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

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

CARRÉ Lorenzo<sup>1</sup>, ZACCAI Giuseppe<sup>1, 2</sup>, DELFOSSE Xavier<sup>3</sup>, GIRARD Eric<sup>1</sup>,

FRANZETTI Bruno<sup>1</sup>

<sup>1</sup> Univ Grenoble Alpes, CNRS, CEA, IBS, Grenoble, France.

<sup>2</sup> Institut Laue-Langevin (ILL), 38042 Grenoble, France

<sup>3</sup> Laboratoire d'Astrophysique, Observatoire de Grenoble, 38400 Saint Martin d'Hères, France

Extremophile biochemistry for exobiology

Corresponding authors: [lorenzo.carre@ibs.fr](mailto:lorenzo.carre@ibs.fr) ; [franzetti@ibs.fr](mailto:franzetti@ibs.fr)

## 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.

## 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 or satellites can host liquid water on the surface or below the surface, the conditions of pressures, temperatures,

1  
2  
3 salinities, pH, etc can however be extreme for a terrestrial type of life, and astrobiology is  
4  
5 interested on eventual life forms in very diverse environments.  
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8 Astrobiology could be defined as “the study of the origin, evolution, distribution (and  
9  
10 future) of life in the universe: extraterrestrial life and life on Earth” (Cottin, 2019). The term  
11  
12 was first proposed in 1953 by the astronomer Gavriil Tikhov (Tikhov, 1953) and the field was  
13  
14 first theorized by the biologist Joshua Lederberg in 1960 under the synonym term of  
15  
16 exobiology (Lederberg, 1960). In fact, to date there is only one kind of life known: terrestrial  
17  
18 life. Every cell on Earth has descended from a common ancestor and shares the same basic  
19  
20 chemistry and biological processes. The discovery of a new kind of life, independent from the  
21  
22 terrestrial life, would be of huge scientific interest.  
23  
24  
25  
26

27 Still, a general definition of life is required if we were to search for extraterrestrial one  
28  
29 (von Hegner, 2019a). Many definitions have been proposed based on thermodynamics,  
30  
31 chemical composition or evolution but as there is only one example of life to date, all  
32  
33 definitions will be geocentric. It has been proposed that terrestrial life could be defined as a  
34  
35 “genome-containing, self-sustaining chemical dissipative system that maintains its localized  
36  
37 level of organization at the expense of producing entropy in the environment; which has  
38  
39 developed its numerous characteristics through pluripotential Darwinian evolution” (von  
40  
41 Hegner, 2019a).  
42  
43  
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45  
46

47 Another way to understand life in regards to astrobiology is through its limits (Takai,  
48  
49 2019). Biological processes are based on the chemical reactions between the molecules  
50  
51 constituting organisms: biomolecules. Physical and chemical properties of biomolecules  
52  
53 determine the range of conditions compatible with their functions and thus the limits of  
54  
55 biological activity. As a consequence, life has long been seen as a fragile thing, needing liquid  
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2  
3 water abundancy, warm temperatures, moderate salinity, no ionizing irradiation, etc (von  
4  
5 Hegner, 2020).  
6  
7

8 In this context, the discovery of extremophiles has pushed farther away the limits of  
9  
10 life. Many natural terrestrial environments have first appeared to be too harsh and  
11  
12 incompatible with persistent life: hyperacidic volcanic hot springs, hyperbaric deep abysses,  
13  
14 subzero polar environments or saturated salt lakes. Nonetheless, organisms permanently  
15  
16 living in these extreme environments and adapted to live under their extreme conditions,  
17  
18 have been discovered. While presence of microscopic infusory-like life in hypersaline lake has  
19  
20 been hypothesized by Darwin during its journey on the Beagle (Darwin, 1839), the term of  
21  
22 extremophiles was first coined in 1974 (Macelroy, 1974). By opposition, non-extremophilic  
23  
24 life can be defined as mesophilic (living between extremes). As life could be found in new  
25  
26 unsuspected environments, it has also appeared that classical definitions of life and  
27  
28 habitability are generally mesocentric and even the definition of an extreme condition,  
29  
30 environment or extremophile may present the mesocentric bias.  
31  
32  
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36  
37 As a consequence, there is an opposition between two points of view, two ways to  
38  
39 understand terrestrial life with consequences on the apparent probability of finding  
40  
41 extraterrestrial life: 1) Life is fragile, needs precise conditions to appear and to develop and is  
42  
43 thus uncommon in the Universe or 2) Life can adapt to many environments and large panel of  
44  
45 physical and chemical conditions and could be more common in the Universe, widening the  
46  
47 criteria of habitability. It should however be noted that the existence of extremophiles does  
48  
49 not infer that life can appear in extreme environments.  
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57 In this review, a description of extraterrestrial environments promising for the search  
58  
59 of life and a description of their extreme conditions will be provided. Then an overview of the  
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1  
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3 impact and relevancy of extremophilic life in astrobiology will be discussed. Focusing on  
4  
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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11 reviewed.  
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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

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2  
3 zones will become amenable to characterization of their atmosphere through transit  
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5 spectroscopy either with James Web Spatial Telescope (JWST) (Doyon *et al.*, 2014; Beichman  
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7 *et al.*, 2014) or by combining high-dispersion spectroscopy with high contrast imaging with  
8  
9 Extremely Large Telescope (ELT) (Snellen *et al.*, 2015), and that a highly ambitious space  
10  
11 missions such as the Large UV/Optical/Infrared Surveyor (LUVOIR), which could be in  
12  
13 operation in the middle of the century, will have as its main objective the characterization of  
14  
15 atmospheres of Earth-like planets around solar type stars. But today, if we know that telluric  
16  
17 planets in habitable zones are very common (Bonfils, Delfosse, *et al.*, 2013; Dressing and  
18  
19 Charbonneau, 2015; Bryson *et al.*, 2020), their environment is globally unknown to us and we  
20  
21 can only imagine their diversity. So our review will focus on the better-known characteristic  
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23 of the bodies of the Solar System.  
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32 Earth is the only planet in the Solar where solid, liquid and gaseous water co-exist on  
33  
34 the surface. A co-existence that is the basis of the cycles that create the climatic conditions  
35  
36 that shape our environment. It has been shown, however, that transient liquid water is  
37  
38 possible on the surface of Mars (Möhlmann and Thomsen, 2011; Chevrier and Rivera-Valentin,  
39  
40 2012; Martín-Torres *et al.*, 2015). Subsurface Martian environments appear to be more  
41  
42 habitable with persistent aqueous environments (Clifford *et al.*, 2010; Jones *et al.*, 2011;  
43  
44 Orosei *et al.*, 2018), raising hope for the existence of a deep Martian biosphere, analogous to  
45  
46 the deep biosphere of Earth's crust (Richard A. Kerr, 1997; Itävaara *et al.*, 2016) or deep  
47  
48 subglacial Antarctic habitats (Michalski *et al.*, 2013; Mikucki *et al.*, 2015).  
49  
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54 Farther away from Earth, liquid water is abundant under the surface of other bodies  
55  
56 of the Solar System. Multiple evidence shows that several icy moons of giant planets possess  
57  
58 liquid water oceans beneath a thick crust of ice. Among them, Enceladus, a moon of Saturn,  
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3 and Europa, a moon of Jupiter, are of particular interest for astrobiology and the search for  
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5 life (Gaidos *et al.*, 1999; Jebbar *et al.*, 2020a)  
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## 10 Mars brines and depths

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17 Several current geological aspects have given birth to the hypothesis of a water ocean  
18 covering large portions of Mars surface approximately 3-4 billion years ago (Brandenburg,  
19 20 1987; Chapman, 2003; Di Achille and Hynek, 2010). This hypothesis proposes that surface  
21 22 23 habitable aquatic environments may have existed on Mars (Grotzinger *et al.*, 2014).  
24 25 Moreover, geochemical traces of past hydrothermal activity on Martian surface lead to the  
26 27 hypothesis that environments similar to acidic terrestrial Laguna Caliente lake (Hynek *et al.*,  
28 29 30 2018) or El Tatio geothermal field (Barbieri and Cavalazzi, 2014), which are both habitable and  
31 32 33 colonized by extremophiles, may also have existed. The permanence of this ancient ocean  
34 35 remains an open question, climate models of Mars having difficulties in reconciling its  
36 37 38 potential existence in these period with the surface temperature estimated to be too cold,  
39 40 41 resulting in most surface water having to be trapped in ice (Head *et al.*, 2018; Palumbo and  
42 43 44 Head, 2018; Turbet and Forget, 2019). This enigma might be solved if the atmosphere of  
45 46 47 ancient Mars was dominated by H<sub>2</sub> and CO<sub>2</sub>. Recent 1D numerical radiative-convective climate  
48 49 50 calculations suggest that this is a viable solution, and may be the only one, to warm the surface  
51 52 53 of Mars above the melting point of water (Turbet *et al.*, 2020) but this remains to be confirmed  
54 55 56 by 3D Global Climate Models computations.  
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3 However, even if the Martian surface was habitable in the past, it is now considered to  
4  
5 be too extreme for life to develop and persist (McCollom, 2006). Major barriers are extreme  
6  
7 aridity, cold temperature, abundance of perchlorates which strong oxidative power would  
8  
9 impair most biomolecules (Quinn *et al.*, 2013; Wadsworth and Cockell, 2017) and intense UV  
10  
11 irradiation which degrades organic matter too. It is also expected that iron, which is abundant  
12  
13 in Martian soils, may catalyzes this degradation process (Stalport *et al.*, 2019). The main place  
14  
15 where to look for life on the Red Planet may, therefore, be below the surface. In particular, a  
16  
17 liquid water body has been recently discovered under the southern ice cap (Orosei *et al.*,  
18  
19 2018).  
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25 In the hope of finding active current biological activity or traces of past life, future  
26  
27 exploration missions such as ESA ExoMars or NASA Mars 2020 will carry equipment such as  
28  
29 rovers designed for studying subsurface environments with a special emphasis on biomarkers  
30  
31 (Simoneit *et al.*, 1996; Vago *et al.*, 2015; Williford *et al.*, 2018). Indeed, the Martian subsurface  
32  
33 is expected to be less harsh than the surface, shielded from sublimation and UV irradiation  
34  
35 and where persistent liquid water within brines could be present and stable (Martínez and  
36  
37 Renno, 2013).  
38  
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41

42 The possibility of a present or past deep Martian biosphere in subsurface waters, based  
43  
44 on chemosynthesis and analogous to terrestrial subsurface ecosystems, has been largely  
45  
46 hypothesized (Boston *et al.*, 1992; Michalski *et al.*, 2013; Grotzinger *et al.*, 2014; Mikucki *et*  
47  
48 *al.*, 2015). However, putative Martian subsurface organisms would have to cope with  
49  
50 desiccation-rehydration cycles and fluctuating salinity, constraints that would select  
51  
52 adaptations to cope with high salinity (Reid *et al.*, 2006; Kendrick and Kral, 2006; Vaclare *et*  
53  
54 *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  
4  
5 (Fox-Powell *et al.*, 2016).  
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7

8       Temperatures are expected to be higher at greater depths under the Martian surface  
9  
10 due to radiogenic heating allowing liquid water to exist (Clifford, 1993; Clifford *et al.*, 2010) as  
11  
12 deep as 310km, at 427°C and a pressure of 4GPa (Jones *et al.*, 2011). By comparison,  
13  
14 subsurface liquid water is only possible on Earth in the first 5km deep in the crust, (Jebbar *et*  
15  
16 *al.*, 2020a) under a maximal pressure of 150MPa. Yet deep biosphere is mostly occupied by  
17  
18 microorganisms in the first 1-2km (Magnabosco *et al.*, 2018) at pressure values around  
19  
20 100MPa. Putative subsurface Martian life at warm temperatures would thus have to cope with  
21  
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  
45  
46 liquid water. However, if subsurface liquid water is also taken into account then the blue  
47  
48 planet appears to be rather dry compared to the icy moons of giant planets (Mosher and  
49  
50 Cheng, 2018). Indeed, several of these moons may possess, under their ice surface layer, huge  
51  
52 liquid water masses that could be called exooceans (Taubner *et al.*, 2020), with liquid water  
53  
54 abundance relative to the total mass of the body exceeding Earth's. Furthermore, Europa,  
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3 Callisto, Titan and Ganymede, subglacial liquid water volumes exceed Earth's total liquid water  
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5 volume.  
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7

8 Europa and Enceladus, in particular, have oceans which are of particular interest for  
9  
10 astrobiology because they would be directly in contact with rock allowing geochemical  
11  
12 processes such as enrichment of water with various organic and inorganic molecules. On  
13  
14 Earth, these interactions between liquid water and rock represent a source of energy and for  
15  
16 existing microorganisms and may have been be critical for the origin of life (Hinman, 2013).  
17  
18 Hence, they are major targets for exploration and study by future space missions (Pappalardo,  
19  
20 2012; Cable *et al.*, 2016).  
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### 30 The subglacial ocean on Europa 31 32 33 34 35

36 First evidence of a liquid water ocean beneath the ice on Europa came from the Galileo  
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38 spacecraft imaging in 1996 of the Jovian moon surface showing a geological young surface and  
39  
40 evidence of hydrogeological activity (Kerr, 1996). Further analysis supported existence of a  
41  
42 subsurface ocean of liquid water (Carr *et al.*, 1998; Pappalardo *et al.*, 1999), whose origin is  
43  
44 the tidal effect of Jupiter's gravity resulting in a massive heating of its satellite (Greenberg,  
45  
46 2009).  
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50  
51 Beneath the ice, the ocean would be global, up to 100-200km deep, depending on the  
52  
53 model used (Anderson *et al.*, 1997, 1998; Pappalardo *et al.*, 1999; Spohn and Schubert, 2003;  
54  
55 Marion *et al.*, 2003; Thomas *et al.*, 2016). Its volume would exceed that of all terrestrial oceans  
56  
57 volume (Chyba and Phillips, 2001). Models depict an ice shell 10km thick (Park *et al.*, 2015)  
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3 with local variations in thickness (Thomas *et al.*, 2016). The layer of ice acts as a barrier against  
4  
5 cosmic rays, thus offering protection to hypothetical current oceanic life or traces of past life.  
6  
7  
8 Europa may also be the only Solar System body other than Earth exhibiting plate tectonics and  
9  
10 subduction (Kattenhorn and Prockter, 2014). Subduction in the ice shell of Europa is a  
11  
12 particularly interesting feature as it could bring molecules from the surface down the liquid  
13  
14 water ocean, molecules such as oxidants produced by photochemistry (Pasek and Greenberg,  
15  
16 2012) or prebiotic molecules delivered by meteorites and comets (Pierazzo and Chyba, 2002;  
17  
18 Kimura and Kitadai, 2015).  
19  
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22  
23 What makes Europa even more interesting for astrobiology is the putative existence  
24  
25 of hydrothermal processes at the bottom of the ocean. Modeling of Europa's geology (Lowell  
26  
27 and DuBose, 2005) and ocean's chemical composition (Zolotov, 2003) argue in favor of abyssal  
28  
29 hydrothermalism. By comparison with Earth's black smokers ecology, hydrothermal vents on  
30  
31 Europa would provide an habitable environment with energy source, chemical and physical  
32  
33 gradients, making Europa's abysses strong candidates for habitable environments with  
34  
35 extraterrestrial life (Gaidos *et al.*, 1999; Chyba and Hand, 2001; Pierazzo and Chyba, 2002;  
36  
37 Zolotov, 2003; Marion *et al.*, 2003).  
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42  
43 The chemical composition of Europa's subglacial ocean has also been modeled. It is  
44  
45 expected to be acidic because of the intake of oxidants produced on the surface by  
46  
47 photochemistry (Pasek and Greenberg, 2012). Nonetheless, pH could vary close to the  
48  
49 putative hydrothermal vents, since hydrothermal fluids which are expected to be alkaline  
50  
51 similarly to Earth's (Zolotov, 2003). Preliminary studies have implied that salinity would be  
52  
53 close to saturation (Hand and Chyba, 2007), similar to terrestrial hypersaline lakes, and  
54  
55 dominated by  $\text{MgSO}_4$  (Kargel *et al.*, 2000; Fanale *et al.*, 2001; Zolotov and Shock, 2001;  
56  
57 McKinnon and Zolensky, 2003). However,  $\text{MgSO}_4$  concentration is supposed to decrease  
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3 significantly with depth and pressure (Schmidt and Manning, 2017). Other works suggest  
4  
5 instead that NaCl would be the dominant salt (Valenti *et al.*, 2012), recently supported by Hubble  
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7 Space Telescope detection of sodium chloride on Europa's surface which may originate from  
8  
9 under the ice layer (Trumbo *et al.*, 2019).  
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## 18 The subglacial ocean on Enceladus

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24 Enceladus is much smaller than Europa. It is also heated by the tidal effect of Saturn  
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26 (Tyler, 2009). First evidence of liquid water beneath the ice came thanks to Cassini-Huygens  
27  
28 mission which flew over the moon in 2005 and 2007. It detected various hydrogeological  
29  
30 activities at the south pole of the moon: cryovolcanism (Porco *et al.*, 2006), surface thermal  
31  
32 emissions (Spencer *et al.*, 2006) and a massive water vapor plume (Hansen *et al.*, 2006). All  
33  
34 these elements argue in favor of a local liquid water ocean beneath the south pole of  
35  
36 Enceladus.  
37  
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41 Other evidence includes gravimetric anomalies (Iess *et al.*, 2014) and chemical analysis  
42  
43 of the plume, which led to sampling the composition of the subglacial ocean. Many chemical  
44  
45 species have been thus identified: the dominant species of the plume is water, CO<sub>2</sub> and CH<sub>4</sub> are  
46  
47 present in significant amounts and small amounts of NH<sub>3</sub>, HCN, propane and acetylene were  
48  
49 also detected (Waite, 2006). Further analysis also showed presence of benzene, methanol,  
50  
51 various alkenes and alkanes (Waite *et al.*, 2009) and even larger organic molecules with  
52  
53 masses above 200Da in ice grains in the plume closer to the icy surface (Postberg *et al.*, 2018).  
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3 The presence of organic molecules has been interpreted as proof that hot aqueous  
4 chemistry (Matson *et al.*, 2007) and hot rock-water interactions (Sekine *et al.*, 2015) are  
5 currently active in the Enceladus subglacial ocean. Accumulation of evidence has  
6 strengthened the hypothesis of the existence of hydrothermal vents at the bottom of the  
7 Enceladus subglacial ocean (Parkinson *et al.*, 2008; Tobie, 2015; Sekine *et al.*, 2015; Waite *et*  
8 *al.*, 2017), making this ocean an even stronger candidate for a habitable extra-terrestrial  
9 environment.  
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20 Enceladus's subglacial ocean is estimated to be about 10km deep (Tyler, 2009), well  
21 protected by the ice sheet from cosmic rays (less *et al.*, 2014). Chemical models of the sub-  
22 glacial ocean based on the study of the plume have been proposed. Enceladus present-day  
23 subglacial ocean would be a solution of NaCl and carbonate (Zolotov, 2007), with some KCl,  
24 MgCl<sub>2</sub> (Brown and Hand, 2013) with a strongly alkaline pH of approximately 11-12, as  
25 determined by models of carbonate speciation (Glein *et al.*, 2015). This would make  
26 Enceladus's ocean similar to the terrestrial soda lakes.  
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### 43 Other icy moons

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48 Titan, a moon of Saturn, is considered by many as an icy body of huge astrobiological  
49 interest. Several attempts have been made to conceptualize putative life or biomes on Titan  
50 (Fortes, 2000; McKay and Smith, 2005). A subglacial ocean hundreds kilometers-deep has  
51 been hypothesized for Titan (Grasset and Sotin, 1996); it would be extremely cold, however,  
52 and composed of water and ammonia (Grindrod *et al.*, 2008; Baland *et al.*, 2011). In spite of  
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3 the fact that various bacteria have been shown to survive in highly concentrated ammonia  
4 solutions (Kelly *et al.*, 2012), these exotic conditions are largely unknown to natural terrestrial  
5  
6 life. If there were life in such subglacial ocean, it is likely that it would be based on a radically  
7  
8 different chemistry than on Earth. However, the existence of hydrothermal vents, as possible  
9  
10 sites for prebiotic chemistry, at the bottom of the subglacial ocean, has also been  
11  
12 hypothesized (Lunine and Rizk, 2007). The surface of Titan is more promising with regard to  
13  
14 prebiotic chemistry because of its complex carbon hydrogeology (Raulin *et al.*, 2012).  
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20 Ganymede, a satellite of Jupiter, is the biggest moon in the Solar System and may have  
21  
22 the largest subglacial ocean (McCord *et al.*, 2001). However, it is also expected that a thick  
23  
24 high-pressure tetragonal ice layer would separate liquid water and rock (Vance *et al.*, 2014),  
25  
26 limiting chemical exchanges.  
27  
28  
29

30 Other icy moons with putative subglacial oceans include Dione, satellite of Saturn  
31  
32 (Beuthe *et al.*, 2017), Mimas, also of Saturn (Rhoden *et al.*, 2017) and Triton, satellite of  
33  
34 Neptune (Ruiz, 2003; Gaeman *et al.*, 2012).  
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### 43 Icy moons environmental conditions

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48 It should be kept in mind that temperature, salinity, pressure, pH and chemical  
49  
50 composition may vary within sub-glacial oceans, leading to multiple types of environment of  
51  
52 various habitability: hydrothermal abyssal fields, seafloors, hypersaline brines and the top of  
53  
54 the oceans close to the ice (Gaidos *et al.*, 1999; Chyba and Phillips, 2001; Marion *et al.*, 2003).  
55  
56 These putative habitable icy moons environments are represented in [Figure 1-B](#).  
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5 While black smokers have been proposed as models for icy moons abyssal  
6 environments, deep hypersaline anoxic basins (DHABs) have been proposed as models for  
7  
8 subglacial general waters (Antunes *et al.*, 2020). In fact, DHABs like the Mediterranean  
9  
10 Discovery, Urania and Kryos basins or Red Sea brine lakes could share some conditions with  
11  
12 icy moons oceans such as anoxia, absence of light, high salinity, high pressure and warm/low  
13  
14 temperatures.  
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20 Subglacial bodies of liquid water are also present on Earth such as the Antarctic lake  
21  
22 Vostok. As they low-temperature environments isolated by ice from the outside, they have  
23  
24 also been proposed as models for icy moons subglacial oceans (Petit *et al.*, 2005; Cockell *et*  
25  
26 *al.*, 2011).  
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30 Laboratory experiments from many scientific fields designed for simulating icy moons  
31  
32 conditions have recently be reviewed (Taubner *et al.*, 2020).  
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## 39 Extremophiles and astrobiology

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45 While the previous Martian and icy moons environments appear rather extreme  
46  
47 compared to classical biological standards, they share multiple features with extreme  
48  
49 terrestrial environments. In this part, extremophilic microorganisms, which have successfully  
50  
51 colonized most of these environments, will be presented in the context of astrobiology and  
52  
53 extraterrestrial habitability.  
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57 Extremophiles are considered as key model organisms for astrobiology by many  
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59 (Seckbach, 2000; Cavicchioli, 2002; Reid *et al.*, 2006; Marion and Schulze-Makuch, 2007;  
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3 Pikuta, 2007; Stojanovic *et al.*, 2008; Canganella and Wiegel, 2011; Merino *et al.*, 2019;  
4  
5 DasSarma *et al.*, 2020; Jebbar *et al.*, 2020a). Their ability to grow at temperatures above 100°C  
6  
7 or under 0°C, at pH under 1 or above 10, at nearly saturating salinity, or at pressure over  
8  
9 100MPa are particularly impressive from our mesophilic anthropocentric point of view. Taken  
10  
11 as a whole, extremophiles encompass all kinds of environments inhabited by present-days  
12  
13 terrestrial life and show that, as long as there is water, most terrestrial environments can be  
14  
15 inhabited and actually are. Existence and diversity of extremophiles argue against the long-  
16  
17 lived belief that life is intrinsically fragile (von Hegner, 2020). In contrast, the idea that life can  
18  
19 work in a various and large range of physical and chemical conditions opens the spectrum of  
20  
21 potentially habitable terrestrial and extraterrestrial environments. On the other hand, a  
22  
23 precise understanding of life at the extremes may also help to tell which extraterrestrial  
24  
25 environments would be too extreme, even for the billion's years-old evolved terrestrial life.  
26  
27 However, as discussed later, feasibility of abiogenesis in extreme environments is still largely  
28  
29 debated. Therefore, it should be stressed that environments putatively habitable by  
30  
31 extremophiles should not be considered necessarily favorable for life to appear.  
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40 In general, it is expected that extraterrestrial environments such as Martian depths  
41  
42 and icy moons oceans are more extreme than terrestrial counterparts. Therefore, putative life  
43  
44 in these environments would have like terrestrial extremophiles to face extreme chemical and  
45  
46 physical conditions. As extreme conditions effects on molecular systems are universal, it  
47  
48 would also be expected that putative extraterrestrial life would use strategies similar to  
49  
50 terrestrial extremophiles to cope with their environment.  
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57 Extremophiles are present in three domains of cellular life, which all share the last  
58  
59 universal common ancestor (LUCA): *bacteria*, *archaea* and *eukarya*. While some  
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1  
2  
3 extremophiles, mainly among fungi, protists and green algae, are indeed eukaryotic (with a  
4 nucleus), prokaryotes (without nucleus) usually dominates in extreme environments.  
5  
6 Macroscopic, which are mostly eukaryotes, are scarce in extreme environments and  
7  
8 microorganisms only can permanently thrive and grow in extreme environments by adapting  
9  
10 their whole biomolecules to the extreme conditions. Moreover, extremophilic prokaryotes  
11  
12 may belong to extensive phylae with extremophiles only, showing a long-evolved adaptation  
13  
14 to extreme conditions.  
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20 Among them, archaea are dominant in extremely hot, acid and hypersaline  
21  
22 environments while bacteria are dominant in cold and alkaline environments. Depending on  
23  
24 the ambient temperature, deep-sea organisms are typically represented by psychrophilic  
25  
26 bacteria or thermophilic archaea. Extremophiles found in marine or continental subsurface  
27  
28 rocks are generally bacteria. Some extremophiles and their representative natural  
29  
30 environments are represented in [Table 1](#).  
31  
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37 Like any other cellular organism, extremophiles may be infected by viruses. In fact,  
38  
39 virions, the extracellular dispersion form of viruses, have been found in black smokers (Geslin  
40  
41 *et al.*, 2003), acidic hot springs (Prangishvili and Garrett, 2005), hypersaline lakes (Sime-  
42  
43 Ngando *et al.*, 2011) and cold polar environments (Wells and Deming, 2006). As they infect  
44  
45 cells inhabiting extreme environments, viruses of extremophiles also possess biomolecules  
46  
47 adapted to these extreme conditions and should be considered as part of extremophilic  
48  
49 biodiversity. While the status of viruses, alive or not, is still debated (Koonin and  
50  
51 Starokadomskyy, 2016; van Regenmortel, 2016), virions are the most abundant biological  
52  
53 objects on earth and their biomass may exceed total biomass of protists (Suttle, 2007). Due  
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3 to their uncontested contribution to biogeochemical cycles and evolutions of cells, viruses  
4  
5 should be considered as a matter of interest for astrobiology (Berliner *et al.*, 2018).  
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## 10 11 12 13 Experimental astrobiology: using extremophiles to test habitability 14 15

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19 Besides theoretical considerations, experimental studies have been used to assess  
20  
21 compatibility between extremophiles physiology and extraterrestrial conditions expected to  
22  
23 be encountered in icy moons subglacial oceans or Martian brines and subsurface.  
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28  
29 As no sunlight is available in icy moons oceans, photosynthesis is expected to be absent  
30  
31 from putative subglacial oceanic life (Gaidos *et al.*, 1999; Chyba and Hand, 2001) and  
32  
33 ecosystems would be rather based on chemosynthesis. The same is expected for putative  
34  
35 subsurface life on Mars (Boston *et al.*, 1992). Without photosynthesis-driven dioxygen  
36  
37 production, these environments would be anoxic. It should be noted that primitive Earth  
38  
39 atmosphere and waters were anoxic until about 2.4 billion years, yet allowing life to appear.  
40  
41  
42 Anoxia should not be considered as an extreme condition for biological systems or a barrier  
43  
44 for life *per se*. On the contrary, since the oxygenation of atmosphere and waters by  
45  
46 photosynthetic microorganisms, aerobic microorganisms have to cope with huge oxidative  
47  
48 stress through various long-evolved molecular systems.  
49  
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53  
54 In terrestrial anaerobic environments, methan-producing microbes (methanogens) are  
55  
56 key organisms to understand such biotops. Methanogens are widespread microorganisms  
57  
58 from the *Archaea* domain encountered in both extreme and moderate environments. They  
59  
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3 are either autotrophs (producing their own organic matter) or heterotrophs (needing organic  
4 matter from the environment). In all cases, CH<sub>4</sub> is a byproduct of their growth, which can be  
5  
6 then metabolized by methanotrophs (bacteria and archaea). Hence, methanogens using CO<sub>2</sub>  
7  
8 as the carbon source and H<sub>2</sub> as the reducing agent (Lyu *et al.*, 2018), and extremophilic ones  
9  
10 in particular, are recurring model organisms in astrobiology which are expected to be able to  
11  
12 thrive in extraterrestrial environments (Reid *et al.*, 2006; Kendrick and Kral, 2006; McKay *et*  
13  
14 *al.*, 2008; Taubner *et al.*, 2015). Moreover, as most methane on Earth is of biological origin  
15  
16 (Liu and Whitman, 2008) it could be considered to some extent as a biomarker (Jebbar *et al.*,  
17  
18 2020b). This must however be balanced by the fact that existence of CH<sub>4</sub> could be a purely  
19  
20 abiotic phenomenon in a case of an H-rich atmosphere. CH<sub>4</sub> being a natural reservoir of carbon  
21  
22 (Woitke *et al.*, 2020). The biomarker status of this molecule depends therefore on the type of  
23  
24 planetary atmosphere.  
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32 For the CO<sub>2</sub>-rich Martian atmosphere, calculations indicate that the presence of  
33  
34 methane is not expected if the atmosphere is at thermodynamic equilibrium (Levine *et al.*,  
35  
36 2010). If emitted, it cannot survive long, with a relatively short lifetime of about 300years. Its  
37  
38 detection, specially within local releases (Formisano *et al.*, 2004; Mumma *et al.*, 2009;  
39  
40 Webster *et al.*, 2015, 2018), therefore argues for a very young source. Martian methane origin  
41  
42 remains an open question, the debate not being settled between geochemical/geological  
43  
44 processes or microbial life (Lyons *et al.*, 2005; Yung *et al.*, 2018) questioning the existence of  
45  
46 methanogens viability on Mars. Experimental works have shown that extremophilic  
47  
48 methanogens could withstand many Martian surface or subsurface extreme conditions:  
49  
50 thermophilic *Methanothermobacter wolfeii* survived long Martian-like desiccation (Kendrick  
51  
52 and Kral, 2006) and low pressure (Mickol and Kral, 2017), psychrophilic *Methanobacterium*  
53  
54 *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  
4  
5 (Morozova *et al.*, 2007), long freezing, high salinity and starvation (Morozova and Wagner,  
6  
7 2007) and psychrophilic *Methanosarcina soligelidi* and *Methanococoides burtonii* could  
8  
9 manage methanogenesis at Martian subsurface pressure and/or at temperatures down to -  
10  
11 5°C (Reid *et al.*, 2006; Schirmack *et al.*, 2014).  
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16 Since H<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub> have been detected in Enceladus's plume (Waite, 2006; Waite  
17  
18 *et al.*, 2009, 2017), it has been proposed that methanogens could be thriving in Enceladus  
19  
20 subglacial ocean. Based on this assumption, recent experimental study have shown that  
21  
22 growth of the thermophilic methanogen *Methanothermococcus okinawensis* is possible under  
23  
24 extrapolated Enceladus hydrothermal vents conditions (Taubner *et al.*, 2018).  
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31 Extremophiles from hypersaline environments have also been considered in simulated  
32  
33 extraterrestrial conditions. As discussed above, many extraterrestrial environments such as  
34  
35 brines of Mars, Enceladus and Europa, may be hypersaline, in which halophilic and  
36  
37 halotolerant microorganisms, for which terrestrial counterparts represent good models,  
38  
39 thrive (DasSarma, 2006; Reid *et al.*, 2006; Sundarasami *et al.*, 2019). Moreover, intracellular  
40  
41 accumulation of salts, particularly manganese salts (Webb *et al.*, 2013), in halophilic cells  
42  
43 provides protection against ionizing radiation (Kish *et al.*, 2009), which is prevalent in some  
44  
45 extraterrestrial environments.  
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49  
50 Experimental studies showed that some halophilic archaea can tolerate high concentrations  
51  
52 of perchlorates similar to what would be faced in Martian brines (Oren *et al.*, 2014; Matsubara  
53  
54 *et al.*, 2017; Laye and DasSarma, 2018), Martial-like UV irradiation (Fendrihan *et al.*, 2009),  
55  
56 desiccation-rehydration cycles (Vauclare *et al.*, 2020), long-term desiccation and freezing  
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3 cycles (Mancinelli *et al.*, 2004) or could grow at Martian subsurface temperature (Reid *et al.*,  
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5  
6 2006; Laye and DasSarma, 2018).  
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## 10 11 12 13 Extremophiles and abiogenesis 14 15 16 17 18 19

20 Existence of extraterrestrial conditions compatible with terrestrial life is not a sufficient  
21  
22 reason to believe extraterrestrial life has actually originated. In fact, extremophilic life is a  
23  
24 complex lifestyle achieved with many mechanisms: large molecular assemblies, efflux/influx  
25  
26 systems, molecular glues and rivets, channeling, etc. Evolution has driven life for at least 3,5  
27  
28 billion years, leaving time to evolve new mechanisms allowing adaptation to new  
29  
30 environments, but discourses on origin of life, on abiogenesis, are only speculative.  
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36  
37 *In silico* predictions of LUCA's genome offered several arguments in favour of a  
38  
39 thermophilic origin of modern cells: G+C-rich content (Di giulio, 2000), which is largely  
40  
41 associated with modern-days thermophily as GC basepair is less susceptible than AT to  
42  
43 thermodenaturation, predicted proteins with amino acid sequences similar to modern-days  
44  
45 thermophilic homologs (Giulio, 2003) and the possession of a gene coding reverse gyrase  
46  
47 enzyme, a hallmark trait of hyperthermophiles (Catchpole and Forterre, 2019) lead to the  
48  
49 model where the last ancestor of all cells have emerged from inorganic natural  
50  
51 compartmentation in hydrothermal vents. (Koonin and Martin, 2005). Others have proposed  
52  
53 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  
4  
5 evolution (Mulkidjanian *et al.*, 2012).  
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8 High pressure encountered in hydrothermal vents environments could have also  
9  
10 facilitated some prebiotic reactions, notably by countering destabilizing effect of high  
11  
12 temperature. It has thus been proposed that LUCA, the last ancestor of all cellular organisms,  
13  
14 was pressure-adapted (Daniel *et al.*, 2006) or that high pressure in hydrothermal vents  
15  
16 environments facilitated synthesis of prebiotic molecules (Hazen *et al.*, 2002; Daniel *et al.*,  
17  
18 2006).  
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22 All these elements place abyssal hydrothermal vents at the center of abiogenesis. Such  
23  
24 claims may be supported by the discovery of putative 3.8-4.3Ga old microorganism fossils in  
25  
26 hydrothermal vents precipitates (Dodd *et al.*, 2017), in spite of the fact that such evidence of  
27  
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  
35  
36 and Lazcano, 1995; Levy and Miller, 1998; Galtier *et al.*, 1999; Bada and Lazcano, 2002; Islas  
37  
38 *et al.*, 2003). In general, some have argued that in the absence of complex long-evolved  
39  
40 adaptations, extreme conditions would have been too harsh for first cells or first  
41  
42 biomolecules, hence excluding the relevance of extremophiles for LUCA lifestyle, RNA world  
43  
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  
50  
51 hydrothermal vents of Enceladus or Europa, which could be habitable for terrestrial  
52  
53 extremophiles does not imply that life actually emerged and adapted there. Extraterrestrial  
54  
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.

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3 By contrast, multiple experimental studies have pushed terrestrial biochemistry in  
4 conditions far beyond their normal context and their expected physical and chemical limits.  
5  
6 An example of such work include enzymatic catalysis in organic solvents (Dordick, 1989;  
7  
8 Klibanov, 1989) or ionic liquids (Kragl *et al.*, 2002). In this context, enzymes from  
9  
10 extremophiles have shown many industrial applications (Horikoshi, 1999; Vieille and Zeikus,  
11  
12 2001; Demirjian *et al.*, 2001; Dumorne; *et al.*, 2017). To some degree, these studies push back  
13  
14 the limits of life, understood as fundamental biochemical processes. However, these works  
15  
16 are generally made outside of the context of astrobiology.  
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23 In this review we would like to defend the position that experimental studies using  
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25 biomolecules such as enzymes from extremophilic terrestrial organisms (extremozymes) are  
26  
27 of particular interest for astrobiology. In fact, studying viability of terrestrial extremophiles in  
28  
29 simulated extraterrestrial environments mainly gives information about one precise  
30  
31 microorganism capacity. In fact, survivability of halophiles in simulated Martian conditions,  
32  
33 for example, has been proven to be dependent on the strain used (Peeters *et al.*, 2010).  
34  
35 Indeed, growth, metabolism and cell division are complex biological phenomenon requiring  
36  
37 multiple enzymes, coordinated processes and are thus largely interdependent. In contrast,  
38  
39 given precise fundamental biochemical processes such as assembly of a macromolecular  
40  
41 edifices, protein synthesis, DNA replication or metabolite production appear simpler to study  
42  
43 and to extrapolate. Even if putative extraterrestrial life is based on the same chemistry as  
44  
45 terrestrial's, large-scale cell processes may significantly differ. Smaller-scale fundamental  
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47 biochemistries however could share more similarities.  
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54 Therefore, studies of biomolecules from extremophiles in simulated planetary  
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56 conditions may offer insights about limits of given biological processes in extraterrestrial  
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58 environments. Moreover, in the context of abiogenesis or early life, in absence of most of the  
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3 long-evolved cellular tools modern cells possess, studying fundamental biological processes  
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5 may give insights on the feasibility of biochemistry in extraterrestrial environments.  
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10 Indeed, some extraterrestrial environments are more extreme than their terrestrial  
11  
12 commonly accepted analogs. Martian surface is not only extremely dry and UV-irradiated like  
13  
14 Atacama Desert but also face generally extremely low temperatures and perchlorates  
15  
16 abundancy. For icy moons, it is often believed that putative hydrothermal vents would be  
17  
18 analogous to terrestrials. However, depth and pressure significantly differ. Deepest  
19  
20 hydrothermal vent on Earth lies at approximately 5km under the sea level with *in situ*  
21  
22 pressure of 50MPa (Connelly *et al.*, 2012). In contrast, some icy moons subglacial oceans are,  
23  
24 according to several models, hundreds kilometers deep (Schmidt and Manning, 2017). Europa  
25  
26 subglacial ocean for example, would be 100-200km deep (Anderson *et al.*, 1997; Pappalardo  
27  
28 *et al.*, 1999; Spohn and Schubert, 2003; Marion *et al.*, 2003; Thomas *et al.*, 2016). Taking  
29  
30 account of the 10km thick ice crust (Park *et al.*, 2015), the 100-200km deep ocean and gravity  
31  
32 field of the moon, the hydrostatic pressure at the bottom of the water, where hydrothermal  
33  
34 vents would be located, is expected to be approximately 130-260MPa (Naganuma and  
35  
36 Uematsu, 1998), which would correspond on Earth to a theoretical oceanic depth of 13-26km  
37  
38 under the sea. There is no known terrestrial hydrothermal vent at such pressure and the  
39  
40 highest pressure at which life has been found is approximately 108MPa at Mariana Trench.  
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49 High pressure processing has been used since 1914 (Hite *et al.*, 1914) to sterilize food  
50  
51 and typically 200-300MPa treatment kills most non-piezophilic bacteria whereas 400-700MPa  
52  
53 treatment kills most spores (Huang *et al.*, 2014). The first known obligate piezophile  
54  
55 microorganism is the black smoker hyperthermophile *Pyrococcus yayanosii* HC1 which has an  
56  
57 optimal growth pressure of 58MPa and is able to grow at pressures as high as 120MPa (Zeng  
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3 *et al.*, 2009; Birrien *et al.*, 2011). However, piezoresistance has been engineered in non-  
4  
5 piezophilic bacteria: selection of pressure-resistant mutants allowed, for example, significant  
6  
7 survival of *Escherichia coli* MG1655 after exposure to 20GPa (Vanlint *et al.*, 2011) but with no  
8  
9 sign of active growth. In similar experiments, some active enzymatic activity has also be  
10  
11 reported at pressures as high as 14GPa (Sharma, 2002; Yayanos, 2002). However, these results  
12  
13 are controversial.  
14  
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16  
17  
18 Yet, little is known about how given biochemical processes, independently of the  
19  
20 cellular context, behave in pressures similar to what would be face in Europa's depths.  
21  
22 Biomolecules from terrestrial abyssal extremophiles may thus provide insights on how these  
23  
24 processes would work under such pressures. More generally, extremophilic molecular  
25  
26 adaptations may help understanding how fundamental biological processes could or not work  
27  
28 under extraterrestrial conditions.  
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### 38 Extreme conditions overview

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44 The scientific field of extremophiles includes both *stricto sensu* extremophilic systems  
45  
46 (needing an extreme condition to work properly) and systems which are just resistant to  
47  
48 conditions which would impair most biological processes. It also includes adaptation based on  
49  
50 the exclusion of extreme condition out of the cell interior when possible and adaptation of all  
51  
52 biomolecules within the cell. Strategies based on special long-term resistance form (such as  
53  
54 spores) with reduced metabolism and paused growth are sometime included in this field.  
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1  
2  
3 Extreme conditions may either be of physical or chemical origin. While chemical  
4  
5 extreme conditions are diverse, including high abundancies of various compounds, physical  
6  
7 extreme conditions are limited to fewer situations. Among them, high or low temperature and  
8  
9 pressure are particularly challenging for life at the molecular scale. Indeed, microorganisms,  
10  
11 which are dominant in extreme environments, cannot form barriers against temperature or  
12  
13 pressure change nor they can maintain internal temperature or pressure different from the  
14  
15 environment. Hence, adaptation of all biomolecules is necessary in order to grow under these  
16  
17 conditions.  
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22  
23 Biological membranes, which separate interior the cell from the external environment,  
24  
25 are permeable to water and not fully impermeable to small solutes. Thus, in hypersaline  
26  
27 environments, osmotic pressure favors loss of intracellular water and increase of intracellular  
28  
29 salinity. The latter makes high salinity a chemical extreme condition different from desiccation  
30  
31 and affecting the internal environment, needing adaptation of all biomolecules.  
32  
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34

35 As described latter, structure and dynamics traits of proteins are the keys for life at  
36  
37 high (HT) or low temperature (LT), high salinity (HS) and high pressure (HP). Because of these  
38  
39 fundamental structural changes, growth or survivability of extremophiles may be reduced or  
40  
41 impossible under mesophilic conditions, making them “true” extremophiles.  
42  
43  
44

45 Effects of these conditions on biomolecules and molecular traits found in  
46  
47 extremophiles to cope with them will be detailed in the next chapters of this review.  
48  
49 Terrestrial environments displaying one or multiple extreme conditions among these are  
50  
51 represented in [Figure 2](#).  
52  
53

54 Other extreme conditions can be handled by organisms at a cellular scale and without  
55  
56 adapting general structure of their biomolecules. In the context of astrobiology, adaptations  
57  
58 at the scale of the cell may appear to be less relevant than adaptations at the scale of the  
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1  
2  
3 structure and dynamics of biomolecules. Nonetheless, these other extreme conditions will be  
4  
5 briefly reviewed in this chapter.  
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7

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

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26 Acidophiles and alkaliphiles are extremophilic organisms needing respectively low (<  
27 3) or high pH (> 9) to grow. Acidic environments on Earth where acidophiles thrive include  
28 volcanic hot springs, solfataric fields, acid mine drainage, bioreactors and coal spoils. Alkaline  
29 environments where alkaliphiles thrive include alkaline hot springs, white smokers, soda lakes  
30 and sewage waters.  
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38 Several attempts to compare acidic terrestrial environments to extraterrestrial ones  
39 have been made. In particular, the acidic metal-rich waters and sediments of Río Tinto have  
40 been compared with ancient aqueous acidic sulfate-rich martian fields (Clifford 1993; Clifford  
41 *et al.* 2010). Enceladus ocean is expected to be alkaline (Glein *et al.*, 2015) but pH of  
42 extraterrestrial environments is still largely debated.  
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50 For terrestrial life, extreme pH is not a condition that cells can afford to totally let in.  
51 Because metabolism lies in generating ATP using pH gradients across the cell membrane,  
52 equilibration of pH between cytoplasm and external acidic or alkaline environment has to be  
53 avoided by cells (Krulwich and Guffanti, 1983; Krulwich, 1995). Therefore, instead of adapting  
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3 proteins and the rest of cellular content to pH < 3, intracellular pH in acidophiles is generally  
4  
5 maintained above 6 (Baker-Austin and Dopson, 2007; Krulwich *et al.*, 2011). The same goes  
6  
7  
8 for alkaliphiles which keep an intracellular pH lower than the environment pH (Krulwich *et al.*,  
9  
10 2011). For example, the model extremely alkaliphilic bacteria *Bacillus pseudofirmus* OF4 grows  
11  
12 at pH 10.5 but keeps a cytoplasmic pH of 8.3 (Sturr *et al.*, 1994) and the most extreme  
13  
14 acidophile organism *Picrophilus torridus* which can grow around pH 0 has an intracellular pH  
15  
16 of 4.6 (Fütterer *et al.*, 2004).  
17  
18

19  
20 Even if terrestrial acidophiles and alkaliphiles possess specific molecular traits, such as  
21  
22 charged cell wall, high membrane impermeability and powerful proton pumps (Krulwich *et*  
23  
24 *al.*, 2011; Kulkarni *et al.*, 2019), extreme pH is an extreme condition that is largely kept out of  
25  
26 the cell, thus not needing an overall large adaptation of proteins.  
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## 29 30 Desiccation 31 32 33 34 35

36 Today, Mars lacks stable surface liquid water. Some terrestrial deserts share several  
37  
38 conditions with Martian surface. In particular, the Atacama desert is considered as one of the  
39  
40 best model environment for present-Mars surface (Dose *et al.*, 2001) as it displays extreme  
41  
42 low abundance of liquid water, extensive UV irradiation and Mars-like soils (Navarro-González  
43  
44 *et al.*, 2003; Fletcher *et al.*, 2012).  
45  
46  
47

48 However, extremely arid terrestrial environments such as Atacama Desert or Antarctic  
49  
50 surface are among the least inhabited places on Earth. If extraterrestrial life shares liquid  
51  
52 water-dependency with Terrestrial life, then it should be considered that current active life would  
53  
54 be harder to find in arid extraterrestrial environments.  
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3 Indeed, most molecular processes in cell require water as a solvent to work. Under  
4  
5 desiccation, reactive oxygen species accumulate, damaging all biomolecules, protein and  
6  
7 nucleic acids undergo direct damage, metabolism is hindered and membrane impermeability  
8  
9 and transport are disrupted (Lebre *et al.*, 2017). Moreover, water activity ( $a_w$ ) decreases,  
10  
11 meaning that less water molecules are available for solvation or catalysis.  
12  
13

14  
15 In this state, life processes are significantly reduced until water returns. Molecular  
16  
17 traits of xerotolerant organisms include accumulation of compatible solutes which replace  
18  
19 water, stabilize membranes and lower oxidative damage and water retention. Under  
20  
21 extended desiccation, xerotolerant organisms undergo reversible cellular states such as  
22  
23 dormancy, sporulation and anhydrobiosis (Crowe *et al.*, 1992). In such state, metabolism and  
24  
25 most biological processes are inhibited or stopped until water is abundant again. Therefore,  
26  
27 xerotolerant organisms may not be described as truly xerophilic.  
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### 38 Ionizing radiation

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43 Highly irradiated environments are uncommon on Earth and most are of anthropic  
44  
45 origin. However, other putatively habitable extraterrestrial environments may be more  
46  
47 irradiated than terrestrial surface. Ionizing radiation, which is either caused by  $\alpha$  or  $\beta$  particles  
48  
49 or by  $\gamma$  or X photons, alters all kinds of biomolecules, mainly by producing radicals. While  
50  
51 radicals lead to both DNA damage, leading to accumulation of mutations and loss of genetic  
52  
53 information, and membrane damage, leading to the loss of cell integrity, protein damage,  
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3 caused by radical-induced carbonylation (Suzuki *et al.*, 2010), is the most important and lethal  
4  
5 effect for microorganisms of ionizing radiation (Daly, 2009).  
6  
7

8 Radioresistant organisms such as the bacteria *Deinococcus radiodurans* (Anderson *et*  
9  
10 *al.*, 1956) and the archaea *Thermococcus gammatolerans* (Jolivet, 2003) can withstand high  
11  
12 doses of ionizing radiation. Proteins (Krisko and Radman, 2010) and DNA (Hutchinson, 1985)  
13  
14 are directly damaged by ionizing radiation. Several molecular traits have been related to  
15  
16 radioresistance, particularly in the model organism *D. radiodurans*: proteome protection,  
17  
18 cytoplasmic accumulation of Mn<sup>2+</sup> and extensive DNA repair (Krisko and Radman, 2013).  
19  
20 Several strategies providing radioresistance, such as Mn<sup>2+</sup> accumulation, are shared with  
21  
22 several archaea (Kish *et al.*, 2009; Webb *et al.*, 2013). However, growth of radioresistant  
23  
24 organisms is still inhibited by ionizing radiation: they are not radiophilic. Nonetheless, as some  
25  
26 extraterrestrial environments are expected to be highly irradiated, they are still considered by  
27  
28 some as an interesting model for astrobiology (Pikuta, 2007; Canganella and Wiegel, 2011;  
29  
30 Merino *et al.*, 2019).  
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37 However, it has to be mentioned that some organisms can actually benefit from  
38  
39 sources of ionizing radiation. Melanized fungi found in remains of Chernobyl Atomic Energy  
40  
41 Station have shown radiotrophic properties: they tend to grow toward sources of ionizing  
42  
43 radiation (Zhdanova *et al.*, 2004) and to produce more biomass as they are irradiated  
44  
45 (Dadachova and Casadevall, 2008). It has been shown that melanine enhances radioresistance  
46  
47 and radiotrophy (Dadachova, Bryan, Huang, *et al.*, 2007; Dadachova, Bryan, Howell, *et al.*,  
48  
49 2007) and that it could acts as a transductor of energy to the benefit of the cell (Dadachova  
50  
51 and Casadevall, 2008). Another example of an organism benefiting of radioactivity is the  
52  
53 bacteria *Candidatus Desulforudis audaxviator* isolated from deep continental environments.  
54  
55 This organisms has been found to live in mono-specie colonies and to use radiolytically  
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3 produced molecules as a source of energy and nutrients making this ecosystem independent  
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5 from surface photosynthesis (Lin *et al.*, 2005, 2006; Chivian *et al.*, 2008). Even if these two  
6  
7 examples of organisms taking advantage of ionizing sources are less radioresistant than model  
8  
9 *D. radiodurans* and *T. gamatolerans*, they still provide examples of how life could adapt in  
10  
11 heavily irradiated extraterrestrial environments (Atri, 2016; Stelmach *et al.*, 2018).  
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## 21 Organic solvents

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26 Organic solvents are highly toxic to biological systems, in particular for membranes and  
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28 protein structures which rely on hydrophobic interactions in a polar medium (Sikkema *et al.*,  
29  
30 1995). Solvent tolerant organisms such as the bacteria *Staphylococcus haemolyticus* can  
31  
32 resume growth after long exposure to 100% toluene, benzene, and p-xylene (Nielsen *et al.*,  
33  
34 2005). In spite of the fact that resistance to organic solvents can be achieved by bacteria and  
35  
36 archaea by various molecular traits mainly based on the exclusion of solvent molecules out of  
37  
38 the cell (Ramos *et al.*, 1997, 2002; Takuichi *et al.*, 1997; Sardesai and Bhosle, 2002; Usami *et*  
39  
40 *al.*, 2005), to our knowledge no organism requires organic solvents to survive: there is no  
41  
42 solvophilic organism.  
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## 53 Heavy metals

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3 Heavy metal-rich environments are uncommon and mainly of anthropic or occasional  
4  
5 geologic origin (Nies, 2000; Krami *et al.*, 2013). Therefore, most are geologically extremely  
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7 recent as they follow industrial revolution. Natural metal-rich environments include  
8  
9 geothermal fields, hydrothermal vents and ultramafic soils (Mergeay, 2006). In these  
10  
11 environments, monoatomic ions of heavy metals such as Cu, Zn, Hg, Cd, As, Ni, Pb, Cr, Co, V,  
12  
13 Ag, Au and Pt and several metal oxides can be highly abundant and toxic to eventual life. Their  
14  
15 toxicity comes mainly from the ability to produce reactive oxygen species (ROS) (Prasad and  
16  
17 Hagemeyer, 1999; Pinto *et al.*, 2003; Shahid *et al.*, 2014), their chaotropicity and their ability  
18  
19 to replace other ions involved in enzymatic activity (Assche and Clijsters, 1990). For example,  
20  
21 many metalloenzymes physiologically possess Ni<sup>2+</sup> in their catalytic center whose replacement  
22  
23 by Zn<sup>2+</sup> makes them inactive (Boer *et al.*, 2014). Moreover, heavy metals disrupt salt and  
24  
25 disulfide bridges of proteins favoring unfolded state (Tamás *et al.*, 2014). Their toxicity is  
26  
27 typically ranked in the following order: Zn<sup>2+</sup> < Ag<sup>+</sup> < Ni<sup>2+</sup> << Cu<sup>2+</sup> < Hg<sup>2+</sup> << UO<sub>2</sub><sup>2-</sup> < Co<sup>2+</sup> < CrO<sub>4</sub><sup>2-</sup>  
28  
29 < Au<sup>3+</sup> < Cd<sup>2+</sup> < Mn<sup>2+</sup> <<< Pb<sup>2+</sup> (Nies, 2000).  
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37 Metalloresistance is mainly achieved by energy dependent efflux of toxic ions (Silver  
38  
39 and Phung, 1996) and by chelating agents (Daniels *et al.*, 1998; Cobbett, 2000). Other  
40  
41 sequestration mechanisms in bacteria (Kothe *et al.*, 2010) include biomineralization  
42  
43 (Bäuerlein, 2003) and cell wall adsorption (Merroun *et al.*, 2005). Periplasmic sensors are also  
44  
45 of particular importance (Trepreau *et al.*, 2014; Nies *et al.*, 2017). Interestingly,  
46  
47 metalloresistance is common in acidophilic archaea and bacteria whose sulfur metabolism  
48  
49 may increase extracellular metal concentration (Dopson *et al.*, 2003, 2014). Nonetheless,  
50  
51 heavy metal concentration is also an extreme condition that can be, to a certain extent, kept  
52  
53 out of the cytoplasm and not requiring an adaptation of all proteins.  
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3 It should however be noted that, in some anoxic environments, many microorganisms,  
4  
5 such as the bacteria *Geobacter metallireducens*, can achieve anaerobic respiration using  
6  
7 metals, metalloids or radionuclides (Lovley *et al.*, 1993). These microorganisms can use  
8  
9 extracellular Fe(III), Mn(IV), Co(III), Cr(VI) or U(VI) as electron acceptors which they  
10  
11 respectively reduce into Fe(II), Mn(II), Co(II), Cr(III) and U(IV) (Gralnick and Newman, 2007;  
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13 Richter *et al.*, 2012).  
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### 23 Nutrient scarcity

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28 Oligotrophic environments are characterized by low nutrient and energy availability  
29  
30 and low biomass. Oligotrophs are slow-growing microorganisms that can persist for long  
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32 under oligotrophic conditions (Hoehler and Jørgensen, 2013; LaRowe and Amend, 2015) but  
33  
34 still they require nutrients and energy sources to grow. To our knowledge, no molecular traits  
35  
36 of biomolecules enabling oligotrophy is known.  
37  
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41 However, organisms adapted to low-energy environments have been considered by  
42  
43 some as interesting models for extraterrestrial life since several extraterrestrial environments  
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45 such as Martian surface appear to be low-energy (Jones *et al.*, 2018).  
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### 53 Other extreme conditions

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3 Some other extreme chemical and physical conditions and their related adapted  
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5 organisms have fallen into the scientific field of extremophiles: oxygen tension, hypobaric  
6  
7 pressure (Schwendner and Schuerger, 2020), vacuum (Stojanovic *et al.*, 2008) and even  
8  
9 chaotropicity (Williams and Hallsworth, 2009; Oren, 2013; Zajc *et al.*, 2014). However, most  
10  
11 of the organisms associated with these extreme conditions are not considered as classical  
12  
13 extremophiles and do not possess clear molecular traits enabling adaptation.  
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# 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

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2  
3 Biology takes place in aqueous solution and interactions with the solvent environment  
4  
5 play an essential role in the folding and stabilization of proteins and nucleic acids and  
6  
7 organization of lipids to form membranes. In fact, the discovery of biological macromolecules  
8  
9 in the late 19th century and their initial characterization in the 20th is tightly interwoven with  
10  
11 the development of solution thermodynamics. Useful definitions:  
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14  
15 The change in *enthalpy*,  $\Delta H$ , during a chemical reaction is the heat absorbed or  
16  
17 released in the breaking and formation of bonds. The heat energy invested by a system in  
18  
19 order to sample all the configurations available to it, at a given temperature, is equal to its  
20  
21 *entropy*,  $S$ , multiplied by the temperature (in Kelvin units). *Gibbs free energy*,  $G$ , represents  
22  
23 the part of the energy in a system that is not used to populate the different entropy  
24  
25 configurations (thermal agitation disorder) and which can be transformed into useful work,  
26  
27 hence its name. At constant pressure and temperature, the free energy released by a reaction  
28  
29 is given by:  
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$$\Delta G = \Delta H - T \Delta S \quad (1)$$

34  
35 A system that exchanges energy with its surroundings evolves in order to maximize its free  
36  
37 energy. The spontaneous sense of a reversible reaction is in the direction for which  $\Delta G < 0$ .  
38  
39 When two states of a system are at equilibrium, their free energy difference is zero,  $\Delta G = 0$ .  
40  
41 In the case of a chemical equilibrium (e.g. between the folded and unfolded state of a protein),  
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43 free energy (and consequently enthalpy and entropy) are calculated from the measured  
44  
45 equilibrium concentrations of the two states.  
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## 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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3 Most importantly, water determines both folding and catalytic functions of proteins  
4  
5 and nucleic acids (Bellissent-Funel *et al.*, 2016) as will be discussed further below.  
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### 13 Proteins

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19 Proteins are the most diverse of all macromolecules, varying in length, size, shape and  
20  
21 physical and chemical properties. Their functions are as diverse with, for example, collagen  
22  
23 structuring the extracellular matrix, muscle and flagella converting force into motion,  
24  
25 membrane proteins acting as signal receptors and, of course, enzymes that are fundamental  
26  
27 for biological systems as they enable catalysis at lower substrate concentrations and with  
28  
29 higher specificity than abiotic catalysis. Depending on the method, 1000-2300 different  
30  
31 proteins can be detected in cells of *E. coli*, one of the most studied organisms, (Han and Lee,  
32  
33 2006; Ishihama *et al.*, 2008; Soufi *et al.*, 2015) whose genome typically encodes up to 4300  
34  
35 putative protein sequences (Serres *et al.*, 2001; Hu *et al.*, 2009).  
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41 Proteins are synthesized by large intracellular macromolecular assemblies called  
42  
43 ribosomes composed of proteins and catalytic RNA (rRNA), which decodes the information of  
44  
45 messenger RNA (mRNA) in the process of translation. mRNA has itself been transcribed from  
46  
47 genomic DNA.  
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50  
51 Proteins are structured (folded) and dynamic polymers whose chemical name is  
52  
53 polypeptide chains. The combination of these two properties is fundamental to their physical  
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55 and chemical properties and their biological function as it allows possibility to build active  
56  
57 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-  
6  
7 dynamics-function relationship. Indeed, their apparent lack of structure is fundamental to  
8  
9 their functions, they can adopt local structures upon binding (Kragelj *et al.*, 2013), they  
10  
11 complement structured proteins (Oldfield and Dunker, 2014) and are fundamental in many  
12  
13 cellular processes (Wright and Dyson, 2015).  
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17  
18 Of particular relevance for effects of extreme conditions and adaptation to extreme  
19  
20 environments is the fact that protein structure and dynamics rely heavily on interactions with  
21  
22 solvents. Consider the free energy difference (eq. 1) between the folded and unfolded state  
23  
24 of polypeptide chain. The  $\Delta H$ ,  $\Delta S$  display a complex dependence on temperature:  $\Delta H$ , because  
25  
26 of bonding within the chain as well as between the chain and solvent;  $\Delta S$ , because of chain  
27  
28 disorder as well as solvent disorder (the hydrophobic effect). This leads to the native state  
29  
30 being stabilized in a narrow temperature range (close to 37°C for mesophilic proteins) falling  
31  
32 off at temperatures above (thermal unfolding) and below (cold denaturation). The maximum  
33  
34 stabilisation free energy is interestingly small. At about 50 kJ/mol it corresponds, for example,  
35  
36 to the enthalpy gained by breaking two or three H-bonds in the protein interior, when  
37  
38 hundreds are involved in internal protein and protein solvent interactions.  
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47 The structure of proteins is in general understood as the combination of four structural  
48  
49 levels, to which a fifth, quinary structure, has however been added for interactions between  
50  
51 different proteins within cells (McConkey, 1982; Cohen and Pielak, 2017).  
52  
53

54 The primary structure is the amino acid sequence. While protein is the functional  
55  
56 molecule, its chemical composition is a polypeptide linear polymer made up of amino acids  
57  
58 (AA) linked by peptide bonds. They share a basic structure with a central  $\alpha$  carbon bonded to  
59  
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1  
2  
3 a hydrogen, an amino group, a carboxyl group and a side chain. All AA found in proteins  
4  
5 (proteinogenic AA) are L-stereoisomers. The side chain determines the nature of the AA  
6  
7 residue in chain. There are twenty AA in the genetic code. However, two other AA that are not  
8  
9 in the universal genetic code can also be found in some proteins: selenocysteine in all three  
10  
11 domains of life and pyrrolysine in methanogenic archaea and some bacteria (Rother and  
12  
13 Krzycki, 2010). Amino acids can be classified according to their characteristics (small/large,  
14  
15 polar/non-polar, charged/uncharged, acid/basic, aromatic or not, etc.) or to their properties  
16  
17 (rigid/flexible, proton donor/acceptor, metal-binding, DNA and RNA binding, Mg<sup>2+</sup> and Ca<sup>2+</sup>-  
18  
19 binding, etc.). AA residue names are generally written with one-letter or three-letters  
20  
21 abbreviations. Primary structures of proteins can be determined by translating open reading  
22  
23 frames in genomic DNA.  
24  
25  
26  
27  
28

29  
30 Secondary structure corresponds to local, defined structural organization in segments  
31  
32 of a protein. The  $\alpha$ -helix and  $\beta$ -sheet are the most common secondary structures, but  $\beta$ -turns  
33  
34 (Hutchinson and Thornton, 1994) and  $\Omega$ -loops (Leszczynski and Rose, 1986; Fetrow, 1995) are  
35  
36 also frequent. Tight turns and flexible loops are usually found between helices and sheets.  
37  
38 Secondary structures result from hydrogen bonds between amino and carboxyl groups of the  
39  
40 same or different AA backbones, thus not directly involving side chains. However, some AA or  
41  
42 AA sequences are known to favor or to block secondary structure formations. For example,  
43  
44 Met, Ala, Leu, Glu and Lys are common in helices whereas Pro and Gly block helices formation  
45  
46 and are common in turns. Secondary structure can often be predicted *in silico* with primary  
47  
48 structure as the sole information.  
49  
50  
51  
52  
53

54  
55 Tertiary structure is the general three-dimensional architecture defined by the spatial  
56  
57 coordinates of each atom of the protein. At this level, prediction by informatics remains really  
58  
59 challenging unless through modelling based on experimental data from a homologous  
60

1  
2  
3 structure. The experimental determination of 3D-structures is made by X-ray crystallography  
4  
5 (XRC), nuclear magnetic resonance (NMR) or cryo-electronic microscopy (cryo-EM). Tertiary  
6  
7 structure involves interactions between side chains: hydrophobic interactions, disulfide  
8  
9 bridges, hydrogen bonds, hydrophilic interactions and ionic bonds. The process under which  
10  
11 linear AA polymers acquire their tertiary structure is called folding. Folding determines protein  
12  
13 properties: affinity with other biomolecules, thermostability, catalytic activity and specificity,  
14  
15 dynamics, etc.  
16  
17  
18  
19

20 Most proteins assemble within homomultimers or heteromultimers (Lynch, 2013).  
21  
22 Quaternary structure is the transitory or stable resulting macromolecular structure made by  
23  
24 association of proteins with other proteins, nucleic acids or other cofactors. Quaternary  
25  
26 structure not only relies on subunits' 3D-shapes but also on the contact surfaces. Interactions  
27  
28 between subunits are mainly based on hydrophobic interactions but can also be stabilized by  
29  
30 electrostatic, hydrogen and occasionally disulfide bonds.  
31  
32  
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36  
37

38 Another level of complexity and variation also lies in post-translational modifications.  
39  
40 Following translation on the ribosome during which they are synthesized, many proteins  
41  
42 undergo covalent chemical modification of carboxy/amino termini or of side chains. These  
43  
44 modifications, which are generally catalyzed by enzymes, are various and include  
45  
46 glycosylation, phosphorylation or addition of aliphatic chains. Although these modifications  
47  
48 are in general not critical for protein structure, they are nonetheless fundamental to many  
49  
50 biochemical and cellular processes.  
51  
52  
53

54 It should also be kept in mind that the state of the proteome, i.e. the set of all proteins  
55  
56 produced by a cell, is tightly regulated. Not only do cells regulate protein synthesis, folding  
57  
58 and post-translational modification but also their repair and degradation are fundamental.  
59  
60

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3 Protein degradation in particular is achieved by central macromolecular assemblies called  
4 proteasomes and allows the essential regulation of protein quantity and quality control  
5  
6  
7  
8 (Sontag *et al.*, 2017).  
9

10  
11  
12  
13 In spite of the fact that countless biologically relevant information has been obtained  
14  
15 with more than 160000 solved protein 3D-structures, classical structural biology approaches  
16  
17 tend to give time-average representations. However, it is now clear that proteins are  
18  
19 dynamical objects. Techniques such as NMR, THz and neutron spectroscopy and neutrons/X-  
20  
21 rays scattering have unraveled various kinds of intramolecular movements within proteins  
22  
23 with different timescales (Xu and Havenith, 2015), see [Figure 3](#). On one hand, flexibility of  
24  
25 proteins is particularly critical for enzymes: substrate selection, entry, binding and catalysis is  
26  
27 permitted by both local and general protein intramolecular movements (Zhao, 2017; Sen *et*  
28  
29 *al.*, 2017; Rout *et al.*, 2018; Fürst *et al.*, 2019). On the other hand, stiffer regions within  
30  
31 structures have been shown to be relevant for proton or electron transfer (Réat *et al.*, 1998;  
32  
33 Sacquin-Mora *et al.*, 2007).  
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## 45 Nucleic acids

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51 Nucleic acids are the biomolecules that harbor the genetic information, which can be  
52  
53 transferred horizontally from organism to organism, or vertically from generation to  
54  
55 generation. They chemically correspond to linear polymers of nucleotides linked by  
56  
57 phosphodiester bonds. Nucleotides are made of a sugar (ribose for RNA and desoxyribose for  
58  
59 DNA) associated with a nitrogenous base which can be a purine (adenine, guanine) or a  
60



1  
2  
3 pyrimidine (cytosine, thymine for DNA or uracil for RNA). The base defines the nucleotide  
4  
5 leading to the one-letter abbreviation: A, C, G, T (or U).  
6  
7

8 DNA is synthesized *in cellulo* on a DNA template by enzymes called DNA-polymerases,  
9  
10 during the process of semi-conservative replication. However, in cells infected by retroviruses,  
11  
12 DNA can also be synthesized from an RNA template by retro-transcriptase enzymes. In  
13  
14 contrast, RNAs are synthesized from a DNA template during transcription in the cell but can  
15  
16 also be replicated from an RNA template in some RNA-virus-infected cells.  
17  
18  
19

20 In Watson and Crick base pairing, bases interact with each other as A-T, A-U, G-C. It  
21  
22 can either lead to the interaction between two separate strands or to the folding of a single  
23  
24 strand. This pairing also leads to the right-handed helical structure of double-stranded nucleic  
25  
26 acids. The DNA double-helix is usually in a right-handed form called B-DNA. However, as a  
27  
28 function of the environment, two other helical structures can be found: the A-form, with  
29  
30 increased diameter, and the left-handed Z-form (Henderson and Krude, 2004).  
31  
32  
33

34 The nucleotide sequence constitutes the primary structure of a nucleic acid, secondary  
35  
36 structure results from base pairing and tertiary structure from the resulting three-dimensional  
37  
38 folding. Nucleic acids can also be chemically modified by enzymes. DNA can be methylated  
39  
40 while RNA can undergo many different post-transcriptional modifications (Nachtergaele and  
41  
42 He, 2017). DNA is also generally topologically negatively supercoiled *in cellulo*.  
43  
44  
45  
46

47 Depending mainly on sequence and length, all double-stranded nucleic acids possess  
48  
49 a melting temperature ( $T_m$ ) above which half of the molecules will be turned into single-  
50  
51 stranded form. However, nucleic acid stability and folding is also a function of salt  
52  
53 concentration as they interact with ions, mainly cations (Von Hippel and Schleich, 1969). tRNA  
54  
55 is for example, has been shown to be stabilized within salt crystals (Tehei *et al.*, 2002).  
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3 While DNA mostly serves as storage of genetic information, RNA molecules may  
4  
5 possess many functions.  
6  
7

8 Among them, ribozymes possess catalytic activity which, like protein enzymes, is  
9  
10 dependent on structure and dynamics (Doherty and Doudna, 2001). Ribosomal RNAs (rRNAs)  
11  
12 are essential and universal ribozymes found in all cells. rRNAs assemble with proteins in a  
13  
14 macromolecular assembly called the ribosome. Ribosome decodes genetic information of  
15  
16 mRNA into protein sequence which they synthesize (Cech, 2000). This process of translation  
17  
18 also requires another type of RNA, transfer RNA (tRNA), which recognizes a three nucleotides  
19  
20 sequence (codon) on mRNA, and brings, to the ribosome, the corresponding amino acid  
21  
22 (according to the genetic code) to be inserted in the growing polypeptide.  
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29

30 Damage to nucleic acids can be spontaneous or induced by chemicals or physical  
31  
32 conditions. These damages can lead to mutations and change or loss of the genetic  
33  
34 information. While most mutations are generally repaired by enzymes, they still permit  
35  
36 phenotypic variation, adaptation and natural selection and, thus, contribute to Darwinian  
37  
38 evolution.  
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## 48 Lipids

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53 Lipids are amphiphilic molecules with polar heads and hydrophobic tails. In aqueous  
54  
55 solution, they spontaneously form diverse structures of which the bilayer is the most relevant  
56  
57 for membrane formation. Note that in certain archaea, the lipid molecules in the outer and  
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1  
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3 inner leaflets are fused by the tails effectively turning the bilayer into a monolayer. The  
4 permeability barrier in biological cellular membranes is the bilayer made up of phospholipids  
5 and glycolipids and other hydrophobic molecules such as sterols into which transmembrane  
6 proteins are inserted. Membranes segregate cell components from extracellular  
7 environments and allow compartmentation. Control of diffusion and active transport across  
8 the membrane enables the possibility for cellular homeostasis and chemical gradients which  
9 can be used to produce energy.  
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20 Lipid bilayers take up different thermodynamic phases as a function of temperature  
21 and pressure, nature and length of hydrophobic tails, positions of unsaturated bonds in the  
22 tails, cyclization etc. Typically, the physiological state of a biological membranes is relatively  
23 fluid while not too disordered, allowing balance between fluidity and rigidity (see [Figure 4](#)) to  
24 favor membrane protein dynamics and function.  
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32 Furthermore, protein-lipid interactions modulate many membrane functions such as  
33 enzymatic activity and microdomain structure (Tien and Ottova-Leitmannova, 2000; Richens  
34 *et al.*, 2015).  
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## 48 Effects of extreme conditions on terrestrial biomolecules

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53 Temperature, salinity and pressure conditions deeply affect structural and dynamical  
54 properties of proteins. Even if some general alterations can be recognized, effects of these  
55 conditions depend on the biomolecule. In particular, it should be kept in mind that, depending  
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3 on their folding, all proteins react differently to these conditions. Therefore, every protein,  
4  
5 even within one organism, possesses its own temperature, salinity and pressure limits.  
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### 10 11 12 13 Effects of low temperature on biomolecules 14

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19 About 75% of the terrestrial biosphere can be considered as cold ( $T < 5^{\circ}\text{C}$ ) with many  
20  
21 environments that are permanently cold such as deep ocean and polar habitats (Cavicchioli,  
22  
23 2006).  
24

25  
26 Temperature is a general physical parameter governing all biomolecules behavior. The  
27  
28 main result of lowering temperature is dynamical, decreasing the motion of atoms in water  
29  
30 and biomolecules. As a consequence, processes requiring flexibility such as catalysis can be  
31  
32 inhibited at low temperature, unless adaptation has led to weaker intramolecular bonding to  
33  
34 allow the flexibility (Tehei *et al.*, 2004). Moreover, as motion is reduced, diffusion is also  
35  
36 slowed down at low temperatures, limiting substrate and cofactors availability. According to  
37  
38 Arrhenius law, a decrease of  $20^{\circ}\text{C}$  for example lowers reaction rates constants by four orders  
39  
40 of magnitude (Russell *et al.*, 1990).  
41  
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45  
46 In addition to this effect, as a result of a decreased motion of atoms, proteins and all  
47  
48 other biomolecules, loose flexibility at cold temperatures. As flexibility is critical for substrate  
49  
50 acceptance and chemical transformation, cold temperature disrupts catalytic potential of  
51  
52 enzymes.  
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3       Upon further temperature decrease, many observations tend to show that proteins  
4  
5 undergo a process called cold denaturation leading to unfolding (see above, section Structure-  
6  
7 Dynamics-Function in Biomolecules). In fact, through the hydrophobic effect, hydration of  
8  
9 non-polar residues by water promoting unfolding is favored at low temperatures (Tsai *et al.*,  
10  
11 2002; Yoshidome and Kinoshita, 2012). In particular, formation of ice-water interfaces  
12  
13 promotes this unfolding (Arsiccio *et al.*, 2020). However, some have argued that, instead,  
14  
15 contribution of hydrophilic groups would be more important (Ben-Naim, 2013). Nonetheless,  
16  
17 folding of proteins is impaired or slowed down by low temperatures. This can lead to the  
18  
19 disassembly of large molecular edifices such as viral capsids (Da Poian *et al.*, 1995). At low  
20  
21 temperatures, ribosome function is also reduced, impairing or slowing down even more  
22  
23 protein folding.  
24  
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32  
33       In contrast to cold-denaturation of proteins, folding of nucleic acids is enhanced at low  
34  
35 temperatures, which stabilize secondary structures, including non-native folding (D'Amico *et*  
36  
37 *al.*, 2006). mRNAs in particular are considered more cold-sensitive than proteins (Narberhaus,  
38  
39 2002). However, increased stability of structures, like DNA hairpins for example, impairs  
40  
41 processes like replication, transcription or translation as the polymerases or ribosomes are  
42  
43 blocked by these structures (Inouye and Phadtare, 2008).  
44  
45  
46

47       Processes involving DNA are also impaired by the increase of negative supercoiling  
48  
49 induced by cold temperature (Wang and Syvanen, 1992; Mizushima *et al.*, 1997).  
50  
51  
52  
53

54       Reduced flexibility of biomolecules also affects membrane structure and functions.  
55  
56 Lowering temperature promotes gel phase transitions in membranes, increasing compacity of  
57  
58 lipid layer, rigidity and reducing permeability and lipid motion (Jebbar *et al.*, 2015, 2020a), see  
59  
60

1  
2  
3 **Figure 4.** This impairs processes such as secretion and transport. Formation of ice crystals can  
4  
5 even perforate cellular membranes (Muldrew and McGann, 1990; Parrilli *et al.*, 2011).  
6  
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8  
9

10 Water properties dramatically change with lowered temperature. Viscosity is  
11  
12 increased and water activity ( $a_w$ ) is decreased, meaning that there is less water available for  
13  
14 catalysis, solvation and interaction with biomolecules.  
15  
16

17  
18 From a general point of view, low temperature causes osmotic stress. When ice forms  
19  
20 in the extracellular environment, osmotic pressure of cytoplasmic water is higher than  
21  
22 extracellular ice leading to the efflux of water out of the cell and thus to a dramatic increase  
23  
24 of many cytoplasmic compounds concentrations (Russell *et al.*, 1990). Concentration of the  
25  
26 extracellular environment may make the environment more extreme (Banciu and Sorokin,  
27  
28 2013). However, due its composition, intracellular water freezes at temperatures under 0°C.  
29  
30 In *S. cerevisiae*, for example, cytosol vitrification typically happens at -17°C (Fonseca *et al.*,  
31  
32 2016). At this point, intracellular ice formation concentrates even more the cytoplasm,  
33  
34 altering pH, salinity, ionic strength, and  $a_w$  (Russell *et al.*, 1990).  
35  
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42 Another general effect of low temperature on water is an increased solubilization of  
43  
44 gas such as oxygen (D'Amico *et al.*, 2006), leading to an enhanced production of ROS species  
45  
46 and thus to an oxidative stress (Abele and Puntarulo, 2004; Vinagre *et al.*, 2012).  
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54 **Effects of high temperature on biomolecules**  
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3 In general, increased temperature means increased molecular movements and overall  
4  
5 increased flexibility (Daniel and Cowan, 2000). A first consequence is that temperature acts as  
6  
7 a catalyzer of most reactions, like the spontaneous degradation of many small molecules in  
8  
9 biological systems. Unstable metabolites like ATP, NAD and acetyl phosphate, for example,  
10  
11 have a short half-life at 95°C (Daniel and Cowan, 2000).  
12  
13  
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18 For protein heat damage, it is possible to distinguish irreversible (degradation) and  
19  
20 reversible (denaturation) damage (Daniel *et al.*, 1996).  
21  
22

23 Protein flexibility enhancement at high temperature can also lead to a decrease of  
24  
25 stability. As discussed before in this review, protein folding relies on various intramolecular  
26  
27 interactions such as hydrophobic contacts, for example. As at high temperature atomic  
28  
29 motions are increased, these bonds loose some strength and exposure of hydrophobic regions  
30  
31 is enhanced, favoring unfolding (Das and Surewicz, 1995). This local denaturation is to some  
32  
33 extent reversible as some protein can spontaneously fold back upon temperature decrease  
34  
35 (Daniel *et al.*, 1996). Exposure of hydrophobic regions may, however, promote, interaction  
36  
37 between unfolded proteins and hence to irreversible aggregation.  
38  
39  
40  
41

42 In short, high temperature promotes unfolding of proteins which can be reversible.  
43  
44  
45  
46

47 Several irreversible chemical modifications of amino acids composing proteins are  
48  
49 enhanced by high temperatures: deamidation of Asn and Gln residues, succinimide formation  
50  
51 at Glu and Asp and oxidation of His, Met, Cys, Trp and Tyr (Ahern and Klibanov, 1985; Zale and  
52  
53 Klibanov, 1986; Daniel *et al.*, 1996; Daniel and Cowan, 2000). These irreversible degradations  
54  
55 not only impair folding but can also lead to cleavage of peptide bond, leading to the loss of  
56  
57 structure and function of proteins.  
58  
59  
60

1  
2  
3 Nucleic acids are also altered by high temperature in both reversible and irreversible  
4  
5 ways. As temperature increases, subtle alterations of DNA double helix occur: the winding  
6  
7 angle expands which lengthens the repeat and reduces the twist in the DNA molecule. A  
8  
9 further effect of temperature is the reversible denaturation of the double helix by unbinding  
10  
11 of base pairs. When turned into single stranded form, DNA is more sensitive to chemical  
12  
13 irreversible damage: hydrolysis which leads to purine deamination and formation of abasic  
14  
15 sites, oxidation leading to the formation of hydroxy-guanine or even to a rupture of the helix,  
16  
17 saturation of double bonds in nucleobases and non-enzymatic methylation (Lindhal, 1993;  
18  
19 Daniel and Cowan, 2000). All these damages impair normal Watson-Crick pairing of  
20  
21 nucleobases and can lead to the accumulation of deleterious mutations if not repaired by the  
22  
23 cell.  
24  
25  
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27  
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29

30 RNA is not only be altered by the previous effects but its differences with DNA make it  
31  
32 even more thermosensitive. Because the sugar moiety in RNA is ribose, which possesses an  
33  
34 additional hydroxyl group, it is sensitive to thermally-enhanced hydrolysis leading to the  
35  
36 cleavage of the backbone (Voet and Voet, 2016). Moreover, as RNA incorporates uracil, which  
37  
38 lacks a methyl group, instead of thymine, base stacking is less stable than in DNA. Both effects  
39  
40 equally diminish thermostability of RNA in comparison to DNA (Wang and Kool, 1995),  
41  
42 dramatically shortening its half-life at high temperatures.  
43  
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49

50 Membrane properties are also directly temperature-dependent. Increasing  
51  
52 temperature promotes fluid phase transitions in the lipid components, increasing disorder,  
53  
54 fluidity, permeability and lipid motion (Jebbar *et al.*, 2015, 2020a), see [Figure 4](#). Increased  
55  
56 molecular agitation of hydrophobic tails means less interactions between them and increased  
57  
58 permeability of sodium and protons, particularly in non-thermophile membranes (Driessen *et*  
59  
60



1  
2  
3 *al.*, 1996; Daniel and Cowan, 2000). As a result, non-thermophilic organisms face problems in  
4  
5 maintaining homeostasis in high temperature environments. Transmembrane gradients are  
6  
7 hence impaired which can turn into bioenergetic failure.  
8  
9

10  
11  
12  
13 At high temperature, water solubilization of gases is decreased, water molecules are  
14  
15 more disordered and interact less with charges on biomolecules, impairing protein solvation  
16  
17 (Elcock, 1998).  
18  
19

## 20 21 22 23 24 25 Effects of high salinity on biomolecules 26

27  
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30  
31 As for other biochemical parameters, cells actively maintain their cytoplasm at a given  
32  
33 salinity in composition and concentration that is different from that in the extracellular  
34  
35 environment. In particular, in all cells, while the Na<sup>+</sup> ion is dominant outside, the K<sup>+</sup> is  
36  
37 dominant within the cell. This is true in human blood, for example, in which an injection of  
38  
39 physiological 0.9% NaCl into the serum will contribute to treat a dehydrated patient, while an  
40  
41 equivalent concentration of KCl would kill the same patient, by overwhelming the membrane  
42  
43 pumps acting on ion exchange. In hypersaline environments such as salt lakes and marshes,  
44  
45 rock salts, brine ice-inclusions and sea brines (Kanekar *et al.*, 2012), the outside osmolarity  
46  
47 poses a further challenge, counterbalanced in the cytoplasm by neutral molecules like glycerol  
48  
49 or ectoine, or, in certain archaea and bacteria, by KCl. While the dominant salt in marine  
50  
51 hypersaline environments is NaCl, other natural and artificial environments can be KCl, MgCl<sub>2</sub>,  
52  
53 MgSO<sub>4</sub> or CaCl<sub>2</sub>-rich (Schneegurt, 2012; Oren, 2013; Jebbar *et al.*, 2020b).  
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3 Salinity-induced toxicity results from two distinct effects: non-specific osmotic stress  
4  
5 and ion-specific toxicity (Serrano, 1996). Depending on the organism, one can be more  
6  
7 important than the other. For example osmotic stress is more toxic in *E. coli* while sodium-  
8  
9 specific toxicity primes in *S. cerevisiae* (Serrano and Gaxiola, 1994).  
10  
11  
12  
13  
14

15 Specific toxicity of ions comes from their excessive interaction with proteins. While  
16  
17 proteins require ions interacting with their charged or polar uncharged residues, excessive  
18  
19 ions abundance can impair protein integrity or interactions, for example, through charge  
20  
21 screening.  
22  
23  
24

25 A change of cytoplasmic ion composition may hence alter these interactions: some  
26  
27 ions with other steric properties will quench previous ones or new destabilizing ionic  
28  
29 interactions will appear (Larsen, 2011; Okur *et al.*, 2017).  
30  
31  
32

33 At molar concentrations, ions can have chaotropic effects, decreasing surface tension  
34  
35 and increasing hydrophobic solubility. Chaotropic ions may either be cations or anions  
36  
37 depending on their hydration and their effect can be countered by kosmotropic ions with  
38  
39 opposite effects, see [Table 2](#).  
40  
41

42 Chaotropic ions (either strongly hydrated cations or weakly hydrated anions) interact  
43  
44 with protein backbone (see [Figure 5](#)) and thus promote exposure of backbone to solvent,  
45  
46 leading to unfolding (Kumar and Venkatesu, 2014; Mazzini and Craig, 2017). Chaotropic  
47  
48 divalent cations  $Mg^{2+}$  or  $Ca^{2+}$  also interact with side-chains of negatively charged amino acids,  
49  
50 quenching their eventual physiological interactions involved in structure or function (Okur *et*  
51  
52 *al.*, 2017). These ions are intracellularly accumulated in athalassohaline environments (Oren,  
53  
54 2013), which can be defined as aquatic environments with ionic proportions different from  
55  
56 seawater.  
57  
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3 Chaotropic anions (weakly hydrated) however interact less with side-chains of  
4 positively charged residues than kosmotropic anions (strongly hydrated) (Okur *et al.*, 2017)  
5  
6 and hence represent a lesser threat to proteins.  
7  
8  
9

10 It has been proposed that chaotropic ions also alter general structure and properties  
11 of water (Marcus, 2009) but this idea has been debated (Ball and Hallsworth, 2015).  
12  
13  
14

15 In general, high concentrations of salt ions directly impair interactions between all  
16 charged biomolecules: proteins, DNA, RNA, lipid heads, metabolites, etc (Eisenberg, 1995).  
17  
18 However, each protein reacts differently depending on its folding and its charge.  
19  
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25 Osmotic stress comes from the binding of water by salt ions, lowering water activity  
26 and causing hydric stress. In a hypersaline environment, water molecules are more bound to  
27 salt ions, hence less diffusive and less available for catalysis, solvation and interaction with  
28 biomolecules.  
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#### 40 Effects of high pressure on biomolecules 41 42 43 44

45 High hydrostatic pressure ( $P > 10\text{MPa}$ ) concerns mainly two environments: deep  
46 waters (88% of the total oceanic volume has a depth  $> 1\text{ km}$ ) and continental and marine  
47 subsurface biosphere, which contains a significant proportion of total earth biomass  
48 (McMahon and Parnell, 2014). Hence, high pressure, like cold temperature, is a common  
49 extreme condition that terrestrial life faces (Jebbar *et al.*, 2015).  
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3 Le Chatelier's principle governs systems under pressure changes: equilibriums of non-  
4  
5 isovolumetric reactions will react to pressure increases by shifting towards a decrease of  
6  
7 volume. Therefore, reactions leading to net increase of charges, such as production of ions,  
8  
9 are favored by pressure as charges can condensate water molecules, which will occupy less  
10  
11 volume (Gross and Jaenicke, 1994; Fumiyoshi *et al.*, 1999). Another consequence is a favored  
12  
13 overall compaction of biomolecules, impairing flexibility and dynamics.  
14  
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20 The first effect of pressure on proteins is the dissociation of large molecular assemblies  
21  
22 such as ribosomes (Gross *et al.*, 1993) or DNA-protein complexes (Kawano *et al.*, 2005). In fact,  
23  
24 quaternary structure often relies on charged residues, which can, upon dissociation, condense  
25  
26 water molecules. Most multimeric assemblies dissociate at 200MPa (Aertsen *et al.*, 2009).  
27  
28  
29

30 Tertiary structure can also be altered and even lost under pressure. Pressure-induced  
31  
32 protein unfolding has long been known (Bridgman, 1914), however, unlike thermal  
33  
34 denaturation, mechanisms behind this process are still unclear. As pressure tends to enhance  
35  
36 compaction of biomolecules, proteins with internal cavities are particularly affected (Girard *et al.*  
37  
38 *et al.*, 2010; Matthews, 2012; Roche *et al.*, 2012). As a general rule: if a protein occupies more  
39  
40 space when folded, which is not the case of all of them, then pressure will favor its unfolding  
41  
42 (Jebbar *et al.*, 2020a). Another effect favoring unfolding of proteins is that exposure of  
43  
44 hydrophobic residue to solvent enables Van Der Waals interactions with water molecules  
45  
46 hence condensing them (Gross *et al.*, 1993). Most proteins unfold at 400MPa (Aertsen *et al.*,  
47  
48  
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50  
51  
52 2009).  
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57 Extensive research has been done about effects of high pressure on DNA-stabilization.  
58  
59 As for other macromolecules, DNA is compacted under pressurization. Preliminary work  
60

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

13  
14  
15 However, since the DNA double-helix needs to be opened for several biological  
16 processes, high pressure-induced stabilization of double-stranded nucleic acids impairs  
17 fundamental biological processes such as replication, transcription and translation  
18 (Macgregor, 2002).

19  
20  
21 As with temperature, membrane rigidity and permeability are pressure-dependent  
22 (Hazel and Eugene Williams, 1990). Increasing pressure promotes gel phase transition of  
23 membranes, increasing compacity of lipid layer, rigidity and reducing permeability and lipid  
24 motion (Jebbar *et al.*, 2015, 2020a), see [Figure 4](#). As effect of pressure on proteins and nucleic  
25 acids appear to be moderated in comparison to other extreme conditions, membrane instead  
26 is considered to be the most pressure-sensitive biomolecule (Oger and Jebbar, 2010).

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3 Cytosolic water is also deeply affected by pressure. Condensation of water molecules  
4  
5 being favored, water tends to lose its structure (Gross and Jaenicke, 1994), especially in the  
6  
7 crowded cytoplasmic environment.  
8  
9

10 According to Henry-Dalton law, gas solubility in water is increased at high pressure  
11  
12 (Gross and Jaenicke, 1994). Dissolution of CO<sub>2</sub> into water in particular releases protons which  
13  
14 leads to acidification of cytosol (Abe and Horikoshi, 1998). This phenomenon is increased by  
15  
16 the pressure-enhanced deprotonation of phosphate groups (Fumiyoshi *et al.*, 1999) and  
17  
18 membrane rigidification which inhibits H<sup>+</sup> export out of the cell (Abe and Horikoshi, 1995).  
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22 Interestingly, in *E. coli*, pressure stress induces synthesis of proteins that are involved  
23  
24 in both cold-shock and heat-shock response (Welch *et al.*, 1993). In fact, pressure acts similarly  
25  
26 to both low and high temperature as it increases stability of membrane and DNA and  
27  
28 destabilizes proteins (Jebbar *et al.*, 2015).  
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## 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

1  
2  
3 thermophilic counterparts. In contrast to mesophilic and thermophilic enzymes, which start  
4  
5 to loose activity as they unfold, psychrophilic enzymes loose activity as temperature increases  
6  
7 before unfolding (D'Amico *et al.*, 2003; Feller, 2013). [Figure 6](#) illustrates stability and activity  
8  
9 dependence of a psychrophilic, mesophilic and thermophilic enzyme, respectively.  
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15 Increased glycine/proline residues ratios appears to be common in psychrophilic  
16  
17 proteins (Zheng *et al.*, 2016). Gly residue (which side chain is replaced by an hydrogen) has an  
18  
19 increased rotational freedom compared to other residues, its abundancy in psychrophilic  
20  
21 proteins may hence provide local flexibility in protein chain. Surface Gly residues can be found  
22  
23 far away from the active site (Fields and Somero, 1998), conferring local flexibility of domains  
24  
25 increasing general wiggling capacities of the enzyme (Deniz, 2018). Gly abundancy can  
26  
27 however also increase in binding pockets (Hashim *et al.*, 2018) increasing flexibility around the  
28  
29 active site which improves turnover number of the enzyme at the expense of its affinity for its  
30  
31 substrate (Fields and Somero, 1998; Georlette *et al.*, 2000). In general, catalytic potential in  
32  
33 the active site is achieved by optimized electrostatics (Smalås *et al.*, 2000).  
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40 In contrast, the Pro residue, which has less movement freedom and bares rigidifying  
41  
42 properties, is scarce in psychrophilic enzymes (Zheng *et al.*, 2016), particularly in protein loops  
43  
44 (Hashim *et al.*, 2018), which are longer and more flexible (Wallon, Lovett, *et al.*, 1997).  
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46

47 As revealed by several X-ray crystal structures, flexibility of psychrophilic enzymes is  
48  
49 largely achieved by a weakening of all intramolecular interactions (Gerday *et al.*, 1997;  
50  
51 Casanueva *et al.*, 2010) as suggested by proteome molecular dynamics (Tehei *et al.*, 2004).  
52  
53 Surface salt bridges involved in tertiary or quaternary structure are less frequent (Wallon,  
54  
55 Lovett, *et al.*, 1997; Riise *et al.*, 2007; Michaux *et al.*, 2008; Bauvois *et al.*, 2008; Parvizpour *et*  
56  
57 *al.*, 2017) and surface charged residues, even if they are often less abundant (Aghajari *et al.*,  
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3 1998), are generally unpaired (Arnórsdóttir *et al.*, 2005; Almog *et al.*, 2008; Pedersen *et al.*,  
4  
5 2009), hence facilitating binding of water and stability of solvation shell. Hydrogen bonds too  
6  
7 are less abundant (Leiros *et al.*, 2007; Michaux *et al.*, 2008; Altermark *et al.*, 2008; Xie *et al.*,  
8  
9 2009). In addition to lowering overall rigidity, reduced hydrophobic interactions in  
10  
11 psychrophilic enzymes (Arnórsdóttir *et al.*, 2005; Huston *et al.*, 2008), especially those  
12  
13 implying aromatic residues (Wallon, Lovett, *et al.*, 1997), also counters hydrophobic effect  
14  
15 caused by low temperature.  
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19  
20 Psychrophilic protein cores are also made less rigid and compact by reducing their  
21  
22 hydrophobicity (Hashim *et al.*, 2018), decreasing the number of Arg and Pro residues (Aghajari  
23  
24 *et al.*, 1998) or by introducing salt bridges across it, which facilitate solvent penetration and  
25  
26 destabilize the core (Parvizpour *et al.*, 2017). In fact, exposure to solvent increases entropy  
27  
28 and thus participate to the gain of flexibility in psychrophilic proteins (Wallon, Lovett, *et al.*,  
29  
30 1997; Arnórsdóttir *et al.*, 2005; Thorvaldsen *et al.*, 2007; Bauvois *et al.*, 2008; Zheng *et al.*,  
31  
32 2016).  
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36  
37 Structural traits in cold-adapted proteins are represented in [Figure 7](#).  
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40 Some studies have found other AA biases associated with psychrophily such as more  
41  
42 Ser and Leu and less Arg, Glu, Thr and acidic residues (Médigue, 2005; D'Amico *et al.*, 2006;  
43  
44 Casanueva *et al.*, 2010; Raymond-Bouchard *et al.*, 2018). However, relations between these  
45  
46 sequence biases and structural modifications are less common than the increased Gly/Pro  
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48 ratio.  
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52 Psychrophiles also produce a specific kind of protein to cope with subzero  
53  
54 temperatures: antifreeze proteins (AFP). AFP are highly soluble and specifically bind ice nuclei  
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56 with their hydrophobic residue by strong Van der Waals interactions (Howard *et al.*, 2011,  
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3 2014), limiting crystallization of the cytoplasm (Scotter *et al.*, 2006; Venketesh and  
4  
5 Dayananda, 2008).  
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10 Since nucleic acid secondary structures are more stable at low temperatures, many  
11 processes involving their opening, such as transcription for DNA and ribosome biogenesis for  
12 RNA, are impaired. This could be managed in psychrophiles by overexpression of helicases  
13 (Lim *et al.*, 2000), which are enzymes able to open nucleic acid double helix in either double  
14 stranded or folded molecules.  
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25 Increased flexibility in psychrophiles is also met at the membrane level. In contrast to  
26 proteins, psychrophilic lipids are not more flexible but possess more steric constraints  
27 reducing the packing of hydrophobic queues and thus leading to a less rigid membrane.  
28 Incorporation of unsaturation in hydrophobic tails of lipids is a common feature as it induces  
29 bends in the tails (Dexter and Cooke, 1984; Okuyama *et al.*, 1993; Jebbar *et al.*, 2020a). Other  
30 trait perturbing compacity and increasing fluidity include methyl-branched lipids (Russell,  
31 1997) and shorter acyl chains (Anesi *et al.*, 2016; Rafiq *et al.*, 2019). Some of these traits are  
32 summarized in [Figure 8](#). Membrane fluidity can also be modulated by insertion of hydrophobic  
33 carotenoid pigments (Jagannadham *et al.*, 2000; Fong *et al.*, 2001a). While polar carotenoid  
34 insertion rigidifies membranes, non-polar carotenoids fluidify them (Subczynski *et al.*, 1992).  
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52 Psychrophilic cytoplasmic content is characterized by an abundance of small  
53 molecules. Like organisms coping with high salinity or desiccation, psychrophilic organisms  
54 counteract reduced water availability by enriching their cytoplasm with several classes of  
55 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  
4  
5 out of the cell and may prevent protein denaturation and aggregation (Phadtare, 2004);  
6  
7 interestingly, neutron spectrometry has shown that trehalose stiffens dynamics and reduces  
8  
9 flexibility in proteins (Cordone *et al.*, 1999), an unfavorable property for psychrophiles.  
10  
11 Trehalose also protects membranes during cold-induced dehydration (Goodrich *et al.*, 1988).  
12  
13 Polyols, such as glycerol and mannitol, are also common molecules produced by psychrophiles  
14  
15 which act as buffering agents for proteins (Jennings, 1984).  
16  
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20 It has also been shown that chaotropic agents are preferentially accumulated by  
21  
22 psychrophiles and that adding such agents in their medium enhances their ability to grow at  
23  
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  
42  
43 represented in bacteria and archaea. The most hyperthermophilic microorganisms are  
44  
45 archaea.  
46  
47

48 At a given temperature, while psychrophilic biomolecules possess increased flexibility  
49  
50 and catalytic potential, thermophilic biomolecules are generally characterized by increased  
51  
52 rigidity, expressed in higher resilience or effective force constants maintaining  
53  
54 macromolecular structure, which would contribute to their stability at high temperatures  
55  
56 (Tehei *et al.*, 2004). As a consequence, these traits make catalysis with thermophilic enzymes  
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3 extremely inefficient at moderate or low temperatures. Despite their exceptional  
4  
5 thermostability, most thermophilic enzymes *in vitro* start to unfold at 80°C. As, according to  
6  
7 the Arrhenius law, reaction speeds keep increasing with temperature, stability of enzymes is  
8  
9 the only upper limit for catalysis in hyperthermophiles. Optimal temperature for activity is  
10  
11 actually reached at a temperature so high that it unfolds most molecules, see [Figure 6](#).  
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18 Thermophilic proteins in particular are more stable and more resilient (Tehei and  
19  
20 Zaccai, 2007; Zaccai, 2013). In fact, resilience has been pointed out as a better term than  
21  
22 rigidity for explaining thermostability (Aguilar *et al.*, 1997). Thermophilic enzymes usually  
23  
24 display significantly reduced catalytic activity at room temperatures, possess higher optimal  
25  
26 temperatures, exceptional thermostability (Vieille *et al.*, 1996; Vieille and Zeikus, 2001) and  
27  
28 some, such as amylopullulanases from the archaea *Pyrococcus furiosus* and *Thermococcus*  
29  
30 *littoralis*, display activity at temperatures as high as 110-125°C (Brown and Kelly, 1993). As  
31  
32 the environment provides much of the energy, catalysis is not a challenge for thermophilic  
33  
34 enzymes.  
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40 In contrast, thermal denaturation being the challenge, thermophilic proteins have  
41  
42 evolved multiple ways to achieve thermostability. At first sight, thermophilic, mesophilic and  
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44 psychrophilic proteins do not display much difference (Vieille and Zeikus, 2001) with sequence  
45  
46 identity of 40-85% (Davies *et al.*, 1993), superimposable tridimensional structures (Gianese *et*  
47  
48 *al.*, 2002) and identical catalytic mechanisms (Zwickl *et al.*, 1990; Bauer *et al.*, 1998). In fact,  
49  
50 thermophilic properties are achieved by a small number of specific mutations which obey to  
51  
52 no clearly understood yet rule (Han *et al.*, 2019) but tend to improve compaction (decreasing  
53  
54 surface/volume ratio), resilience and enhance hydrophobic interactions within the protein  
55  
56 core (Haney *et al.*, 1999; Feller, 2010).  
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3 Comparative studies of protein dynamics and primary, secondary, tertiary and  
4 quaternary structures have given some insights about the ways to achieve thermostability  
5 (Irimia *et al.*, 2004; Koutsopoulos *et al.*, 2005a, 2005b; Karlström *et al.*, 2006). It appears that  
6 the most recurring molecular traits in thermophilic proteins are salt bridges and ion-pair  
7 networks lowering exposure of the protein to the solvent (Goldman, 1995; Yip *et al.*, 1995;  
8 Kumar *et al.*, 2000). Indeed, as at high temperatures water molecules interact less with  
9 charged residues, these residues can form stabilizing interactions (Elcock, 1998).

10  
11 For thermophilic multimeric assemblies, increased hydrophobicity of subunit  
12 interfaces (Wallon, Kryger, *et al.*, 1997; Hatanaka *et al.*, 1997) and introduction of  
13 intermolecular disulfide bridges (Dick *et al.*, 2016) favor stability of the quaternary structure.  
14 Within globular proteins, interactions between aromatic residues (Phe, Trp, Tyr) have also  
15 been proposed as a way to achieve thermostability (Karlström *et al.*, 2006). Probably as a way  
16 to protect them from oxidation which is enhanced at high temperature, Cys residues involved  
17 in disulfide bridges are often buried within the protein (Choi *et al.*, 1999; Kumar and Nussinov,  
18 2001). As Gly and Pro residues respectively induce flexibility or rigidity in protein backbone,  
19 many thermophilic proteins display decreased Gly/Pro ratio (Vieille and Zeikus, 2001).  
20 However Gly are more abundant in alpha helices where they increase stability (Petski, 2001).  
21 Flexible surface loops can also be shortened or deleted in thermophilic proteins (Thompson  
22 and Eisenberg, 1999; Yamagata *et al.*, 2001; Tanaka *et al.*, 2004), hence decreasing  
23 surface/volume ratio (Petski, 2001).

24  
25 **Figure 7** summarizes some major interactions and structural features enabling  
26 thermostability in thermophilic proteins.

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3 In contrast to the negatively supercoiled DNA in mesophiles, all hyperthermophiles  
4  
5 have the reverse gyrase enzyme that positively supercoils DNA *in vitro* (de La Tour *et al.*, 1990).  
6  
7 This enzyme is specific to thermophiles, possessed by all both bacterial and archaeal  
8  
9 hyperthermophiles and is absent from mesophiles (Catchpole and Forterre, 2019). However,  
10  
11 the importance of this enzyme for hyperthermophilic life has been long debated (Forterre,  
12  
13 2002; Atomi *et al.*, 2004; Heine and Chandra, 2009). Nonetheless, it is now clear that growth  
14  
15 above 90°C requires reverse gyrase (Lipscomb *et al.*, 2017). The *in vivo* function of reverse  
16  
17 gyrase is yet still unclear. Reverse gyrase may prevent thermal denaturation (Ogawa *et al.*,  
18  
19 2015), maintain constant helical repeat in the DNA molecule (Forterre *et al.*, 1996), assure  
20  
21 DNA recombination (Jamroze *et al.*, 2014) or repair thermally-depurinated bases (Kampmann  
22  
23 and Stock, 2004).  
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30 Other major traits found in some thermophiles for DNA protection include:  
31  
32 compaction of DNA by cationic histone-like proteins (Green *et al.*, 1983; Grayling *et al.*, 1996),  
33  
34 stabilization by high intracellular ions concentrations (Hensel and König, 1988) and by  
35  
36 polyamines such as putrescine, spermidine, norspermidine, thermospermine and spermine  
37  
38 (Friedman and Oshima, 1989).  
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42 We could also mention the case of the virion of SIRV2, a virus infecting the  
43  
44 hyperthermophilic archaeon *Sulfolobus islandicus*. In this virion, tight polar contacts between  
45  
46 viral proteins and viral DNA force him to adopt A-form instead of canonical B-form, limiting  
47  
48 contact with solvent and hence protecting it (DiMaio *et al.*, 2015). This way to protect DNA in  
49  
50 absence of active repair system is also found in some bacterial spores which are particularly  
51  
52 resistant to heat (Setlow, 1992).  
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3 Some post-transcriptional chemical modifications of RNA have also been found to  
4  
5 enhance thermostability, such as methylation of rRNA (Noon *et al.*, 1998) and tRNA (Edmonds  
6  
7 *et al.*, 1991; Kowalak *et al.*, 1994).  
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11  
12 Similarly to their proteins, lipids of thermophiles and hyperthermophiles are more rigid  
13  
14 and stable than their mesophilic counterparts. Lipids of hyperthermophilic archaea possess  
15  
16 several features increasing membrane thermostability. Firstly, instead of an ester bond, polar  
17  
18 head and hydrophobic tails are bound by an ether bond, which is less susceptible to hydrolysis.  
19  
20 While most lipids in mesophiles possess one head for two hydrophobic tails,  
21  
22 hyperthermophilic archaea have lipids with two polar heads, one at either end of the  
23  
24 molecule, which can either occupy the same side or opposite sides of the membrane (De Rosa  
25  
26 and Gambacorta, 1988). Such lipids form a monolayer in the membrane, like a bilayer of  
27  
28 molecules whose tail ends are fused together, which is a structure believed to be particularly  
29  
30 rigid. Other determinants of thermostability in lipids are hydrophobic tails of increased length  
31  
32 and branching isoprenoid cyclization and crosslinking of isoprenoid chains (Gliozzi *et al.*, 1983;  
33  
34 Tolner *et al.*, 1997). These traits increase interactions between lipids and other membrane  
35  
36 components, enhancing overall rigidity, stability and impermeability of hyperthermophilic  
37  
38 membrane (Gambacorta *et al.*, 1995).  
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47 Recently, it has been shown that insertion of squalane, an apolar molecule found in  
48  
49 membranes of hydrothermal vent hyperthermophilic archaea, may contribute to membrane  
50  
51 thermostability by inducing non-lamellar phase (Salvador-Castell *et al.*, 2020).  
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54  
55 In spite of the fact that these features are mostly archaeal traits, some  
56  
57 hyperthermophilic bacteria also possess lipids with one of more of these properties (Koga,  
58  
59 2012; Vinçon-Laugier *et al.*, 2017).  
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5 See [Figure 8](#) for a summary of main thermophilic lipid traits (Jebbar *et al.*, 2015, 2020a).  
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10 As with DNA protection, hyperthermophiles also produce various small soluble organic  
11 molecules which improve stability or refolding of proteins (Lamosa *et al.*, 2000; Faria *et al.*,  
12 2004; Empadinhas and Costa, 2006; Mukaiyama *et al.*, 2008). These thermoprotectants are  
13 typically molecules derived from sugar, amino acid or energetic metabolism with kosmotropic  
14 properties (Faria *et al.*, 2004; Schiefner *et al.*, 2004).  
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## 28 Halophilic molecular traits

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34 Organisms able to grow at salinities above sea average are generally considered  
35 halotolerant. True halophiles are organisms with an absolute requirement for high salt  
36 concentrations in order to grow. Two strategies for salt adaptation are generally observed,  
37 depending on which osmolyte counterbalances high extracellular salt: salt-in-cytoplasm and  
38 organic-osmolyte cytoplasm (Jebbar *et al.*, 2020a). While the latter enables to some extent  
39 exclusion of salinity out of the cytoplasm, the fact that the most extreme halophiles all display  
40 salt-in strategy leads to the idea that organic-osmolyte strategy may not be sufficient in  
41 extremely saline environments. The salt-in-cytoplasm strategy has been proposed in  
42 halophilic protozoa (Harding, 2018) and allows extreme halophilic archaea such as  
43 *Halobacterium salinarum* to grow in salt-saturated environments (Ventosa and Oren, 1996).  
44 Moreover, the salt-in strategy implies adaptation of the whole proteome to the extreme  
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3 condition. Halophiles may also use a combination of both strategies to cope with their  
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5 environment.  
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8 Halophiles counter the outside osmolarity of  $\text{Na}^+$  by accumulating  $\text{K}^+$  in the cytoplasm  
9  
10 (Ginzburg and Ginzburg, 1976). Intracellular concentration of  $\text{K}^+$  typically reaches 2-5.5M in  
11  
12 extremely halophilic archaea while  $\text{Na}^+$  concentration is generally maintained under 2M  
13  
14 (Oren, 2002).  
15  
16

17  
18 In order to function in such a hypersaline cytoplasm, halophilic proteins possess  
19  
20 several molecular traits allowing stability, activity and dynamics under these conditions. It was  
21  
22 initially believed that the solvation shell in these proteins excluded salt, until a battery of  
23  
24 complementary biochemical and biophysical methods established the opposite:  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$   
25  
26 ions are, in fact, recruited into the solvation shell and into the structure, and are essential for  
27  
28 stabilization and dynamics (Bonneté *et al.*, 1993). Remarkably, even if halophilic malate  
29  
30 dehydrogenase, the model studied, is from a salt-in-cytoplasm archaeon, it can also be  
31  
32 stabilized similarly to mesophilic proteins by high concentrations of kosmotropic salts  
33  
34 (Bonneté *et al.*, 1994).  
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42 Compared to mesophilic homologs, halophilic proteins are enriched in acidic residues  
43  
44 (Asp and Glu). Surfaces of halophilic proteins in particular present more acidic patches (Dym  
45  
46 *et al.*, 1995) and clusters of salt bridges, especially in interfaces between subunits of  
47  
48 multimeric proteins (Richard *et al.*, 2000). This enrichment leads to highly negatively charged  
49  
50 proteins (Kutnowski *et al.*, 2018; Niero *et al.*, 2020; Shirodkar *et al.*, 2020). In hypersaline  
51  
52 solvents, such accumulation of charges has been proven to stabilize proteins. The reason lies  
53  
54 in both increased ion-binding and solvation shell-structuring properties. In facts, more surface  
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56 negatively charged residues can bind more solvent cations (Ebel *et al.*, 2002). On the other  
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3 hand, anions appear to be strongly bind by fewer sites (Ebel *et al.*, 2002). This ion-rich shell  
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5 may limit water condensation on protein surface which would lead to excessive interaction  
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7 between proteins and, hence, to their aggregation (Costenaro *et al.*, 2002). Nonetheless, as  
8  
9 shown by neutron diffraction experiments, aspartic acid is also particularly efficient for tightly  
10  
11 binding water molecules in hypersaline solutions (Lenton *et al.*, 2016). Thus, acidic surfaces of  
12  
13 halophilic proteins strongly bind water (Frolow *et al.*, 1996) into a dense, highly structured  
14  
15 solvation shell with multiple layers (Britton *et al.*, 2006) and disruption of pentagonal water  
16  
17 networks (Talon *et al.*, 2014). In comparison, positively charged and uncharged protein  
18  
19 surfaces appear to bind less tightly water molecules (Kim *et al.*, 2016). Models of interactions  
20  
21 between salt, water and proteins have proposed that acidic surface composition may provide  
22  
23 specific binding sites for water and salt ions on protein leading to a highly structured and salty  
24  
25 solvation shell (Madern *et al.*, 2000). Because of this shell, surfaces are less accessible and  
26  
27 overall interaction with bulk solvent is reduced, enhancing solubility and stability in low water  
28  
29 activity environment (Tadeo *et al.*, 2009).  
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37 In contrast, halophilic proteins tend to have fewer Lys residues, slightly more small  
38  
39 hydrophobic residues such as Gly, Ala and Val and less haliphatic residues like Phe (Madern *et*  
40  
41 *al.*, 1995, 2000). Computational analysis of halophilic sequences also revealed a preference  
42  
43 for small, polar and charged residue (Gln, His, Pro) and lower amounts of Asn, Cys, Ile, Met,  
44  
45 Ser (Nath, 2016). As a consequence of these sequence bias, halophilic proteins tend to display  
46  
47 increased flexibility (Paul *et al.*, 2008) and polarity and avoid positively charged residues and  
48  
49 hydrophobicity (Nath, 2016), weakening hydrophobic contacts (Siglioccolo *et al.*, 2011) and  
50  
51 leading to more polar and charged cores (Nayek *et al.*, 2014).  
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57 Since they have fewer Lys residues, halophilic proteins use more Arg residues in salt  
58  
59 bridges which are crucial for stability in hypersaline environments (Madern *et al.*, 2000). On  
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3 average, halophilic proteins have two times more salt bridges than mesophilic homologs, both  
4 buried or exposed at surface, stabilized within salt bridges networks (Nayek *et al.*, 2014).  
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8 Major structural traits of halophilic proteins are represented in [Figure 7](#).  
9

10 Interestingly, halophilic enzymes show resistance to organic solvent-induced unfolding  
11 (Niero *et al.*, 2020; Shirodkar *et al.*, 2020). Moreover, compared to their mesophilic homologs,  
12 halophilic enzymes tend to renature easier after heat-induced (Tokunaga *et al.*, 2004) or  
13 methanol-induced denaturation (Tokunaga *et al.*, 2017). This property is believed to originate  
14 from their high solubility countering the hydrophobic-based aggregation which normally  
15 makes denaturation irreversible (Tokunaga *et al.*, 2017).  
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25 Most of these traits make halophilic proteins highly unstable in low salinity  
26 environments: most unfold under 2-3M KCl (Eisenberg, 1995). Nonetheless some proteins  
27 found in halophiles show both properties of halophilic proteins, such as high solvation shell  
28 salt concentration, and non-halophilic proteins, such as the water-binding potential, making  
29 them resistant to both salinity-induced denaturation and to low-salinity denaturation  
30 (Coquelle *et al.*, 2010).  
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42 As halophilic proteins avoid positively charged residues, interaction with the  
43 negatively-charged DNA is achieved through cations tightly bound by negatively charged  
44 residues deep in the protein (Kutnowski *et al.*, 2018).  
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52 Membrane of halophiles possess various traits which may be helpful for living in  
53 hypersaline environments. In particular, to keep  $K^+$  in their cytoplasm and to maintain  $Na^+$   
54 exclusion, membrane permeability is critical. Main adaptation is that halophilic lipids possess  
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3 sulfate groups with negative charges shielding salt cations (Russell, 1989), limiting H<sup>+</sup> and Na<sup>+</sup>  
4 permeability and increasing stability (van de Vossenberg *et al.*, 1999).  
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8 Carotenoid insertion is observed in both halophilic archaea (Calegari-Santos *et al.*,  
9 2016) and bacteria (Köcher and Müller, 2011). These hydrophobic pigments inserted in  
10 membrane act as membrane rivets, increasing its rigidity and impermeability (Lazrak *et al.*,  
11 1988) and thus preventing cell lysis (Köcher and Müller, 2011). While polar carotenoid  
12 insertion rigidifies membranes, non-polar carotenoids fluidify them (Subczynski *et al.*, 1992).  
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23 Neutron scattering is particularly sensitive to intracellular water dynamics and as  
24 hence be used to study traits of water in halophilic cells (Jasnin *et al.*, 2010). Experiments on  
25 *Haloarcula marismortui*, an halophilic archaeaon from the Dead Sea, revealed a fraction of  
26 exceptionally slow water in the hypersaline cytoplasm, hypothetically organized by structured  
27 K<sup>+</sup> ions (Tehei *et al.*, 2007; Frölich *et al.*, 2008).  
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38 The organic-osmolyte compatible solute strategy is characterized by the intracellular  
39 accumulation of small organic molecules such as betaine (Galinski and Trüper, 1982; Imhoff  
40 and Rodriguez-Valera, 1984; Nyssola *et al.*, 2000) and ectoines (Ono *et al.*, 1998; Vargas *et*  
41 *al.*, 2006). Compatible solutes in halophiles are highly soluble molecules, polar but uncharged  
42 at intracellular pH, with kosmotropic properties that stabilize the solvation shell of proteins  
43 (Galinski, 1993; Zaccai *et al.*, 2016).  
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## 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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3 observed for individual piezophilic proteins (Shrestha *et al.*, 2015), and a more compact and  
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5 less dynamic solvation shell may hence be an adaptive strategy for piezophiles.  
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11 Enhanced flexibility compensates pressure-induced rigidification while compaction of  
12  
13 the volume occupied by water molecules bound to proteins could stabilize them.  
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16 High pressure environments tend to constrain protein sequence evolution towards the  
17  
18 reduction of side-chain volume (Gunbin *et al.*, 2009) and assembly of multimeric assemblies  
19  
20 tend to display reduced volume changes in deep sea organisms (Morita, 2003). An explanation  
21  
22 could be that folding or assembly processes which are less dependent on volume changes are  
23  
24 less pressure-sensitive.  
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28 Sequence analysis revealed that sequence bias in piezophiles is actually dependent on  
29  
30 the thermophilic/psychrophilic status. Nonetheless, in comparison to non-psychrophilic  
31  
32 homologs, psychrophilic proteins tend to have more polar, hydrophilic and small amino acids  
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34 (Di Giulio, 2005; Nath and Subbiah, 2016), counting for the reduction of side-chain volume.  
35  
36 For non-psychrophilic piezophiles, hydrophobic, non-polar and aliphatic amino acids are also  
37  
38 over-represented in comparison to non-psychrophilic relatives (Nath and Subbiah, 2016).  
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43 Loosely packed protein structures also seem to offer a way to decrease deleterious  
44  
45 volumetric effects of high pressure (Nagae *et al.*, 2012). Indeed, when structure and activity  
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47 rely on internal cavities, larger internal volumes in piezophilic proteins permit to maintain  
48  
49 some internal cavities at abyssal pressures while, in comparison, non-piezophilic counterparts  
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51 will lose too much internal volume. Major structural traits of pressure-adapted proteins are  
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53 represented in [Figure 7](#).  
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57 As quaternary structure is primarily affected by high pressure, it has been found that  
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59 multimeric assemblies, such as SSB DNA-binding proteins from piezophilic *Shewanella* strains  
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3 (Chilukuri *et al.*, 2002) and large TET peptidases assemblies from piezophilic *Pyrococcus*  
4  
5 *horikoshii* (Rosenbaum *et al.*, 2012), are more resistant to pressure-induced dissociation.  
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10 Some nucleic acid piezophilic traits have been found. For example, an increased  
11 length of helices in 16s rRNA improves interaction with proteins and overall resistance to  
12 pressure-induced dissociation of ribosomes (Lauro *et al.*, 2007).  
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20 Piezophilic membrane traits have been described. The common feature of piezophilic  
21 membrane is increased fluidity (Siliakus *et al.*, 2017) which is achieved with molecular traits  
22 shared with psychrophiles: more unsaturated (DeLong and Yayanos, 1985; Hazel and Eugene  
23 Williams, 1990) and shorter (Bartlett and Bidle, 1999) hydrophobic tails. Branched lipids,  
24 which increase membrane fluidity, have also been found to be produced by deep sea  
25 microorganism as a response to high pressure (Scoma *et al.*, 2019). In abyssal hydrothermal  
26 environments however, high temperature may lead to a preference for typical thermophile  
27 lipids and to highly stable and rigid membranes.  
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42 Accumulation of compatible solutes in piezophiles may also help to cope with high  
43 pressure.  $\beta$ -hydroxybutyrate has been shown to be accumulated in the piezophilic bacterial  
44 response to high pressure (Martin *et al.*, 2002) and other small organic molecules may  
45 enhance protein stability under pressure in piezophilic cells (Jebbar *et al.*, 2015).  
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52 Interestingly, insertion of squalane, an apolar molecule found in hydrothermal vent  
53 archaea, in membranes not only enhances thermal stability but also resistance to pressure  
54 (Salvador-Castell *et al.*, 2020).  
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3 It should be noted that high pressure triggers both cold-shock (Fujii *et al.*, 1999) and  
4 heat-shock responses (Sato *et al.*, 2015) in piezophilic *Shewanella* bacterial strains. Both  
5  
6 pathways induce production of proteins or small molecules related to pressure-resistance.  
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## 10 11 12 13 14 15 16 17 18 Living with many extremes: Polyextremophily and limits of life 19 20 21 22

23 Terrestrial extreme environments often possess more than one extreme physical or  
24 chemical condition, see [Figure 2](#). Inhabitants of these poly-extreme environments are thus  
25 called polyextremophiles. Abyssal black smokers thermophilic life also faces high pressure and  
26 high concentrations of toxic heavy metals which the hydrothermal fluid is enriched with  
27 (Seegerer *et al.*, 1993; Luther *et al.*, 2001; Damm, 2013) leading to the selection of  
28 metalloresistance (Vetriani *et al.*, 2005) whereas thermophiles in continental volcanic hot  
29 springs are exposed to high acidity and are thus also acidophilic (Seegerer *et al.*, 1993; Burton  
30 and Norris, 2000; Siering *et al.*, 2006; Seckbach *et al.*, 2013), soda lakes halophiles are also  
31 alkaliphilic (Jones *et al.*, 1998; Sorokin *et al.*, 2014), Río Tinto river microorganisms face acidic  
32 pH and high heavy metals concentrations (Amils *et al.*, 2014) and many brines, either abyssal,  
33 subglacial or on the surface, display combinations of high salinity and high pressure,  
34 chaotropicity, ultraviolet radiation, heavy metal abundancy, extreme pH and extreme  
35 temperatures (Hallsworth, 2019; Varrella *et al.*, 2020).  
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54 As it was described before, extraterrestrial environments can also have one or more  
55 extreme condition in regard of terrestrial life, see [Figure 1](#). Mars subsurface hypersaline  
56 environments can be, depending on the depth, cold or hot and under pressure; icy moons  
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3 oceans can present, depending on the depth and the proximity with a hydrothermal vent,  
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5 extreme temperatures, extreme pH and high pressure. Therefore, terrestrial environments  
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7 where polyextremophilic thrive can be considered as good models for extraterrestrial  
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9 environments (Capece *et al.*, 2013). However, some extreme conditions have synergic  
10  
11 deleterious effects on biological systems and some natural polyextreme environments are  
12  
13 well known for being the harshest for life, sometimes over the limits of life. If extremely dry  
14  
15 environments are excluded from this concern, as liquid water presence is a *sine qua non*  
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17 condition for terrestrial life processes, some natural aqueous environments are devoid of  
18  
19 significant life and therefore considered as the most extreme for life.  
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25 Dallol volcanic hypersaline volcanic springs presents ponds with high temperature,  
26  
27 saturated salinity, high abundance of iron and extreme acidity (Cavalazzi *et al.*, 2019;  
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29 Kotopoulou *et al.*, 2019). In this unique environment, considered by some as a potential  
30  
31 analog of ancient Mars (Gómez *et al.*, 2019), even if lipids biomarkers have been detected  
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33 (Carrizo *et al.*, 2019) and some ultra-small archaea and bacteria may have been observed close  
34  
35 to the most extreme ponds (Gómez *et al.*, 2019), two strict barriers for life have been  
36  
37 identified: combination between extreme salinity and extreme acidity and combination  
38  
39 between extreme salinity and extreme chaotropicity resulting from  $Mg^{2+}/Ca^{2+}$  abundancy  
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41 (Belilla *et al.*, 2019).  
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47 Other examples of poly-extreme environment devoid of life are the Mg-rich deep  
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49 hypersaline anoxic basins (DHAB). These deep sea brines, which can host deep-sea halophilic  
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51 and piezotolerant microorganisms (van der Wielen, 2005), are considered by some as good  
52  
53 models for icy moons subglacial oceans (Antunes *et al.*, 2020). In spite of the fact that some  
54  
55 evidence of microbial activity have been found in Discovery Basin (van der Wielen, 2005), the  
56  
57 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  
4  
5 where no life has been found yet (Cono *et al.*, 2019). At such low water activity values and  
6  
7 high concentrations of  $Mg^{2+}$ , life appears to be only possible if kosmotropic ions are present  
8  
9 to counteract toxicity of  $Mg^{2+}$ , like  $Na^+$  in the Kryos DHAB (Yakimov *et al.*, 2015; Steinle *et al.*,  
10  
11 2018).  
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14  
15 In these two examples, chaotropicity induced by natural divalent cation abundancy  
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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  $Mg^{2+}$   
20  
21 and  $Ca^{2+}$  in Dead Sea is progressively making it too hostile for halophiles (Oren, 2010).  
22  
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25 Although these natural terrestrial environments provide good insights on limits of  
26  
27 terrestrial life with regard to water activity and chaotropicity, other poly-extreme conditions  
28  
29 that could be present in extraterrestrial environments are still largely unknown. Some  
30  
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  
34  
35 deleterious effect would be too much for biomolecules and molecular traits would be  
36  
37 mutually incompatible. In other cases, natural environments with a given combination could  
38  
39 just not exist. These combinations include psychroacidophily (combination of low-  
40  
41 temperature and low pH adaptations), haloacidophily (high salinity and low pH) and  
42  
43 piezoalkaliphily (high pressure and high pH) (Bowers *et al.*, 2009; Capece *et al.*, 2013; Harrison  
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45 *et al.*, 2013).  
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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 (Siglioccolo *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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3 halotolerant (Kaye and Baross, 2000) and moderately halophilic (Kaye *et al.*, 2004) bacteria as  
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5 well as halophilic archaea (Takai *et al.*, 2001; Ellis *et al.*, 2008) have been identified in  
6  
7 continental or abyssal hydrothermal environments, it has also been proposed that hot  
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9 hydrothermal chimneys may host hypersaline hot microenvironments allowing colonization  
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11 by halophiles (Takai *et al.*, 2001).  
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15 To date, the most halophilic hyperthermophile is *Thermococcus waiotapuensis sp. nov.*  
16  
17 which grows at temperatures as high as 90°C and salinity as high as 2.2M NaCl (González *et*  
18  
19 *al.*, 1999) and the most thermophilic extreme halophile is *Natronolimnobius aegyptiacus*  
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21 which grows at saturated concentration of Na<sup>+</sup> and at temperature as high as 56°C (Bowers  
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23 and Wiegel, 2011; Zhao *et al.*, 2018).  
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### 33 Thermopiezophily

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38 Generally, pressure and temperature have opposite volumetric effects on  
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40 macromolecular systems: while temperature increases flexibility and pushes objects to  
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42 occupy more space, pressure increases rigidity and tends to reduce volumes of objects. One  
43  
44 major effect of high pressure is the elevation of boiling temperature of water. In abyssal  
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46 hydrothermal environments, pressure hence widens the range of liquid water conditions,  
47  
48 opening new environments for a water-based life to develop. There is no known  
49  
50 hyperthermophile with an optimal growth temperature above 100°C. However, the most  
51  
52 thermophilic organism *Methanopyrus kandleri*, which optimally grows at 98°C (Kurr *et al.*,  
53  
54 1991), has been shown to grow at temperatures as high as 122°C (Takai *et al.*, 2008) which  
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3 can only be achieved at abyssal pressures. It has also been shown that for both abyssal archaea  
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5 *M. kandleri* and abyssal bacteria *Marinitoga piezophile* optimal growth temperature is  
6  
7 proportional to pressure (Miroshnichenko and Bonch-Osmolovskaya, 2006; Takai *et al.*, 2008).  
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10 Some individual enzymes can however be more efficient at temperatures above 100°C.  
11  
12 Protease from *Methanococcus jannaschii* for example works optimally at 116°C and displays  
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14 activity at temperatures as high as 130°C (Michels and Clark, 1997). Thermophilic and  
15  
16 piezophilic molecular traits may be synergetic (Capece *et al.*, 2013), such as insertion of  
17  
18 squalane in lipid bilayers which improves membrane stability under both high temperature  
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20 and high pressure (Salvador-Castell *et al.*, 2020). Nonetheless, structural signatures of  
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22 thermopiezophily have yet to be described.  
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27 The fact that high pressure induces heat-shock response in both piezophilic (Sato *et*  
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29 *al.*, 2015) and pressure-sensitive microorganisms (Iwahashi *et al.*, 1991; Aertsen *et al.*, 2004)  
30  
31 goes along with the idea that some thermophilic traits can also cope with high pressure. In  
32  
33 particular, molecular systems coping with protein instability may be useful for both heat-  
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35 induced and pressure-induced unfolding.  
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## 45 Psychrohalophily

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51 Polar cold environments are often associated with high salinity. In spite of the fact that  
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53 salt solubility in water decreases with temperature, freezing of water excludes salt from the  
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55 ice thus concentrating the remaining liquid which becomes hypersaline. Moreover, high  
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57 salinity lowers freezing point of water to the point that saturated NaCl brines are liquid at  
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3 temperatures as low as  $-21^{\circ}\text{C}$  (Brady, 1992). The saltiest liquid water body on earth is also the  
4  
5 coldest: Antarctic Don Juan pond remains liquid at temperatures as low as  $-50^{\circ}\text{C}$  because of  
6  
7 its high  $\text{CaCl}_2$  concentration (Harris and Cartwright, 2013). As with water above  $100^{\circ}\text{C}$   
8  
9 requiring high pressure to remain liquid, subzero liquid water is possible at high salinity,  
10  
11 opening the window for life. The most psychrophilic halophile is *Psychromonas ingrahamii*  
12  
13 which grows at temperatures as low as  $-12^{\circ}\text{C}$  at 3.3M NaCl (Auman *et al.*, 2006) and the most  
14  
15 halophilic psychrophile is *Planococcus halocryophilus* Or1, which grows at salinities as high as  
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17 18% at  $-15^{\circ}\text{C}$  (Mykytczuk *et al.*, 2013). While true psychrophiles found in hypersaline cold  
18  
19 environment are bacteria, Antarctic hypersaline Deep Lake is dominated by cold-tolerant  
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21 halophilic archaea (DeMaere *et al.*, 2013; Williams *et al.*, 2017).  
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28 At the biomolecular level, psychrophily and halophily appear to have some degree of  
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30 synergy (Capece *et al.*, 2013). Intracellular accumulation of chaotropes such as  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  or  
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32  $\text{Ca}^{2+}$ , which is easy in hypersaline environments, facilitates life at low temperature as they  
33  
34 counter the cold-induced stabilization of proteins (Chin *et al.*, 2010). As both low temperature  
35  
36 and salinity cause osmotic stress, compatibles solutes such as polyols may also be critical to  
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38 psychrohalophily. Both salt-in-cytoplasm and compatible solute strategies for halophily thus  
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40 enhance psychrophily.  
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45 At the membrane level, cold temperature rigidifies membranes and increases their  
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47 impermeability, which serves resistance to high salinity. Moreover, carotenoids are observed  
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49 in both halophiles and psychrophiles where they serve membrane fluidity control. However,  
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51 depending on their nature, they have either rigidifying or fluidifying properties (Subczynski *et*  
52  
53 *al.*, 1992). As psychrophilic bacteria increase production of carotenoids as response to  
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55 increased salinity (Fong *et al.*, 2001b), they may represent a tool for adjusting membrane  
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57 fluidity in cold hypersaline environments.  
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3 At less extreme values of cold and salinity, plants possess common pathways for  
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5 resisting to drought, coldness and salinity (Xiong *et al.*, 2002; Zhu, 2002; Seki *et al.*, 2003).  
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### 13 Psychropiezophily

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19 Deep ocean cold and pressurized waters represent most of the biosphere volume.  
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21 While organisms of hydrothermal vents benefit from compensating effects of high pressure  
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23 and high temperature on molecular flexibility, deep sea organism molecules are rigidified by  
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25 both low temperature and high pressure (Capece *et al.*, 2013).  
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29 Remarkably, high pressure induces cold-shock response in both mesophilic (Welch *et*  
30  
31 *al.*, 1993; Wemekamp-Kamphuis *et al.*, 2002) and piezophilic bacteria (Fujii *et al.*, 1999). This  
32  
33 pathway is critical to psychropiezophily. In particular, it enables production of unsaturated  
34  
35 lipids to counter cold-induced and pressure-induced crystallization (Kato, 2010).  
36  
37 Cryoprotectant extracellular polysaccharides (EPS) production also appears to be induced by  
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39 high pressure conditions in deep-sea bacteria (Marx *et al.*, 2009; Bælum *et al.*, 2012), however  
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41 the exact function of EPS for pressure-tolerance is still unclear.  
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### 51 Halopiezophily

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57 Most piezophiles have been isolated at low salinity and most halophiles at atmospheric  
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59 pressure (Harrison *et al.*, 2013). Hypersaline deep environments are limited to DHABs.  
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3 However, nature of salt may significantly reduce the window for life. For example, high  
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5 concentrations of  $MgCl_2$  in Discovery and Hephaestus DHABs strongly limits life independently  
6  
7 from pressure (Hallsworth *et al.*, 2007; Cono *et al.*, 2019; Antunes *et al.*, 2020).  
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10 Halophilic and halotolerant bacteria are commonly found in deep sea environments  
11  
12 (Takami *et al.*, 1999; Lu *et al.*, 2001) where they generally face low temperature too.  
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15 Nonetheless, halophilic piezophilic and piezotolerant bacterial enzymes from 3-3.5km  
16  
17 deep Medee, Bannock and Kryos DHABs have been described (Alcaide *et al.*, 2015).  
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19 Interestingly, these enzymes displayed various combinations of traits observed in proteins of  
20  
21 thermophiles (such as aromatic interactions or less glycine), halophiles (such as acidic surface)  
22  
23 and pizeophiles or piezotolerant (such as less salt bridges, small amino acids) organisms  
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25 (Alcaide *et al.*, 2015).  
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30 Some halophiles also seem to be rather piezotolerant. For example, the extreme  
31  
32 halophilic archaea *Halobacterium salinarium* NRC-1 which is found in surface hypersaline  
33  
34 environments has been shown to survive 400MPa exposure (Kish *et al.*, 2012) and moderate  
35  
36 halophilic seawater bacteria *Micrococcus roseus* shows some resistance to 207MPa exposure  
37  
38 (Tanaka *et al.*, 2001).  
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42 Adaptation to both high pressure and high salinity may use compatible solutes  
43  
44 conferring both haloresistance and piezotolerance such as  $\beta$ -hydroxybutyrate (Martin *et al.*,  
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46 2002).  
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## 55 Extremophilic molecular traits as biomarkers

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3 The search for extraterrestrial life is not limited to the search of environments  
4 compatible with life but can also be extended to the search of traces of a current or past  
5 biological activity. These traces are commonly called biomarkers or biosignatures. Biomarkers  
6 may either be remotely observed on or found *in situ* by space missions. However, reliability  
7 and detectability of a biomarker seems to be mutually incompatible. For example, CH<sub>4</sub> and  
8 dioxygen have been proposed as biomarkers and can be remotely detected in extraterrestrial  
9 atmospheres. As discussed before, CH<sub>4</sub> is mostly biogenic on Earth but can accumulate  
10 abiotically in other planetary contexts. As significant accumulation of dioxygen in terrestrial  
11 atmosphere was caused and is maintained by photosynthesis, it has been proposed that  
12 oxygen-related species could be used as biomarker (Mendillo *et al.*, 2018; Meadows *et al.*,  
13 2018). However, like CH<sub>4</sub>, oxygen gases may also be produced abiotically in significant  
14 amounts (Luger and Barnes, 2015; Narita *et al.*, 2015). Even simple organic molecules that  
15 constitute essential bricks of life have been proven to be easily produced in abiotic conditions.  
16 The famous Murchison meteorite for example contains for example various amino acids  
17 (Kvenvolden *et al.*, 1971; Cronin and Pizzarello, 1983), nucleobases (Martins *et al.*, 2008) and  
18 even sugar-like polyols (Cooper *et al.*, 2001) that could all be produced by abiotic or prebiotic  
19 chemistry. On the other hand, detection of macromolecules and polymers with several  
20 features like complexity, chirality or sequence, like proteins or nucleic acids, would constitute  
21 a strong evidence for biological activity but would be more difficult to detect in large amounts.  
22 Moreover, terrestrial complex biomolecules can be considered as extremely fragile in  
23 comparison to their constituents or the products of cell metabolism. Proteins and RNA for  
24 example are known to be difficult to handle in laboratory conditions, requiring various  
25 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  
9  
10 greater stability, a point which as recently be reviewed (Jebbar *et al.*, 2020a). As mentioned  
11  
12 before, lipids and proteins of thermophiles are exceptionally stable in numerous chemical and  
13  
14 physical conditions, which would make them easier to detect in extraterrestrial environments.  
15  
16 Moreover, many extremophiles accumulate large amounts of compatibles solutes and small  
17  
18 organic molecules to cope with extreme conditions. With the proper geological and chemical  
19  
20 setting, these molecules could be stable over long periods and hence represent interesting  
21  
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  
30  
31 distinctive increase of red light reflection a the planetary level which has remotely detected  
32  
33 (Sagan *et al.*, 1993). Red edge on reflectance spectra of exoplanets and exomoons has hence  
34  
35 been proposed as a surface biomarkers (Seager *et al.*, 2005; O'Malley-James and Kaltenecker,  
36  
37 2019), and may be extended to other wavelengths, depending on the light received by the  
38  
39 extraterrestrial body which may influence the type of pigments needed by putative organisms  
40  
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  
46  
47 UVs, giving to hypersaline lakes a distinctive red color. As pigment-based radioprotection and  
48  
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  
9  
10 could produce biomarkers. For example, in volcanic or hydrothermal environments, which  
11  
12 present various combinations of extreme conditions (high temperature, low pH, high  
13  
14 pressure, heavy metal abundancy, etc), many organisms metabolize sulfur-containing  
15  
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  
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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  
8  
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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18 Each type of biomolecule (protein, nucleic acids, lipids) from extremophiles displays its  
19  
20 own set of molecular traits associated with extremophily. Individual proteins may present one  
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22 or many of these traits allowing function under extreme conditions. Usually, no clear signature  
23  
24 of extremophilic adaptation can be recognized, only trends. These adaptations tend to  
25  
26 counter the effect of extreme conditions, for example through increased flexibility of  
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28 psychrophilic proteins to counter rigidification induced by low temperatures or increased  
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30 solubility of halophilic proteins to counter enhanced aggregation under hypersalinity. For  
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32 proteins, relatively small changes of overall structure can be observed in crystal structures. In  
33  
34 contrast, huge differences with mesophilic proteins can be observed at the dynamical level by  
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36 techniques such as neutron scattering, spectroscopy or NMR. In particular, adaptation of the  
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38 solvation shell, which should be understood as a part of the proteins, is fundamental to cope  
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40 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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5 whereas low temperature and high pressure in deep sea environments both increase rigidity  
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7 of biomolecules. While some strategies may be shared between two extreme condition,  
8  
9 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  
18  
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  
22  
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  
26  
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  
38  
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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3 properties. At the scale of the proteome however, numerous changes are required for  
4  
5 organisms, leading to the need of a longer evolution.  
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8 Can macroscopic life appear in extreme environments? Most extremophiles are indeed  
9  
10 microorganisms, mostly bacteria and archaea. Extreme environments may host macroscopic  
11  
12 life, such as shrimps and tube worms around black smokers, but ancestors of these  
13  
14 macroorganisms have originated in mesophilic environments. Furthermore, the hypothesis of  
15  
16 abiogenesis taking place in extreme environments is largely debated.  
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20 Can macroscopic life appear without photosynthesis? Even if claims about life billion  
21  
22 years ago are controversial, it is generally believed that multicellular and macroscopic life have  
23  
24 appeared long after photosynthesis. Before photosynthesis, ecosystems must have relied on  
25  
26 chemosynthesis, just like many modern extreme environments, such as great depths or  
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28 extremely hot environments, which are largely devoid of photosynthetic organisms. Because  
29  
30 metabolic yields are low compared to photosynthesis, it is not certain that macroscopic life  
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32 could appear in ecosystems with chemosynthesis only. Hence, putative life in Martian  
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34 subsurface or icy moon abyssal environments may be limited to microorganisms.  
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40 Where should one search for extraterrestrial life? In the absence of known  
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42 extraterrestrial mesophilic environments, extreme extraterrestrial environments such as  
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44 Martian depths or icy moons abysses may be the most habitable. As adaptation to extreme  
45  
46 conditions limits the possibility for extremophiles to thrive under mesophilic conditions,  
47  
48 environmental variations of physical and chemical conditions may be a limiting factor. Thus,  
49  
50 stable extreme environments should be favored over those displaying variations. As pointed  
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52 by many, the search for hydrothermal should also be a priority. Liquid water may however not  
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54 be enough, making the search for salt-enriched liquid water environment a priority too. In  
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56 fact, biochemistry relies on a solvent which is an ionic solution rather than pure water and  
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3 many biological processes such as folding and metabolism require ions to work. In particular,  
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5 hydrothermal processes not only favors organic matter enrichment but also accumulation of  
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7 ions in water. In terrestrial life, solvent is not pure water but an ionic solution where charges  
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9 enable biological processes such as metabolism. Interestingly, hydrothermalism favors both  
10  
11 organic matter and mineral ions enrichment of water.  
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15 In this context, the search for rocky exoplanets with surface mesophilic conditions may  
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17 unravel new habitable environments.  
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## 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.

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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.

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).

## 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  $L_{\beta}$  and  $L_{\alpha}$ . 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  $P_{\beta}$  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

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| 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 $T > 50^{\circ}\text{C}$<br>Hyperthermophiles grow at $T > 80^{\circ}\text{C}$ | Growth reported at $122^{\circ}\text{C}$ ( <i>Methanopyrus kandleri</i> )                                 | Marine hydrothermal vents, volcanic hot springs and lakes                                | Icy moons putative hydrothermal vents   | Evidence of survival after exposure to $130^{\circ}\text{C}$ ( <i>Geogemma barossii</i> Strain 121) |
| Low Temperature (Impossible to exclude)            | Psychrophile can grow at $T < 15^{\circ}\text{C}$   | Growth at $-18^{\circ}\text{C}$ ( <i>Rhodotulura glutinis</i> )   | Polar ocean, deep sea, ice interiors, Antarctic surface                                  | Mars surface and subsurface rocks and brines<br>Icy moons subglacial oceans top, middle and bottom waters | Evidence of metabolism at $-33^{\circ}\text{C}$ ( <i>Paenisporosarcina sp. B5</i> )                 |
| High Salinity (Partially possible to exclude)      | Halophiles grow at salinity $> 8,8\%$<br>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<br>Icy Moons Oceans   |   |
| High hydrostatic Pressure (Impossible to exclude)  | Piezophiles grow at $P > 10\text{Mpa}$<br>Hyperpiezophiles grow at $P > 50\text{MPa}$               | Growth at $130\text{MPa}$ ( <i>Thermococcus piezophilus</i> CDGST)  | Deep sea, DHAB, oceanic and continental subsurfaces                                      | Mars deep brines and rocks<br>Icy Moons abysses   | Non-piezophilic bacteria have been engineered to survive exposure to $20\text{GPa}$                 |
| Other true extremophily types                      |   |   |  |   |   |
| Low pH (Partially possible to exclude)             | Acidophiles grow at $\text{pH} < 5$<br>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 $\text{pH} > 9$<br>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<br>Icy moons subglacial brines                                     | No true xerophile known yet   |

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|   | Chaotropes abundancy (Possible to exclude)                 | Chaotolerant organisms tolerate high concentration of MgCl <sub>2</sub> or CaCl <sub>2</sub> without kosmotropic counterion | Growth at 2.1M MgCl <sub>2</sub> ( <i>Aspergillus proliferans</i> ) or 2.0M CaCl <sub>2</sub> ( <i>Aspergillus sydowii</i> )  | DHABs, athalassohaline lakes   | Europa subglacial ocean, Martian surface         | No true chaophile known yet                          |
|   | Heavy metal abundancy (Possible to exclude)                | Metalloresistant organisms can grow at millimolar concentrations of heavy metal ions  | Growth at 20mM Zn <sup>2+</sup> , 20mM Co <sup>2+</sup> or 5mM Cd <sup>2+</sup> ( <i>Ralstonia</i> sp. CHR4)<br>> 48h survival on pure copper surface ( <i>Kocuria palustris</i> and <i>Brachybacterium conglomeratum</i> ) | Contaminated soils, Rio Tinto,   | Ancient Martian surface                          | No true metallophile known yet                       |
|   | Organic solvents abundancy (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 abundancy 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<br>← Stabilize proteins<br>← Decrease hydrophobic solubility<br>← Increase surface tension   | Chaotropic = Salting in →<br>Promote denaturation →<br>Increase hydrophobic solubility →<br>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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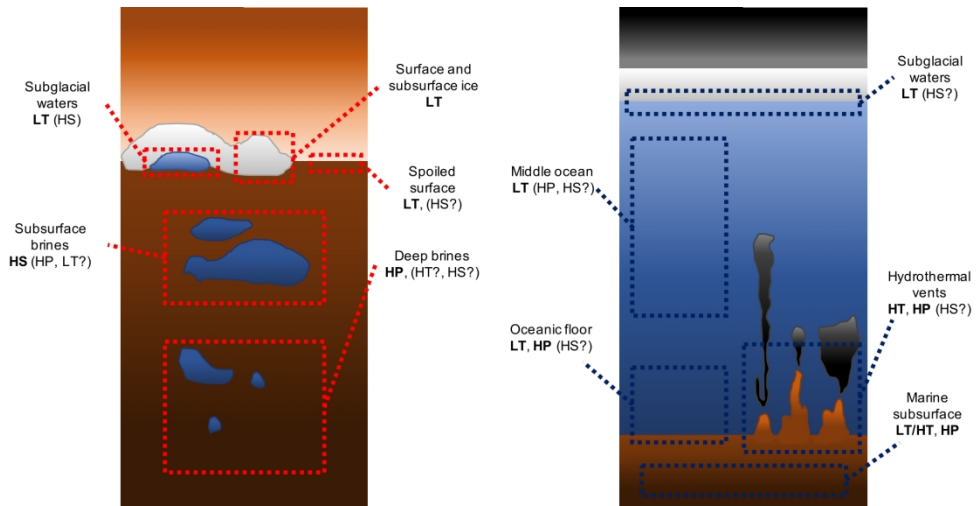


Figure 1: Extreme extraterrestrial environments

Martian (A) and icy moons (B) putative habitable extraterrestrial environments. Putative extreme conditions are shown, 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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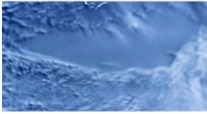

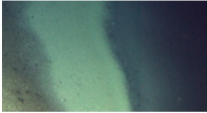

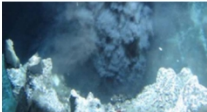




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| 7  | <b>Subzero brines</b>          |    | <b>LT HS</b>       |
| 8  | Don Juan Pond                  |   |                    |
| 9  |                                |   |                    |
| 10 |                                |   |                    |
| 11 | <b>Subglacial lakes</b>        |    | <b>LT (HP, HS)</b> |
| 12 | Lake Vostok, lake              |   |                    |
| 13 | Willhans, lake Ellsworth       |   |                    |
| 14 |                                |   |                    |
| 15 | <b>Sea ice, permafrost,</b>    |    | <b>LT</b>          |
| 16 | <b>polar regions</b>           |   |                    |
| 17 |                                |   |                    |
| 18 | <b>DHAB, cold seeps,</b>       |    | <b>HS (HP, LT)</b> |
| 19 | <b>hypersaline sdiments</b>    |   |                    |
| 20 | Discovery basin, Kryos         |   |                    |
| 21 | basin                          |   |                    |
| 22 |                                |   |                    |
| 23 | <b>Deep ocean, trenches</b>    |    | <b>HP (LT, HS)</b> |
| 24 | Marianna trench                |   |                    |
| 25 |                                |   |                    |
| 26 | <b>Hydrothermal vents</b>      |   | <b>HT (HP)</b>     |
| 27 | Pito seamount, Lost City       |   |                    |
| 28 | hydrothermal field             |   |                    |
| 29 |                                |   |                    |
| 30 | <b>Marine and continental</b>  |  | <b>HP (LT/HT)</b>  |
| 31 | <b>subsurface</b>              |   |                    |
| 32 |                                |   |                    |
| 33 | <b>Volcanic hot springs,</b>   |  | <b>HT</b>          |
| 34 | <b>hydrothermal fields</b>     |   |                    |
| 35 | Yellowstone hot springs        |   |                    |
| 36 | field, Solfataric fields       |   |                    |
| 37 |                                |   |                    |
| 38 | <b>Hypersaline hot springs</b> |  | <b>HT HS</b>       |
| 39 | Dallol volcano                 |   |                    |
| 40 |                                |   |                    |
| 41 | <b>Hypersaline lakes and</b>   |  | <b>HS (HT)</b>     |
| 42 | <b>sediments</b>               |   |                    |
| 43 | Dead sea, lake Natron          |   |                    |
| 44 |                                |   |                    |

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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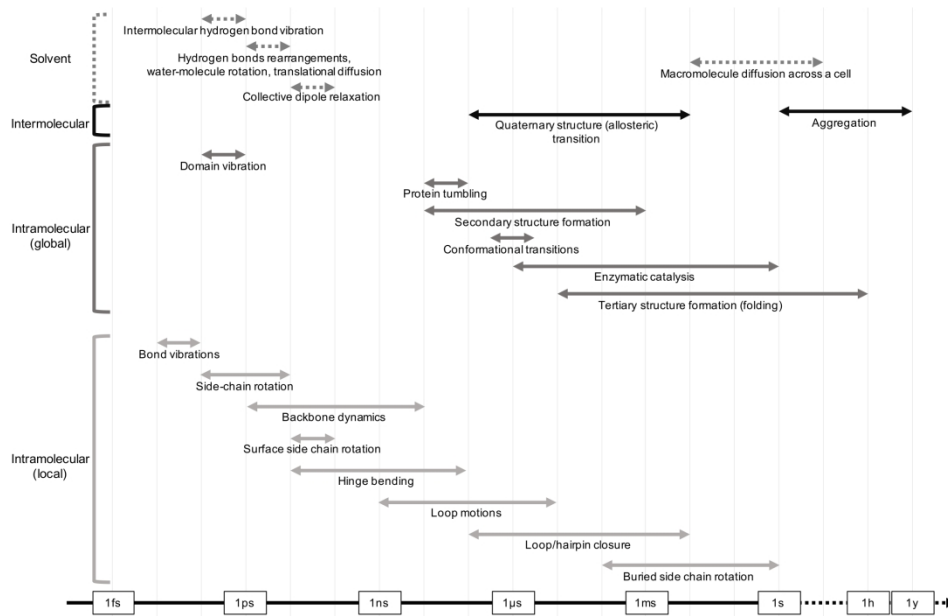


Figure 3: Dynamical properties of proteins and solvent

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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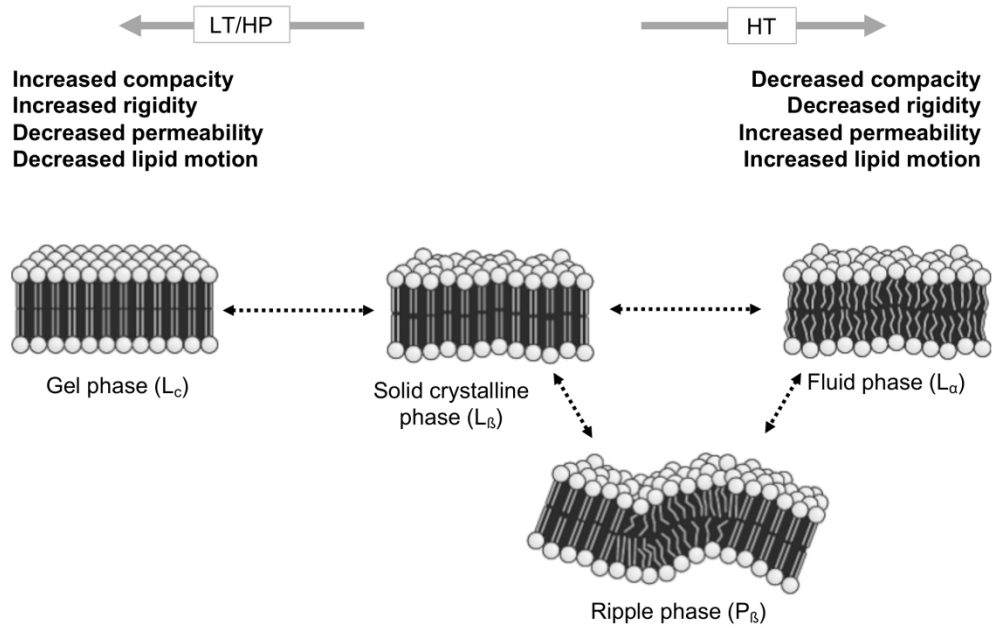


Figure 4: Phase transitions of lipid membranes

This figure represents simple lipid bilayers, not biological membranes. The functional state of lipids in biological membranes is close to a disordered phase, between  $L_\beta$  and  $L_\alpha$ . 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  $P_\beta$  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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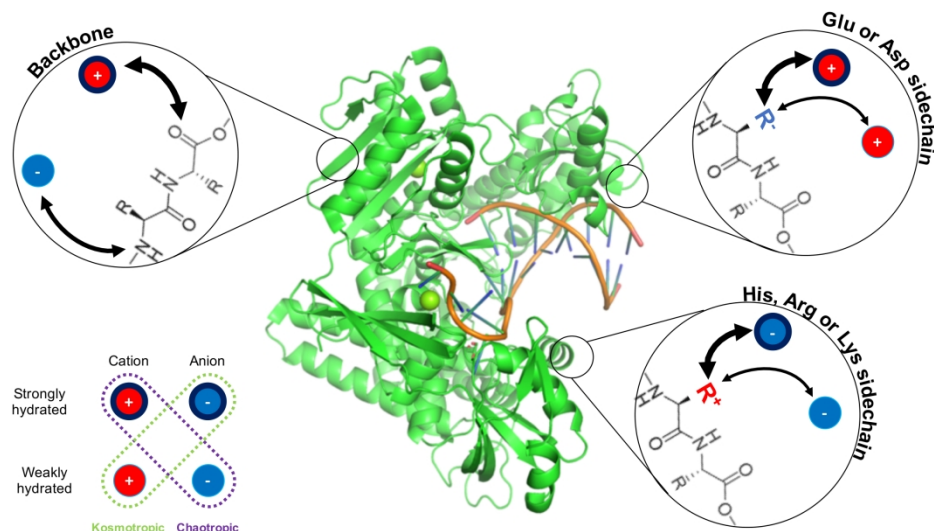


Figure 5: Interactions between ions and proteins

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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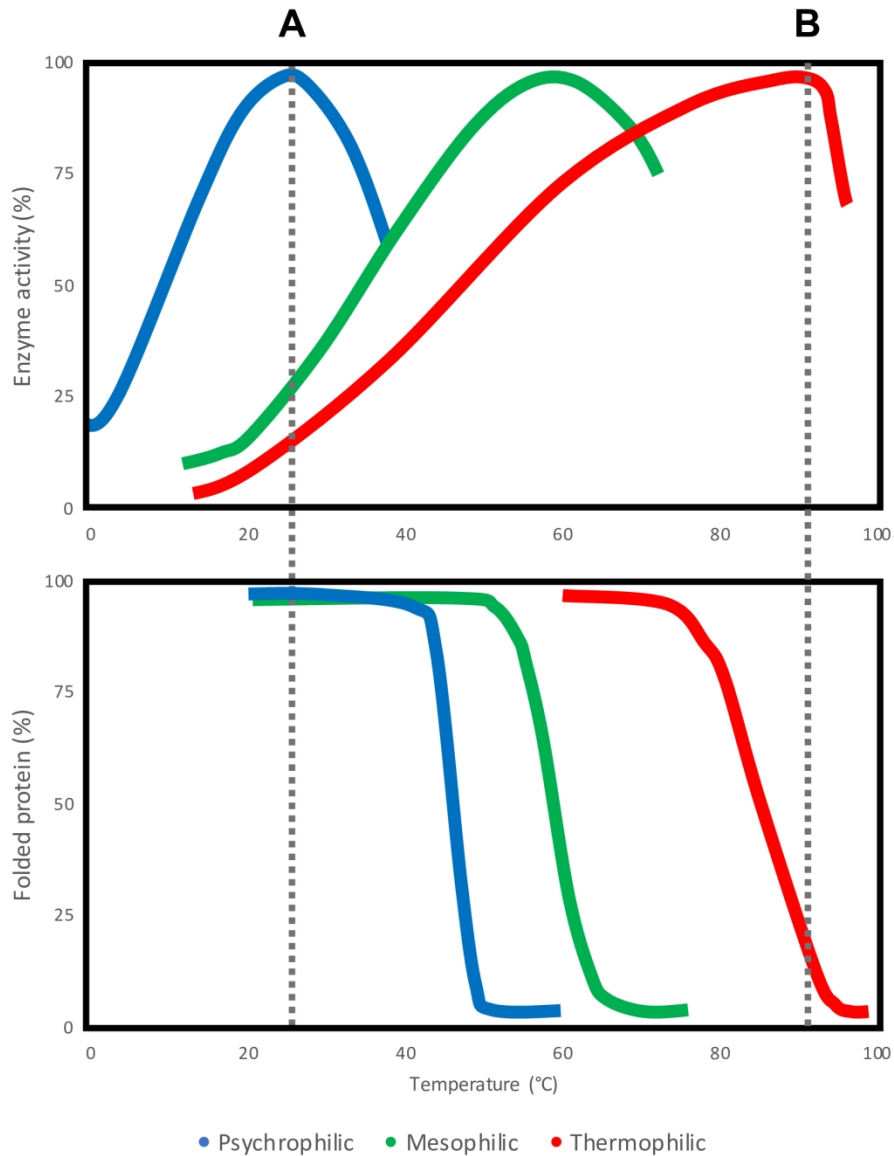


Figure 6: Theoretical stability/activity thermal curves of three homologous extremophilic enzymes

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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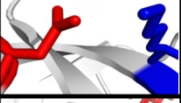
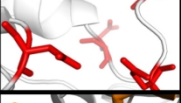
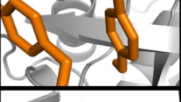


| Interactions and involved residues   | Thermophilic protein                                       | Psychrophilic protein                               | Halophilic protein               | Piezophilic protein |
|--|--|---|----------------------------------|---------------------|
|  <b>Hydrophobic effect</b><br>Trp, Tyr, Phe, Leu, Ile, Val, Ala                               | Overall increased hydrophobicity, surface less hydrophobic | Less hydrophobic interactions                       | Overall decreased hydrophobicity | ?                   |
|  <b>Salt bridges</b><br>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       |
|  <b>Hydrogen bonds</b><br>Ser, Thr, Tyr, Lys, Arg, Trp, His and backbone + His, Trp, backbone | Form surface networks                                      | Less abundant                                       | ?                                | ?                   |
|  <b>π-π interaction</b><br>Trp, Tyr, Phe  | More abundant  | Less abundant                                       | Less abundant                    | ?                   |
|  <b>π-cation interaction</b><br>Trp, Tyr, Phe + Arg, His, Lys                                 | More Arg-based   | Less abundant                                       | Less abundant                    | ?                   |
|  <b>Disulfide bridge</b><br>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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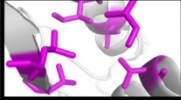


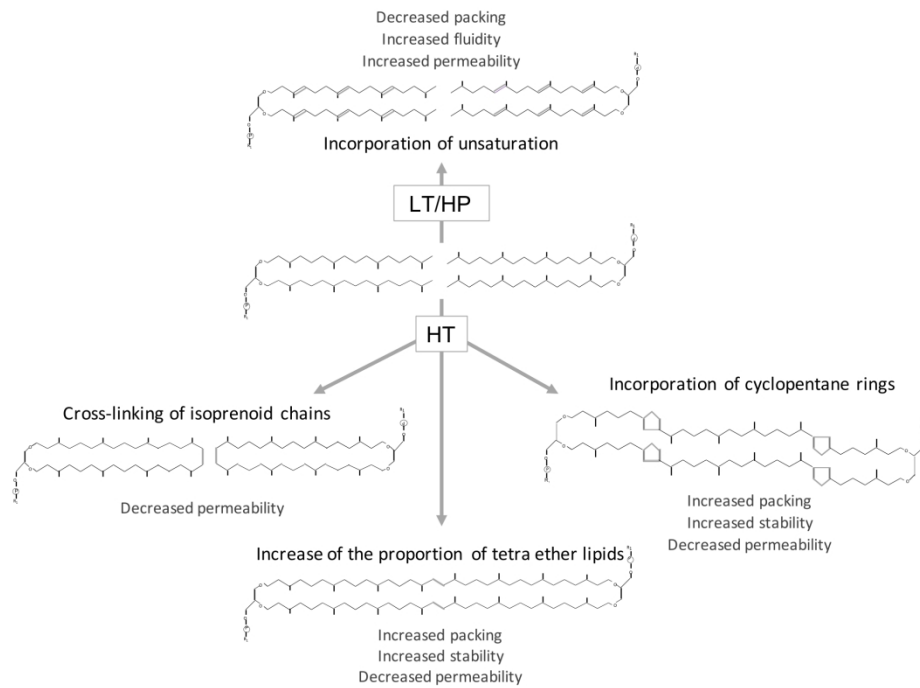
| Other features  |                 | Thermophilic protein                                       | Psychrophilic protein                                   | Halophilic protein                            | Piezophilic protein      |
|---|-----------------|--|---|---|--------------------------|
|  | Loops           | 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

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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30 Figure 8: Molecular traits of lipids in extreme conditions

31  
32 Adaptive membrane changes in archaea. Lipids may adopt multiple traits at the same time. Traits reducing  
33 membrane permeability may also protect against other extreme conditions such as low pH. Adapted from  
34 (Jebbar et al. 2015).

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37 801x588mm (72 x 72 DPI)