archaea. Lipids may adopt multiple traits at the same time. Traits reducing membrane permeability may also protect against other extreme conditions such as low pH. Adapted from (Jebbar et al. 2015).

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