



HAL
open science

Relevance of Earth-Bound Extremophiles in the Search for Extraterrestrial Life.

Lorenzo Carré, Giuseppe Zaccai, Xavier Delfosse, Eric Girard, Bruno Franzetti

► **To cite this version:**

Lorenzo Carré, Giuseppe Zaccai, Xavier Delfosse, Eric Girard, Bruno Franzetti. Relevance of Earth-Bound Extremophiles in the Search for Extraterrestrial Life.. *Astrobiology*, 2022, 22 (3), pp.322-367. 10.1089/ast.2021.0033 . hal-03819312

HAL Id: hal-03819312

<https://hal.science/hal-03819312v1>

Submitted on 18 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL
open science

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

Lorenzo Carré, Giuseppe Zaccai, Xavier Delfosse, Éric Girard, Bruno Franzetti

► **To cite this version:**

Lorenzo Carré, Giuseppe Zaccai, Xavier Delfosse, Éric Girard, Bruno Franzetti. Relevance of earth-bound extremophiles in the search for extra-terrestrial life. *Astrobiology*, Mary Ann Liebert, inPress. hal-03400442

HAL Id: hal-03400442

<https://hal.archives-ouvertes.fr/hal-03400442>

Submitted on 25 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Astrobiology

Astrobiology Manuscript Central: <http://mc.manuscriptcentral.com/astrobiology>

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

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

SCHOLARONE™
Manuscripts

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

CARRÉ Lorenzo¹, ZACCAI Giuseppe^{1, 2}, DELFOSSE Xavier³, GIRARD Eric¹,
FRANZETTI Bruno¹

¹ Univ Grenoble Alpes, CNRS, CEA, IBS, Grenoble, France.

² Institut Laue-Langevin (ILL), 38042 Grenoble, France

³ Laboratoire d'Astrophysique, Observatoire de Grenoble, 38400 Saint Martin d'Hères, France

Extremophile biochemistry for exobiology

Corresponding authors: lorenzo.carre@ibs.fr ; 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 of 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.
6
7

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
44
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
56
57
58
59
60

1
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
33
34
35

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.
50
51
52
53
54
55

56
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
60

1
2
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
6
7 biomolecules and biological processes are altered by extreme conditions. Molecular traits
8
9 found in extremophiles allowing life under one or multiple extreme conditions will then be
10
11 reviewed.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3 zones will become amenable to characterization of their atmosphere through transit
4
5 spectroscopy either with James Web Spatial Telescope (JWST) (Doyon *et al.*, 2014; Beichman
6
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
22
23 of the bodies of the Solar System.
24
25
26
27
28
29
30
31

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
50
51
52
53

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,
59
60

1
2
3 and Europa, a moon of Jupiter, are of particular interest for astrobiology and the search for
4
5 life (Gaidos *et al.*, 1999; Jebbar *et al.*, 2020a)
6
7
8
9

10 Mars brines and depths

11
12
13
14
15
16

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 H₂ and CO₂. 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.
57
58
59
60

1
2
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).
20
21
22
23
24

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
39
40
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,
55
56
57
58
59
60

1
2
3 similarly to the salinities of terrestrial hypersaline lakes such as Great Salt Lake or Dead Sea
4
5 (Fox-Powell *et al.*, 2016).
6
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.
23
24
25
26

27 These present-days putative habitable Martian environments are represented in
28
29 [Figure 1-A](#).
30
31
32
33
34
35
36
37

38 [Subglacial oceans of giant planets icy moons](#)

39
40
41
42
43

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,
55
56
57
58
59
60

1
2
3 Callisto, Titan and Ganymede, subglacial liquid water volumes exceed Earth's total liquid water
4
5 volume.
6
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).
21
22
23
24
25
26
27
28
29

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
37
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).
47
48
49

50 Beneath the ice, the ocean would be global, up to 100-200km deep, depending on the
51
52 model used (Anderson *et al.*, 1997, 1998; Pappalardo *et al.*, 1999; Spohn and Schubert, 2003;
53
54 Marion *et al.*, 2003; Thomas *et al.*, 2016). Its volume would exceed that of all terrestrial oceans
55
56 volume (Chyba and Phillips, 2001). Models depict an ice shell 10km thick (Park *et al.*, 2015)
57
58
59
60

1
2
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
20
21

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).
38
39
40
41

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 MgSO_4 (Kargel *et al.*, 2000; Fanale *et al.*, 2001; Zolotov and Shock, 2001;
56
57 McKinnon and Zolensky, 2003). However, MgSO_4 concentration is supposed to decrease
58
59
60

1
2
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
6
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).
10
11
12
13
14
15
16
17

18 The subglacial ocean on Enceladus

19
20
21
22
23

24 Enceladus is much smaller than Europa. It is also heated by the tidal effect of Saturn
25
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
38
39

40
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, CO₂ and CH₄ are
46
47 present in significant amounts and small amounts of NH₃, 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).
54
55
56
57
58
59
60

1
2
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.
10
11
12
13
14
15
16
17
18
19

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 MgCl₂ (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.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43 Other icy moons

44
45
46
47

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
53
54
55
56
57
58
59
60

1
2
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).
15
16
17
18
19

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).
35
36
37
38
39
40
41
42

43 Icy moons environmental conditions 44 45 46 47

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](#).
57
58
59
60

1
2
3
4
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.
15
16
17
18
19

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).
27
28
29

30 Laboratory experiments from many scientific fields designed for simulating icy moons
31
32 conditions have recently be reviewed (Taubner *et al.*, 2020).
33
34
35
36
37
38

39 Extremophiles and astrobiology

40
41
42
43
44

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.
54
55
56

57 Extremophiles are considered as key model organisms for astrobiology by many
58
59 (Seckbach, 2000; Cavicchioli, 2002; Reid *et al.*, 2006; Marion and Schulze-Makuch, 2007;
60

1
2
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.
32
33
34
35
36
37
38
39

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.
51
52
53
54
55
56

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
60

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.
15
16
17
18
19

20 Among them, archaea are dominant in extremely hot, acid and hypersaline
21 environments while bacteria are dominant in cold and alkaline environments. Depending on
22
23 the ambient temperature, deep-sea organisms are typically represented by psychrophilic
24
25 bacteria or thermophilic archaea. Extremophiles found in marine or continental subsurface
26
27 rocks are generally bacteria. Some extremophiles and their representative natural
28
29 environments are represented in [Table 1](#).
30
31
32
33
34
35
36

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
54
55
56
57
58
59
60

1
2
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).
6
7
8
9

10 11 12 13 Experimental astrobiology: using extremophiles to test habitability 14 15 16 17 18

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.
24
25
26
27
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
43 Anoxia should not be considered as an extreme condition for biological systems or a barrier
44
45 for life *per se*. On the contrary, since the oxygenation of atmosphere and waters by
46
47 photosynthetic microorganisms, aerobic microorganisms have to cope with huge oxidative
48
49 stress through various long-evolved molecular systems.
50
51
52

53 In terrestrial anaerobic environments, methan-producing microbes (methanogens) are
54
55 key organisms to understand such biotops. Methanogens are widespread microorganisms
56
57 from the *Archaea* domain encountered in both extreme and moderate environments. They
58
59
60

1
2
3 are either autotrophs (producing their own organic matter) or heterotrophs (needing organic
4 matter from the environment). In all cases, CH₄ is a byproduct of their growth, which can be
5
6 then metabolized by methanotrophs (bacteria and archaea). Hence, methanogens using CO₂
7
8 as the carbon source and H₂ 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 CH₄ could be a purely
19
20 abiotic phenomenon in a case of an H-rich atmosphere. CH₄ 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.
25
26
27
28
29
30
31

32 For the CO₂-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),
55
56
57
58
59
60

1
2
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).
12
13
14

15
16 Since H₂, CO₂ and CH₄ 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).
25
26
27
28
29

30
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.
46
47
48

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
57
58
59
60

1
2
3 cycles (Mancinelli *et al.*, 2004) or could grow at Martian subsurface temperature (Reid *et al.*,
4
5
6 2006; Laye and DasSarma, 2018).
7
8
9

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.
31
32
33
34
35

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
54
55
56
57
58
59
60

1
2
3 *et al.*, 2008) and land hydrothermal fields have also been proposed as the place for precellular
4
5 evolution (Mulkidjanian *et al.*, 2012).
6
7

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).
19
20
21

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).
29
30
31

32
33
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).
45
46
47

48
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).
56
57
58
59
60

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.

1
2
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.
17
18
19
20
21
22

23 In this review we would like to defend the position that experimental studies using
24
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
46
47 biochemistries however could share more similarities.
48
49
50
51
52
53

54 Therefore, studies of biomolecules from extremophiles in simulated planetary
55
56 conditions may offer insights about limits of given biological processes in extraterrestrial
57
58 environments. Moreover, in the context of abiogenesis or early life, in absence of most of the
59
60

1
2
3 long-evolved cellular tools modern cells possess, studying fundamental biological processes
4
5 may give insights on the feasibility of biochemistry in extraterrestrial environments.
6
7
8
9

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.
41
42
43
44
45
46
47
48

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
58
59
60

1
2
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
15

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.
29
30
31
32
33
34
35
36
37

38 Extreme conditions overview

39
40
41
42
43

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.
55
56
57
58
59
60

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.
18
19
20
21

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
33
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
59
60

1
2
3 structure and dynamics of biomolecules. Nonetheless, these other extreme conditions will be
4
5 briefly reviewed in this chapter.
6
7

8 A general overview of extreme conditions, extremophily types, associated terrestrial
9
10 environments and extraterrestrial environments putatively displaying these conditions is
11
12 presented in [Table 1](#).
13
14
15
16
17
18
19
20

21 Extreme pH

22
23
24
25

26 Acidophiles and alkaliphiles are extremophilic organisms needing respectively low (<
27
28 3) or high pH (> 9) to grow. Acidic environments on Earth where acidophiles thrive include
29
30 volcanic hot springs, solfataric fields, acid mine drainage, bioreactors and coal spoils. Alkaline
31
32 environments where alkaliphiles thrive include alkaline hot springs, white smokers, soda lakes
33
34 and sewage waters.
35
36
37

38 Several attempts to compare acidic terrestrial environments to extraterrestrial ones
39
40 have been made. In particular, the acidic metal-rich waters and sediments of Río Tinto have
41
42 been compared with ancient aqueous acidic sulfate-rich martian fields (Clifford 1993; Clifford
43
44 *et al.* 2010). Enceladus ocean is expected to be alkaline (Glein *et al.*, 2015) but pH of
45
46 extraterrestrial environments is still largely debated.
47
48
49

50 For terrestrial life, extreme pH is not a condition that cells can afford to totally let in.
51
52 Because metabolism lies in generating ATP using pH gradients across the cell membrane,
53
54 equilibration of pH between cytoplasm and external acidic or alkaline environment has to be
55
56 avoided by cells (Krulwich and Guffanti, 1983; Krulwich, 1995). Therefore, instead of adapting
57
58
59
60

1
2
3 proteins and the rest of cellular content to $\text{pH} < 3$, 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 for alkaliphiles which keep an intracellular pH lower than the environment pH (Krulwich *et al.*,
8
9 2011). For example, the model extremely alkaliphilic bacteria *Bacillus pseudofirmus* OF4 grows
10
11 at pH 10.5 but keeps a cytoplasmic pH of 8.3 (Sturr *et al.*, 1994) and the most extreme
12
13 acidophile organism *Picrophilus torridus* which can grow around pH 0 has an intracellular pH
14
15 of 4.6 (Fütterer *et al.*, 2004).
16
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.
27
28
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.
55
56
57
58
59
60

1
2
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.
28
29
30
31
32
33
34
35
36
37

38 Ionizing radiation

39
40
41
42

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 α or β particles
48
49 or by γ 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,
54
55
56
57
58
59
60

1
2
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 Mn²⁺ and extensive DNA repair (Krisko and Radman, 2013).
19
20 Several strategies providing radioresistance, such as Mn²⁺ 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).
31
32
33
34
35
36

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
56
57
58
59
60

1
2
3 produced molecules as a source of energy and nutrients making this ecosystem independent
4
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).
12
13
14
15
16
17
18
19
20

21 Organic solvents

22
23
24
25
26 Organic solvents are highly toxic to biological systems, in particular for membranes and
27
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.
43
44
45
46
47
48
49
50
51
52

53 Heavy metals

54
55
56
57
58
59
60

1
2
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
6
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 Ni²⁺ in their catalytic center whose replacement
22
23 by Zn²⁺ 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: Zn²⁺ < Ag⁺ < Ni²⁺ << Cu²⁺ < Hg²⁺ << UO₂²⁻ < Co²⁺ < CrO₄²⁻
28
29 < Au³⁺ < Cd²⁺ < Mn²⁺ <<< Pb²⁺ (Nies, 2000).
30
31
32
33
34
35
36

37
38 Metalloresistance is mainly achieved by energy dependent efflux of toxic ions (Silver
39
40 and Phung, 1996) and by chelating agents (Daniels *et al.*, 1998; Cobbett, 2000). Other
41
42 sequestration mechanisms in bacteria (Kothe *et al.*, 2010) include biomineralization
43
44 (Bäuerlein, 2003) and cell wall adsorption (Merroun *et al.*, 2005). Periplasmic sensors are also
45
46 of particular importance (Trepreau *et al.*, 2014; Nies *et al.*, 2017). Interestingly,
47
48 metalloresistance is common in acidophilic archaea and bacteria whose sulfur metabolism
49
50 may increase extracellular metal concentration (Dopson *et al.*, 2003, 2014). Nonetheless,
51
52 heavy metal concentration is also an extreme condition that can be, to a certain extent, kept
53
54 out of the cytoplasm and not requiring an adaptation of all proteins.
55
56
57
58
59
60

1
2
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;
12
13 Richter *et al.*, 2012).
14
15
16
17
18
19
20
21
22

23 Nutrient scarcity

24
25
26
27
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
31
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
38
39

40
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
44
45 such as Martian surface appear to be low-energy (Jones *et al.*, 2018).
46
47
48
49
50
51
52

53 Other extreme conditions

54
55
56
57
58
59
60

1
2
3 Some other extreme chemical and physical conditions and their related adapted
4
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.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
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:
12
13

14
15 The change in *enthalpy*, ΔH , 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:
30
31
32
33

$$\Delta G = \Delta H - T \Delta S \quad (1)$$

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),
42
43 free energy (and consequently enthalpy and entropy) are calculated from the measured
44
45 equilibrium concentrations of the two states.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

1
2
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.
6
7
8
9
10
11
12

13 Proteins

14
15
16
17
18
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).
36
37
38
39
40

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.
48
49

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
54
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
58
59
60

1
2
3 results in a loss of function. Even intrinsically disordered proteins (IDPs) or IDP domains in
4
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).
14
15
16

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 ΔH , ΔS display a complex dependence on temperature: ΔH , because
25
26 of bonding within the chain as well as between the chain and solvent; ΔS , 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.
39
40
41
42
43
44
45
46

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 α carbon bonded to
59
60

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, Mg²⁺ and Ca²⁺-
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 α -helix and β -sheet are the most common secondary structures, but β -turns
33
34 (Hutchinson and Thornton, 1994) and Ω -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
33
34
35
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

1
2
3 Protein degradation in particular is achieved by central macromolecular assemblies called
4
5 proteasomes and allows the essential regulation of protein quantity and quality control
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).
34
35
36
37
38
39
40
41
42
43
44

45 Nucleic acids

46
47
48
49

50
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).
56
57
58
59
60

1
2
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.
23
24
25
26
27
28
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.
39
40
41
42
43
44
45
46
47

48 Lipids

49
50
51
52

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
58
59
60

1
2
3 inner leaflets are fused by the tails effectively turning the bilayer into a monolayer. The
4
5 permeability barrier in biological cellular membranes is the bilayer made up of phospholipids
6
7 and glycolipids and other hydrophobic molecules such as sterols into which transmembrane
8
9 proteins are inserted. Membranes segregate cell components from extracellular
10
11 environments and allow compartmentation. Control of diffusion and active transport across
12
13 the membrane enables the possibility for cellular homeostasis and chemical gradients which
14
15 can be used to produce energy.
16
17
18

19
20 Lipid bilayers take up different thermodynamic phases as a function of temperature
21
22 and pressure, nature and length of hydrophobic tails, positions of unsaturated bonds in the
23
24 tails, cyclization etc. Typically, the physiological state of a biological membranes is relatively
25
26 fluid while not too disordered, allowing balance between fluidity and rigidity (see [Figure 4](#)) to
27
28 favor membrane protein dynamics and function.
29
30
31

32
33 Furthermore, protein-lipid interactions modulate many membrane functions such as
34
35 enzymatic activity and microdomain structure (Tien and Ottova-Leitmannova, 2000; Richens
36
37 *et al.*, 2015).
38
39
40
41
42
43
44
45
46
47

48 Effects of extreme conditions on terrestrial biomolecules

49
50
51
52

53
54 Temperature, salinity and pressure conditions deeply affect structural and dynamical
55
56 properties of proteins. Even if some general alterations can be recognized, effects of these
57
58 conditions depend on the biomolecule. In particular, it should be kept in mind that, depending
59
60

1
2
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.
6
7
8
9

10 11 12 13 Effects of low temperature on biomolecules 14

15
16
17
18
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°C for example lowers reaction rates constants by four orders
39
40 of magnitude (Russell *et al.*, 1990).
41
42
43
44

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.
53
54
55
56
57
58
59
60

1
2
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
25
26
27
28
29
30
31

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
7
8
9

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

17 From a general point of view, low temperature causes osmotic stress. When ice forms
18 in the extracellular environment, osmotic pressure of cytoplasmic water is higher than
19 extracellular ice leading to the efflux of water out of the cell and thus to a dramatic increase
20 of many cytoplasmic compounds concentrations (Russell *et al.*, 1990). Concentration of the
21 extracellular environment may make the environment more extreme (Banciu and Sorokin,
22 2013). However, due its composition, intracellular water freezes at temperatures under 0°C.
23 In *S. cerevisiae*, for example, cytosol vitrification typically happens at -17°C (Fonseca *et al.*,
24 2016). At this point, intracellular ice formation concentrates even more the cytoplasm,
25 altering pH, salinity, ionic strength, and a_w (Russell *et al.*, 1990).
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 Another general effect of low temperature on water is an increased solubilization of
43 gas such as oxygen (D'Amico *et al.*, 2006), leading to an enhanced production of ROS species
44 and thus to an oxidative stress (Abele and Puntarulo, 2004; Vinagre *et al.*, 2012).
45
46
47
48
49
50
51
52
53
54

55 **Effects of high temperature on biomolecules**
56
57
58
59
60

1
2
3 In general, increased temperature means increased molecular movements and overall
4 increased flexibility (Daniel and Cowan, 2000). A first consequence is that temperature acts as
5 a catalyzer of most reactions, like the spontaneous degradation of many small molecules in
6 biological systems. Unstable metabolites like ATP, NAD and acetyl phosphate, for example,
7 have a short half-life at 95°C (Daniel and Cowan, 2000).
8
9
10
11
12
13
14
15
16
17

18 For protein heat damage, it is possible to distinguish irreversible (degradation) and
19 reversible (denaturation) damage (Daniel *et al.*, 1996).
20
21
22

23 Protein flexibility enhancement at high temperature can also lead to a decrease of
24 stability. As discussed before in this review, protein folding relies on various intramolecular
25 interactions such as hydrophobic contacts, for example. As at high temperature atomic
26 motions are increased, these bonds loose some strength and exposure of hydrophobic regions
27 is enhanced, favoring unfolding (Das and Surewicz, 1995). This local denaturation is to some
28 extent reversible as some protein can spontaneously fold back upon temperature decrease
29 (Daniel *et al.*, 1996). Exposure of hydrophobic regions may, however, promote, interaction
30 between unfolded proteins and hence to irreversible aggregation.
31
32
33
34
35
36
37
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 enhanced by high temperatures: deamidation of Asn and Gln residues, succinimide formation
49 at Glu and Asp and oxidation of His, Met, Cys, Trp and Tyr (Ahern and Klibanov, 1985; Zale and
50 Klibanov, 1986; Daniel *et al.*, 1996; Daniel and Cowan, 2000). These irreversible degradations
51 not only impair folding but can also lead to cleavage of peptide bond, leading to the loss of
52 structure and function of proteins.
53
54
55
56
57
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
26
27
28
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
44
45
46
47
48
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
28
29
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⁺ ion is dominant outside, the K⁺ 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, MgCl₂,
52
53 MgSO₄ or CaCl₂-rich (Schneegurt, 2012; Oren, 2013; Jebbar *et al.*, 2020b).
54
55
56
57
58
59
60

1
2
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
58
59
60

1
2
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
20
21
22
23
24

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.
29
30
31
32
33
34
35
36
37
38
39

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).
50
51
52
53
54
55
56
57
58
59
60

1
2
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
15
16
17
18
19

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
49
50
51
52 2009).
53
54
55
56

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

1
2
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).

1
2
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 CO₂ 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⁺ export out of the cell (Abe and Horikoshi, 1995).
19
20
21

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).
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.
10
11
12
13
14

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).
34
35
36
37
38
39

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).
45
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.*,
58
59
60

1
2
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.
16
17
18

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).
33
34
35

36
37 Structural traits in cold-adapted proteins are represented in [Figure 7](#).
38

39
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
47
48 ratio.
49
50

51
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
55
56 with their hydrophobic residue by strong Van der Waals interactions (Howard *et al.*, 2011,
57
58
59
60

1
2
3 2014), limiting crystallization of the cytoplasm (Scotter *et al.*, 2006; Venketesh and
4
5 Dayananda, 2008).
6
7
8
9

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.
15
16
17
18
19
20
21
22
23
24

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).
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

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)
56
57
58
59
60

1
2
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
17
18
19

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).
25
26
27
28
29
30
31
32

33 Thermophilic molecular traits

34
35
36
37
38

39 Thermophiles and hyperthermophiles are organisms defined by optimal growth
40
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
57
58
59
60

1
2
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](#).
12
13
14
15
16
17

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.
35
36
37
38
39

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
43
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).
57
58
59
60

1
2
3 Comparative studies of protein dynamics and primary, secondary, tertiary and
4
5 quaternary structures have given some insights about the ways to achieve thermostability
6
7 (Irimia *et al.*, 2004; Koutsopoulos *et al.*, 2005a, 2005b; Karlström *et al.*, 2006). It appears that
8
9 the most recurring molecular traits in thermophilic proteins are salt bridges and ion-pair
10
11 networks lowering exposure of the protein to the solvent (Goldman, 1995; Yip *et al.*, 1995;
12
13 Kumar *et al.*, 2000). Indeed, as at high temperatures water molecules interact less with
14
15 charged residues, these residues can form stabilizing interactions (Elcock, 1998).
16
17
18
19

20 For thermophilic multimeric assemblies, increased hydrophobicity of subunit
21
22 interfaces (Wallon, Kryger, *et al.*, 1997; Hatanaka *et al.*, 1997) and introduction of
23
24 intermolecular disulfide bridges (Dick *et al.*, 2016) favor stability of the quaternary structure.
25
26 Within globular proteins, interactions between aromatic residues (Phe, Trp, Tyr) have also
27
28 been proposed as a way to achieve thermostability (Karlström *et al.*, 2006). Probably as a way
29
30 to protect them from oxidation which is enhanced at high temperature, Cys residues involved
31
32 in disulfide bridges are often buried within the protein (Choi *et al.*, 1999; Kumar and Nussinov,
33
34 2001). As Gly and Pro residues respectively induce flexibility or rigidity in protein backbone,
35
36 many thermophilic proteins display decreased Gly/Pro ratio (Vieille and Zeikus, 2001).
37
38 However Gly are more abundant in alpha helices where they increase stability (Petski, 2001).
39
40 Flexible surface loops can also be shortened or deleted in thermophilic proteins (Thompson
41
42 and Eisenberg, 1999; Yamagata *et al.*, 2001; Tanaka *et al.*, 2004), hence decreasing
43
44 surface/volume ratio (Petski, 2001).
45
46
47
48
49
50
51

52 **Figure 7** summarizes some major interactions and structural features enabling
53
54 thermostability in thermophilic proteins.
55
56
57
58
59
60

1
2
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).
24
25
26
27
28
29

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).
39
40
41

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).
53
54
55
56
57
58
59
60

1
2
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).
8
9

10
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).
39
40
41
42
43
44
45

46
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).
52
53

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).
60

1
2
3
4
5 See [Figure 8](#) for a summary of main thermophilic lipid traits (Jebbar *et al.*, 2015, 2020a).
6
7
8
9

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).
15
16
17
18
19
20
21
22
23
24
25
26
27

28 Halophilic molecular traits

29
30
31
32
33

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
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60 Moreover, the salt-in strategy implies adaptation of the whole proteome to the extreme

1
2
3 condition. Halophiles may also use a combination of both strategies to cope with their
4
5 environment.
6
7

8 Halophiles counter the outside osmolarity of Na^+ by accumulating K^+ in the cytoplasm
9
10 (Ginzburg and Ginzburg, 1976). Intracellular concentration of K^+ typically reaches 2-5.5M in
11
12 extremely halophilic archaea while 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: Na^+ , K^+ , 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).
35
36
37
38
39
40
41

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
55
56 negatively charged residues can bind more solvent cations (Ebel *et al.*, 2002). On the other
57
58
59
60

1
2
3 hand, anions appear to be strongly bind by fewer sites (Ebel *et al.*, 2002). This ion-rich shell
4
5 may limit water condensation on protein surface which would lead to excessive interaction
6
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).
30
31
32
33
34
35

36
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).
52
53
54
55

56
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
60

1
2
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).
5
6
7

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).
16
17
18
19
20
21
22
23
24

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).
31
32
33
34
35
36
37
38
39
40
41

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).
45
46
47
48
49
50
51

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
55
56
57
58
59
60

1
2
3 sulfate groups with negative charges shielding salt cations (Russell, 1989), limiting H⁺ and Na⁺
4 permeability and increasing stability (van de Vossenberg *et al.*, 1999).
5
6
7

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).
13
14
15
16
17
18
19
20
21
22

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 archaeon from the Dead Sea, revealed a fraction of
26 exceptionally slow water in the hypersaline cytoplasm, hypothetically organized by structured
27 K⁺ ions (Tehei *et al.*, 2007; Frölich *et al.*, 2008).
28
29
30
31
32
33
34
35
36
37

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).
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3 observed for individual piezophilic proteins (Shrestha *et al.*, 2015), and a more compact and
4
5 less dynamic solvation shell may hence be an adaptive strategy for piezophiles.
6
7
8
9

10
11 Enhanced flexibility compensates pressure-induced rigidification while compaction of
12
13 the volume occupied by water molecules bound to proteins could stabilize them.
14

15
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.
25

26
27 Sequence analysis revealed that sequence bias in piezophiles is actually dependent on
28
29 the thermophilic/psychrophilic status. Nonetheless, in comparison to non-psychrophilic
30
31 homologs, psychrophilic proteins tend to have more polar, hydrophilic and small amino acids
32
33 (Di Giulio, 2005; Nath and Subbiah, 2016), counting for the reduction of side-chain volume.
34
35 For non-psychrophilic piezophiles, hydrophobic, non-polar and aliphatic amino acids are also
36
37 over-represented in comparison to non-psychrophilic relatives (Nath and Subbiah, 2016).
38
39
40

41
42 Loosely packed protein structures also seem to offer a way to decrease deleterious
43
44 volumetric effects of high pressure (Nagae *et al.*, 2012). Indeed, when structure and activity
45
46 rely on internal cavities, larger internal volumes in piezophilic proteins permit to maintain
47
48 some internal cavities at abyssal pressures while, in comparison, non-piezophilic counterparts
49
50 will lose too much internal volume. Major structural traits of pressure-adapted proteins are
51
52 represented in [Figure 7](#).
53
54

55
56 As quaternary structure is primarily affected by high pressure, it has been found that
57
58 multimeric assemblies, such as SSB DNA-binding proteins from piezophilic *Shewanella* strains
59
60

1
2
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.
6
7
8
9

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).
13
14
15
16
17
18
19

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.
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 Accumulation of compatible solutes in piezophiles may also help to cope with high
43 pressure. β -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).
46
47
48
49
50
51

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).
55
56
57
58
59
60

1
2
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.
7
8
9
10
11
12
13
14
15
16

17 Living with many extremes: Polyextremophily and limits of life

18
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).
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53

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
57
58
59
60

1
2
3 oceans can present, depending on the depth and the proximity with a hydrothermal vent,
4
5 extreme temperatures, extreme pH and high pressure. Therefore, terrestrial environments
6
7 where polyextremophilic thrive can be considered as good models for extraterrestrial
8
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*
16
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.
20
21
22
23

24
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;
28
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
32
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
40
41 (Belilla *et al.*, 2019).
42
43
44
45
46

47 Other examples of poly-extreme environment devoid of life are the Mg-rich deep
48
49 hypersaline anoxic basins (DHAB). These deep sea brines, which can host deep-sea halophilic
50
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
58
59
60

1
2
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).
12
13

14
15 In these two examples, chaotropicity induced by natural divalent cation abundancy
16
17 strongly limits life and may, similarly, represent a barrier for life in Martian brines (Fox-Powell
18
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
23
24

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
32
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
44
45 *et al.*, 2013).
46
47
48
49
50

51
52 In the next part of this review, combinations between high/low temperature, high
53
54 salinity and high pressure, will be discussed in regard to their effect on biomolecule and their
55
56 eventual polyextremophiles.
57
58
59
60

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

1
2
3 halotolerant (Kaye and Baross, 2000) and moderately halophilic (Kaye *et al.*, 2004) bacteria as
4
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
8
9 hydrothermal chimneys may host hypersaline hot microenvironments allowing colonization
10
11 by halophiles (Takai *et al.*, 2001).
12
13

14
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*
20
21 which grows at saturated concentration of Na⁺ and at temperature as high as 56°C (Bowers
22
23 and Wiegel, 2011; Zhao *et al.*, 2018).
24
25
26
27
28
29
30
31
32

33 Thermopiezophily

34
35
36
37

38 Generally, pressure and temperature have opposite volumetric effects on
39
40 macromolecular systems: while temperature increases flexibility and pushes objects to
41
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
45
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
55
56
57
58
59
60

1
2
3 can only be achieved at abyssal pressures. It has also been shown that for both abyssal archaea
4
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).
8
9

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
13
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
19
20 and high pressure (Salvador-Castell *et al.*, 2020). Nonetheless, structural signatures of
21
22 thermopiezophily have yet to be described.
23
24
25

26
27 The fact that high pressure induces heat-shock response in both piezophilic (Sato *et*
28
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-
34
35 induced and pressure-induced unfolding.
36
37
38
39
40
41
42
43
44

45 Psychrohalophily

46
47
48
49

50
51 Polar cold environments are often associated with high salinity. In spite of the fact that
52
53 salt solubility in water decreases with temperature, freezing of water excludes salt from the
54
55 ice thus concentrating the remaining liquid which becomes hypersaline. Moreover, high
56
57 salinity lowers freezing point of water to the point that saturated NaCl brines are liquid at
58
59
60

1
2
3 temperatures as low as -21°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°C because of
6
7 its high CaCl_2 concentration (Harris and Cartwright, 2013). As with water above 100°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°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
16
17 18% at -15°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
20
21 halophilic archaea (DeMaere *et al.*, 2013; Williams *et al.*, 2017).
22
23
24
25
26

27
28 At the biomolecular level, psychrophily and halophily appear to have some degree of
29
30 synergy (Capece *et al.*, 2013). Intracellular accumulation of chaotropes such as Na^+ , Mg^{2+} or
31
32 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
37
38 psychrohalophily. Both salt-in-cytoplasm and compatible solute strategies for halophily thus
39
40 enhance psychrophily.
41
42
43

44
45 At the membrane level, cold temperature rigidifies membranes and increases their
46
47 impermeability, which serves resistance to high salinity. Moreover, carotenoids are observed
48
49 in both halophiles and psychrophiles where they serve membrane fluidity control. However,
50
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
54
55 increased salinity (Fong *et al.*, 2001b), they may represent a tool for adjusting membrane
56
57 fluidity in cold hypersaline environments.
58
59
60

1
2
3 At less extreme values of cold and salinity, plants possess common pathways for
4
5 resisting to drought, coldness and salinity (Xiong *et al.*, 2002; Zhu, 2002; Seki *et al.*, 2003).
6
7
8
9
10
11
12

13 Psychropiezophily

14
15
16
17
18
19 Deep ocean cold and pressurized waters represent most of the biosphere volume.
20
21 While organisms of hydrothermal vents benefit from compensating effects of high pressure
22
23 and high temperature on molecular flexibility, deep sea organism molecules are rigidified by
24
25 both low temperature and high pressure (Capece *et al.*, 2013).
26
27

28
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
38
39 high pressure conditions in deep-sea bacteria (Marx *et al.*, 2009; Bælum *et al.*, 2012), however
40
41 the exact function of EPS for pressure-tolerance is still unclear.
42
43
44
45
46
47
48
49
50

51 Halopiezophily

52
53
54
55
56
57 Most piezophiles have been isolated at low salinity and most halophiles at atmospheric
58
59 pressure (Harrison *et al.*, 2013). Hypersaline deep environments are limited to DHABs.
60

1
2
3 However, nature of salt may significantly reduce the window for life. For example, high
4
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).
8
9

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.
13
14

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).
18
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
24
25 (Alcaide *et al.*, 2015).
26
27
28
29

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).
39
40
41

42 Adaptation to both high pressure and high salinity may use compatible solutes
43
44 conferring both haloresistance and piezotolerance such as β -hydroxybutyrate (Martin *et al.*,
45
46 2002).
47
48
49
50
51
52
53
54

55 Extremophilic molecular traits as biomarkers

56
57
58
59
60

1
2
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, CH₄ and
8 dioxygen have been proposed as biomarkers and can be remotely detected in extraterrestrial
9 atmospheres. As discussed before, CH₄ 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 CH₄, 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

1
2
3 numerous biological repairing or recycling processes, such as chaperones and proteasomes,
4
5 to cope with this instability.
6
7

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.
23
24
25

26
27 Another example of biomarker related to extremophiles is the case of pigments.
28
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
50
51 accumulated by putative life in heavily irradiated extraterrestrial surface environments. It
52
53
54
55
56
57
58
59
60

1
2
3 should nonetheless be kept in mind that remote detection of extraterrestrial pigments may
4
5 be technically difficult or even impossible (Björn *et al.*, 2009).
6
7

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
17
18 (Chela-Flores, 2019). While this type metabolism is not limited *per se* to extreme
19
20 environments, it is still more abundant in acidic hot springs and deep sea hydrothermal vents.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3 at the cellular level and require less to no molecular trait. In contrast, high and low
4
5 temperature, high salinity and high pressure are extreme conditions commonly faced by life
6
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
10
11 properties such as flexibility, stability or activity. In particular, alteration of solvation shell,
12
13 which is fundamental for structure and dynamics of biomolecules, is critical.
14
15

16
17
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
21
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
27
28 psychrophilic proteins to counter rigidification induced by low temperatures or increased
29
30 solubility of halophilic proteins to counter enhanced aggregation under hypersalinity. For
31
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
35
36 techniques such as neutron scattering, spectroscopy or NMR. In particular, adaptation of the
37
38 solvation shell, which should be understood as a part of the proteins, is fundamental to cope
39
40 with extreme temperature, salinity or pressure.
41
42
43
44
45

46
47 While most extremophilic systems are studied in regard to one extreme condition,
48
49 terrestrial extreme environments actually generally display more than one such condition. The
50
51 same goes for extraterrestrial environments which can also be called polyextreme. Some
52
53 combinations of extreme conditions are however unknown on Earth and associated with no
54
55 organism to date. Combinations of extreme conditions may either be compensating or
56
57 synergistically deleterious for biological system. High pressure and high temperature present
58
59
60

1
2
3 in abyssal hydrothermal vents for example have opposite effects on molecular flexibility
4
5 whereas low temperature and high pressure in deep sea environments both increase rigidity
6
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.
10
11

12
13 Most data about extremophilic adaptation have been obtained through study of
14
15 simple systems such as monomeric proteins. However, complex biological processes such as
16
17 DNA replication, transcription, translation, protein degradation or metabolism largely rely on
18
19 large molecular assemblies. Systems like multimeric enzymes, DNA/RNA polymerases,
20
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
24
25 conditions may bring interesting data about habitability of extraterrestrial environments and
26
27 how life could manage extreme conditions out of Earth.
28
29
30
31
32
33
34

35 Existence and diversity of extremophiles have opened the window of habitability,
36
37 suggesting that life would be possible in extraterrestrial environments similar to extreme
38
39 terrestrial ones. Yet, some open questions still remain.
40
41

42 Can putative extremophilic biomarkers be find in extraterrestrial environments? The
43
44 study of extremophiles has shown that they may produce biomarkers relevant for
45
46 astrobiological concern. In particular, biomolecules in thermophiles and halophiles display
47
48 exceptional stability and resilience and thus could make interesting biomarkers.
49
50
51

52 How fast can life adapt to extreme conditions? It has been showed here that few
53
54 molecular traits allow single biomolecules such as proteins to work under extreme conditions.
55
56 These modifications may appear simple but they lead to dramatical changes of dynamical
57
58
59
60

1
2
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.
6
7

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.
17
18

19
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
27
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
31
32 could appear in ecosystems with chemosynthesis only. Hence, putative life in Martian
33
34 subsurface or icy moon abyssal environments may be limited to microorganisms.
35
36
37
38

39
40 Where should one search for extraterrestrial life? In the absence of known
41
42 extraterrestrial mesophilic environments, extreme extraterrestrial environments such as
43
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
51
52 by many, the search for hydrothermal should also be a priority. Liquid water may however not
53
54 be enough, making the search for salt-enriched liquid water environment a priority too. In
55
56 fact, biochemistry relies on a solvent which is an ionic solution rather than pure water and
57
58
59
60

1
2
3 many biological processes such as folding and metabolism require ions to work. In particular,
4
5 hydrothermal processes not only favors organic matter enrichment but also accumulation of
6
7 ions in water. In terrestrial life, solvent is not pure water but an ionic solution where charges
8
9 enable biological processes such as metabolism. Interestingly, hydrothermalism favors both
10
11 organic matter and mineral ions enrichment of water.
12
13
14

15 In this context, the search for rocky exoplanets with surface mesophilic conditions may
16
17 unravel new habitable environments.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acknowledgments

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

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

Author contributions

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

Disclosure statement

1
2
3 The authors are not aware of any affiliations, memberships, funding, or financial holdings that
4
5 might be perceived as affecting the objectivity of this review.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only; Not for Distribution

References

- Abe, F., Horikoshi, K. (1995). Hydrostatic pressure promotes the acidification of vacuoles in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* 130:307–312.
- Abe, F., Horikoshi, K. (1998). Analysis of intracellular pH in the yeast *Saccharomyces cerevisiae* under elevated hydrostatic pressure: a study in baro- (piezo-) physiology. *Extremophiles* 2:223–228.
- Abele, D., Puntarulo, S. (2004). Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 138:405–415.
- Aertsen, A., Meersman, F., Hendrickx, M.E.G., Vogel, R.F., Michiels, C.W. (2009). Biotechnology under high pressure: applications and implications. *Trends in Biotechnology* 27:434–441.
- Aertsen, A., Vanoirbeek, K., Spiegeleer, P.D., Sermon, J., Hauben, K., Farewell, A., Nyström, T., Michiels, C.W. (2004). Heat Shock Protein-Mediated Resistance to High Hydrostatic Pressure in *Escherichia coli*. *Appl Environ Microbiol* 70:2660–2666.
- Aghajari, N., Feller, G., Gerday, C., Haser, R. (1998). Structures of the psychrophilic *Alteromonas haloplanctis* α -amylase give insights into cold adaptation at a molecular level. *Structure* 6:1503–1516.
- Aguilar, C.F., Sanderson, I., Moracci, M., Ciaramella, M., Nucci, R., Rossi, M., Pearl, L.H. (1997). Crystal structure of the β -glycosidase from the hyperthermophilic archeon *Sulfolobus solfataricus*: resilience as a key factor in thermostability¹¹ Edited by R. Huber. *Journal of Molecular Biology* 271:789–802.
- Ahern, T.J., Klibanov, A.M. (1985). The mechanisms of irreversible enzyme inactivation at 100C. *Science* 228:1280–1284.
- Alcaide, M., Stogios, P.J., Lafraya, Á., Tchigvintsev, A., Flick, R., Bargiela, R., Chernikova, T.N., Reva, O.N., Hai, T., Leggewie, C.C., Katzke, N., Cono, V.L., Matesanz, R., Jebbar, M., Jaeger, K.-E., Yakimov, M.M., Yakunin, A.F., Golyshin, P.N., Golyshina, O.V., Savchenko, A., Ferrer, M. (2015). Pressure adaptation is linked to thermal adaptation in salt-saturated marine habitats. *Environmental Microbiology* 17:332–345.
- Almog, O., Kogan, A., Leeuw, M. de, Gdalevsky, G.Y., Cohen-Luria, R., Parola, A.H. (2008). Structural insights into cold inactivation of tryptophanase and cold adaptation of subtilisin S41. *Biopolymers* 89:354–359.
- Altermark, B., Helland, R., Moe, E., Willassen, N.P., Smalås, A.O. (2008). Structural adaptation of endonuclease I from the cold-adapted and halophilic bacterium *Vibrio salmonicida*. *Acta Cryst D* 64:368–376.
- Amils, R., Fernández-Remolar, D., the IPBSL Team (2014). Río Tinto: A Geochemical and Mineralogical Terrestrial Analogue of Mars. *Life* 4:511–534.
- Anderson, J.D., Lau, E.L., Sjogren, W.L., Schubert, G., Moore, W.B. (1997). Europa's Differentiated Internal Structure: Inferences from Two Galileo Encounters. *Science* 276:1236–1239.
- Anderson, A.W., Nordon, H.C., Cain, R., Parrish, G., Duggan, D., Anderson, A.O., Nordan, H., Parish, G., Cullum-Dugan, D. (1956). Studies on a radio-resistant micrococcus. I, Isolation, morphology, cultural characteristics, and resistance to gamma radiation.
- Anderson, J.D., Schubert, G., Jacobson, R.A., Lau, E.L., Moore, W.B., Sjogren, W.L. (1998). Europa's Differentiated Internal Structure: Inferences from Four Galileo Encounters. *Science*

1
2
3 281:2019–2022.

4 Anesi, A., Obertegger, U., Hansen, G., Sukenik, A., Flaim, G., Guella, G. (2016).
5 Comparative Analysis of Membrane Lipids in Psychrophilic and Mesophilic Freshwater
6 Dinoflagellates. *Front Plant Sci* 7.

7
8 Anglada-Escudé, G., Amado, P.J., Barnes, J., Berdiñas, Z.M., Butler, R.P., Coleman, G.A.L.,
9 de la Cueva, I., Dreizler, S., Endl, M., Giesers, B., Jeffers, S.V., Jenkins, J.S., Jones, H.R.A.,
10 Kiraga, M., Kürster, M., López-González, M.J., Marvin, C.J., Morales, N., Morin, J., Nelson,
11 R.P., Ortiz, J.L., Ofir, A., Paardekoooper, S.-J., Reiners, A., Rodríguez, E., Rodríguez-López,
12 C., Sarmiento, L.F., Strachan, J.P., Tsapras, Y., Tuomi, M., Zechmeister, M. (2016). A
13 terrestrial planet candidate in a temperate orbit around Proxima Centauri. *Nature* 536:437–
14 440.

15
16 Anschutz, P., Blanc, G., Chatin, F., Geiller, M., Pierret, M.-C. (1999). Hydrographic changes
17 during 20 years in the brine-filled basins of the Red Sea. *Deep Sea Research Part I:
18 Oceanographic Research Papers* 46:1779–1792.

19 Antunes, A., Olsson-Francis, K., McGenity, T.J. (2020). Exploring Deep-Sea Brines as
20 Potential Terrestrial Analogues of Oceans in the Icy Moons of the Outer Solar System. In:
21 *Astrobiology: Current, Evolving, and Emerging Perspectives*, Caister Academic Press, p .

22 Arnórsdóttir, J., Kristjánsson, M.M., Ficner, R. (2005). Crystal structure of a subtilisin-like
23 serine proteinase from a psychrotrophic *Vibrio* species reveals structural aspects of cold
24 adaptation. *The FEBS Journal* 272:832–845.

25
26 Arsiccio, A., McCarty, J., Pisano, R., Shea, J.-E. (2020). Heightened Cold-Denaturation of
27 Proteins at the Ice–Water Interface. *J Am Chem Soc* 142:5722–5730.

28 Assche, F.V., Clijsters, H. (1990). Effects of metals on enzyme activity in plants. *Plant, Cell
29 & Environment* 13:195–206.

30
31 Atomi, H., Matsumi, R., Imanaka, T. (2004). Reverse Gyrase Is Not a Prerequisite for
32 Hyperthermophilic Life. *Journal of Bacteriology* 186:4829–4833.

33 Atri, D. (2016). On the possibility of galactic cosmic ray-induced radiolysis-powered life in
34 subsurface environments in the Universe. *Journal of The Royal Society Interface*
35 13:20160459.

36 Auman, A.J., Breezee, J.L., Gosink, J.J., Kämpfer, P., Staley, J.T. (2006). *Psychromonas*
37 *ingrahamii* sp. nov., a novel gas vacuolate, psychrophilic bacterium isolated from Arctic polar
38 sea ice. *International Journal of Systematic and Evolutionary Microbiology*, 56:1001–1007.

39 Bada, J.L., Lazcano, A. (2002). Some Like It Hot, But Not the First Biomolecules. *Science*
40 296:1982–1983.

41
42 Badiea, E.A., Sayed, A.A., Maged, M., Fouad, W.M., Said, M.M., Esmat, A.Y. (2019). A
43 novel thermostable and halophilic thioredoxin reductase from the Red Sea Atlantis II hot
44 brine pool. *PLOS ONE* 14:e0217565.

45
46 Bælum, J., Borglin, S., Chakraborty, R., Fortney, J.L., Lamendella, R., Mason, O.U., Auer,
47 M., Zemla, M., Bill, M., Conrad, M.E., Malfatti, S.A., Tringe, S.G., Holman, H.-Y., Hazen,
48 T.C., Jansson, J.K. (2012). Deep-sea bacteria enriched by oil and dispersant from the
49 Deepwater Horizon spill. *Environmental Microbiology* 14:2405–2416.

50 Baker-Austin, C., Dopson, M. (2007). Life in acid: pH homeostasis in acidophiles. *Trends in
51 Microbiology* 15:165–171.

52 Baland, R.-M., Van Hoolst, T., Yseboodt, M., Karatekin, Ö. (2011). Titan’s obliquity as
53 evidence of a subsurface ocean? *Astronomy & Astrophysics* 530:A141.

54 Ball, P. (2008). Water as an Active Constituent in Cell Biology. *Chemical Reviews* 108:74–
55 108.

56
57 Ball, P., Hallsworth, J.E. (2015). Water structure and chaotropicity: their uses, abuses and
58 biological implications. *Phys Chem Chem Phys* 17:8297–8305.

59 Banciu, H.L., Sorokin, D.Y. (2013). Adaptation in Haloalkaliphiles and Natronophilic
60

- 1
2
3 Bacteria. In: Seckbach J, Oren A, Stan-Lotter H (eds) *Polyextremophiles: Life Under*
4 *Multiple Forms of Stress*, Cellular Origin, Life in Extreme Habitats and Astrobiology.
5 Springer Netherlands: Dordrecht, pp 121–178.
- 6 Barbieri, R., Cavalazzi, B. (2014). How Do Modern Extreme Hydrothermal Environments
7 Inform the Identification of Martian Habitability? The Case of the El Tatio Geysir Field.
8 *Challenges* 5:430–443.
- 9 Barge, L.M., White, L.M. (2017). Experimentally Testing Hydrothermal Vent Origin of Life
10 on Enceladus and Other Icy/Ocean Worlds. *Astrobiology* 17:820–833.
- 11 Bartlett, D.H., Bidle, K.A. (1999). Membrane-Based Adaptations of Deep-Sea Piezophiles.
12 In: Seckbach J (ed) *Enigmatic Microorganisms and Life in Extreme Environments*, Cellular
13 Origin and Life in Extreme Habitats. Springer Netherlands: Dordrecht, pp 501–512.
- 14 Bauer, M.W., Driskill, L.E., Kelly, R.M. (1998). Glycosyl hydrolases from hyperthermophilic
15 microorganisms. *Current Opinion in Biotechnology* 9:141–145.
- 16 Bäuerlein, E. (2003). Biomineralization of Unicellular Organisms: An Unusual Membrane
17 Biochemistry for the Production of Inorganic Nano- and Microstructures. *Angewandte*
18 *Chemie International Edition* 42:614–641.
- 19 Bauvois, C., Jacquamet, L., Huston, A.L., Borel, F., Feller, G., Ferrer, J.-L. (2008). Crystal
20 Structure of the Cold-active Aminopeptidase from *Colwellia psychrerythraea*, a Close
21 Structural Homologue of the Human Bifunctional Leukotriene A4 Hydrolase. *J Biol Chem*
22 283:23315–23325.
- 23 Beichman, C., Benneke, B., Knutson, H., Smith, R., Lagage, P.O., Dressing, C., Latham, D.,
24 Lunine, J., Birkmann, S., Ferruit, P., Giardino, G., Kempton, E., Carey, S., Krick, J., Deroo,
25 P.D., Mandell, A., Ressler, M.E., Shporer, A., Swain, M., Vasisht, G., Ricker, G., Bouwman,
26 J., Crossfield, I., Greene, T., Howell, S., Christiansen, J., Ciardi, D., Clampin, M.,
27 Greenhouse, M., Sozzetti, A., Goudfrooij, P., Hines, D., Keyes, T., Lee, J., McCullough, P.,
28 Robberto, M., Stansberry, J., Valenti, J., Rieke, M., Rieke, G., Fortney, J., Bean, J.,
29 Kreidberg, L., Ehrenreich, D., Deming, D., Albert, L., Doyon, R., Sing, D. (2014).
30 Observations of transiting exoplanets with the James Webb Space Telescope (JWST).
31 *Publications of the Astronomical Society of the Pacific* 126:1134–1173.
- 32 Belilla, J., Moreira, D., Jardillier, L., Reboul, G., Benzerara, K., López-García, J.M.,
33 Bertolino, P., López-Archilla, A.I., López-García, P. (2019). Hyperdiverse archaea near life
34 limits at the polyextreme geothermal Dallol area. *Nat Ecol Evol* 3:1552–1561.
- 35 Bellissent-Funel, M.-C., Hassanali, A., Havenith, M., Henchman, R., Pohl, P., Sterpone, F.,
36 van der Spoel, D., Xu, Y., Garcia, A.E. (2016). Water Determines the Structure and Dynamics
37 of Proteins. *Chemical Reviews* 116:7673–7697.
- 38 Ben-Naim, A. (2013). Theory of cold denaturation of proteins. 2013.
- 39 Berliner, A.J., Mochizuki, T., Stedman, K.M. (2018). Astrovirology: Viruses at Large in the
40 Universe. *Astrobiology* 18:207–223.
- 41 Beuthe, M., Rivoldini, A., Trinh, A. (2017). Enceladus's and Dione's floating ice shells
42 supported by minimum stress isostasy. *Geophysical Research Letters*:10,088–10,096.
- 43 Birrien, J.-L., Zeng, X., Jebbar, M., Cambon-Bonavita, M.-A., Querellou, J., Oger, P.,
44 Bienvenu, N., Xiao, X., Prieur, D. (2011). *Pyrococcus yayanosii* sp. nov., an obligate
45 piezophilic hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent.
46 *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*
47 61:2827–2881.
- 48 Björn, L., Papageorgiou, G., Dravins, D., Govindjee, G. (2009). Detectability of life and
49 photosynthesis on exoplanets. *Current Science* 96:1171–1175.
- 50 Boer, J.L., Mulrooney, S.B., Hausinger, R.P. (2014). Nickel-dependent metalloenzymes.
51 *Archives of Biochemistry and Biophysics* 544:142–152.
- 52 Bonfils, X., Curto, G.L., Correia, A.C.M., Laskar, J., Udry, S., Delfosse, X., Forveille, T.,
53
54
55
56
57
58
59
60

- 1
2
3 Astudillo-Defru, N., Benz, W., Bouchy, F., Gillon, M., Hébrard, G., Lovis, C., Mayor, M.,
4 Moutou, C., Naef, D., Neves, V., Pepe, F., Perrier, C., Queloz, D., Santos, N.C., Ségransan,
5 D. (2013). The HARPS search for southern extra-solar planets - XXXIV. A planetary system
6 around the nearby M dwarf GJ 163, with a super-Earth possibly in the habitable zone. *A&A*
7 556:A110.
8
9 Bonfils, X., Delfosse, X., Udry, S., Forveille, T., Mayor, M., Perrier, C., Bouchy, F., Gillon,
10 M., Lovis, C., Pepe, F., Queloz, D., Santos, N.C., Ségransan, D., Bertaux, J.-L. (2013). The
11 HARPS search for southern extra-solar planets - XXXI. The M-dwarf sample. *A&A*
12 549:A109.
13
14 Bonneté, F., Ebel, C., Zaccai, G., Eisenberg, H. (1993). Biophysical study of halophilic
15 malate dehydrogenase in solution: revised subunit structure and solvent interactions of native
16 and recombinant enzyme. *J Chem Soc, Faraday Trans* 89:2659–2666.
17
18 Bonneté, F., Madern, D., Zaccai, G. (1994). Stability against denaturation mechanisms in
19 halophilic malate dehydrogenase ‘adapt’ to solvent conditions. *J Mol Biol* 244:436–447.
20
21 Boston, P.J., Ivanov, M.V., P. McKay, C. (1992). On the possibility of chemosynthetic
22 ecosystems in subsurface habitats on Mars. *Icarus* 95:300–308.
23
24 Bowers, K.J., Mesbah, N.M., Wiegel, J. (2009). Biodiversity of poly-extremophilic Bacteria:
25 Does combining the extremes of high salt, alkaline pH and elevated temperature approach a
26 physico-chemical boundary for life? *Saline Systems* 5:9.
27
28 Bowers, K.J., Wiegel, J. (2011). Temperature and pH optima of extremely halophilic archaea:
29 a mini-review. *Extremophiles* 15:119–128.
30
31 Bowman, J.S., Deming, J.W. (2014). Alkane hydroxylase genes in psychrophile genomes and
32 the potential for cold active catalysis. *BMC genomics* 15:1120.
33
34 Brady, J.B. (1992). Does Ice Dissolve or Does Halite Melt? *Journal of Geological Education*
35 40:116–118.
36
37 Brandenburg, J.E. (1987). The Paleo-ocean of Mars
38
39 Bridgman, P.W. (1914). The Coagulation of Albumen by Pressure. *J Biol Chem* 19:511–512.
40
41 Britton, K.L., Baker, P.J., Fisher, M., Ruzheinikov, S., Gilmour, D.J., Bonete, M.-J., Ferrer,
42 J., Pire, C., Esclapez, J., Rice, D.W. (2006). Analysis of protein solvent interactions in
43 glucose dehydrogenase from the extreme halophile *Haloferax mediterranei*. *PNAS* 103:4846–
44 4851.
45
46 Brown, M.E., Hand, K.P. (2013). SALTS AND RADIATION PRODUCTS ON THE
47 SURFACE OF EUROPA. *AJ* 145:110.
48
49 Brown, S.H., Kelly, R.M. (1993). Characterization of Amylolytic Enzymes, Having Both α -
50 1,4 and α -1,6 Hydrolytic Activity, from the Thermophilic Archaea *Pyrococcus furiosus* and
51 *Thermococcus litoralis*. *Appl Environ Microbiol* 59:2614–2621.
52
53 Bryson, S., Kunimoto, M., Kopparapu, R.K., Coughlin, J.L., Borucki, W.J., Koch, D.,
54 Aguirre, V.S., Allen, C., Zamudio, K.A., *et al.* (2020). The Occurrence of Rocky Habitable
55 Zone Planets Around Solar-Like Stars from Kepler Data. *arXiv:201014812 [astro-ph]*.
56
57 Burton, N.P., Norris, P.R. (2000). Microbiology of acidic, geothermal springs of Montserrat:
58 environmental rDNA analysis. *Extremophiles* 4:315–320.
59
60 Cable, M.L., Clark, K., Lunine, J.I., Postberg, F., Reh, K., Spilker, L., Waite, J.H. (2016).
61 Enceladus Life Finder: the search for life in a habitable moon.
62
63 Calegari-Santos, R., Diogo, R.A., Fontana, J.D., Bonfim, T.M.B. (2016). Carotenoid
64 Production by Halophilic Archaea Under Different Culture Conditions. *Curr Microbiol*
65 72:641–651.
66
67 Canganella, F., Wiegel, J. (2011). Extremophiles: from abyssal to terrestrial ecosystems and
68 possibly beyond. *Naturwissenschaften* 98:253–279.
69
70 Capece, M.C., Clark, E., Saleh, J.K., Halford, D., Heinl, N., Hoskins, S., Rothschild, L.J.
71 (2013). Polyextremophiles and the Constraints for Terrestrial Habitability. In: Seckbach J,

- Oren A, Stan-Lotter H (eds) *Polyextremophiles: life under multiple forms of stress*, Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer Netherlands: Dordrecht Vol 27, pp 3–59.
- Caro, G.M.M., Dartois, E. (2013). Prebiotic chemistry in icy grain mantles in space. An experimental and observational approach. *Chem Soc Rev* 42:2173–2185.
- Carr, M.H., Belton, M.J.S., Chapman, C.R., Davies, M.E., Geissler, P., Greenberg, R., McEwen, A.S., Tufts, B.R., Greeley, R., Sullivan, R., Head, J.W., Pappalardo, R.T., Klaasen, K.P., Johnson, T.V., Kaufman, J., Senske, D., Moore, J., Neukum, G., Schubert, G., Burns, J.A., Thomas, P., Veverka, J. (1998). Evidence for a subsurface ocean on Europa. *Nature* 391:363–365.
- Carrizo, D., Sánchez-García, L., Rodriguez, N., Gómez, F. (2019). Lipid Biomarker and Carbon Stable Isotope Survey on the Dallol Hydrothermal System in Ethiopia. *Astrobiology* 19:1474–1489.
- Casanueva, A., Tuffin, M., Cary, C., Cowan, D.A. (2010). Molecular adaptations to psychrophily: the impact of ‘omic’ technologies. *Trends in Microbiology* 18:374–381.
- Catchpole, R.J., Forterre, P. (2019). The Evolution of Reverse Gyrase Suggests a Nonhyperthermophilic Last Universal Common Ancestor. *Mol Biol Evol* 36:2737–2747.
- Cavalazzi, B., Barbieri, R., Gómez, F., Capaccioni, B., Olsson-Francis, K., Pondrelli, M., Rossi, A. p., Hickman-Lewis, K., Agangi, A., Gasparotto, G., Glamoclija, M., Ori, G. g., Rodriguez, N., Hagos, M. (2019). The Dallol Geothermal Area, Northern Afar (Ethiopia)—An Exceptional Planetary Field Analog on Earth. *Astrobiology* 19:553–578.
- Cavicchioli, R. (2002). Extremophiles and the Search for Extraterrestrial Life. *Astrobiology* 2:281–292.
- Cavicchioli, R. (2006). Cold-adapted archaea. *Nature Reviews Microbiology* 4:331–343.
- Cavicchioli, R. (2015). On the concept of a psychrophile. *The ISME Journal*.
- Cech, T.R. (2000). The Ribosome Is a Ribozyme. *Science* 289:878–879.
- Chapman, M.G. (2003). Sub-ice volcanoes and ancient oceans/lakes: a Martian challenge. *Global and Planetary Change* 35:185–198.
- Chela-Flores, J. (2019). Testing S isotopes as biomarkers for Mars. *International Journal of Astrobiology* 18:436–439.
- Chevrier, V.F., Rivera-Valentin, E.G. (2012). Formation of recurring slope lineae by liquid brines on present-day Mars. *Geophysical Research Letters* 39.
- Chilukuri, L., Bartlett, D., Fortes, G. (2002). Comparison of high pressure-induced dissociation of single-stranded DNA-binding protein (SSB) from high pressure-sensitive and high pressure-adapted marine *Shewanella* species. *Extremophiles* 6:377–383.
- Chin, J.P., Megaw, J., Magill, C.L., Nowotarski, K., Williams, J.P., Bhaganna, P., Linton, M., Patterson, M.F., Underwood, G.J.C., Mswaka, A.Y., Hallsworth, J.E. (2010). Solutes determine the temperature windows for microbial survival and growth. *PNAS* 107:7835–7840.
- Chivian, D., Brodie, E.L., Alm, E.J., Culley, D.E., Dehal, P.S., DeSantis, T.Z., Gihring, T.M., Lapidus, A., Lin, L.-H., Lowry, S.R., Moser, D.P., Richardson, P.M., Southam, G., Wanger, G., Pratt, L.M., Andersen, G.L., Hazen, T.C., Brockman, F.J., Arkin, A.P., Onstott, T.C. (2008). Environmental Genomics Reveals a Single-Species Ecosystem Deep Within Earth. *Science* 322:275–278.
- Choi, I.-G., Bang, W.-G., Kim, S.-H., Yu, Y.G. (1999). Extremely Thermostable Serine-type Protease from *Aquifex pyrophilus* MOLECULAR CLONING, EXPRESSION, AND CHARACTERIZATION. *J Biol Chem* 274:881–888.
- Chyba, C.F., Hand, K.P. (2001). Life Without Photosynthesis. *Science* 292:2026–2027.
- Chyba, C.F., Phillips, C.B. (2001). Possible ecosystems and the search for life on Europa. *Proceedings of the National Academy of Sciences* 98:801–804.
- Cleaves, H.J. (2014). Prebiotic Synthesis of Biochemical Compounds. In: *Astrobiology: An*

1
2
3 *Evolutionary Approach*, CRC Press, p .

4 Cleaves, H.J., Chalmers, J.H. (2004). Extremophiles May Be Irrelevant to the Origin of Life.
5 *Astrobiology* 4:1–9.

6 Clifford, S.M. (1993). A model for the hydrologic and climatic behavior of water on Mars.
7 *Journal of Geophysical Research: Planets* 98:10973–11016.

8 Clifford, S.M., Lasue, J., Heggy, E., Boisson, J., McGovern, P., Max, M.D. (2010). Depth of
9 the Martian cryosphere: Revised estimates and implications for the existence and detection of
10 subpermafrost groundwater. *Journal of Geophysical Research* 115.

11 Cobbett, C.S. (2000). Phytochelatin biosynthesis and function in heavy-metal detoxification.
12 *Current Opinion in Plant Biology* 3:11–16.

13 Cockell, C.S., Bagshaw, E., Balme, M., Doran, P., McKay, C.P., Miljkovic, K., Pearce, D.,
14 Siegert, M.J., Tranter, M., Voytek, M., Wadham, J. (2011). Subglacial environments and the
15 search for life beyond the Earth. In: Siegert MJ, Kennicutt MC, Bindschadler RA (eds)
16 *Geophysical Monograph Series*, American Geophysical Union: Washington, D. C. Vol 192,
17 pp 129–148.

18 Cohen, R.D., Pielak, G.J. (2017). A cell is more than the sum of its (dilute) parts: A brief
19 history of quinary structure. *Protein Science* 26:403–413.

20 Connelly, D.P., Copley, J.T., Murton, B.J., Stansfield, K., Tyler, P.A., German, C.R., Dover,
21 C.L.V., Amon, D., Furlong, M., Grindlay, N., Hayman, N., Hühnerbach, V., Judge, M., Bas,
22 T.L., McPhail, S., Meier, A., Nakamura, K., Nye, V., Pebody, M., Pedersen, R.B., Plouviez,
23 S., Sands, C., Searle, R.C., Stevenson, P., Taws, S., Wilcox, S. (2012). Hydrothermal vent
24 fields and chemosynthetic biota on the world’s deepest seafloor spreading centre. *Nat*
25 *Commun* 3:1–9.

26 Cono, V.L., Bortoluzzi, G., Messina, E., Spada, G.L., Smedile, F., Giuliano, L., Borghini, M.,
27 Stumpp, C., Schmitt-Kopplin, P., Harir, M., O’Neill, W.K., Hallsworth, J.E., Yakimov, M.
28 (2019). The discovery of Lake Hephæstus , the youngest athalassohaline deep-sea formation
29 on Earth. *Sci Rep* 9:1–11.

30 Cooper, G., Kimmich, N., Belisle, W., Sarinana, J., Brabham, K., Garrel, L. (2001).
31 Carbonaceous meteorites as a source of sugar-related organic compounds for the early Earth.
32 *Nature* 414:879–883.

33 Coquelle, N., Talon, R., Juers, D.H., Girard, É., Kahn, R., Madern, D. (2010). Gradual
34 Adaptive Changes of a Protein Facing High Salt Concentrations. *Journal of Molecular*
35 *Biology* 404:493–505.

36 Cordone, L., Ferrand, M., Vitrano, E., Zaccai, G. (1999). Harmonic Behavior of Trehalose-
37 Coated Carbon-Monoxo-Myoglobin at High Temperature. *Biophysical Journal* 76:1043–
38 1047.

39 Costenaro, L., Zaccai, G., Ebel, C. (2002). Link between Protein–Solvent and Weak
40 Protein–Protein Interactions Gives Insight into Halophilic Adaptation. *Biochemistry*
41 41:13245–13252.

42 Cottin, H. (2019). What is astrobiology ?

43 Cronin, J.R., Pizzarello, S. (1983). Amino acids in meteorites. *Advances in Space Research*
44 3:5–18.

45 Crowe, J.H., Hoekstra, F.A., Crowe, L.M. (1992). Anhydrobiosis. *Annual Review of*
46 *Physiology* 54:579–599.

47 Da Poian, A.T., Oliveira, A.C., Silva, J.L. (1995). Cold Denaturation of an Icosahedral Virus.
48 The Role of Entropy in Virus Assembly. *Biochemistry* 34:2672–2677.

49 Dadachova, E., Bryan, R.A., Howell, R.C., Schweitzer, A.D., Aisen, P., Nosanchuk, J.D.,
50 Casadevall, A. (2007). The radioprotective properties of fungal melanin are a function of its
51 chemical composition, stable radical presence and spatial arrangement: Radioprotective
52 properties of fungal melanin. *Pigment Cell & Melanoma Research* 21:192–199.

- 1
2
3 Dadachova, E., Bryan, R.A., Huang, X., Moadel, T., Schweitzer, A.D., Aisen, P., Nosanchuk,
4 J.D., Casadevall, A. (2007). Ionizing Radiation Changes the Electronic Properties of Melanin
5 and Enhances the Growth of Melanized Fungi (J Rutherford, Ed.). *PLoS ONE* 2:e457.
6 Dadachova, E., Casadevall, A. (2008). Ionizing radiation: how fungi cope, adapt, and exploit
7 with the help of melanin. *Current Opinion in Microbiology* 11:525–531.
8 Dalmasso, C., Oger, P., Courtine, D., Georges, M., Takai, K., Maignien, L., Alain, K. (2016).
9 Complete Genome Sequence of the Hyperthermophilic and Piezophilic Archeon
10 *Thermococcus piezophilus* CDGST, Able To Grow under Extreme Hydrostatic Pressures.
11 *Genome Announc* 4.
12 Daly, M.J. (2009). A new perspective on radiation resistance based on *Deinococcus*
13 *radiodurans*. *Nature Reviews Microbiology* 7:237–245.
14 D’Amico, S., Collins, T., Marx, J.-C., Feller, G., Gerday, C. (2006). Psychrophilic
15 microorganisms: challenges for life. *EMBO reports* 7:385–389.
16 D’Amico, S., Marx, J.-C., Gerday, C., Feller, G. (2003). Activity-Stability Relationships in
17 Extremophilic Enzymes. *J Biol Chem* 278:7891–7896.
18 Damm, K.L.V. (2013). Controls on the Chemistry and Temporal Variability of Seafloor
19 Hydrothermal Fluids. In: *Seafloor Hydrothermal Systems: Physical, Chemical, Biological,*
20 *and Geological Interactions*, American Geophysical Union (AGU), pp 222–247.
21 Daniel, R.M., Cowan, D.A. (2000). Biomolecular stability and life at high temperatures.
22 *Cellular and Molecular Life Sciences CMLS* 57:250–264.
23 Daniel, R., Dines, M., Petach, H. (1996). The denaturation and degradation of stable enzymes
24 at high temperatures. *Biochem J* 317:1–11.
25 Daniel, I., Oger, P., Winter, R. (2006). Origins of life and biochemistry under high-pressure
26 conditions. *Chemical Society Reviews* 35:858–875.
27 Daniels, M.J., Turner-Cavet, J.S., Selkirk, R., Sun, H., Parkinson, J.A., Sadler, P.J., Robinson,
28 N.J. (1998). Coordination of Zn²⁺ (and Cd²⁺) by Prokaryotic Metallothionein. *Journal of*
29 *Biological Chemistry* 273:22957–22961.
30 Darwin, C. (1839). *The Voyage of the Beagle*. P.F. Collier.
31 Das, K.P., Surewicz, W.K. (1995). Temperature-induced exposure of hydrophobic surfaces
32 and its effect on the chaperone activity of α -crystallin. *FEBS Letters* 369:321–325.
33 DasSarma, S. (2006). Extreme Halophiles Are Models for Astrobiology. *Microbe Magazine*
34 1:120–126.
35 DasSarma, S., DasSarma, P., Laye, V.J., Schwieterman, E.W. (2020). Extremophilic models
36 for astrobiology: haloarchaeal survival strategies and pigments for remote sensing.
37 *Extremophiles* 24:31–41.
38 Davies, G.J., Gamblin, S.J., Littlechild, J.A., Watson, H.C. (1993). The structure of a
39 thermally stable 3-phosphoglycerate kinase and a comparison with its mesophilic equivalent.
40 *Proteins: Structure, Function, and Bioinformatics* 15:283–289.
41 De Rosa, M., Gambacorta, A. (1988). The lipids of archaebacteria. *Progress in Lipid*
42 *Research* 27:153–175.
43 Delfosse, X., Bonfils, X., Forveille, T., Udry, S., Mayor, M., Bouchy, F., Gillon, M., Lovis,
44 C., Neves, V., Pepe, F., Perrier, C., Queloz, D., Santos, N.C., Ségransan, D. (2013). The
45 HARPS search for southern extra-solar planets - XXXIII. Super-Earths around the M-dwarf
46 neighbors Gl 433 and Gl 667C. *A&A* 553:A8.
47 DeLong, E.F., Yayanos, A.A. (1985). Adaptation of the membrane lipids of a deep-sea
48 bacterium to changes in hydrostatic pressure. *Science* 228:1101–1103.
49 DeMaere, M.Z., Williams, T.J., Allen, M.A., Brown, M.V., Gibson, J.A.E., Rich, J., Lauro,
50 F.M., Dyllal-Smith, M., Davenport, K.W., Woyke, T., Kyrpides, N.C., Tringe, S.G.,
51 Cavicchioli, R. (2013). High level of intergenera gene exchange shapes the evolution of
52 haloarchaea in an isolated Antarctic lake. *PNAS* 110:16939–16944.
53
54
55
56
57
58
59
60

- 1
2
3 Demirjian, D.C., Moris-Varas, F., Cassidy, C.S. (2001). Enzymes from extremophiles.
4 *Current Opinion in Chemical Biology* 5:144–151.
- 5 Deniz, A.A. (2018). Enzymes can adapt to cold by wiggling regions far from their active site.
6 *Nature* 558:195–196.
- 7
8 Dexter, Y., Cooke, R.C. (1984). Fatty acids, sterols and carotenoids of the psychrophile
9 *Mucor Strictus* and some mesophilic *Mucor* species. *Transactions of the British Mycological*
10 *Society Journal* 3:455–461.
- 11 Di Achille, G., Hynek, B.M. (2010). Ancient ocean on Mars supported by global distribution
12 of deltas and valleys. *Nature Geoscience* 3:459–463.
- 13 Di giulio, M. (2000). The Universal Ancestor Lived in a Thermophilic or Hyperthermophilic
14 Environment. *Journal of Theoretical Biology* 203:203–213.
- 15 Di Giulio, M. (2005). A comparison of proteins from *Pyrococcus furiosus* and *Pyrococcus*
16 *abyssi*: barophily in the physicochemical properties of amino acids and in the genetic code.
17 *Gene* 346:1–6.
- 18 Dick, M., Weiergräber, O.H., Classen, T., Bisterfeld, C., Bramski, J., Gohlke, H., Pietruszka,
19 J. (2016). Trading off stability against activity in extremophilic aldolases. *Scientific Reports*
20 6:17908.
- 21
22 DiMaio, F., Yu, X., Rensen, E., Krupovic, M., Prangishvili, D., Egelman, K.L. (2015). A
23 virus that infects a hyperthermophile encapsidates A-form DNA. *Science* 348:910–914.
- 24 Dodd, M.S., Papineau, D., Grenne, T., Slack, J.F., Rittner, M., Pirajno, F., O’Neil, J., Little,
25 C.T.S. (2017). Evidence for early life in Earth’s oldest hydrothermal vent precipitates. *Nature*
26 543:60–64.
- 27
28 Doherty, E.A., Doudna, J.A. (2001). Ribozyme Structures and Mechanisms. :20.
- 29 Dopson, M., Baker-Austin, C., Koppineedi, P.R., Bond, P.L. (2003). Growth in sulfidic
30 mineral environments: metal resistance mechanisms in acidophilic micro-organisms.
31 *Microbiology (Reading, Engl)* 149:1959–1970.
- 32 Dopson, M., Ossandon, F.J., Lövgren, L., Holmes, D.S. (2014). Metal resistance or tolerance?
33 Acidophiles confront high metal loads via both abiotic and biotic mechanisms. *Front*
34 *Microbiol* 5.
- 35
36 Dordick, J.S. (1989). Enzymatic catalysis in monophasic organic solvents. *Enzyme and*
37 *Microbial Technology* 11:194–211.
- 38 Dose, K., Bieger-Dose, A., Ernst, B., Feister, U., Gómez-Silva, B., Klein, A., Risi, S., Stridde,
39 C. (2001). Survival of Microorganisms under the Extreme Conditions of the Atacama Desert.
40 *Orig Life Evol Biosph* 31:287–303.
- 41
42 Doyon, R., Lafrenière, D., Albert, L., Artigau, E., Meyer, M., Jayawardhana, R. (2014).
43 Transit Spectroscopy with NIRISS on JWST. *Search for Life Beyond the Solar System*
44 *Exoplanets, Biosignatures & Instruments*:3.6.
- 45 Dressing, C.D., Charbonneau, D. (2015). The Occurrence of Potentially Habitable Planets
46 Orbiting M Dwarfs Estimated from the Full Kepler Dataset and an Empirical Measurement of
47 the Detection Sensitivity. *The Astrophysical Journal* 807:45.
- 48
49 Driessen, A.J.M., van de Vossenberg, J.L.C.M., Konings, W.N. (1996). Membrane
50 composition and ion-permeability in extremophiles. *FEMS Microbiol Rev* 18:139–148.
- 51 Dubins, D.N., Lee, A., Macgregor, R.B., Chalikian, T.V. (2001). On the Stability of Double
52 Stranded Nucleic Acids. *Journal of the American Chemical Society* 123:9254–9259.
- 53 Dumorne, K., Cordova, D.C., Astorga-Elo, M., Renganathan, P. (2017). Extremozymes: A
54 Potential Source for Industrial Applications. *Journal of Microbiology and Biotechnology*
55 27:649–659.
- 56
57 Dym, O., Mevarech, M., Sussman, J.L. (1995). Structural Features That Stabilize Halophilic
58 Malate Dehydrogenase from an Archaeobacterium. *Science* 267:1344–1346.
- 59 Ebel, C., Costenaro, L., Pascu, M., Faou, P., Kernel, B., Proust-De Martin, F., Zaccai, G.
- 60

- (2002). Solvent Interactions of Halophilic Malate Dehydrogenase. *Biochemistry* 41:13234–13244.
- Edmonds, C.G., Crain, P.F., Gupta, R., Hashizume, T., Hocart, C.H., Kowalak, J.A., Pomerantz, S.C., Stetter, K.O., McCloskey, J.A. (1991). Posttranscriptional modification of tRNA in thermophilic archaea (Archaeobacteria). *Journal of bacteriology* 173:3138–3148.
- Eisenberg, H. (1995). Life in Unusual Environments: Progress in Understanding the Structure and Function of Enzymes from Extreme Halophilic Bacteria. *Archives of Biochemistry and Biophysics* 318:1–5.
- Elcock, A.H. (1998). The stability of salt bridges at high temperatures: implications for hyperthermophilic proteins | Edited by B. Honig. *Journal of Molecular Biology* 284:489–502.
- Ellis, D.G., Bizzoco, R.W., Kelley, S.T. (2008). Halophilic Archaea determined from geothermal steam vent aerosols. *Environmental Microbiology* 10:1582–1590.
- Empadinhas, N., Costa, M.S. da (2006). Diversity and biosynthesis of compatible solutes in hyper/thermophiles. *Diversidade e biossíntese de solutos compatíveis em hiper/termófilas*.
- Fanale, F.P., Li, Y.-H., Carlo, E.D., Farley, C., Sharma, S.K., Horton, K., Granahan, J.C. (2001). An experimental estimate of Europa's "ocean" composition independent of Galileo orbital remote sensing. *Journal of Geophysical Research: Planets* 106:14595–14600.
- Faria, T.Q., Lima, J.C., Bastos, M., Maçanita, A.L., Santos, H. (2004). Protein Stabilization by Osmolytes from Hyperthermophiles EFFECT OF MANNOSYLGLYCERATE ON THE THERMAL UNFOLDING OF RECOMBINANT NUCLEASE A FROM STAPHYLOCOCCUS AUREUS STUDIED BY PICOSECOND TIME-RESOLVED FLUORESCENCE AND CALORIMETRY. *J Biol Chem* 279:48680–48691.
- Feller, G. (2010). Protein stability and enzyme activity at extreme biological temperatures. *J Phys: Condens Matter* 22:323101.
- Feller, G. (2013). Psychrophilic Enzymes: From Folding to Function and Biotechnology. *Scientifica* 2013:e512840.
- Feller, G., Gerday, C. (2003). Psychrophilic enzymes: hot topics in cold adaptation. *Nat Rev Microbiol* 1:200–208.
- Fendrihan, S., Bérces, A., Lammer, H., Musso, M., Rontó, G., Polacsek, T.K., Holzinger, A., Kolb, C., Stan-Lotter, H. (2009). Investigating the Effects of Simulated Martian Ultraviolet Radiation on *Halococcus dombrowskii* and Other Extremely Halophilic Archaeobacteria. *Astrobiology* 9:104–112.
- Fetrow, J.S. (1995). Omega loops; nonregular secondary structures significant in protein function and stability. *The FASEB Journal* 9:708–717.
- Fields, P.A., Somero, G.N. (1998). Hot spots in cold adaptation: Localized increases in conformational flexibility in lactate dehydrogenase A4 orthologs of Antarctic notothenioid fishes. *PNAS* 95:11476–11481.
- Fletcher, L.E., Valdivia-Silva, J.E., Perez-Montaña, S., Condori-Apaza, R.M., Conley, C.A., McKay, C.P. (2012). Variability of organic material in surface horizons of the hyper-arid Mars-like soils of the Atacama Desert. *Advances in Space Research* 49:271–279.
- Fong, N., Burgess, M., Barrow, K., Glenn, D. (2001a). Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. *Appl Microbiol Biotechnol* 56:750–756.
- Fong, N., Burgess, M., Barrow, K., Glenn, D. (2001b). Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. *Appl Microbiol Biotechnol* 56:750–756.
- Fonseca, F., Meneghel, J., Cenard, S., Passot, S., Morris, G.J. (2016). Determination of Intracellular Vitrification Temperatures for Unicellular Micro Organisms under Conditions Relevant for Cryopreservation. *PLoS One* 11.

- 1
2
3 Formisano, V., Atreya, S., Encrenaz, T., Ignatiev, N., Giuranna, M. (2004). Detection of
4 Methane in the Atmosphere of Mars. *Science* 306:1758–1761.
5 Forterre, P. (2002). A hot story from comparative genomics: reverse gyrase is the only
6 hyperthermophile-specific protein. *Trends in Genetics* 18:236–237.
7 Forterre, P., Bergerat, A., Lopez-Garcia, P. (1996). The unique DNA topology and DNA
8 topoisomerases of hyperthermophilic archaea. *FEMS microbiology reviews* 18:237–248.
9 Fortes, A.D. (2000). Exobiological Implications of a Possible Ammonia–Water Ocean inside
10 Titan. *Icarus* 146:444–452.
11 Fox-Powell, M.G., Hallsworth, J.E., Cousins, C.R., Cockell, C.S. (2016). Ionic Strength Is a
12 Barrier to the Habitability of Mars. *Astrobiology* 16:427–442.
13 Friedman, S.M., Oshima, T. (1989). Polyamines of Sulfur-Dependent Archaeobacteria and
14 Their Role in Protein Synthesis. *J Biochem* 105:1030–1033.
15 Frölich, A., Gabel, F., Jasnin, M., Lehnert, U., Oesterhelt, D., Stadler, A.M., Tehei, M., Weik,
16 M., Wood, K., Zaccai, G. (2008). From shell to cell: neutron scattering studies of biological
17 water dynamics and coupling to activity. *Faraday Discuss* 141:117–130.
18 Frolow, F., Harell, M., Sussman, J.L., Mevarech, M., Shoham, M. (1996). Insights into
19 protein adaptation to a saturated salt environment from the crystal structure of a halophilic
20 2Fe-2S ferredoxin. *Nat Struct Mol Biol* 3:452–458.
21 Fujii, S., Nakasone, K., Horikoshi, K. (1999). An Expression of a Cold Shock Inducible Gene
22 CspA Under Hydrostatic Pressure in Deep-Sea Barophilic Bacterium, *Shewanella* sp. Strain
23 DSS12. In: Ludwig H (ed) *Advances in High Pressure Bioscience and Biotechnology*,
24 Springer: Berlin, Heidelberg, pp 21–24.
25 Fumiyoshi, A., Chiaki, K., Koki, H. (1999). Pressure-regulated metabolism in
26 microorganisms. *Trends in Microbiology* 7:447–453.
27 Fürst, M.J., Fiorentini, F., Fraaije, M.W. (2019). Beyond active site residues: overall
28 structural dynamics control catalysis in flavin-containing and heme-containing
29 monooxygenases. *Current Opinion in Structural Biology* 59:29–37.
30 Fütterer, O., Angelov, A., Liesegang, H., Gottschalk, G., Schleper, C., Schepers, B., Dock, C.,
31 Antranikian, G., Liebl, W. (2004). Genome sequence of *Picrophilus torridus* and its
32 implications for life around pH 0. *PNAS* 101:9091–9096.
33 Gaeman, J., Hier-Majumder, S., Roberts, J.H. (2012). Sustainability of a subsurface ocean
34 within Triton’s interior. *Icarus* 220:339–347.
35 Gaidos, E.J., Neelson, K.H., Kirschvink, J. (1999). Life in ice-Covered Oceans. 284:3.
36 Galinski, E.A. (1993). Compatible solutes of halophilic eubacteria: molecular principles,
37 water-solute interaction, stress protection. *Experientia* 49:487–496.
38 Galinski, E.A., Trüper, H.G. (1982). Betaine, a compatible solute in the extremely halophilic
39 phototrophic bacterium *Ectothiorhodospira halochloris*. *FEMS Microbiol Lett* 13:357–360.
40 Galtier, N., Tourasse, N., Gouy, M. (1999). A Nonhyperthermophilic Common Ancestor to
41 Extant Life Forms. *Science* 283:220–221.
42 Gambacorta, A., Gliozzi, A., De Rosa, M. (1995). Archaeal lipids and their biotechnological
43 applications. *World Journal of Microbiology & Biotechnology* 11:115–131.
44 Gargaud, M., Martin, H., López-García, P., Montmerle, T., Pascal, R. (2012). The Messages
45 from the Oldest Terrestrial Rocks. In: Gargaud M, Martin H, López-García P, Montmerle T,
46 Pascal R (eds) *Young Sun, Early Earth and the Origins of Life: Lessons for Astrobiology*,
47 Springer: Berlin, Heidelberg, pp 167–210.
48 Gáspári, Z., Perczel, A. (2010). Protein Dynamics as Reported by NMR. In: *Annual Reports*
49 *on NMR Spectroscopy*, Elsevier Vol 71, pp 35–75.
50 Georgette, D., Jónsson, Z.O., Petegem, F.V., Chessa, J.-P., Beeumen, J.V., Hübscher, U.,
51 Gerday, C. (2000). A DNA ligase from the psychrophile *Pseudoalteromonas haloplanktis*
52 gives insights into the adaptation of proteins to low temperatures. *European Journal of*
53
54
55
56
57
58
59
60

1
2
3 *Biochemistry* 267:3502–3512.

4 Gerday, C., Aittaleb, M., Arpigny, J.L., Baise, E., Chessa, J.-P. (1997). Psychrophilic
5 enzymes: a thermodynamic challenge. *Biochimica et Biophysica Acta* 1342:119–131.

6 Geslin, C., Romancer, M.L., Erauso, G., Gaillard, M., Perrot, G., Prieur, D. (2003). PAV1, the
7 First Virus-Like Particle Isolated from a Hyperthermophilic Euryarchaeote, “*Pyrococcus*
8 *abyssi*”. *Journal of Bacteriology* 185:3888–3894.

9 Gianese, G., Bossa, F., Pascarella, S. (2002). Comparative structural analysis of psychrophilic
10 and meso- and thermophilic enzymes. *Proteins: Structure, Function, and Bioinformatics*
11 47:236–249.

12 Gillon, M., Triaud, A.H.M.J., Demory, B.-O., Jehin, E., Agol, E., Deck, K.M., Lederer, S.M.,
13 de Wit, J., Burdanov, A., Ingalls, J.G., Bolmont, E., Leconte, J., Raymond, S.N., Selsis, F.,
14 Turbet, M., Barkaoui, K., Burgasser, A., Burleigh, M.R., Carey, S.J., Chaushev, A.,
15 Copperwheat, C.M., Delrez, L., Fernandes, C.S., Holdsworth, D.L., Kotze, E.J., Van Grootel,
16 V., Almleaky, Y., Benkhaldoun, Z., Magain, P., Queloz, D. (2017). Seven temperate
17 terrestrial planets around the nearby ultracool dwarf star TRAPPIST-1. *Nature* 542:456–460.

18 Ginzburg, M., Ginzburg, B.Z. (1976). Regulation of cell volume and ion concentrations in
19 Halobacterium. *J Membrin Biol* 26:153–171.

20 Girard, E., Marchal, S., Perez, J., Finet, S., Kahn, R., Fourme, R., Marassio, G., Dhaussy, A.-
21 C., Prangé, T., Giffard, M., Dulin, F., Bonneté, F., Lange, R., Abraini, J.H., Mezouar, M.,
22 Colloc'h, N. (2010). Structure-Function Perturbation and Dissociation of Tetrameric Urate
23 Oxidase by High Hydrostatic Pressure. *Biophysical Journal* 98:2365–2373.

24 Girard, E., Prangé, T., Dhaussy, A.-C., Migianu-Griffoni, E., Lecouvey, M., Chervin, J.-C.,
25 Mezouar, M., Kahn, R., Fourme, R. (2007). Adaptation of the base-paired double-helix
26 molecular architecture to extreme pressure. *Nucleic Acids Res* 35:4800–4808.

27 Giulio, M.D. (2003). The Universal Ancestor was a Thermophile or a Hyperthermophile:
28 Tests and Further Evidence. *Journal of Theoretical Biology* 221:425–436.

29 Glein, C.R., Baross, J.A., Waite, J.H. (2015). The pH of Enceladus’ ocean. *Geochimica et*
30 *Cosmochimica Acta* 162:202–219.

31 Gliozzi, A., Paoli, G., De Rosa, M., Gambacorta, A. (1983). Effect of isoprenoid cyclization
32 on the transition temperature of lipids in thermophilic archaeobacteria. *Biochimica et*
33 *Biophysica Acta*:234–242.

34 Goldman, A. (1995). How to make my blood boil. *Structure* 3:1277–1279.

35 Gómez, F., Cavalazzi, B., Rodríguez, N., Amils, R., Ori, G.G., Olsson-Francis, K., Escudero,
36 C., Martínez, J.M., Miruts, H. (2019). Ultra-small microorganisms in the polyextreme
37 conditions of the Dallol volcano, Northern Afar, Ethiopia. *Scientific Reports* 9:7907.

38 González, J.M., Sheckells, D., Viebahn, M., Krupatkina, D., Borges, K.M., Robb, F.T.
39 (1999). *Thermococcus waiotapuensis* sp. nov., an extremely thermophilic archaeon isolated
40 from a freshwater hot spring. *Arch Microbiol* 172:95–101.

41 Goodrich, R.P., Handel, T.M., Baldeschwieler, J.D. (1988). Modification of lipid phase
42 behavior with membrane-bound cryoprotectants. *Biochim Biophys Acta* 938:143–154.

43 Galnack, J.A., Newman, D.K. (2007). Extracellular respiration. *Mol Microbiol* 65:1–11.

44 Grasset, O., Sotin, C. (1996). The Cooling Rate of a Liquid Shell in Titan’s Interior. *Icarus*
45 123:101–112.

46 Grayling, R.A., Sandman, K., Reeve, J.N. (1996). Histones and chromatin structure in
47 hyperthermophilic Archaea. *FEMS microbiology reviews* 18:203–214.

48 Green, G.R., Searcy, D.G., DeLange, R.J. (1983). Histone-like protein in the
49 Archaeobacterium *Sulfolobus acidocaldarius*. *Biochimica et Biophysica Acta (BBA) - Gene*
50 *Structure and Expression* 741:251–257.

51 Greenberg, R. (2009). *Europa – The Ocean Moon: Search For An Alien Biosphere*. Springer
52 Science & Business Media.
53
54
55
56
57
58
59
60

- 1
2
3 Gress, T., Mirault, P. (2016). *La philosophie au risque de l'intelligence extraterrestre*.
4 Librairie Philosophique J. Vrin.
- 5 Grindrod, P.M., Fortes, A.D., Nimmo, F., Feltham, D.L., Brodholt, J.P., Vočadlo, L. (2008).
6 The long-term stability of a possible aqueous ammonium sulfate ocean inside Titan. *Icarus*
7 197:137–151.
- 8 Gross, M., Jaenicke, R. (1994). Proteins under pressure. The influence of high hydrostatic
9 pressure on structure, function and assembly of proteins and protein complexes. *European*
10 *Journal of Biochemistry* 221:617–630.
- 11 Gross, M., Lehle, K., Jaenicke, R., Nierhaus, K.H. (1993). Pressure-induced dissociation of
12 ribosomes and elongation cycle intermediates. *European Journal of Biochemistry* 218:463–
13 468.
- 14 Grotzinger, J.P., Sumner, D.Y., Kah, L.C., Stack, K., Gupta, S., Edgar, L., Rubin, D., Lewis,
15 K., Team, M.S., *et al.* (2014). A Habitable Fluvio-Lacustrine Environment at Yellowknife
16 Bay, Gale Crater, Mars. *Science* 343.
- 17 Gunbin, K.V., Afonnikov, D.A., Kolchanov, N.A. (2009). Molecular evolution of the
18 hyperthermophilic archaea of the Pyrococcus genus: analysis of adaptation to different
19 environmental conditions. *BMC Genomics* 10:639.
- 20 Gunter, T.E., Gunter, K.K. (1972). Pressure dependence of the helix–coil transition
21 temperature for polynucleic acid helices. *Biopolymers* 11:667–678.
- 22 Hallsworth, J.E. (2019). Microbial unknowns at the saline limits for life. *Nature Ecology &*
23 *Evolution* 3:1503–1504.
- 24 Hallsworth, J.E., Yakimov, M.M., Golyshin, P.N., Gillion, J.L.M., D'Auria, G., Alves,
25 F.D.L., Cono, V.L., Genovese, M., McKew, B.A., Hayes, S.L., Harris, G., Giuliano, L.,
26 Timmis, K.N., McGenity, T.J. (2007). Limits of life in MgCl₂-containing environments:
27 chaotropicity defines the window. *Environmental Microbiology* 9:801–813.
- 28 Han, M.-J., Lee, S.Y. (2006). The Escherichia coli Proteome: Past, Present, and Future
29 Prospects. *Microbiol Mol Biol Rev* 70:362–439.
- 30 Han, H., Ling, Z., Khan, A., Virk, A.K., Kulshrestha, S., Li, X. (2019). Improvements of
31 thermophilic enzymes: From genetic modifications to applications. *Bioresource Technology*
32 279:350–361.
- 33 Hand, K.P., Chyba, C.F. (2007). Empirical constraints on the salinity of the european ocean
34 and implications for a thin ice shell. *Icarus* 189:424–438.
- 35 Haney, P.J., Badger, J.H., Buldak, G.L., Reich, C.I., Woese, C.R., Olsen, G.J. (1999).
36 Thermal adaptation analyzed by comparison of protein sequences from mesophilic and
37 extremely thermophilic Methanococcus species. *PNAS* 96:3578–3583.
- 38 Hansen, C.J., Esposito, L., Stewart, A.I.F., Colwell, J., Hendrix, A., Pryor, W., Shemansky,
39 D., West, R. (2006). Enceladus' Water Vapor Plume. *Science* 311:1422–1425.
- 40 Harding, T. (2018). Molecular Adaptations in Extremely Halophilic Protists.
- 41 Harris, H.J.H., Cartwright, K. (2013). Hydrology of the Don Juan Basin, Wright Valley,
42 Antarctica. In: *Dry Valley Drilling Project*, American Geophysical Union (AGU), pp 161–
43 184.
- 44 Harrison, J.P., Gheeraert, N., Tsigelnitskiy, D., Cockell, C.S. (2013). The limits for life under
45 multiple extremes. *Trends in Microbiology* 21:204–212.
- 46 Hashim, N.H.F., Mahadi, N.M., Ilias, R.M., Feroz, S.R., Abu Bakar, F.D., Murad, A.M.A.
47 (2018). Biochemical and structural characterization of a novel cold-active esterase-like
48 protein from the psychrophilic yeast Glaciozyma antarctica. *Extremophiles* 22:607–616.
- 49 Hatanaka, H., Tanimura, R., Katoh, S., Inagaki, F. (1997). Solution structure of ferredoxin
50 from the thermophilic Cyanobacterium Synechococcus elongatus and its
51 thermostability. Edited by P.E. Wright. *Journal of Molecular Biology* 268:922–933.
- 52 Hazel, J.R., Eugene Williams, E. (1990). The role of alterations in membrane lipid
53
54
55
56
57
58
59
60

- composition in enabling physiological adaptation of organisms to their physical environment. *Progress in Lipid Research* 29:167–227.
- Hazen, R.M., Boctor, N., Brandes, J.A., Cody, G.D., Hemley, R.J., Sharma, A., Yoder, H.S. (2002). High pressure and the origin of life. *J Phys: Condens Matter* 14:11489–11494.
- Head, J.W., Forget, F., Wordsworth, R., Turbet, M., Cassanelli, J., Palumbo, A. (2018). Two Oceans on Mars?: History, Problems, and Prospects. *Lunar and Planetary Science Conference*:2194.
- Hedén, C.G., Lindahl, T., Toplin, I. (1964). The Stability of Deoxyribonucleic Acid Solutions under High Pressure. *Acta Chemica Scandinavica* 18:1150–1156.
- von Hegner, I. (2019a). An ab initio definition of life pertaining to Astrobiology. *Unpublished*.
- von Hegner, I. (2019b). Astrobiology and Astrophilosophy: Subsuming or bifurcating disciplines? *Philosophy and Cosmology* 23.
- von Hegner, I. (2020). Extremophiles: a special or general case in the search for extra-terrestrial life? *Extremophiles* 24:167–175.
- Heine, M., Chandra, S.B.C. (2009). The linkage between reverse gyrase and hyperthermophiles: A review of their invariable association. *J Microbiol* 47:229.
- Henderson, R., Krude, T. (2004). *DNA: Changing Science and Society*. Cambridge University Press.
- Hensel, R., König, H. (1988). Thermoadaptation of methanogenic bacteria by intracellular ion concentration. *FEMS Microbiol Lett* 49:75–79.
- Hinman, N.W. (2013). Water-Rock Interaction and Life. *Procedia Earth and Planetary Science* 7:354–359.
- Hite, B.H., Giddings, N.J., Weakley, C.E. (1914). *The Effect of Pressure on Certain Micro-Organisms Encountered in the Preservation of Fruits and Vegetables*. West Virginia University Agricultural Experiment Station.
- Hoehler, T.M., Jørgensen, B.B. (2013). Microbial life under extreme energy limitation. *Nature Reviews Microbiology* 11:83–94.
- Horikoshi, K. (1999). Alkaliphiles: Some Applications of Their Products for Biotechnology. *Microbiol Mol Biol Rev* 63:735–750.
- Hörst, S. m., Yelle, R. v., Buch, A., Carrasco, N., Cernogora, G., Dutuit, O., Quirico, E., Sciamma-O'Brien, E., Smith, M. a., Somogyi, Á., Szopa, C., Thissen, R., Vuitton, V. (2012). Formation of Amino Acids and Nucleotide Bases in a Titan Atmosphere Simulation Experiment. *Astrobiology* 12:809–817.
- Howard, E.I., Blakeley, M.P., Haertlein, M., Haertlein, I.P.-, Mitschler, A., Fisher, S.J., Siah, A.C.-, Salvay, A.G., Popov, A., Dieckmann, C.M.-, Petrova, T., Podjarny, A. (2011). Neutron structure of type-III antifreeze protein allows the reconstruction of AFP–ice interface. *Journal of Molecular Recognition* 24:724–732.
- Howard, E., Blakeley, M.P., Salvay, A.G., Podjarny, A. (2014). Use of neutron scattering techniques for Antifreeze Protein mechanistic studies. *Neutron News* 25:24–27.
- Hu, P., Janga, S.C., Babu, M., Díaz-Mejía, J.J., Butland, G., Yang, W., Pogoutse, O., Guo, X., Phanse, S., Wong, P., Chandran, S., Christopoulos, C., Nazarians-Armavil, A., Nasser, N.K., Musso, G., Ali, M., Nazemof, N., Eroukova, V., Golshani, A., Paccanaro, A., Greenblatt, J.F., Moreno-Hagelsieb, G., Emili, A. (2009). Global functional atlas of Escherichia coli encompassing previously uncharacterized proteins. *PLoS Biol* 7:e96.
- Huang, H.-W., Lung, H.-M., Yang, B.B., Wang, C.-Y. (2014). Responses of microorganisms to high hydrostatic pressure processing. *Food Control* 40:250–259.
- Hughes, F., Steiner, R.F. (1966). Effects of pressure on the helix–coil transitions of the poly A–poly U system. *Biopolymers* 4:1081–1090.
- Huston, A.L., Haeggström, J.Z., Feller, G. (2008). Cold adaptation of enzymes: Structural,

- kinetic and microcalorimetric characterizations of an aminopeptidase from the Arctic psychrophile *Colwellia psychrerythraea* and of human leukotriene A4 hydrolase. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1784:1865–1872.
- Hutchinson, F. (1985). Chemical changes induced in DNA by ionizing radiation. *Prog Nucleic Acid Res Mol Biol* 32:115–154.
- Hutchinson, E.G., Thornton, J.M. (1994). A revised set of potentials for beta-turn formation in proteins. *Protein Sci* 3:2207–2216.
- Hynek, B.M., Rogers, K.L., Antunovich, M., Avard, G., Alvarado, G.E. (2018). Lack of Microbial Diversity in an Extreme Mars Analog Setting: Poás Volcano, Costa Rica. *Astrobiology* 18:923–933.
- Iess, L., Stevenson, D.J., Parisi, M., Hemingway, D., Jacobson, R.A., Lunine, J.I., Nimmo, F., Armstrong, J.W., Asmar, S.W., Ducci, M., Tortora, P. (2014). The Gravity Field and Interior Structure of Enceladus. *Science* 344:78–80.
- Imhoff, J.F., Rodriguez-Valera, F. (1984). Betaine is the main compatible solute of halophilic eubacteria. *Journal of Bacteriology* 160:478–479.
- Inouye, M., Phadtare, S. (2008). The Cold Shock Response. *EcoSal Plus* 3.
- Irimia, A., Vellieux, F.M.D., Madern, D., Zaccai, G., Karshikoff, A., Tibbelin, G., Ladenstein, R., Lien, T., Birkeland, N.-K. (2004). The 2.9Å Resolution Crystal Structure of Malate Dehydrogenase from *Archaeoglobus fulgidus*: Mechanisms of Oligomerisation and Thermal Stabilisation. *Journal of Molecular Biology* 335:343–356.
- Ishihama, Y., Schmidt, T., Rappsilber, J., Mann, M., Hartl, F.U., Kerner, M.J., Frishman, D. (2008). Protein abundance profiling of the *Escherichia coli* cytosol. *BMC Genomics* 9:102.
- Islas, S., Velasco, A.M., Becerra, A., Delaye, L., Lazcano, A. (2003). Hyperthermophily and the origin and earliest evolution of life. *Int Microbiol* 6:87–94.
- Islas, S., Velasco, A.M., Becerra, A., Delaye, L., Lazcano, A. (2007). Extremophiles and the Origin of Life. *Physiology and Biochemistry of Extremophiles*:3–10.
- Itävaara, M., Salavirta, H., Marjamaa, K., Ruskeeniemi, T. (2016). Geomicrobiology and Metagenomics of Terrestrial Deep Subsurface Microbiomes. In: *Advances in Applied Microbiology*, Elsevier Vol 94, pp 1–77.
- Iwahashi, H., Kaul, S.C., Obuchi, K., Komatsu, Y. (1991). Induction of barotolerance by heat shock treatment in yeast. *FEMS Microbiol Lett* 80:325–328.
- Jagannadham, M.V., Chattopadhyay, M.K., Subbalakshmi, C., Vairamani, M., Narayanan, K., Mohan Rao, C., Shivaji, S. (2000). Carotenoids of an Antarctic psychrotolerant bacterium, *Sphingobacterium antarcticus*, and a mesophilic bacterium, *Sphingobacterium multivorum*. *Arch Microbiol* 173:418–424.
- Jamroze, A., Perugino, G., Valenti, A., Rashid, N., Rossi, M., Akhtar, M., Ciaramella, M. (2014). The Reverse Gyrase from *Pyrobaculum calidifontis*, a Novel Extremely Thermophilic DNA Topoisomerase Endowed with DNA Unwinding and Annealing Activities. *J Biol Chem* 289:3231–3243.
- Jasnin, M., Stadler, A., Tehei, M., Zaccai, G. (2010). Specific cellular water dynamics observed in vivo by neutron scattering and NMR. *Phys Chem Chem Phys* 12:10154–10160.
- Javaux, E.J., Dehant, V. (2010). Habitability: from stars to cells. *The Astronomy and Astrophysics Review* 18:383–416.
- Jebbar, M., Franzetti, B., Girard, E., Oger, P. (2015). Microbial diversity and adaptation to high hydrostatic pressure in deep-sea hydrothermal vents prokaryotes. *Extremophiles* 19:721–740.
- Jebbar, M., Hickman-Lewis, K., Cavalazzi, B., Taubner, R.-S., Rittmann, S.K.-M.R., Antunes, A. (2020a). Microbial Diversity and Biosignatures: An Icy Moons Perspective. *Space Sci Rev* 216:10.
- Jebbar, M., Hickman-Lewis, K., Cavalazzi, B., Taubner, R.-S., Rittmann, S., Antunes, A.

- (2020b). Microbial Diversity and Biosignatures: An Icy Moons Perspective. *Space Science Reviews* 216.
- Jennings, D.H. (1984). Polyol metabolism in fungi. *Adv Microb Physiol* 25:149–193.
- Jolivet, E. (2003). *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY* 53:847–851.
- Jones, R.M., Goordial, J.M., Orcutt, B.N. (2018). Low Energy Subsurface Environments as Extraterrestrial Analogs. *Front Microbiol* 9.
- Jones, B.E., Grant, W.D., Duckworth, A.W., Owenson, G.G. (1998). Microbial diversity of soda lakes. *Extremophiles* 2:191–200.
- Jones, E.G., Lineweaver, C.H. (2010). To What Extent Does Terrestrial Life “Follow The Water”? *Astrobiology* 10:349–361.
- Jones, E.G., Lineweaver, C.H., Clarke, J.D. (2011). An Extensive Phase Space for the Potential Martian Biosphere. *Astrobiology* 11:1017–1033.
- Kampmann, M., Stock, D. (2004). Reverse gyrase has heat-protective DNA chaperone activity independent of supercoiling. *Nucleic Acids Res* 32:3537–3545.
- Kanekar, P.P., Kanekar, S.P., Kelkar, A.S., Dhakephalkar, P.K. (2012). Halophiles – Taxonomy, Diversity, Physiology and Applications. In: Satyanarayana T, Johri BN (eds) *Microorganisms in Environmental Management: Microbes and Environment*, Springer Netherlands: Dordrecht, pp 1–34.
- Kargel, J.S., Kaye, J.Z., Head, J.W., Marion, G.M., Sassen, R., Crowley, J.K., Ballesteros, O.P., Grant, S.A., Hogenboom, D.L. (2000). Europa’s Crust and Ocean: Origin, Composition, and the Prospects for Life. *Icarus* 148:226–265.
- Karlström, M., Steen, I.H., Madern, D., Fedöy, A.-E., Birkeland, N.-K., Ladenstein, R. (2006). The crystal structure of a hyperthermostable subfamily II isocitrate dehydrogenase from *Thermotoga maritima*. *The FEBS Journal* 273:2851–2868.
- Kasting, J.F., Whitmire, D.P., Reynolds, R.T. (1993). Habitable Zones around Main Sequence Stars. *Icarus* 101:108–128.
- Kato, C. (2010). Distribution of piezophiles. In: *Extremophiles handbook*, pp 543–655.
- Kato, C., Li, L., Nogi, Y., Nakamura, Y., Tamaoka, J., Horikoshi, K. (1998). Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 meters. *Applied and environmental microbiology* 64:1510–1513.
- Kattenhorn, S.A., Prockter, L.M. (2014). Evidence for subduction in the ice shell of Europa. *Nature Geoscience* 7:762–767.
- Kawano, H., Nakasone, K., Abe, F., Kato, C., Yoshida, Y., Usami, R., Horikoshi, K. (2005). Protein–DNA Interactions under High-Pressure Conditions, Studied by Capillary Narrow-Tube Electrophoresis. *Bioscience, Biotechnology, and Biochemistry* 69:1415–1417.
- Kaye, J.Z., Baross, J.A. (2000). High incidence of halotolerant bacteria in Pacific hydrothermal-vent and pelagic environments. *FEMS Microbiol Ecol* 32:249–260.
- Kaye, J.Z., Márquez, M.C., Ventosa, A., Baross, J.A. (2004). *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *International Journal of Systematic and Evolutionary Microbiology*, 54:499–511.
- Kelly, L.C., Cockell, C.S., Summers, S. (2012). Diverse microbial species survive high ammonia concentrations. *International Journal of Astrobiology* 11:125–131.
- Kendrick, M.G., Kral, T.A. (2006). Survival of Methanogens During Desiccation: Implications for Life on Mars. *Astrobiology* 6:546–551.
- Kerr, R.A. (1996). Does Europa’s Ice Hide an Ocean? *Science* 274:2015–2015.
- Kiang, N.Y. (2008). The Color of Plants on Other Worlds. *Scientific American* 298:48–55.

- 1
2
3 Kim, H.S., Martel, A., Girard, E., Moulin, M., Härtlein, M., Madern, D., Blackledge, M.,
4 Franzetti, B., Gabel, F. (2016). SAXS/SANS on Supercharged Proteins Reveals Residue-
5 Specific Modifications of the Hydration Shell. *Biophysical Journal* 110:2185–2194.
- 6 Kimura, J., Kitadai, N. (2015). Polymerization of Building Blocks of Life on Europa and
7 Other Icy Moons. *Astrobiology* 15:430–441.
- 8 Kish, A., Griffin, P.L., Rogers, K.L., Fogel, M.L., Hemley, R.J., Steele, A. (2012). High-
9 pressure tolerance in *Halobacterium salinarum* NRC-1 and other non-piezophilic prokaryotes.
10 *Extremophiles* 16:355–361.
- 11 Kish, A., Kirkali, G., Robinson, C., Rosenblatt, R., Jaruga, P., Dizdaroglu, M., DiRuggiero, J.
12 (2009). Salt shield: intracellular salts provide cellular protection against ionizing radiation in
13 the halophilic archaeon, *Halobacterium salinarum* NRC-1. *Environmental Microbiology*
14 11:1066–1078.
- 15 Kivelson, M.G., Khurana, K.K., Russell, C.T., Volwerk, M., Walker, R.J., Zimmer, C. (2000).
16 Galileo Magnetometer Measurements: A Stronger Case for a Subsurface Ocean at Europa.
17 *Science* 289:1340–1343.
- 18 Klibanov, A.M. (1989). Enzymatic catalysis in anhydrous organic solvents. *Trends in*
19 *Biochemical Sciences* 14:141–144.
- 20 Köcher, S., Müller, V. (2011). The Nature and Function of Carotenoids in the Moderately
21 Halophilic Bacterium *Halobacillus halophilus*. In: Ventosa A, Oren A, Ma Y (eds)
22 *Halophiles and Hypersaline Environments: Current Research and Future Trends*, Springer:
23 Berlin, Heidelberg, pp 303–317.
- 24 Koga, Y. (2012). Thermal Adaptation of the Archaeal and Bacterial Lipid Membranes.
25 *Archaea* 2012:e789652.
- 26 Koonin, E.V., Martin, W. (2005). On the origin of genomes and cells within inorganic
27 compartments. *Trends in Genetics* 21:647–654.
- 28 Koonin, E.V., Starokadomskyy, P. (2016). Are viruses alive? The replicator paradigm sheds
29 decisive light on an old but misguided question. *Studies in History and Philosophy of Science*
30 *Part C: Studies in History and Philosophy of Biological and Biomedical Sciences* 59:125–
31 134.
- 32 Kothe, E., Dimkpa, C., Haferburg, G., Schmidt, A., Schmidt, A., Schütze, E. (2010).
33 Streptomycete Heavy Metal Resistance: Extracellular and Intracellular Mechanisms. In: *Soil*
34 *Heavy Metals*, Soil Biology. Springer Berlin Heidelberg: Berlin, Heidelberg Vol 19, pp 225–
35 235.
- 36 Kotopoulou, E., Delgado Huertas, A., Garcia-Ruiz, J.M., Dominguez-Vera, J.M., Lopez-
37 Garcia, J.M., Guerra-Tschuschke, I., Rull, F. (2019). A Polyextreme Hydrothermal System
38 Controlled by Iron: The Case of Dallol at the Afar Triangle. *ACS Earth Space Chem* 3:90–99.
- 39 Koutsopoulos, S., Oost, J. van der, Norde, W. (2005a). Conformational studies of a
40 hyperthermostable enzyme. *The FEBS Journal* 272:5484–5496.
- 41 Koutsopoulos, S., Oost, J. van der, Norde, W. (2005b). Temperature-dependent structural and
42 functional features of a hyperthermostable enzyme using elastic neutron scattering. *Proteins:*
43 *Structure, Function, and Bioinformatics* 61:377–384.
- 44 Kowalak, J.A., Dalluge, J.J., McCloskey, J.A., Stetter, K.O. (1994). The role of
45 posttranscriptional modification in stabilization of transfer RNA from hyperthermophiles.
46 *Biochemistry* 33:7869–7876.
- 47 Kragelj, J., Ozenne, V., Blackledge, M., Jensen, M.R. (2013). Conformational Propensities of
48 Intrinsically Disordered Proteins from NMR Chemical Shifts. *ChemPhysChem* 14:3034–
49 3045.
- 50 Kragl, U., Eckstein, M., Kaftzik, N. (2002). Enzyme catalysis in ionic liquids. *Current*
51 *Opinion in Biotechnology* 13:565–571.
- 52 Krami, L.K., Amiri, F., Sefiyanian, A., Shariff, A.R.B.M., Tabatabaie, T., Pradhan, B. (2013).
- 53
54
55
56
57
58
59
60

- 1
2
3 Spatial patterns of heavy metals in soil under different geological structures and land uses for
4 assessing metal enrichments. *Environ Monit Assess* 185:9871–9888.
- 5 Krisko, A., Radman, M. (2010). Protein damage and death by radiation in *Escherichia coli*
6 and *Deinococcus radiodurans*. *PNAS* 107:14373–14377.
- 7 Krisko, A., Radman, M. (2013). Biology of Extreme Radiation Resistance: The Way of
8 *Deinococcus radiodurans*. *Cold Spring Harb Perspect Biol* 5:a012765.
- 9 Krulwich, T.A. (1995). Alkaliphiles: 'basic' molecular problems of pH tolerance and
10 bioenergetics. *Molecular microbiology* 15:403–410.
- 11 Krulwich, T.A., Guffanti, A.A. (1983). Physiology of Acidophilic and Alkaliphilic Bacteria.
12 In: *Advances in Microbial Physiology*, Elsevier Vol 24, pp 173–214.
- 13 Krulwich, T.A., Sachs, G., Padan, E. (2011). Molecular aspects of bacterial pH sensing and
14 homeostasis. *Nature Reviews Microbiology* 9:330–343.
- 15 Kulkarni, S., Dhakar, K., Joshi, A. (2019). Alkaliphiles: Diversity and Bioprospection. In:
16 *Microbial Diversity in the Genomic Era*, Elsevier, pp 239–263.
- 17 Kumar, S., Nussinov, R. (2001). How do thermophilic proteins deal with heat? *CMLS, Cell*
18 *Mol Life Sci* 58:1216–1233.
- 19 Kumar, S., Tsai, C.-J., Ma, B., Nussinov, R. (2000). Contribution of Salt Bridges Toward
20 Protein Thermostability. *Journal of Biomolecular Structure and Dynamics* 17:79–85.
- 21 Kumar, A., Venkatesu, P. (2014). Does the stability of proteins in ionic liquids obey the
22 Hofmeister series? *International Journal of Biological Macromolecules* 63:244–253.
- 23 Kurr, M., Huber, R., König, H., Jannasch, H.W., Fricke, H., Trincone, A., Kristjansson, J.K.,
24 Stetter, K.O. (1991). *Methanopyrus kandleri*, gen. and sp. nov. represents a novel group of
25 hyperthermophilic methanogens, growing at 110°C. *Arch Microbiol* 156:239–247.
- 26 Kusube, M., Kyaw, T.S., Tanikawa, K., Chastain, R.A., Hardy, K.M., Cameron, J., Bartlett,
27 D.H. (2017). *Colwellia marinimaniae* sp. nov., a hyperpiezophilic species isolated from an
28 amphipod within the Challenger Deep, Mariana Trench. *International Journal of Systematic*
29 *and Evolutionary Microbiology*, 67:824–831.
- 30 Kutnowski, N., Shmueli, H., Dahan, I., Shmulevich, F., Davidov, G., Shahar, A., Eichler, J.,
31 Zarivach, R., Shaanan, B. (2018). The 3-D structure of VNG0258H/RosR – A haloarchaeal
32 DNA-binding protein in its ionic shell. *Journal of Structural Biology* 204:191–198.
- 33 Kvenvolden, K.A., Lawless, J.G., Ponnampereuma, C. (1971). Nonprotein Amino Acids in the
34 Murchison Meteorite. *PNAS* 68:486–490.
- 35 de La Tour, C.B., Portemer, C., Nadal, M., Stetter, K.O., Forterre, P., Duguet, M. (1990).
36 Reverse gyrase, a hallmark of the hyperthermophilic archaeobacteria. *Journal of bacteriology*
37 172:6803–6808.
- 38 Lamosa, P., Burke, A., Peist, R., Huber, R., Liu, M.-Y., Silva, G., Rodrigues-Pousada, C.,
39 LeGall, J., Maycock, C., Santos, H. (2000). Thermostabilization of Proteins by Diglycerol
40 Phosphate, a New Compatible Solute from the Hyperthermophile *Archaeoglobus fulgidus*.
41 *Appl Environ Microbiol* 66:1974–1979.
- 42 LaRowe, D.E., Amend, J.P. (2015). Power limits for microbial life. *Front Microbiol* 6.
- 43 Larsen, H. (2011). Biochemical aspects of extreme halophilism. *Advances in microbial*
44 *physiology* 1:97–132.
- 45 Lauro, F.M., Chastain, R.A., Blankenship, L.E., Yayanos, A.A., Bartlett, D.H. (2007). The
46 Unique 16S rRNA Genes of Piezophiles Reflect both Phylogeny and Adaptation. *Appl*
47 *Environ Microbiol* 73:838–845.
- 48 Laye, V.J., DasSarma, S. (2018). An Antarctic Extreme Halophile and Its Polyextremophilic
49 Enzyme: Effects of Perchlorate Salts. *Astrobiology* 18:412–418.
- 50 Lazrak, T., Wolff, G., Albrecht, A.-M., Nakatani, Y., Ourisson, G., Kates, M. (1988).
51 Bacterioruberins reinforce reconstituted *Halobacterium* lipid membranes. *Biochimica et*
52 *Biophysica Acta (BBA) - Biomembranes* 939:160–162.
- 53
54
55
56
57
58
59
60

- 1
2
3 Lebre, P.H., De Maayer, P., Cowan, D.A. (2017). Xerotolerant bacteria: surviving through a
4 dry spell. *Nature Reviews Microbiology* 15:285–296.
- 5 Lederberg, J. (1960). Exobiology: Approaches to Life beyond the Earth. *Science* 132:393–
6 400.
- 7
8 Leiros, H.-K.S., Pey, A.L., Innselset, M., Moe, E., Leiros, I., Steen, I.H., Martinez, A. (2007).
9 Structure of Phenylalanine Hydroxylase from *Colwellia psychrerythraea* 34H, a Monomeric
10 Cold Active Enzyme with Local Flexibility around the Active Site and High Overall Stability.
11 *J Biol Chem* 282:21973–21986.
- 12 Lenton, S., Walsh, D.L., Rhys, N.H., Soper, A.K., Dougan, L. (2016). Structural evidence for
13 solvent-stabilisation by aspartic acid as a mechanism for halophilic protein stability in high
14 salt concentrations. *Phys Chem Chem Phys* 18:18054–18062.
- 15 Leszczynski, J.F., Rose, G.D. (1986). Loops in globular proteins: a novel category of
16 secondary structure. *Science* 234:849–855.
- 17 Levine, J.S., Summers, M.E., Ewell, M. (2010). Methane on Mars: Thermodynamic
18 Equilibrium and Photochemical Calculations. In: *Geophysical Research Letters*, Vol 29, pp
19 24-1-24–4.
- 20
21 Levy, M., Miller, S.L. (1998). The stability of the RNA bases: Implications for the origin of
22 life. *Proceedings of the National Academy of Sciences* 95:7933–7938.
- 23 Lim, J., Thomas, T., Cavicchioli, R. (2000). Low temperature regulated DEAD-box RNA
24 helicase from the antarctic archaeon, *Methanococcoides burtonii* 11 Edited by J. H. Miller.
25 *Journal of Molecular Biology* 297:553–567.
- 26
27 Lin, L.-H., Hall, J., Lippmann-Pipke, J., Ward, J.A., Lollar, B.S., DeFlaun, M., Rothmel, R.,
28 Moser, D., Gihring, T.M., Mislowack, B., Onstott, T.C. (2005). Radiolytic H₂ in continental
29 crust: Nuclear power for deep subsurface microbial communities. *Geochemistry, Geophysics,*
30 *Geosystems* 6.
- 31
32 Lin, L.-H., Wang, P.-L., Rumble, D., Lippmann-Pipke, J., Boice, E., Pratt, L.M., Lollar, B.S.,
33 Brodie, E.L., Hazen, T.C., Andersen, G.L., DeSantis, T.Z., Moser, D.P., Kershaw, D.,
34 Onstott, T.C. (2006). Long-Term Sustainability of a High-Energy, Low-Diversity Crustal
35 Biome. *Science* 314:479–482.
- 36 Lindhal, T. (1993). Instability and decay of the primary structure of DNA. *Nature* 362:709–
37 716.
- 38
39 Lipscomb, G.L., Hahn, E.M., Crowley, A.T., Adams, M.W.W. (2017). Reverse gyrase is
40 essential for microbial growth at 95 °C. *Extremophiles* 21:603–608.
- 41 Liu, Y., Whitman, W.B. (2008). Metabolic, Phylogenetic, and Ecological Diversity of the
42 Methanogenic Archaea. *Annals of the New York Academy of Sciences* 1125:171–189.
- 43 Lovley, D.R., Giovannoni, S.J., White, D.C., Champine, J.E., Phillips, E.J.P., Gorby, Y.A.,
44 Goodwin, S. (1993). *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable
45 of coupling the complete oxidation of organic compounds to the reduction of iron and other
46 metals. *Arch Microbiol* 159:336–344.
- 47
48 Lowell, R.P., DuBose, M. (2005). Hydrothermal systems on Europa. *Geophysical Research*
49 *Letters* 32.
- 50
51 Lu, J., Nogi, Y., Takami, H. (2001). *Oceanobacillus iheyensis* gen. nov., sp. nov., a deep-sea
52 extremely halotolerant and alkaliphilic species isolated from a depth of 1050 m on the Iheya
53 Ridge. *FEMS Microbiol Lett* 205:291–297.
- 54 Luger, R., Barnes, R. (2015). Extreme Water Loss and Abiotic O₂ Buildup on Planets
55 Throughout the Habitable Zones of M Dwarfs. *Astrobiology* 15:119–143.
- 56 Lunine, J.I., Rizk, B. (2007). Titan. In: Baross J, Sullivan I Woodruff T (eds) *Planets and*
57 *Life: The Emerging Science of Astrobiology*, Cambridge University Press: Cambridge, pp
58 424–443.
- 59
60 Luther, G.W., Rozan, T.F., Taillefert, M., Nuzzio, D.B., Meo, C.D., Shank, T.M., Lutz, R.A.,

- 1
2
3 Cary, S.C. (2001). Chemical speciation drives hydrothermal vent ecology. *Nature* 410:813–
4 816.
- 5 Lynch, M. (2013). Evolutionary diversification of the multimeric states of proteins. *PNAS*
6 110:E2821–E2828.
- 7
8 Lynden-Bell, R.M., Morris, S.C., Barrow, J.D., Finney, J.L., Harper, C. (2010). *Water and*
9 *Life: The Unique Properties of H₂O*, 1st edn. CRC Press: Boca Raton, FL.
- 10
11 Lyons, J.R., Manning, C., Nimmo, F. (2005). Formation of methane on Mars by fluid-rock
12 interaction in the crust. *Geophysical Research Letters* 32.
- 13
14 Lyu, Z., Shao, N., Akinyemi, T., Whitman, W.B. (2018). Methanogenesis. *Current Biology*
15 28:R727–R732.
- 16
17 Macelroy, R.D. (1974). Some comments on the evolution of extremophiles. *Biosystems* 6:74–
18 75.
- 19
20 Macgregor, R.B. (1996). Chain length and oligonucleotide stability at high pressure.
21 *Biopolymers* 38:321–328.
- 22
23 Macgregor, R.B. (1998). Effect of hydrostatic pressure on nucleic acids. *Biopolymers* 48:253–
24 263.
- 25
26 Macgregor, R.B. (2002). The interactions of nucleic acids at elevated hydrostatic pressure.
27 *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*
28 1595:266–276.
- 29
30 Madern, D., Ebel, C., Zaccai, G. (2000). Halophilic adaptation of enzymes. *Extremophiles*
31 4:91–98.
- 32
33 Madern, D., Pfister, C., Zaccai, G. (1995). Mutation at a Single Acidic Amino Acid Enhances
34 the Halophilic Behaviour of Malate Dehydrogenase from Haloarcula Marismortui in
35 Physiological Salts. *European Journal of Biochemistry* 230:1088–1095.
- 36
37 Magnabosco, C., Lin, L.-H., Dong, H., Bomberg, M., Ghiorse, W., Stan-Lotter, H., Pedersen,
38 K., Kieft, T.L., van Heerden, E., Onstott, T.C. (2018). The biomass and biodiversity of the
39 continental subsurface. *Nature Geoscience* 11:707–717.
- 40
41 Mancinelli, R.L., Fahlen, T.F., Landheim, R., Klovstad, M.R. (2004). Brines and evaporites:
42 analogs for Martian life. *Advances in Space Research* 33:1244–1246.
- 43
44 Marcus, Y. (2009). Effect of Ions on the Structure of Water: Structure Making and Breaking.
45 *Chem Rev* 109:1346–1370.
- 46
47 Marion, G.M., Fritsen, C.H., Eicken, H., Payne, M.C. (2003). The Search for Life on Europa:
48 Limiting Environmental Factors, Potential Habitats, and Earth Analogues. *Astrobiology*
49 3:785–811.
- 50
51 Marion, G.M., Schulze-Makuch, D. (2007). Astrobiology and the Search for Life in the
52 Universe. In: *Physiology and Biochemistry of Extremophiles*, American Society of
53 Microbiology, pp 351–358.
- 54
55 Marteinson, V.T., Birrien, J.-L., Reysenbach, A.-L., Vernet, M., Marie, D., Gambacorta, A.,
56 Messner, P., Sleytr, U.B., Prieur, D. (1999). Thermococcus barophilus sp. nov., a new
57 barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a
58 deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary*
59 *Microbiology*, 49:351–359.
- 60
61 Martin, W., Baross, J., Kelley, D., Russell, M.J. (2008). Hydrothermal vents and the origin of
62 life. *Nat Rev Microbiol* 6:805–814.
- 63
64 Martin, D., Bartlett, D.H., Roberts, M.F. (2002). Solute accumulation in the deep-sea
65 bacterium Photobacterium profundum. *Extremophiles* 6:507–514.
- 66
67 Martinez, N., Michoud, G., Cario, A., Ollivier, J., Franzetti, B., Jebbar, M., Oger, P., Peters, J.
68 (2016). High protein flexibility and reduced hydration water dynamics are key pressure
69 adaptive strategies in prokaryotes. *Sci Rep* 6:1–11.
- 70
71 Martínez, G.M., Renno, N.O. (2013). Water and Brines on Mars: Current Evidence and

1
2
3 Implications for MSL. *Space Sci Rev* 175:29–51.

4 Martins, Z., Botta, O., Fogel, M.L., Sephton, M.A., Glavin, D.P., Watson, J.S., Dworkin, J.P.,
5 Schwartz, A.W., Ehrenfreund, P. (2008). Extraterrestrial nucleobases in the Murchison
6 meteorite. *Earth and Planetary Science Letters* 270:130–136.

7 Martín-Torres, F.J., Zorzano, M.-P., Valentín-Serrano, P., Harri, A.-M., Genzer, M.,
8 Kemppinen, O., Rivera-Valentin, E.G., Jun, I., Wray, J., Madsen, M.B., Goetz, W., McEwen,
9 A.S., Hardgrove, C., Renno, N., Chevrier, V.F., Mischna, M., Navarro-González, R.,
10 Martínez-Frías, J., Conrad, P., McConnochie, T., Cockell, C., Berger, G., Vasavada, A.R.,
11 Sumner, D., Vaniman, D. (2015). Transient liquid water and water activity at Gale crater on
12 Mars. *Nature Geosci* 8:357–361.

13 Marx, J.G., Carpenter, S.D., Deming, J.W. (2009). Production of cryoprotectant extracellular
14 polysaccharide substances (EPS) by the marine psychrophilic bacterium *Colwellia*
15 *psychrerythraea* strain 34H under extreme conditions. *Can J Microbiol* 55:63–72.

16 Matson, D.L., Castillo, J.C., Lunine, J., Johnson, T.V. (2007). Enceladus' plume:
17 Compositional evidence for a hot interior. *Icarus* 187:569–573.

18 Matsubara, T., Fujishima, K., Saltikov, C.W., Nakamura, S., Rothschild, L.J. (2017). Earth
19 analogues for past and future life on Mars: isolation of perchlorate resistant halophiles from
20 Big Soda Lake. *International Journal of Astrobiology* 16:218–228.

21 Matthews, B.W. (2012). Proteins under pressure. *PNAS* 109:6792–6793.

22 Mayor, M., Queloz, D. (1995). A Jupiter-mass companion to a solar-type star. *Nature*
23 378:355–359.

24 Mazzini, V., Craig, V. (2017). What is the fundamental ion-specific series for anions and
25 cations? Ion specificity in standard partial molar volumes of electrolytes and electrostriction
26 in water and non-aqueous solvents. *Chem Sci* 8.

27 McCollom, T.M. (2006). The Habitability of Mars: Past and Present. In: Blondel P, Mason
28 JW (eds) *Solar System Update*, Springer Praxis Books. Springer-Verlag: Berlin, Heidelberg,
29 pp 159–175.

30 McConkey, E.H. (1982). Molecular evolution, intracellular organization, and the quinary
31 structure of proteins. *PNAS* 79:3236–3240.

32 McCord, T.B., Hansen, G.B., Hibbitts, C.A. (2001). Hydrated Salt Minerals on Ganymede's
33 Surface: Evidence of an Ocean Below. *Science* 292:1523–1525.

34 McKay, C.P., Porco, C.C., Altheide, T., Davis, W.L., Kral, T.A. (2008). The Possible Origin
35 and Persistence of Life on Enceladus and Detection of Biomarkers in the Plume. *Astrobiology*
36 8:909–919.

37 McKay, C.P., Smith, H.D. (2005). Possibilities for methanogenic life in liquid methane on the
38 surface of Titan. *Icarus* 178:274–276.

39 McKinnon, W.B., Zolensky, M.E. (2003). Sulfate Content of Europa's Ocean and Shell:
40 Evolutionary Considerations and Some Geological and Astrobiological Implications.
41 *Astrobiology* 3:879–897.

42 McMahan, S., Parnell, J. (2014). Weighing the deep continental biosphere. *FEMS Microbiol*
43 *Ecol* 87:113–120.

44 Meadows, V.S., Reinhard, C.T., Arney, G.N., Parenteau, M.N., Schwieterman, E.W.,
45 Domagal-Goldman, S.D., Lincowski, A.P., Stapelfeldt, K.R., Rauer, H., DasSarma, S.,
46 Hegde, S., Narita, N., Deitrick, R., Lustig-Yaeger, J., Lyons, T.W., Siegler, N., Grenfell, J.L.
47 (2018). Exoplanet Biosignatures: Understanding Oxygen as a Biosignature in the Context of
48 Its Environment. *Astrobiology* 18:630–662.

49 Médigue, C. (2005). Coping with cold: The genome of the versatile marine Antarctica
50 bacterium *Pseudoalteromonas haloplanktis* TAC125. *Genome Research* 15:1325–1335.

51 Mendillo, M., Withers, P., Dalba, P.A. (2018). Atomic oxygen ions as ionospheric biomarkers
52 on exoplanets. *Nature Astronomy* 2:287–291.

- 1
2
3 Mergeay, M. (2006). Metallophiles and Acidophiles in Metal-Rich Environments. In:
4 *Extremophiles (Life under extreme conditions)*, Eolss Publishers: Oxford, United Kingdom, p
5 9.
6
7 Merino, N., Aronson, H.S., Bojanova, D.P., Feyhl-Buska, J., Wong, M.L., Zhang, S.,
8 Giovannelli, D. (2019). Living at the Extremes: Extremophiles and the Limits of Life in a
9 Planetary Context. *Front Microbiol* 10.
10 Merroun, M.L., Raff, J., Rossberg, A., Hennig, C., Reich, T., Selenska-Pobell, S. (2005).
11 Complexation of Uranium by Cells and S-Layer Sheets of *Bacillus sphaericus* JG-A12. *Appl*
12 *Environ Microbiol* 71:5532–5543.
13 Mesbah, N.M., Wiegel, J. (2005). Halophilic Thermophile: A Novel Group of Extremophiles.
14 In: *Microbial Diversity: Current Perspectives and Potential Applications.*, I. K. International
15 Pvt Ltd, pp 99–118.
16 Michalski, J.R., Cuadros, J., Niles, P.B., Parnell, J., Rogers, A.D., Wright, S.P. (2013).
17 Groundwater activity on Mars and implications for a deep biosphere. *Nature Geosci* 6:133–
18 138.
19
20 Michaux, C., Massant, J., Kerff, F., Frère, J.-M., Docquier, J.-D., Vandenberghe, I., Samyn,
21 B., Pierrard, A., Feller, G., Charlier, P., Beeumen, J.V., Wouters, J. (2008). Crystal structure
22 of a cold-adapted class C β -lactamase. *The FEBS Journal* 275:1687–1697.
23 Michels, P.C., Clark, D.S. (1997). Pressure-enhanced activity and stability of a
24 hyperthermophilic protease from a deep-sea methanogen. *Appl Environ Microbiol* 63:3985–
25 3991.
26
27 Mickol, R.L., Kral, T.A. (2017). Low Pressure Tolerance by Methanogens in an Aqueous
28 Environment: Implications for Subsurface Life on Mars. *Orig Life Evol Biosph* 47:511–532.
29 Mikucki, J.A., Auken, E., Tulaczyk, S., Virginia, R.A., Schamper, C., Sørensen, K.I., Doran,
30 P.T., Dugan, H., Foley, N. (2015). Deep groundwater and potential subsurface habitats
31 beneath an Antarctic dry valley. *Nat Commun* 6:1–9.
32 Miller, S.L., Lazcano, A. (1995). The origin of life—did it occur at high temperatures? *J Mol*
33 *Evol* 41:689–692.
34
35 Miroshnichenko, M.L., Bonch-Osmolovskaya, E.A. (2006). Recent developments in the
36 thermophilic microbiology of deep-sea hydrothermal vents. *Extremophiles* 10:85–96.
37 Mizushima, T., Kataoka, K., Ogata, Y., Inoue, R., Sekimizu, K. (1997). Increase in negative
38 supercoiling of plasmid DNA in *Escherichia coli* exposed to cold shock. *Molecular*
39 *Microbiology* 23:381–386.
40 Möhlmann, D., Thomsen, K. (2011). Properties of cryobrines on Mars. *Icarus* 212:123–130.
41 Morita, T. (2003). Structure-based Analysis of High Pressure Adaptation of α -Actin. *J Biol*
42 *Chem* 278:28060–28066.
43 Morozova, D., Möhlmann, D., Wagner, D. (2007). Survival of Methanogenic Archaea from
44 Siberian Permafrost under Simulated Martian Thermal Conditions. *Orig Life Evol Biosph*
45 37:189–200.
46 Morozova, D., Wagner, D. (2007). Stress response of methanogenic archaea from Siberian
47 permafrost compared with methanogens from nonpermafrost habitats. *FEMS Microbiol Ecol*
48 61:16–25.
49 Mosher, D., Cheng, J. (2018). Earth is a desert planet compared to these ocean worlds in the
50 solar system. *Business Insider*.
51 Mukaiyama, A., Koga, Y., Takano, K., Kanaya, S. (2008). Osmolyte effect on the stability
52 and folding of a hyperthermophilic protein. *Proteins: Structure, Function, and Bioinformatics*
53 71:110–118.
54 Muldrew, K., McGann, L.E. (1990). Mechanisms of intracellular ice formation. *Biophys J*
55 57:525–532.
56 Mulkidjanian, A.Y., Bychkov, A.Y., Dibrova, D.V., Galperin, M.Y., Koonin, E.V. (2012).

- 1
2
3 Origin of first cells at terrestrial, anoxic geothermal fields. *Proceedings of the National*
4 *Academy of Sciences* 109:E821–E830.
- 5 Mumma, M.J., Villanueva, G.L., Novak, R.E., Hewagama, T., Bonev, B.P., DiSanti, M.A.,
6 Mandell, A.M., Smith, M.D. (2009). Strong Release of Methane on Mars in Northern
7 Summer 2003. *Science* 323:1041–1045.
- 8 Mykyteczuk, N.C.S., Foote, S.J., Omelon, C.R., Southam, G., Greer, C.W., Whyte, L.G.
9 (2013). Bacterial growth at $-15\text{ }^{\circ}\text{C}$; molecular insights from the permafrost bacterium
10 *Planococcus halocryophilus* Or1. *The ISME Journal* 7:1211–1226.
- 11 Nachtergaele, S., He, C. (2017). The emerging biology of RNA post-transcriptional
12 modifications. *RNA Biology* 14:156–163.
- 13 Nagae, T., Kato, C., Watanabe, N. (2012). Structural analysis of 3-isopropylmalate
14 dehydrogenase from the obligate piezophile *Shewanella benthica* DB21MT-2 and the
15 nonpiezophile *Shewanella oneidensis* MR-1. *Acta Cryst F* 68:265–268.
- 16 Naganuma, T., Uematsu, H. (1998). Dive Europa: A Search-for-Life Initiative. *Biological*
17 *Sciences in Space* 12:126–130.
- 18 Narberhaus, F. (2002). mRNA-mediated detection of environmental conditions. *Arch*
19 *Microbiol* 178:404–410.
- 20 Narita, N., Enomoto, T., Masaoka, S., Kusakabe, N. (2015). Titania may produce abiotic
21 oxygen atmospheres on habitable exoplanets. *Scientific Reports* 5:13977.
- 22 Nath, A. (2016). Insights into the sequence parameters for halophilic adaptation. *Amino Acids*
23 48:751–762.
- 24 Nath, A., Subbiah, K. (2016). Insights into the molecular basis of piezophilic adaptation:
25 Extraction of piezophilic signatures. *Journal of Theoretical Biology* 390:117–126.
- 26 Navarro-González, R., Rainey, F.A., Molina, P., Bagaley, D.R., Hollen, B.J., Rosa, J. de la,
27 Small, A.M., Quinn, R.C., Grunthaner, F.J., Cáceres, L., Gomez-Silva, B., McKay, C.P.
28 (2003). Mars-Like Soils in the Atacama Desert, Chile, and the Dry Limit of Microbial Life.
29 *Science* 302:1018–1021.
- 30 Nayek, A., Sen Gupta, P., Banerjee, S., Mondal, B., Bandyopadhyay, A. (2014). Salt-Bridge
31 Energetics in Halophilic Proteins. *PloS one* 9:e93862.
- 32 Nielsen, L.E., Kadavy, D.R., Rajagopal, S., Drijber, R., Nickerson, K.W. (2005). Survey of
33 Extreme Solvent Tolerance in Gram-Positive Cocci: Membrane Fatty Acid Changes in
34 *Staphylococcus haemolyticus* Grown in Toluene. *Applied and Environmental Microbiology*
35 71:5171–5176.
- 36 Niero, M., Righetto, I., Beneventi, E., Polverino de Laureto, P., Fraaije, M.W., Filippini, F.,
37 Bergantino, E. (2020). Unique Features of a New Baeyer–Villiger Monooxygenase from a
38 Halophilic Archaeon. *Catalysts* 10:128.
- 39 Nies, D.H. (2000). Heavy metal-resistant bacteria as extremophiles: molecular physiology and
40 biotechnological use of *Ralstonia* sp. CH34. *Extremophiles* 4:77–82.
- 41 Nies, D.H., Covès, J., Sawers, R.G. (2017). Cross-Talk Between Nickel and Other Metals in
42 Microbial Systems. In: Zamble D, Rowinska-Zyrek M, Kozlowski H (eds) *Metallobiology*,
43 Royal Society of Chemistry: Cambridge, pp 306–338.
- 44 Nogi, Y., Hosoya, S., Kato, C., Horikoshi, K. (2007). *Psychromonas hadalis* sp. nov., a novel
45 piezophilic bacterium isolated from the bottom of the Japan Trench. *International Journal of*
46 *Systematic and Evolutionary Microbiology*, 57:1360–1364.
- 47 Noon, K.R., Bruenger, E., McCloskey, J.A. (1998). Posttranscriptional Modifications in 16S
48 and 23S rRNAs of the Archaeal Hyperthermophile *Sulfolobus solfataricus*. *Journal of*
49 *Bacteriology* 180:2883–2888.
- 50 Nyssola, A., Kerovuo, J., Kaukinen, P., von Weymarn, N., Reinikainen, T. (2000). Extreme
51 Halophiles Synthesize Betaine from Glycine by Methylation. *Journal of Biological Chemistry*
52 275:22196–22201.

- Ogawa, T., Yogo, K., Furuike, S., Sutoh, K., Kikuchi, A., Kinoshita, K. (2015). Direct observation of DNA overwinding by reverse gyrase. *PNAS* 112:7495–7500.
- Oger, P.M., Jebbar, M. (2010). The many ways of coping with pressure. *Research in Microbiology* 161:799–809.
- Okur, H.I., Hladílková, J., Rembert, K.B., Cho, Y., Heyda, J., Dzubiella, J., Cremer, P.S., Jungwirth, P. (2017). Beyond the Hofmeister Series: Ion-Specific Effects on Proteins and Their Biological Functions. *J Phys Chem B* 121:1997–2014.
- Okuyama, H., Okajima, N., Sasaki, S., Higashi, S. (1993). The cis/trans isomerization of the double bond of a fatty acid as a strategy for adaptation to changes in ambient temperature in the psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *Biochimica et Biophysica Acta* 1084:13–20.
- Oldfield, C.J., Dunker, A.K. (2014). Intrinsically Disordered Proteins and Intrinsically Disordered Protein Regions. *Annual Review of Biochemistry* 83:553–584.
- O'Malley-James, J.T., Kaltenecker, L. (2019). Expanding the Timeline for Earth's Photosynthetic Red Edge Biosignature. *ApJL* 879:L20.
- O'Malley-James, J.T., Lutz, S. (2013). From Life to Exolife: The Interdependence of Astrobiology and Evolutionary Biology. In: Pontarotti P (ed) *Evolutionary Biology: Exobiology and Evolutionary Mechanisms*, Springer: Berlin, Heidelberg, pp 95–108.
- O'Malley-James, J. t., Raven, J. a., Cockell, C. s., Greaves, J. s. (2012). Life and Light: Exotic Photosynthesis in Binary and Multiple-Star Systems. *Astrobiology* 12:115–124.
- Ono, H., Okuda, M., Tongpim, S., Imai, K., Shinmyo, A., Sakuda, S., Kaneko, Y., Murooka, Y., Takano, M. (1998). Accumulation of compatible solutes, ectoine and hydroxyectoine, in a moderate halophile, *Halomonas elongata* KS3 isolated from dry salty land in Thailand. *Journal of Fermentation and Bioengineering* 85:362–368.
- Oren, A. (Ed.) (2002). Intracellular Salt Concentrations and Ion Metabolism in Halophilic Microorganisms. In: *Halophilic Microorganisms and their Environments*, Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer Netherlands: Dordrecht, pp 207–231.
- Oren, A. (2010). The dying Dead Sea: The microbiology of an increasingly extreme environment. *Lakes & Reservoirs: Science, Policy and Management for Sustainable Use* 15:215–222.
- Oren, A. (2013). Life in Magnesium- and Calcium-Rich Hypersaline Environments: Salt Stress by Chaotropic Ions. In: Seckbach J, Oren A, Stan-Lotter H (eds) *Polyextremophiles*, Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer Netherlands: Dordrecht Vol 27, pp 215–232.
- Oren, A., Eleri Bardavid, R., Mana, L. (2014). Perchlorate and halophilic prokaryotes: implications for possible halophilic life on Mars. *Extremophiles* 18:75–80.
- Orosei, R., Lauro, S.E., Pettinelli, E., Cicchetti, A., Coradini, M., Cosciotti, B., Paolo, F.D., Flamini, E., Mattei, E., Pajola, M., Soldovieri, F., Cartacci, M., Cassenti, F., Frigeri, A., Giuppi, S., Martufi, R., Masdea, A., Mitri, G., Nenna, C., Noschese, R., Restano, M., Seu, R. (2018). Radar evidence of subglacial liquid water on Mars. *Science* 361:490–493.
- Palumbo, A.M., Head, J.W. (2018). Early Mars Climate History: Characterizing a “Warm and Wet” Martian Climate With a 3-D Global Climate Model and Testing Geological Predictions. *Geophysical Research Letters* 45:10,249–10,258.
- Panja, A.S., Maiti, S., Bandyopadhyay, B. (2020). Protein stability governed by its structural plasticity is inferred by physicochemical factors and salt bridges. *Sci Rep* 10:1–9.
- Pappalardo, R.T. (2012). *Europa Study 2012 Report*.
- Pappalardo, R.T., Belton, M.J.S., Breneman, H.H., Carr, M.H., Chapman, C.R., Collins, G.C., Denk, T., Fagents, S., Geissler, P.E., Giese, B., Greeley, R., Greenberg, R., Head, J.W., Helfenstein, P., Hoppa, G., Kadel, S.D., Klaasen, K.P., Klemaszewski, J.E., Magee, K., McEwen, A.S., Moore, J.M., Moore, W.B., Neukum, G., Phillips, C.B., Prockter, L.M.,

- 1
2
3 Schubert, G., Senske, D.A., Sullivan, R.J., Tufts, B.R., Turtle, E.P., Wagner, R., Williams,
4 K.K. (1999). Does Europa have a subsurface ocean? Evaluation of the geological evidence.
5 *Journal of Geophysical Research: Planets* 104:24015–24055.
- 6 Park, R.S., Bills, B., Buffington, B.B., Folkner, W.M., Konopliv, A.S., Martin-Mur, T.J.,
7 Mastrodemos, N., McElrath, T.P., Riedel, J.E., Watkins, M.M. (2015). Improved detection of
8 tides at Europa with radiometric and optical tracking during flybys. *Planetary and Space*
9 *Science* 112:10–14.
- 10 Parkinson, C.D., Liang, M.-C., Yung, Y.L., Kirschvink, J.L. (2008). Habitability of
11 Enceladus: Planetary Conditions for Life. *Origins of Life and Evolution of Biospheres*
12 38:355–369.
- 13 Parrilli, E., Sannino, F., Marino, G., Tutino, M.L. (2011). Life in icy habitats: new insights
14 supporting panspermia theory. *Rendiconti Lincei* 22:375–383.
- 15 Parvizpour, S., Razmara, J., Shamsir, M.S., Illias, R.M., Murad, A.M.A. (2017). The role of
16 alternative salt bridges in cold adaptation of a novel psychrophilic laminarinase. *Journal of*
17 *Biomolecular Structure and Dynamics* 35:1685–1692.
- 18 Pasek, M.A., Greenberg, R. (2012). Acidification of Europa’s Subsurface Ocean as a
19 Consequence of Oxidant Delivery. *Astrobiology* 12:151–159.
- 20 Paul, S., Bag, S.K., Das, S., Harvill, E.T., Dutta, C. (2008). Molecular signature of
21 hypersaline adaptation: insights from genome and proteome composition of halophilic
22 prokaryotes. *Genome Biology* 9:R70.
- 23 Pedersen, H.L., Willassen, N.P., Leiros, I. (2009). The first structure of a cold-adapted
24 superoxide dismutase (SOD): biochemical and structural characterization of iron SOD from
25 *Aliivibrio salmonicida*. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 65:84–92.
- 26 Peeters, Z., Vos, D., ten Kate, I.L., Selch, F., van Sluis, C.A., Sorokin, D.Yu., Muijzer, G.,
27 Stan-Lotter, H., van Loosdrecht, M.C.M., Ehrenfreund, P. (2010). Survival and death of the
28 haloarchaeon *Natronorubrum* strain HG-1 in a simulated martian environment. *Advances in*
29 *Space Research* 46:1149–1155.
- 30 Peretti, F.-X. de (2019). De la pluralité des mondes. In: Grenoble World Trade Center, p .
31 Petit, J.R., Alekhina, I., Bulat, S. (2005). Lake Vostok, Antarctica: Exploring a Subglacial
32 Lake and Searching for Life in an Extreme Environment. In: Gargaud M, Barbier B, Martin
33 H, Reisse J (eds) *Lectures in Astrobiology, Advances in Astrobiology and Biogeophysics*.
34 Springer Berlin Heidelberg: Berlin, Heidelberg, pp 227–288.
- 35 Petski, G.A. (2001). Structural Basis of Thermostability in Hyperthermophilic Proteins, or
36 ‘There’s More Than One Way to Skin a Cat’. *Methods In Enzymology*.
- 37 Phadtare, S. (2004). Recent developments in bacterial cold-shock response. *Curr Issues Mol*
38 *Biol* 6:125–136.
- 39 Pierazzo, E., Chyba, C.F. (2002). Cometary Delivery of Biogenic Elements to Europa. *Icarus*
40 157:120–127.
- 41 Pikuta, E.V.H. (2007). Astrobiological Significance of Microbial Extremophiles. In: San
42 Diego, CA, United States, p .
- 43 Pinto, E., Sigaud-kutner, T., Leitao, M.A., Okamoto, O.K., Morse, D., Colepicolo, P. (2003).
44 Heavy Metal-Induced Oxidative Stress in Algae. *Journal of Phycology* 39:1008–1018.
- 45 Porco, C.C., Helfenstein, P., Thomas, P.C., Ingersoll, A.P., Wisdom, J., West, R., Neukum,
46 G., Denk, T., Wagner, R., Roatsch, T., Kieffer, S., Turtle, E., McEwen, A., Johnson, T.V.,
47 Rathbun, J., Veverka, J., Wilson, D., Perry, J., Spitalé, J., Brahic, A., Burns, J.A., DelGenio,
48 A.D., Dones, L., Murray, C.D., Squyres, S. (2006). Cassini Observes the Active South Pole of
49 Enceladus. *Science* 311:1393–1401.
- 50 Postberg, F., Khawaja, N., Abel, B., Choblet, G., Glein, C.R., Gudipati, M.S., Henderson,
51 B.L., Hsu, H.-W., Kempf, S., Klenner, F., Moragas-Klostermeyer, G., Magee, B., Nölle, L.,
52 Perry, M., Reviol, R., Schmidt, J., Srama, R., Stolz, F., Tobie, G., Trierloff, M., Waite, J.H.

- (2018). Macromolecular organic compounds from the depths of Enceladus. *Nature* 558:564–568.
- Prangishvili, D., Garrett, R.A. (2005). Viruses of hyperthermophilic Crenarchaea. *Trends in Microbiology* 13:535–542.
- Prasad, M.N.V., Hagemeyer, J. (1999). *Heavy Metal Stress in Plants*. Springer Berlin Heidelberg: Berlin, Heidelberg.
- Pucci, F., Rooman, M. (2017). Physical and molecular bases of protein thermal stability and cold adaptation. *Current Opinion in Structural Biology* 42:117–128.
- Quinn, R.C., Martucci, H.F.H., Miller, S.R., Bryson, C.E., Grunthaner, F.J., Grunthaner, P.J. (2013). Perchlorate Radiolysis on Mars and the Origin of Martian Soil Reactivity. *Astrobiology* 13:515–520.
- Rafiq, M., Hassan, N., Rehman, M., Hasan, F. (2019). Adaptation Mechanisms and Applications of Psychrophilic Fungi. In: Tiquia-Arashiro SM, Grube M (eds) *Fungi in Extreme Environments: Ecological Role and Biotechnological Significance*, Springer International Publishing: Cham, pp 157–174.
- Rambaux, N., Hoolst, T.V., Karatekin, Ö. (2011). Librational response of Europa, Ganymede, and Callisto with an ocean for a non-Keplerian orbit. *A&A* 527:A118.
- Ramos, J.L., Duque, E., Gallegos, M.-T., Godoy, P., Ramos-González, M.I., Rojas, A., Terán, W., Segura, A. (2002). Mechanisms of Solvent Tolerance in Gram-Negative Bacteria. *Annual Review of Microbiology* 56:743–768.
- Ramos, J.L., Duque, E., Rodríguez-Herva, J.-J., Godoy, P., Haïdour, A., Reyes, F., Fernández-Barrero, A. (1997). Mechanisms for solvent tolerance in bacteria. *Journal of Biological Chemistry* 272:3887–3890.
- Raulin, F., Brassé, C., Poch, O., Coll, P. (2012). Prebiotic-like chemistry on Titan. *Chem Soc Rev* 41:5380–5393.
- Rayan, G., Macgregor, R.B. (2005). Comparison of the Heat- and Pressure-Induced Helix–Coil Transition of Two DNA Copolymers. *The Journal of Physical Chemistry B* 109:15558–15565.
- Raymond-Bouchard, I., Goordial, J., Zolotarov, Y., Ronholm, J., Stromvik, M., Bakermans, C., Whyte, L.G. (2018). Conserved genomic and amino acid traits of cold adaptation in subzero-growing Arctic permafrost bacteria. *FEMS Microbiol Ecol* 94.
- Réat, V., Patzelt, H., Ferrand, M., Pfister, C., Oesterhelt, D., Zaccai, G. (1998). Dynamics of different functional parts of bacteriorhodopsin: H-2H labeling and neutron scattering. *PNAS* 95:4970–4975.
- van Regenmortel, M.H.V. (2016). The metaphor that viruses are living is alive and well, but it is no more than a metaphor. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences* 59:117–124.
- Reid, I.N., Sparks, W.B., Lubow, S., McGrath, M., Livio, M., Valenti, J., Sowers, K.R., Shukla, H.D., MacAuley, S., Miller, T., Suvanasuthi, R., Belas, R., Colman, A., Robb, F.T., DasSarma, P., Müller, J.A., Coker, J.A., Cavicchioli, R., Chen, F., DasSarma, S. (2006). Terrestrial models for extraterrestrial life: methanogens and halophiles at Martian temperatures. *International Journal of Astrobiology* 5:89–97.
- Rhoden, A.R., Henning, W., Hurford, T.A., Patthoff, D.A., Tajeddine, R. (2017). The implications of tides on the Mimas ocean hypothesis. *Journal of Geophysical Research: Planets* 122:400–410.
- Richard A. Kerr (1997). Geomicrobiology: Life Goes to Extremes in the Deep Earth--and Elsewhere? *Science* 276:703–704.
- Richard, S.B., Madern, D., Garcin, E., Zaccai, G. (2000). Halophilic Adaptation: Novel Solvent Protein Interactions Observed in the 2.9 and 2.6 Å Resolution Structures of the Wild Type and a Mutant of Malate Dehydrogenase from *Haloarcula marismortui*. *Biochemistry*

39:992–1000.

Richens, J.L., Lane, J.S., Bramble, J.P., O'Shea, P. (2015). The electrical interplay between proteins and lipids in membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1848:1828–1836.

Richter, K., Schicklberger, M., Gescher, J. (2012). Dissimilatory Reduction of Extracellular Electron Acceptors in Anaerobic Respiration. *Appl Environ Microbiol* 78:913–921.

Riise, E.K., Lorentzen, M.S., Helland, R., Smalås, A.O., Leiros, H.-K.S., Willassen, N.P. (2007). The first structure of a cold-active catalase from *Vibrio salmonicida* at 1.96 Å reveals structural aspects of cold adaptation. *Acta Cryst D* 63:135–148.

Roche, J., Caro, J.A., Norberto, D.R., Barthe, P., Roumestand, C., Schlessman, J.L., Garcia, A.E., Garcia-Moreno E., B., Royer, C.A. (2012). Cavities determine the pressure unfolding of proteins. *Proceedings of the National Academy of Sciences* 109:6945–6950.

Rosenbaum, E., Gabel, F., Durá, M.A., Finet, S., Cléry-Barraud, C., Masson, P., Franzetti, B. (2012). Effects of hydrostatic pressure on the quaternary structure and enzymatic activity of a large peptidase complex from *Pyrococcus horikoshii*. *Archives of Biochemistry and Biophysics* 517:104–110.

Rother, M., Krzycki, J.A. (2010). Selenocysteine, Pyrrolysine, and the Unique Energy Metabolism of Methanogenic Archaea. *Archaea* 2010.

Rout, M.K., Lee, B.L., Lin, A., Xiao, W., Spyrapopoulos, L. (2018). Active Site Gate Dynamics Modulate the Catalytic Activity of the Ubiquitination Enzyme E2-25K. *Sci Rep* 8:1–15.

Ruiz, J. (2003). Heat flow and depth to a possible internal ocean on Triton. *Icarus* 166:436–439.

Russell, N.J. (1989). Adaptive modifications in membranes of halotolerant and halophilic microorganisms. *Journal of Bioenergetics and Biomembranes* 21:93–113.

Russell, N.J. (1997). Psychrophilic bacteria—Molecular adaptations of membrane lipids. *Comparative Biochemistry and Physiology Part A: Physiology* 118:489–493.

Russell, N.J., Harrison, P., Johnston, I.A., Jaenicke, R., Zuber, M., Franks, F., Wynn-Williams, D. (1990). Cold Adaptation of Microorganisms [and Discussion]. *Philosophical Transactions of the Royal Society B: Biological Sciences* 326:595–611.

Sacquin-Mora, S., Sebban, P., Derrien, V., Frick, B., Lavery, R., Alba-Simionesco, C. (2007). Probing the Flexibility of the Bacterial Reaction Center: The Wild-Type Protein Is More Rigid Than Two Site-Specific Mutants. *Biochemistry* 46:14960–14968.

Sagan, C., Thompson, W.R., Carlson, R., Gurnett, D., Hord, C. (1993). A search for life on Earth from the Galileo spacecraft. *Nature* 365:715–721.

Salvador-Castell, M., Brooks, N.J., Peters, J., Oger, P. (2020). Induction of non-lamellar phases in archaeal lipids at high temperature and high hydrostatic pressure by apolar polyisoprenoids. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1862:183130.

Sardesai, Y., Bhosle, S. (2002). Tolerance of bacteria to organic solvents. *Research in Microbiology*:263–268.

Sato, H., Nakasone, K., Yoshida, T., Kato, C., Maruyama, T. (2015). Increases of heat shock proteins and their mRNAs at high hydrostatic pressure in a deep-sea piezophilic bacterium, *Shewanella violacea*. *Extremophiles* 19:751–762.

Schiefner, A., Holtmann, G., Diederichs, K., Welte, W., Bremer, E. (2004). Structural Basis for the Binding of Compatible Solutes by ProX from the Hyperthermophilic Archaeon *Archaeoglobus fulgidus*. *J Biol Chem* 279:48270–48281.

Schirmack, J., Böhm, M., Brauer, C., Löhmansröben, H.-G., de Vera, J.-P., Möhlmann, D., Wagner, D. (2014). Laser spectroscopic real time measurements of methanogenic activity under simulated Martian subsurface analog conditions. *Planetary and Space Science* 98:198–204.

- Schmidt, C., Manning, C.E. (2017). Pressure-induced ion pairing in MgSO₄ solutions: Implications for the oceans of icy worlds. *Geochemical Perspectives Letters*:66–74.
- Schneegurt, M.A. (Ed.) (2012). Media and Conditions for the Growth of Halophilic and Halotolerant Bacteria and Archaea. In: *Advances in Understanding the Biology of Halophilic Microorganisms*, Springer Netherlands: Dordrecht, p .
- Schwendner, P., Schuerger, A.C. (2020). Exploring Microbial Activity in Low-pressure Environments. *Current Issues in Molecular Biology*:163–196.
- Scoma, A., Garrido-Amador, P., Nielsen, S.D., Røy, H., Kjeldsen, K.U. (2019). The Polyextremophilic Bacterium *Clostridium paradoxum* Attains Piezophilic Traits by Modulating Its Energy Metabolism and Cell Membrane Composition. *Appl Environ Microbiol* 85.
- Scotter, A.J., Marshall, C.B., Graham, L.A., Gilbert, J.A., Garnham, C.P., Davies, P.L. (2006). The basis for hyperactivity of antifreeze proteins. *Cryobiology* 53:229–239.
- Seager, S., Turner, E. I., Schafer, J., Ford, E. b. (2005). Vegetation's Red Edge: A Possible Spectroscopic Biosignature of Extraterrestrial Plants. *Astrobiology* 5:372–390.
- Seckbach, J. (2000). Extremophilies as Models for Extraterrestrial Life. *Bioastronomy* 99 213.
- Seckbach, J., Oren, A., Stan-Lotter, H. (2013). *Polyextremophiles: life under multiple forms of stress*. Springer Science+Business Media: Dordrecht.
- Seeger, A.H., Burggraf, S., Fiala, G., Huber, G., Huber, R., Pley, U., Stetter, K.O. (1993). Life in hot springs and hydrothermal vents. *Origins Life Evol Biosphere* 23:77–90.
- Seki, M., Kamei, A., Yamaguchi-Shinozaki, K., Shinozaki, K. (2003). Molecular responses to drought, salinity and frost: common and different paths for plant protection. *Current Opinion in Biotechnology* 14:194–199.
- Sekine, Y., Shibuya, T., Postberg, F., Hsu, H.-W., Suzuki, K., Masaki, Y., Kuwatani, T., Mori, M., Hong, P.K., Yoshizaki, M., Tachibana, S., Sirono, S. (2015). High-temperature water–rock interactions and hydrothermal environments in the chondrite-like core of Enceladus. *Nature Communications* 6:8604.
- Selsis, F., Kasting, J.F., Levrard, B., Paillet, J., Ribas, I., Delfosse, X. (2007). Habitable planets around the star Gliese 581? *A&A* 476:1373–1387.
- Sen, K., Horrell, S., Kekilli, D., Yong, C.W., Keal, T.W., Atakisi, H., Moreau, D.W., Thorne, R.E., Hough, M.A., Strange, R.W. (2017). Active-site protein dynamics and solvent accessibility in native *Achromobacter cycloclastes* copper nitrite reductase. *IUCrJ* 4:495–505.
- Serrano, R. (1996). Salt Tolerance in Plants and Microorganisms: Toxicity Targets and Defense Responses. In: Jeon KW (ed) *International Review of Cytology*, Academic Press Vol 165, pp 1–52.
- Serrano, R., Gaxiola, R. (1994). Microbial Models and Salt Stress Tolerance in Plants. *Critical Reviews in Plant Sciences* 13:121–138.
- Serres, M.H., Gopal, S., Nahum, L.A., Liang, P., Gaasterland, T., Riley, M. (2001). A functional update of the *Escherichia coli* K-12 genome. *Genome Biol* 2:research0035.1-research0035.7.
- Setlow, P. (1992). DNA in dormant spores of *Bacillus* species is in an A-like conformation. *Mol Microbiol* 6:563–567.
- Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M., Pinelli, E. (2014). Heavy-Metal-Induced Reactive Oxygen Species: Phytotoxicity and Physicochemical Changes in Plants. In: Whitacre DM (ed) *Reviews of Environmental Contamination and Toxicology Volume 232*, Springer International Publishing: Cham Vol 232, pp 1–44.
- Sharma, A. (2002). Microbial Activity at Gigapascal Pressures. *Science* 295:1514–1516.
- Shcherbakova, V., Oshurkova, V., Yoshimura, Y. (2015). The Effects of Perchlorates on the Permafrost Methanogens: Implication for Autotrophic Life on Mars. *Microorganisms* 3:518–534.

- 1
2
3 Shirodkar, P.V., Muraleedharan, U.D., Damare, S., Raghukumar, S. (2020). A Mesohaline
4 Thraustochytrid Produces Extremely Halophilic Alpha-Amylases. *Mar Biotechnol.*
5 Shrestha, U.R., Bhowmik, D., Copley, J.R.D., Tyagi, M., Leão, J.B., Chu, X. (2015). Effects
6 of pressure on the dynamics of an oligomeric protein from deep-sea hyperthermophile. *PNAS*
7 112:13886–13891.
8 Siering, P.L., Clarke, J.M., Wilson, M.S. (2006). Geochemical and Biological Diversity of
9 Acidic, Hot Springs in Lassen Volcanic National Park. *Geomicrobiology Journal* 23:129–
10 141.
11 Siglioccolo, A., Paiardini, A., Piscitelli, M., Pascarella, S. (2011). Structural adaptation of
12 extreme halophilic proteins through decrease of conserved hydrophobic contact surface. *BMC*
13 *Struct Biol* 11:50.
14 Sikkema, J., De Bont, J.A., Poolman, B. (1995). Mechanisms of membrane toxicity of
15 hydrocarbons. *Microbiological reviews* 59:201–222.
16 Siliakus, M.F., van der Oost, J., Kengen, S.W.M. (2017). Adaptations of archaeal and
17 bacterial membranes to variations in temperature, pH and pressure. *Extremophiles* 21:651–
18 670.
19 Silver, S., Phung, L.T. (1996). Bacterial heavy metal resistance: New Surprises. *Annual*
20 *Review of Microbiology* 50:753–789.
21 Sime-Ngando, T., Lucas, S., Robin, A., Tucker, K.P., Colombet, J., Bettarel, Y., Desmond,
22 E., Gribaldo, S., Forterre, P., Breitbart, M., Prangishvili, D. (2011). Diversity of virus-host
23 systems in hypersaline Lake Retba, Senegal: Viral diversity in Lake Retba. *Environmental*
24 *Microbiology* 13:1956–1972.
25 Simoneit, B.R.T., Summons, R.E., Jahnke, L.L. (1996). Biomarkers as tracers for life on early
26 earth and mars. *Origins of Life and Evolution of the Biosphere* 26:515–516.
27 Smalås, A.O., Leiros, H.K., Os, V., Willassen, N.P. (2000). Cold adapted enzymes.
28 *Biotechnol Annu Rev* 6:1–57.
29 Snellen, I., Kok, R. de, Birkby, J.L., Brandl, B., Brogi, M., Keller, C., Kenworthy, M.,
30 Schwarz, H., Stuik, R. (2015). Combining high-dispersion spectroscopy with high contrast
31 imaging: Probing rocky planets around our nearest neighbors. *A&A* 576:A59.
32 Sontag, E.M., Samant, R.S., Frydman, J. (2017). Mechanisms and Functions of Spatial
33 Protein Quality Control. *Annual Review of Biochemistry* 86:97–122.
34 Sorokin, D.Y., Berben, T., Melton, E.D., Overmars, L., Vavourakis, C.D., Muyzer, G. (2014).
35 Microbial diversity and biogeochemical cycling in soda lakes. *Extremophiles* 18:791–809.
36 Soufi, B., Krug, K., Harst, A., Macek, B. (2015). Characterization of the E. coli proteome and
37 its modifications during growth and ethanol stress. *Front Microbiol* 6.
38 Spencer, J.R., Pearl, J.C., Segura, M., Flasar, F.M., Mamoutkine, A., Romani, P., Buratti,
39 B.J., Hendrix, A.R., Spilker, L.J., Lopes, R.M.C. (2006). Cassini Encounters Enceladus:
40 Background and the Discovery of a South Polar Hot Spot. *Science* 311:1401–1405.
41 Spohn, T., Schubert, G. (2003). Oceans in the icy Galilean satellites of Jupiter? *Icarus*
42 161:456–467.
43 Stalport, F., Rouquette, L., Poch, O., Dequaire, T., Chaouche-Mechidal, N., Payart, S., Szopa,
44 C., Coll, P., Chaput, D., Jaber, M., Raulin, F., Cottin, H. (2019). The Photochemistry on
45 Space Station (PSS) Experiment: Organic Matter under Mars-like Surface UV Radiation
46 Conditions in Low Earth Orbit. *Astrobiology* 19:1037–1052.
47 Steinle, L., Knittel, K., Felber, N., Casalino, C., de Lange, G., Tessarolo, C., Stadnitskaia, A.,
48 Sinninghe Damsté, J.S., Zopfi, J., Lehmann, M.F., Treude, T., Niemann, H. (2018). Life on
49 the edge: active microbial communities in the Kryos MgCl₂-brine basin at very low water
50 activity. *The ISME Journal* 12:1414–1426.
51 Stelmach, K.B., Neveu, M., Vick-Majors, T.J., Mickol, R.L., Chou, L., Webster, K.D., Tilley,
52 M., Zacchei, F., Escudero, C., Flores Martinez, C.L., Labrado, A., Fernández, E.J.G. (2018).

- 1
2
3 Secondary Electrons as an Energy Source for Life. *Astrobiology* 18:73–85.
- 4 Stojanovic, D., Fojkar, O., Drobac-Cik, A., Cajko, K., Dulic, T., Svircev, Z. (2008).
- 5 Extremophiles: Link between earth and astrobiology. *Zbornik Matice srpske za prirodne*
- 6 *nauke*:5–16.
- 7 Sturr, M.G., Guffanti, A.A., Krulwich, T.A. (1994). Growth and bioenergetics of alkaliphilic
- 8 *Bacillus firmus* OF4 in continuous culture at high pH. *Journal of Bacteriology* 176:3111–
- 9 3116.
- 10 Subczynski, W.K., Markowska, E., Gruszecki, W.I., Siewewiesiuk, J. (1992). Effects of polar
- 11 carotenoids on dimyristoylphosphatidylcholine membranes: a spin-label study. *Biochimica et*
- 12 *Biophysica Acta (BBA) - Biomembranes* 1105:97–108.
- 13 Sundarasami, A., Sridhar, A., Mani, K. (2019). Chapter 13 - Halophilic archaea as beacon for
- 14 exobiology: Recent advances and future challenges. In: Meena SN, Naik MM (eds) *Advances*
- 15 *in Biological Science Research*, Academic Press, pp 197–214.
- 16 Suttle, C.A. (2007). Marine viruses — major players in the global ecosystem. *Nature Reviews*
- 17 *Microbiology* 5:801–812.
- 18 Suzuki, Y.J., Carini, M., Butterfield, D.A. (2010). Protein Carbonylation. *Antioxid Redox*
- 19 *Signal* 12:323–325.
- 20 Tadeo, X., López-Méndez, B., Castaño, D., Trigueros, T., Millet, O. (2009). Protein
- 21 Stabilization and the Hofmeister Effect: The Role of Hydrophobic Solvation. *Biophysical*
- 22 *Journal* 97:2595–2603.
- 23 Takai, K. (2019). Limits of Terrestrial Life and Biosphere. In: Yamagishi A, Kakegawa T,
- 24 Usui T (eds) *Astrobiology: From the Origins of Life to the Search for Extraterrestrial*
- 25 *Intelligence*, Springer: Singapore, pp 323–344.
- 26 Takai, K., Komatsu, T., Inagaki, F., Horikoshi, K. (2001). Distribution of Archaea in a Black
- 27 Smoker Chimney Structure. *Appl Environ Microbiol* 67:3618–3629.
- 28 Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H.,
- 29 Nakagawa, S., Nunoura, T., Horikoshi, K. (2008). Cell proliferation at 122°C and isotopically
- 30 heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation.
- 31 *Proc Natl Acad Sci U S A* 105:10949–10954.
- 32 Takami, H., Kobata, K., Nagahama, T., Kobayashi, H., Inoue, A., Horikoshi, K. (1999).
- 33 Biodiversity in deep-sea sites located near the south part of Japan. *Extremophiles* 3:97–102.
- 34 Takuichi, T., Toshiyuki, T., Ryuichiro, K. (1997). Change of cell membrane hydrophobicity
- 35 in a bacterium tolerant to toxic alcohols. *Canadian journal of microbiology* 43:292–295.
- 36 Talon, R., Coquelle, N., Madern, D., Girard, E. (2014). An experimental point of view on
- 37 hydration/solvation in halophilic proteins. *Front Microbiol* 5.
- 38 Tamás, M.J., Sharma, S.K., Ibstedt, S., Jacobson, T., Christen, P. (2014). Heavy Metals and
- 39 Metalloids As a Cause for Protein Misfolding and Aggregation. *Biomolecules* 4:252–267.
- 40 Tanaka, T., Burgess, J., Wright, P. (2001). High-pressure adaptation by salt stress in a
- 41 moderately halophilic bacterium obtained from open seawater. *Applied Microbiology and*
- 42 *Biotechnology* 57:200–204.
- 43 Tanaka, Y., Tsumoto, K., Yasutake, Y., Umetsu, M., Yao, M., Fukada, H., Tanaka, I.,
- 44 Kumagai, I. (2004). How Oligomerization Contributes to the Thermostability of an Archaeon
- 45 Protein PROTEIN I-ISOASPARTYL-O-METHYLTRANSFERASE FROM SULFOLOBUS
- 46 TOKODAI. *J Biol Chem* 279:32957–32967.
- 47 Taubner, R.-S., Olsson-francis, K., Vance, S., Ramkissoon, N., Postberg, F., de Vera, J.-P.,
- 48 Antunes, A., Camprubí Casas, E., Sekine, Y., Noack, L., Barge, L., Goodman, J., Jebbar, M.,
- 49 Journaux, B., Karatekin, Ö., Klenner, F., Rabbow, E., Rettberg, P., Rückriemen-Bez, T.,
- 50 Soderlund, K. (2020). Experimental and Simulation Efforts in the Astrobiological Exploration
- 51 of Exooceans. *Space Science Reviews* 216.
- 52 Taubner, R.-S., Pappenreiter, P., Zwicker, J., Smrzka, D., Pruckner, C., Kolar, P., Bernacchi,
- 53
54
55
56
57
58
59
60

- 1
2
3 S., Seifert, A.H., Krajete, A., Bach, W., Peckmann, J., Paulik, C., Firneis, M.G., Schleper, C.,
4 Rittmann, S.K.-M.R. (2018). Biological methane production under putative Enceladus-like
5 conditions. *Nature Communications* 9:748.
- 6 Taubner, R.-S., Schleper, C., Firneis, M.G., Rittmann, S.K.-M.R. (2015). Assessing the
7 Ecophysiology of Methanogens in the Context of Recent Astrobiological and Planetological
8 Studies. *Life* 5:1652–1686.
- 9 Tehei, M., Franzetti, B., Madern, D., Ginzburg, M., Ginzburg, B.Z., Giudici-Ortoni, M.-T.,
10 Bruschi, M., Zaccai, G. (2004). Adaptation to extreme environments: macromolecular
11 dynamics in bacteria compared in vivo by neutron scattering. *EMBO reports* 5:66–70.
- 12 Tehei, M., Franzetti, B., Maurel, M.-C., Vergne, J., Hountondji, C., Zaccai, G. (2002). The
13 search for traces of life: the protective effect of salt on biological macromolecules.
14 *Extremophiles* 6:427–430.
- 15 Tehei, M., Franzetti, B., Wood, K., Gabel, F., Fabiani, E., Jasnin, M., Zamponi, M.,
16 Oesterhelt, D., Zaccai, G., Ginzburg, M., Ginzburg, B.-Z. (2007). Neutron scattering reveals
17 extremely slow cell water in a Dead Sea organism. *PNAS* 104:766–771.
- 18 Tehei, M., Zaccai, G. (2007). Adaptation to high temperatures through macromolecular
19 dynamics by neutron scattering. *The FEBS Journal*:4034–4043.
- 20 Thomas, P.C., Tajeddine, R., Tiscareno, M.S., Burns, J.A., Joseph, J., Loredó, T.J.,
21 Helfenstein, P., Porco, C. (2016). Enceladus's measured physical libration requires a global
22 subsurface ocean. *Icarus* 264:37–47.
- 23 Thompson, M.J., Eisenberg, D. (1999). Transproteomic evidence of a loop-deletion
24 mechanism for enhancing protein thermostability 1 Edited by I. B. Honig. *Journal of*
25 *Molecular Biology* 290:595–604.
- 26 Thorvaldsen, S., Hjerde, E., Fenton, C., Willassen, N.P. (2007). Molecular characterization of
27 cold adaptation based on ortholog protein sequences from Vibrionaceae species.
28 *Extremophiles* 11:719–732.
- 29 Tien, H.T., Ottova-Leitmannova, A. (2000). *Membrane Biophysics: As Viewed from*
30 *Experimental Bilayer Lipid Membranes*. Elsevier.
- 31 Tikhov, G.A. (1953). Astrobiology. *Molodaya gvardia (Young Guard) Moscow: Publishing*
32 *House*.
- 33 Tobie, G. (2015). Enceladus' hot springs. *Nature* 519:162–163.
- 34 Tokunaga, H., Ishibashi, M., Arakawa, T., Tokunaga, M. (2004). Highly efficient renaturation
35 of beta-lactamase isolated from moderately halophilic bacteria. *FEBS Lett* 558:7–12.
- 36 Tokunaga, H., Maeda, J., Arakawa, T., Tokunaga, M. (2017). Reversible Activation of
37 Halophilic β -lactamase from Methanol-Induced Inactive Form: Contrast to Irreversible
38 Inactivation of Non-Halophilic Counterpart. *Protein J* 36:228–237.
- 39 Tolner, B., Poolman, B., Konings, W.N. (1997). Adaptation of microorganisms and their
40 transport systems to high temperatures. *Comparative Biochemistry and Physiology Part A:*
41 *Physiology* 118:423–428.
- 42 Trepreau, J., Grosse, C., Mouesca, J.-M., Sarret, G., Girard, E., Petit-Haertlein, I.,
43 Kuennemann, S., Desbourdes, C., de Rosny, E., Maillard, A.P., Nies, D.H., Covès, J. (2014).
44 Metal sensing and signal transduction by CnrX from *Cupriavidus metallidurans* CH34: role of
45 the only methionine assessed by a functional, spectroscopic, and theoretical study.
46 *Metallomics* 6:263–273.
- 47 Trumbo, S.K., Brown, M.E., Hand, K.P. (2019). Sodium chloride on the surface of Europa.
48 *Science Advances* 5:eaaw7123.
- 49 Tsai, C.-J., Maizel, J.V., Nussinov, R. (2002). The hydrophobic effect: a new insight from
50 cold denaturation and a two-state water structure. *Critical Reviews in Biochemistry and*
51 *Molecular Biology* 37:55–69.
- 52 Turbet, M., Boulet, C., Karman, T. (2020). Measurements and semi-empirical calculations of
53
54
55
56
57
58
59
60

- 1
2
3 CO₂ + CH₄ and CO₂ + H₂ collision-induced absorption across a wide range of wavelengths
4 and temperatures. Application for the prediction of early Mars surface temperature. *Icarus*
5 346:113762.
- 6 Turbet, M., Forget, F. (2019). The paradoxes of the Late Hesperian Mars ocean. *Sci Rep* 9:1–
7 5.
- 8 Tyler, R.H. (2009). Ocean tides heat Enceladus. *Geophysical Research Letters* 36.
- 9 Usami, R., Fukushima, T., Mizuki, T., Yoshida, Y., Inoue, A., Horikoshi, K. (2005). Organic
10 solvent tolerance of halophilic archaea, Haloarcula strains: Effects of NaCl concentration on
11 the tolerance and polar lipid composition. *Journal of Bioscience and Bioengineering* 99:169–
12 174.
- 13 Vago, J., Witasse, O., Svedhem, H., Baglioni, P., Haldemann, A., Gianfiglio, G., Blancquaert,
14 T., McCoy, D., de Groot, R. (2015). ESA ExoMars program: The next step in exploring Mars.
15 *Sol Syst Res* 49:518–528.
- 16 Valenti, P., Bodnar, R.J., Schmidt, C. (2012). Experimental determination of H₂O–NaCl
17 liquid to 25mass% NaCl and 1.4GPa: Application to the Jovian satellite Europa. *Geochimica*
18 *et Cosmochimica Acta* 92:117–128.
- 19 Vance, S., Bouffard, M., Choukroun, M., Sotin, C. (2014). Ganymede's internal structure
20 including thermodynamics of magnesium sulfate oceans in contact with ice. *Planetary and*
21 *Space Science* 96:62–70.
- 22 Vanlint, D., Mitchell, R., Bailey, E., Meersman, F., McMillan, P.F., Michiels, C.W., Aertsen,
23 A. (2011). Rapid Acquisition of Gigapascal-High-Pressure Resistance by *Escherichia coli*.
24 *mBio* 2.
- 25 Vargas, C., Jebbar, M., Carrasco, R., Blanco, C., Calderón, M.I., Iglesias-Guerra, F., Nieto,
26 J.J. (2006). Ectoines as compatible solutes and carbon and energy sources for the halophilic
27 bacterium *Chromohalobacter salexigens*. *Journal of Applied Microbiology* 100:98–107.
- 28 Varrella, S., Tangherlini, M., Corinaldesi, C. (2020). Deep Hypersaline Anoxic Basins as
29 Untapped Reservoir of Polyextremophilic Prokaryotes of Biotechnological Interest. *Marine*
30 *Drugs* 18:91.
- 31 Vauclore, P., Natali, F., Kleman, J.P., Zaccai, G., Franzetti, B. (2020). Surviving salt
32 fluctuations: stress and recovery in *Halobacterium salinarum*, an extreme halophilic
33 Archaeon. *Sci Rep* 10:1–10.
- 34 Venketesh, S., Dayananda, C. (2008). Properties, Potentials, and Prospects of Antifreeze
35 Proteins. *Critical Reviews in Biotechnology* 28:57–82.
- 36 Ventosa, A., Oren, A. (1996). *Halobacterium salinarum* nom. corrig., a Name To Replace
37 *Halobacterium salinarium* (Elazari-Volcani) and To Include *Halobacterium halobium* and
38 *Halobacterium cutirubrum*. *International Journal of Systematic and Evolutionary*
39 *Microbiology*, 46:347–347.
- 40 Vetriani, C., Chew, Y.S., Miller, S.M., Yagi, J., Coombs, J., Lutz, R.A., Barkay, T. (2005).
41 Mercury Adaptation among Bacteria from a Deep-Sea Hydrothermal Vent. *Appl Environ*
42 *Microbiol* 71:220–226.
- 43 Vieille, C., Burdette, D.S., Zeikus, J.G. (1996). Thermozyms. In: El-Gewely MR (ed)
44 *Biotechnology Annual Review*, Elsevier Vol 2, pp 1–83.
- 45 Vieille, C., Zeikus, G.J. (2001). Hyperthermophilic Enzymes: Sources, Uses, and Molecular
46 Mechanisms for Thermostability. *Microbiol Mol Biol Rev* 65:1–43.
- 47 Vinagre, C., Madeira, D., Narciso, L., Cabral, H.N., Diniz, M. (2012). Effect of temperature
48 on oxidative stress in fish: Lipid peroxidation and catalase activity in the muscle of juvenile
49 seabass, *Dicentrarchus labrax*. *Ecological Indicators* 23:274–279.
- 50 Vinçon-Laugier, A., Cravo-Laureau, C., Mitteau, I., Grossi, V. (2017). Temperature-
51 Dependent Alkyl Glycerol Ether Lipid Composition of Mesophilic and Thermophilic Sulfate-
52 Reducing Bacteria. *Front Microbiol* 8:1532.
- 53
54
55
56
57
58
59
60

- 1
2
3 Voet, D., Voet, J.G. (2016). *Biochimie*. De Boeck Superieur.
- 4 Von Hippel, P.H., Schleich, T. (1969). Ion effects on the solution structure of biological
5 macromolecules. *Acc Chem Res* 2:257–265.
- 6 van de Vossenberg, J.L.C.M.C., Driessen, A.J.M., Grant, D., Konings, W.N. (1999). Lipid
7 membranes from halophilic and alkali-halophilic Archaea have a low H⁺ and Na⁺
8 permeability at high salt concentration. *Extremophiles* 3:253–257.
- 9 Wadsworth, J., Cockell, C.S. (2017). Perchlorates on Mars enhance the bacteriocidal effects
10 of UV light. *Sci Rep* 7:1–8.
- 11 Waite, J.H. (2006). Cassini Ion and Neutral Mass Spectrometer: Enceladus Plume
12 Composition and Structure. *Science* 311:1419–1422.
- 13 Waite, J.H., Glein, C.R., Perryman, R.S., Teolis, B.D., Magee, B.A., Miller, G., Grimes, J.,
14 Perry, M.E., Miller, K.E., Bouquet, A., Lunine, J.I., Brockwell, T., Bolton, S.J. (2017).
15 Cassini finds molecular hydrogen in the Enceladus plume: Evidence for hydrothermal
16 processes. *Science* 356:155–159.
- 17 Waite, J.H., Lewis, W.S., Magee, B.A., Lunine, J.I., McKinnon, W.B., Glein, C.R., Mousis,
18 O., Young, D.T., Brockwell, T., Westlake, J., Nguyen, M.-J., Teolis, B.D., Niemann, H.B., Jr,
19 R.L.M., Perry, M., Ip, W.-H. (2009). Liquid water on Enceladus from observations of
20 ammonia and 40 Ar in the plume. *Nature* 460:487–490.
- 21 Wallon, G., Kryger, G., Lovett, S.T., Oshima, T., Ringe, D., Petsko, G.A. (1997). Crystal
22 structures of Escherichia coli and Salmonella typhimurium 3-isopropylmalate dehydrogenase
23 and comparison with their thermophilic counterpart from Thermus thermophilus 11 Edited by
24 A. R. Fersht. *Journal of Molecular Biology* 266:1016–1031.
- 25 Wallon, G., Lovett, S.T., Magyar, C., Svingor, A., Szilagyi, A., Zavodszky, P., Ringe, D.,
26 Petsko, G.A. (1997). Sequence and homology model of 3-isopropylmalate dehydrogenase
27 from the psychrotrophic bacterium Vibrio sp. I5 suggest reasons for thermal instability.
28 *Protein engineering* 10:665–672.
- 29 Wang, S., Kool, E.T. (1995). Origins of the large differences in stability of DNA and RNA
30 helices: C-5 methyl and 2'-hydroxyl effects. *Biochemistry* 34:4125–4132.
- 31 Wang, J.-Y., Syvanen, M. (1992). DNA twist as a transcriptional sensor for environmental
32 changes. *Molecular Microbiology* 6:1861–1866.
- 33 Webb, K.M., Yu, J., Robinson, C.K., Noboru, T., Lee, Y.C., DiRuggiero, J. (2013). Effects of
34 intracellular Mn on the radiation resistance of the halophilic archaeon Halobacterium
35 salinarum. *Extremophiles* 17:485–497.
- 36 Webster, C.R., Mahaffy, P.R., Atreya, S.K., Flesch, G.J., Mischna, M.A., Meslin, P.-Y.,
37 Farley, K.A., Conrad, P.G., Christensen, L.E., Pavlov, A.A., Martín-Torres, J., Zorzano, M.-
38 P., McConnochie, T.H., Owen, T., Eigenbrode, J.L., Glavin, D.P., Steele, A., Malespin, C.A.,
39 Archer, P.D., Sutter, B., Coll, P., Freissinet, C., McKay, C.P., Moores, J.E., Schwenzer, S.P.,
40 Bridges, J.C., Navarro-Gonzalez, R., Gellert, R., Lemmon, M.T., Team, the M.S. (2015).
41 Mars methane detection and variability at Gale crater. *Science* 347:415–417.
- 42 Webster, C.R., Mahaffy, P.R., Atreya, S.K., Moores, J.E., Flesch, G.J., Malespin, C., McKay,
43 C.P., Martinez, G., Smith, C.L., Martin-Torres, J., Gomez-Elvira, J., Zorzano, M.-P., Wong,
44 M.H., Trainer, M.G., Steele, A., Archer, D., Sutter, B., Coll, P.J., Freissinet, C., Meslin, P.-Y.,
45 Gough, R.V., House, C.H., Pavlov, A., Eigenbrode, J.L., Glavin, D.P., Pearson, J.C.,
46 Keymeulen, D., Christensen, L.E., Schwenzer, S.P., Navarro-Gonzalez, R., Pla-García, J.,
47 Rafkin, S.C.R., Vicente-Retortillo, Á., Kahanpää, H., Viudez-Moreiras, D., Smith, M.D.,
48 Harri, A.-M., Genzer, M., Hassler, D.M., Lemmon, M., Crisp, J., Sander, S.P., Zurek, R.W.,
49 Vasavada, A.R. (2018). Background levels of methane in Mars' atmosphere show strong
50 seasonal variations. *Science* 360:1093–1096.
- 51 Welch, T.J., Farewell, A., Neidhardt, F.C., Bartlett, D.H. (1993). Stress response of
52 Escherichia coli to elevated hydrostatic pressure. *Journal of Bacteriology* 175:7170–7177.
- 53
54
55
56
57
58
59
60

- 1
2
3 Wells, L.E., Deming, J.W. (2006). Characterization of a cold-active bacteriophage on two
4 psychrophilic marine hosts. *Aquatic Microbial Ecology* 45:15–29.
- 5 Wemekamp-Kamphuis, H.H., Karatzas, A.K., Wouters, J.A., Abee, T. (2002). Enhanced
6 Levels of Cold Shock Proteins in *Listeria monocytogenes* LO28 upon Exposure to Low
7 Temperature and High Hydrostatic Pressure. *Appl Environ Microbiol* 68:456–463.
- 8 van der Wielen, P.W.J.J. (2005). The Enigma of Prokaryotic Life in Deep Hypersaline
9 Anoxic Basins. *Science* 307:121–123.
- 10 Williams, J.P., Hallsworth, J.E. (2009). Limits of life in hostile environments: no barriers to
11 biosphere function? *Environ Microbiol* 11:3292–3308.
- 12 Williams, T.J., Liao, Y., Ye, J., Kuchel, R.P., Poljak, A., Raftery, M.J., Cavicchioli, R.
13 (2017). Cold adaptation of the Antarctic haloarchaea *Halohasta litchfieldiae* and *Halorubrum*
14 *lacusprofundi*. *Environmental Microbiology* 19:2210–2227.
- 15 Williford, K.H., Farley, K.A., Stack, K.M., Allwood, A.C., Beaty, D., Beegle, L.W., Bhartia,
16 R., Brown, A.J., de la Torre Juarez, M., Hamran, S.-E., Hecht, M.H., Hurowitz, J.A.,
17 Rodriguez-Manfredi, J.A., Maurice, S., Milkovich, S., Wiens, R.C. (2018). Chapter 11 - The
18 NASA Mars 2020 Rover Mission and the Search for Extraterrestrial Life. In: Cabrol NA,
19 Grin EA (eds) *From Habitability to Life on Mars*, Elsevier, pp 275–308.
- 20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Woitke, P., Herbort, O., Helling, C., Stüeken, E., Dominik, M., Barth, P., Samra, D. (2020).
Coexistence of CH₄, CO₂ and H₂O in exoplanet atmospheres. *arXiv:201012241 [astro-ph]*.
- Wordsworth, R.D. (2016). The Climate of Early Mars. *Annual Review of Earth and Planetary
Sciences* 44:381–408.
- Wright, P.E., Dyson, H.J. (2015). Intrinsically disordered proteins in cellular signalling and
regulation. *Nat Rev Mol Cell Biol* 16:18–29.
- Xia, Y.-L., Sun, J.-H., Ai, S.-M., Li, Y., Du, X., Sang, P., Yang, L.-Q., Fu, Y.-X., Liu, S.-Q.
(2018). Insights into the role of electrostatics in temperature adaptation: a comparative study
of psychrophilic, mesophilic, and thermophilic subtilisin-like serine proteases. *RSC Advances*
8:29698–29713.
- Xie, B.-B., Bian, F., Chen, X.-L., He, H.-L., Guo, J., Gao, X., Zeng, Y.-X., Chen, B., Zhou,
B.-C., Zhang, Y.-Z. (2009). Cold Adaptation of Zinc Metalloproteases in the Thermolysin
Family from Deep Sea and Arctic Sea Ice Bacteria Revealed by Catalytic and Structural
Properties and Molecular Dynamics NEW INSIGHTS INTO RELATIONSHIP BETWEEN
CONFORMATIONAL FLEXIBILITY AND HYDROGEN BONDING. *J Biol Chem*
284:9257–9269.
- Xiong, L., Schumaker, K.S., Zhu, J.-K. (2002). Cell Signaling during Cold, Drought, and Salt
Stress. *The Plant Cell* 14:S165–S183.
- Xu, Y., Havenith, M. (2015). Perspective: Watching low-frequency vibrations of water in
biomolecular recognition by THz spectroscopy. *J Chem Phys* 143:170901.
- Yakimov, M.M., Cono, V.L., Spada, G.L., Bortoluzzi, G., Messina, E., Smedile, F., Arcadi,
E., Borghini, M., Ferrer, M., Schmitt-Kopplin, P., Hertkorn, N., Cray, J.A., Hallsworth, J.E.,
Golyshin, P.N., Giuliano, L. (2015). Microbial community of the deep-sea brine Lake Kryos
seawater–brine interface is active below the chaotropicity limit of life as revealed by recovery
of mRNA. *Environmental Microbiology* 17:364–382.
- Yamagata, Y., Ogasahara, K., Hioki, Y., Lee, S.J., Nakagawa, A., Nakamura, H., Ishida, M.,
Kuramitsu, S., Yutani, K. (2001). Entropic Stabilization of the Tryptophan Synthase α -
Subunit from a Hyperthermophile, *Pyrococcus furiosus* X-RAY ANALYSIS AND
CALORIMETRY. *J Biol Chem* 276:11062–11071.
- Yayanos, A.A. (2002). Are Cells Viable at Gigapascal Pressures? *Science* 297:295–295.
- Yip, K.S.P., Stillman, T.J., Britton, K.L., Artymiuk, P.J., Baker, P.J., Sedelnikova, S.E.,
Engel, P.C., Pasquo, A., Chiaraluce, R., Consalvi, V., others (1995). The structure of
Pyrococcus furiosus glutamate dehydrogenase reveals a key role for ion-pair networks in

- maintaining enzyme stability at extreme temperatures. *Structure* 3:1147–1158.
- Yoshidome, T., Kinoshita, M. (2012). Physical origin of hydrophobicity studied in terms of cold denaturation of proteins: comparison between water and simple fluids. *Phys Chem Chem Phys* 14:14554–14566.
- Yung, Y.L., Chen, P., Nealson, K., Atreya, S., Beckett, P., Blank, J.G., Ehlmann, B., Eiler, J., Etiope, G., Ferry, J.G., Forget, F., Gao, P., Hu, R., Kleinböhl, A., Klusman, R., Lefèvre, F., Miller, C., Mischna, M., Mumma, M., Newman, S., Oehler, D., Okumura, M., Oremland, R., Orphan, V., Popa, R., Russell, M., Shen, L., Sherwood Lollar, B., Staehle, R., Stamenković, V., Stolper, D., Templeton, A., Vandaele, A.C., Viscardy, S., Webster, C.R., Wennberg, P.O., Wong, M.L., Worden, J. (2018). Methane on Mars and Habitability: Challenges and Responses. *Astrobiology* 18:1221–1242.
- Zaccai, G. (2011). Molecular adaptations to life in high salt: lessons from *Haloarcula marismortui*. In: *Origins and Evolution of Life : An Astrobiological Perspective*, Cambridge University Press, pp 375–388.
- Zaccai, G. (2013). Ecology of Protein Dynamics.
- Zaccai, G. (2020). Molecular dynamics in cells: A neutron view. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1864:129475.
- Zaccai, G., Bagyan, I., Combet, J., Cuello, G.J., Demé, B., Fichou, Y., Gallat, F.-X., Galvan Josa, V.M., von Gronau, S., Haertlein, M., Martel, A., Moulin, M., Neumann, M., Weik, M., Oesterhelt, D. (2016). Neutrons describe ectoine effects on water H-bonding and hydration around a soluble protein and a cell membrane. *Scientific Reports* 6:31434.
- Zajc, J., Džeroski, S., Kocev, D., Oren, A., Sonjak, S., Tkavc, R., Gunde-Cimerman, N. (2014). Chaophilic or chaotolerant fungi: a new category of extremophiles? *Front Microbiol* 5.
- Zale, S.E., Klibanov, A.M. (1986). Why does ribonuclease irreversibly inactivate at high temperatures? *Biochemistry* 25:5432–5444.
- Zeng, X., Birrien, J.-L., Fouquet, Y., Cherkashov, G., Jebbar, M., Querellou, J., Oger, P., Cambon-Bonavita, M.-A., Xiao, X., Prieur, D. (2009). *Pyrococcus* CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life. *ISME J* 3:873–876.
- Zhao, Q. (2017). On the indirect relationship between protein dynamics and enzyme activity. *Progress in Biophysics and Molecular Biology* 125:52–60.
- Zhao, B., Hu, Q., Guo, X., Liao, Z., Sarmiento, F., Mesbah, N.M., Yan, Y., Li, J., Wiegel, J. (2018). *Natronolimnobius aegyptiacus* sp. nov., an extremely halophilic alkalithermophilic archaeon isolated from the athalassohaline Wadi An Natrun, Egypt. *Int J Syst Evol Microbiol* 68:498–506.
- Zhdanova, N.N., Tugay, T., Dighton, J., Zheltonozhsky, V., Mcdermott, P. (2004). Ionizing radiation attracts soil fungi. *Mycological Research* 108:1089–1096.
- Zheng, Y., Li, Y., Liu, W., Chen, C.-C., Ko, T.-P., He, M., Xu, Z., Liu, M., Luo, H., Guo, R.-T., Yao, B., Ma, Y. (2016). Structural insight into potential cold adaptation mechanism through a psychrophilic glycoside hydrolase family 10 endo- β -1,4-xylanase. *Journal of Structural Biology* 193:206–211.
- Zhu, J.-K. (2002). Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology* 53:247–273.
- Zolotov, M.Y. (2003). Energy for biologic sulfate reduction in a hydrothermally formed ocean on Europa. *Journal of Geophysical Research* 108.
- Zolotov, M.Y. (2007). An oceanic composition on early and today's Enceladus. *Geophysical Research Letters* 34.
- Zolotov, M.Y., Shock, E.L. (2001). Composition and stability of salts on the surface of Europa and their oceanic origin. *Journal of Geophysical Research: Planets* 106:32815–

1
2
3 32827.

4 Zwickl, P., Fabry, S., Bogedain, C., Haas, A., Hensel, R. (1990). Glyceraldehyde-3-phosphate
5 dehydrogenase from the hyperthermophilic archaeobacterium *Pyrococcus woesei*:
6 characterization of the enzyme, cloning and sequencing of the gene, and expression in
7 *Escherichia coli*. *Journal of Bacteriology* 172:4329–4338.

8
9 Zwier, M.C., Chong, L.T. (2010). Reaching biological timescales with all-atom molecular
10 dynamics simulations. *Current Opinion in Pharmacology* 10:745–752.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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_{β} and L_{α} . Reversible phase transitions can be triggered by low temperature (LT), high pressure (HP) or high temperature (HT). Locally, some types of lipids are found in the ripple phase P_{β} which is an intermediary between the gel and fluid phases. Bilayer pictures taken from: <http://www.vwalter.fr/ressources/scheme/>
Adapted from (Jebbar et al. 2015).

Figure 5: Interactions between ions and proteins

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

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

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

Figure 7: Structural features of extremophilic proteins

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

Figure 8: Molecular traits of lipids in extreme conditions

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1: General overview of extremophily

For Peer Review Only; Not for Distribution

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

| | | | | | | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------|------------------------------------------------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 | Ionizing radiation (Impossible to exclude) | Radioresistant organisms survive exposure to 500Gy | Survival after exposure to 30kGy (<i>Thermococcus gammatolerans</i>) | Anthropically contaminated environments, Guyamas basin hydrothermal vent | Martian surface | Less radioresistant but radiotrophic fungi are known |
| | Chaotropes abundancy (Possible to exclude) | Chaotolerant organisms tolerate high concentration of MgCl ₂ or CaCl ₂ without kosmotropic counterion | Growth at 2.1M MgCl ₂ (<i>Aspergillus proliferans</i>) or 2.0M CaCl ₂ (<i>Aspergillus sydowii</i>) | DHABs, athalassohaline lakes | Europa subglacial ocean, Martian surface | No true chaophile known yet |
| | Heavy metal abundancy (Possible to exclude) | Metalloresistant organisms can grow at millimolar concentrations of heavy metal ions | Growth at 20mM Zn ²⁺ , 20mM Co ²⁺ or 5mM Cd ²⁺ (<i>Ralstonia</i> sp. CHR4) > 48h survival on pure copper surface (<i>Kocuria palustris</i> and <i>Brachybacterium conglomeratum</i>) | Contaminated soils, 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 ← Stabilize proteins ← Decrease hydrophobic solubility ← Increase surface tension | Chaotropic = Salting in → Promote denaturation → Increase hydrophobic solubility → Decrease surface tension → |
| Anions | $\text{CO}_3^{2-} > \text{SO}_4^{2-} > \text{HPO}_4^{2-} > \text{CH}_3\text{COO}^- > \text{Cl}^- > \text{NO}_3^- > \text{ClO}_3^- > \text{ClO}_4^- > \text{SCN}^-$ | |
| | ← Strongly hydrated | Weakly hydrated → |
| Cations | $\text{NH}_4^+ > \text{K}^+ > \text{Na}^+ > \text{H}^+ > \text{Mg}^{2+} > \text{Ca}^{2+} > \text{Al}^{3+}$ | |
| | ← Weakly hydrated cations | Strongly hydrated cations → |

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

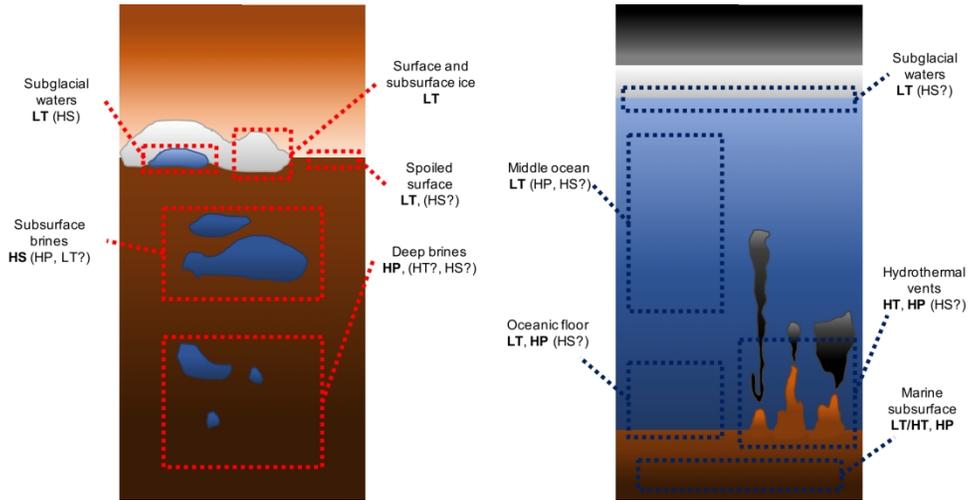


Figure 1: Extreme extraterrestrial environments

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.

719x358mm (72 x 72 DPI)

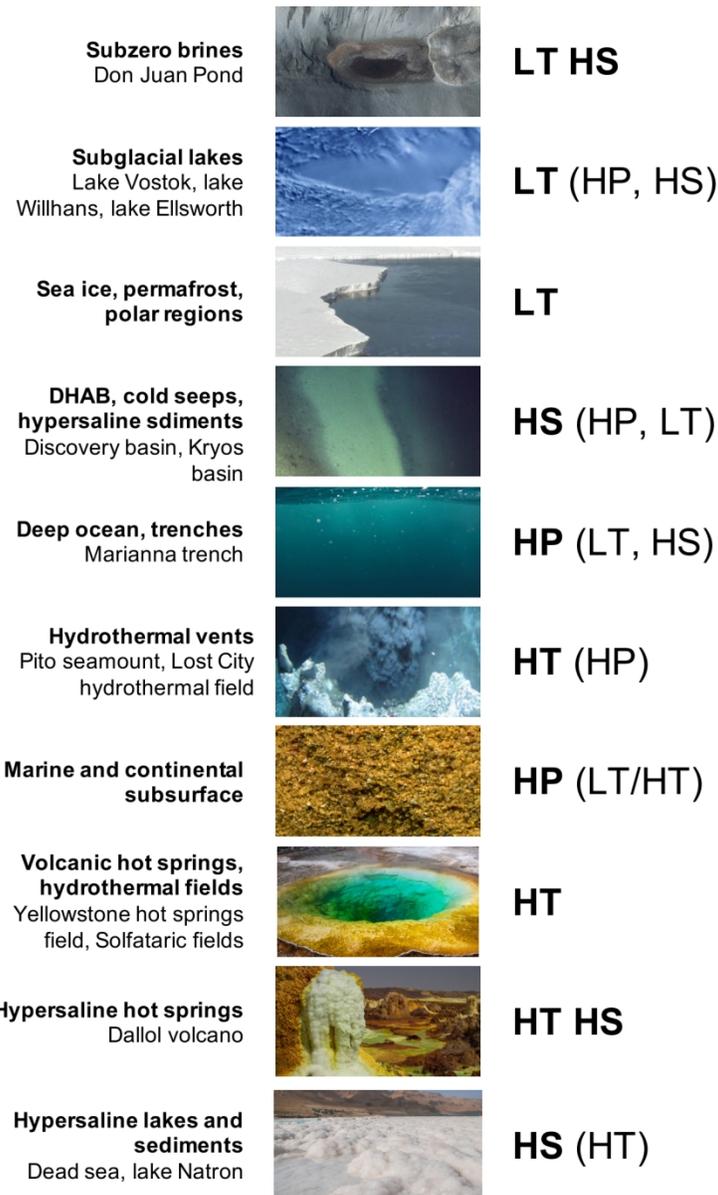


Figure 2: Terrestrial environments with permanent extreme conditions

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).

490x754mm (72 x 72 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

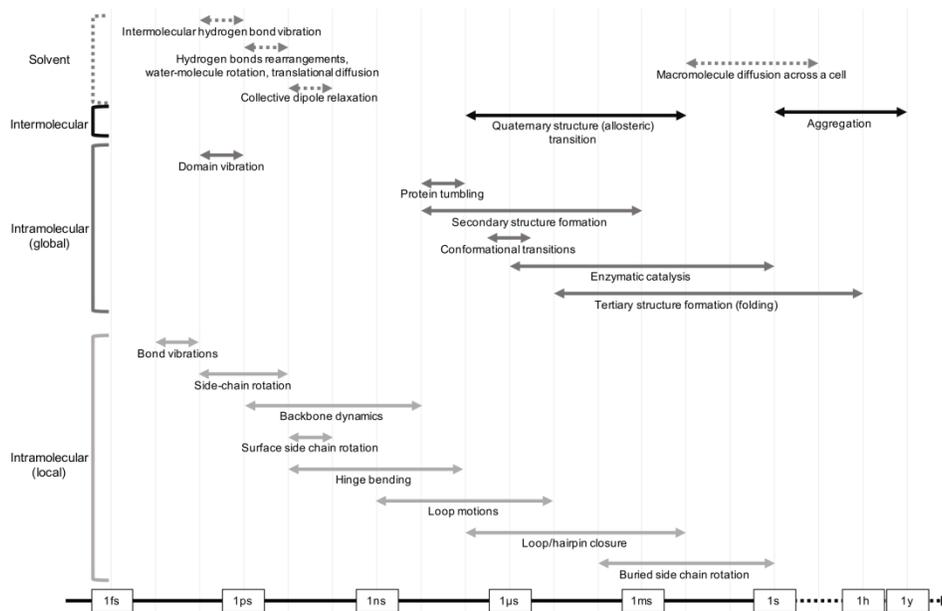


Figure 3: Dynamical properties of proteins and solvent

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).

858x530mm (72 x 72 DPI)

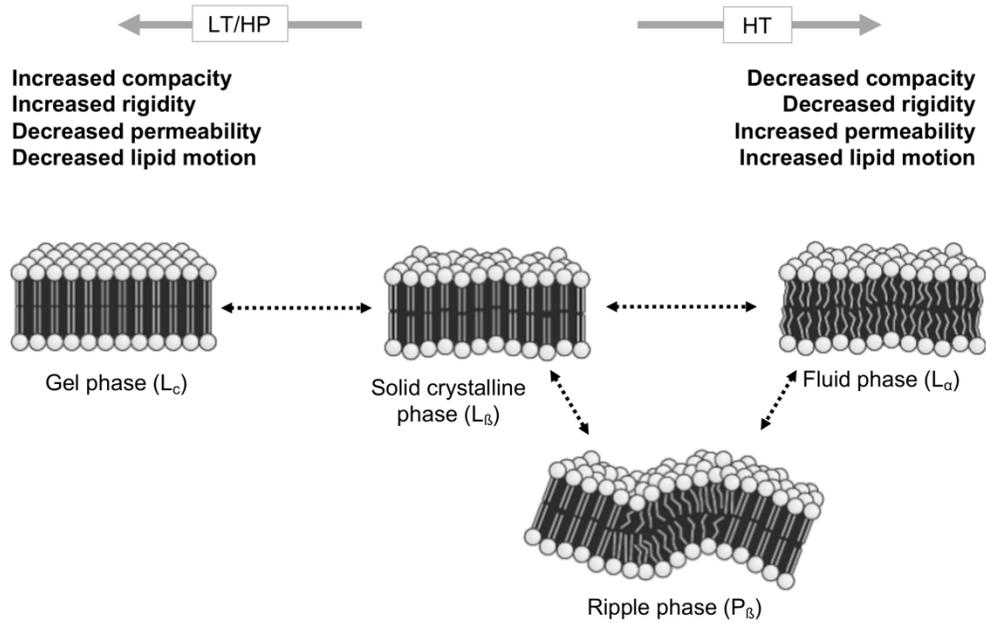


Figure 4: Phase transitions of lipid membranes

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

785x500mm (72 x 72 DPI)

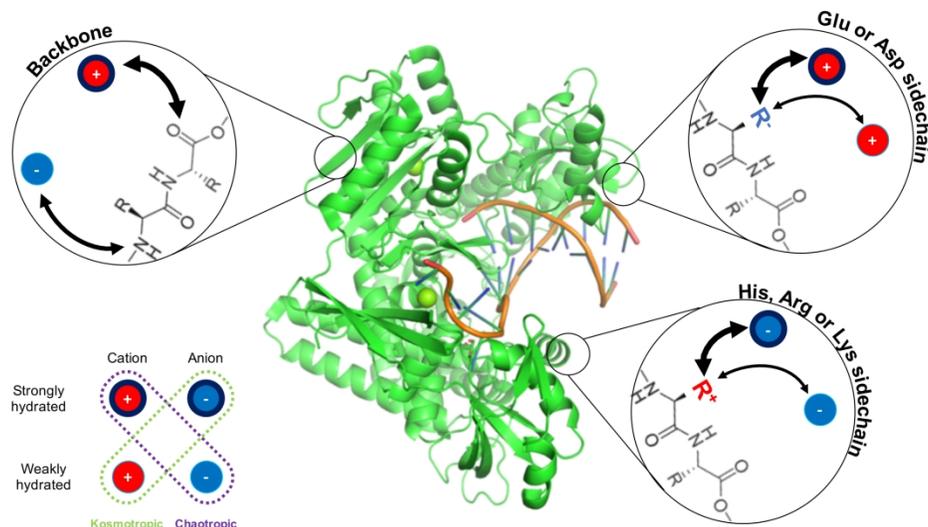


Figure 5: Interactions between ions and proteins

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).

984x581mm (72 x 72 DPI)

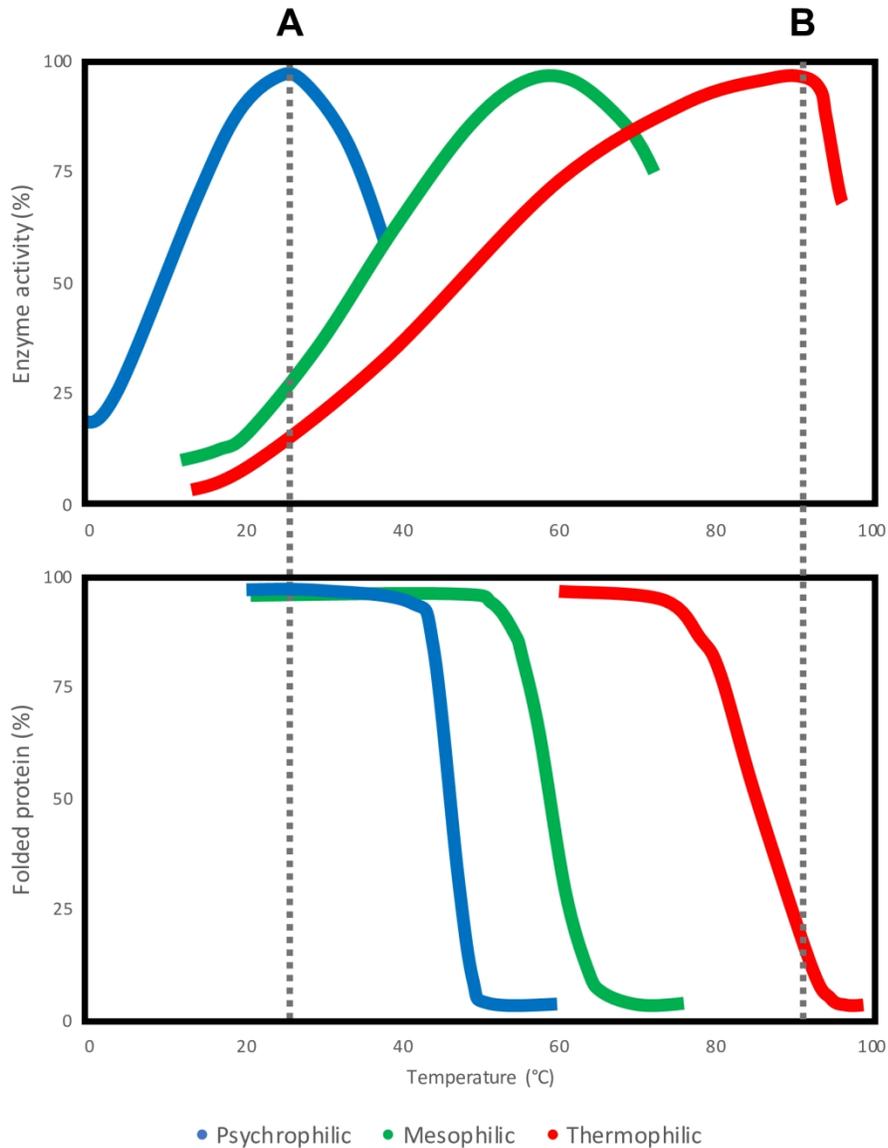


Figure 6: Theoretical stability/activity thermal curves of three homologous extremophilic enzymes

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).

155x199mm (300 x 300 DPI)

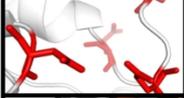
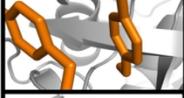
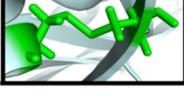
| Interactions and involved residues | Thermophilic protein | Psychrophilic protein | Halophilic protein | Piezophilic protein |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------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

Figure 7: Structural features of extremophilic proteins

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

683x464mm (72 x 72 DPI)

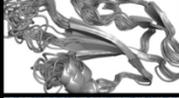
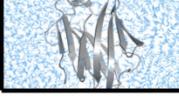
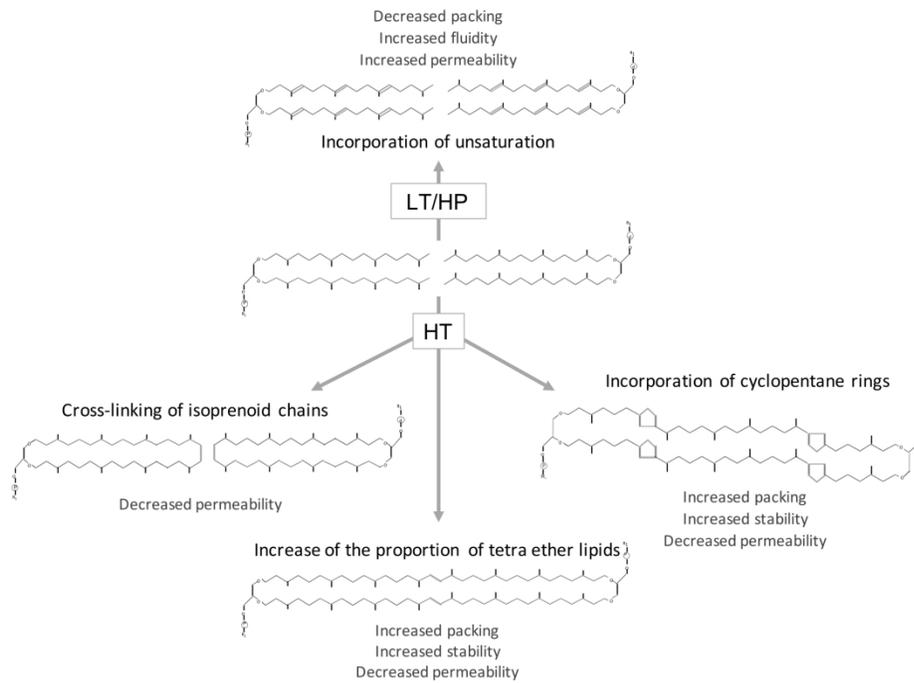
| Other features | | Thermophilic protein | Psychrophilic protein | Halophilic protein | Piezophilic protein |
|-----------------------------------------------------------------------------------|-----------------|------------------------------------------------------------|---------------------------------------------------------|-------------------------------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

682x325mm (72 x 72 DPI)



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).

35
36
37 801x588mm (72 x 72 DPI)