REVIEW ARTICLE

The ambivalent role of water at the origins of life

Andrey do Nascimento Vieira[1], Karl Kleinermanns[2], William F. Martin[1] and Martina Preiner[1]

1 Institute for Molecular Evolution, University of Düsseldorf, Germany
2 Institute for Physical Chemistry, University of Düsseldorf, Germany

Life as we know it would not exist without water. However, water molecules not only serve as a solvent and reactant but can also promote hydrolysis, which counteracts the formation of essential organic molecules. This conundrum constitutes one of the central issues in origin of life. Hydrolysis is an important part of energy metabolism for all living organisms but only because, inside cells, it is a controlled reaction. How could hydrolysis have been regulated under prebiotic settings? Lower water activities possibly provide an answer: geochemical sites with less free and more bound water can supply the necessary conditions for protometabolic reactions. Such conditions occur in serpentinising systems, hydrothermal sites that synthesise hydrogen gas via rock–water interactions. Here, we summarise the parallels between biotic and abiotic means of controlling hydrolysis in order to narrow the gap between biochemical and geochemical reactions and briefly outline how hydrolysis could even have played a constructive role at the origin of molecular self-organisation.

Keywords: geochemistry; hydrolysis; hydrothermal vents; mineral catalysis; molecular self-organisation; origin of life; protometabolism; serpentinising systems; water activity; water–rock interactions

Water is essential for all known forms of life [1]. As the solvent for life, it provides protons (H^+) and hydroxyl groups (OH^-) for myriad reactions but it creates a central problem when it comes to life's origin: hydrolysis. Water molecules dissociate chemical bonds and thereby break larger molecules or polymers into their monomeric components. In free solution, condensation reactions that generate water are thermodynamically unfavourable. Both protons and hydroxide ions can catalyse hydrolysis reactions, making them highly pH-dependent processes [2]. Water molecules can easily cleave ester and amide bonds and thus hydrolyse nucleic acids and proteins or they affect the half-life of reactants. In hydrolysis, OH^- usually replaces another moiety in the molecule (e.g., phosphate, amino or thiol group) by nucleophilic substitution. In *Escherichia coli* metabolism, for example, the most common reactant is H^+, followed by water, which participates as a substrate or product in over 500 reactions [3].

Cells counter hydrolysis by a number of mechanisms, including energy metabolism [4]. The main polymers of cells, proteins and nucleic acids are susceptible to hydrolysis. Their synthesis consumes about 80% of an anaerobic cell's energy budget [5]. Energy metabolism continuously supports ATP-dependent polymer synthesis, thereby ensuring that the rate of polymer synthesis is faster than the rate of hydrolysis. At the origin of life roughly 4 billion years ago, however, before polymerisation of nucleotides, amino acids or sugars could be coupled to an elaborate energy metabolism, there must have been other means to avoid the natural tendency towards hydrolysis [6,7]. In serpentinising systems such as alkaline hydrothermal vents (see Box 1 'Serpentinisation'), for example, the chemically reactive environment can provide a steady supply of monomers from simple inorganic compounds (CO_2, carbonates, hydrogen) via rock–water interactions [8–11], although the exact source of these monomers is a matter of debate [12].


Box 1. Serpentinisation

Serpentinisation is a geochemical process that occurs when ultramafic rocks in the upper mantle interact with seawater drawn from cracks in the crust [13]. The main gas-phase product of this process is molecular hydrogen (H_2), resulting from the reduction of water protons with iron minerals.

The upper part of the Earth's mantle consists mainly of peridotite, which is composed mostly of pyroxene (chain silicate forming minerals) and olivine [14]. Olivine is an iron-magnesium silicate, a solid solution between the magnesium silicate forsterite (Mg_2SiO_4) and the iron silicate fayalite (Fe_2SiO_4). Below 400 °C forsterite dissolves in water [15–18]:

$$Mg_2SiO_4 + 4\ H^+ \rightarrow 2\ Mg^{2+} + SiO_2(aq) + 2\ H_2O$$

At sufficient concentration of dissolved species, serpentine ($Mg_3Si_2O_5(OH)_4$) and brucite ($Mg(OH)_2$) nucleate and precipitate [15]

$$3\ Mg^{2+} + 2\ SiO_2(aq) + 5\ H_2O \rightarrow Mg_3Si_2O_5(OH)_4 + 6\ H^+$$
$$Mg^{2+} + 2\ H_2O \rightarrow Mg(OH)_2 + 2\ H^+$$
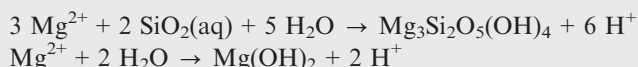
Both reactions hence consume water and produce H^+ which promotes the dissolution of forsterite. Fayalite reacts with hydrothermal water to magnetite (Fe_3O_4) and H_2.

$$3\ Fe_2SiO_4 + 2\ H_2O \rightarrow 2\ Fe_3O_4 + 3\ SiO_2(aq) + 2\ H_2$$

This reaction also consumes water, but magnetite is a minor component of serpentinisation, although it is the main product of Fe^{2+} oxidation. The majority of water is consumed through the reactions to serpentine and brucite. The equilibrium pH of the hydrothermal fluid is nearly neutral at temperatures near 300 °C but increases to about pH 11 at 50 °C because the solubility of brucite increases at lower temperatures, releasing dissolved Mg^{2+} and OH^- ions [18].

The mineral content in serpentinising systems strongly varies with the environment in which each system is situated. If sufficient amounts of H_2 accumulate in surroundings that bear Ni^{2+} containing compounds, native NiFe alloys such as awaruite (Ni_3Fe) can form [19,20].

$$(FeO) + 3\ (NiO) + 4\ H_2 \rightarrow Ni_3Fe + 4\ H_2O$$

Similarly, iron or nickel sulphides can form in systems with a higher proportion of H_2S instead of H_2 [19,21]. The amount of H_2 generated by serpentinisation depends upon temperature and the water:rock ratio of the reacting formation. For example, at 100 °C, serpentinisation starting with harzburgite, an ultramafic olivine-containing rock, generates about 0.9 moles of H_2 per kg of rock at a water:rock ratio of 0.2 (an excess of rock over water) but increases to about 130 moles of H_2 as the water:rock ratio increases to 10 [18].

Serpentinisation is a very widespread process and was probably more abundant on the early Earth than it is now [16].

Recent findings reveal striking parallels between the first steps of biochemical CO_2 fixation with hydrogen (H_2) and carbon fixation under geochemical conditions similar to those in serpentinising systems [22]. The organic acids formate, acetate and pyruvate are formed readily from H_2 and CO_2 in the presence of H_2O with the help of metals and mineral catalysts also found in serpentinising systems [22,23]. Metal ion-assisted reactions between pyruvate and glyoxylate lead to more complex organic acids observed in metabolism, for example fumarate, malate or α-ketoglutarate [24]. In presence of reduced nitrogen compounds such as ammonia [25] or hydroxylamine [24], pyruvate,

oxaloacetate and acetate can react to the simple amino acids alanine, aspartic acid and glycine. Such reduced nitrogen compounds can be obtained under hydrothermal conditions through the hydrogenation of dinitrogen (N_2) over mineral catalysts [26–28]. Heterocyclic monomers as nucleobases can either be derived from formamide (which itself is the product of the reaction between formic acid and ammonia) [29] or via a condensation reaction of amino acids, a route closer to biological pathways [30]. Both approaches require, however, low water activity. Although the direct synthesis of amino acids or nucleobases starting from N_2 and CO_2 coupled to serpentinisation has not yet been

demonstrated [11,12], under the high pressure and high temperature conditions provided by serpentinising systems, activating N_2, CO_2 and H_2 simultaneously on mineral surfaces could, in principle, lead to complex biomolecules and monomers including amino acids, cofactors and nucleobases, which could foster the formation of protometabolic autocatalytic networks [31]. Thus, the synthesis of simple biological organics from H_2 and CO_2 using hydrothermal catalysts is facile [22], yet the synthesis of more complex biomolecules from H_2, CO_2 and N_2 has yet to be reported.

But even if biomonomers can be formed in serpentinising systems, as long as polymerisation products are hydrolysed faster than they are synthesised, no molecular complexity will ensue, because the formation of complex monomers themselves can be prevented by hydrolysis. This calls for a closer look at water activity in geochemical, biological and primordial protometabolic settings. In this paper, we address the issue of hydrolysis in an origin context at the interface of geochemistry and biochemistry, considering its mechanisms and their control under environmental and cellular conditions.

Hydrolysis and water activity in biology

The water content of the cytosol varies with cell size and metabolism but it can range from a few dozen to many thousand femtolitres (10^{-15}) [32]. It consists of bound and bulk ('free') water, water activity being the mole fraction of bulk water (see Box 2 'What is water activity?'). Cytosol is saturated with a wide range of molecules making it a crowded environment with concentrations up to hundreds of $g{\cdot}L^{-1}$ of amino acids, peptides, proteins, nucleic acids, nucleobases, monosaccharides, sugars, etc. [33,34]. For example, the concentration of amino acids in the cytosol, both free and polymerised as protein, is about 550 $g{\cdot}L^{-1}$ [5]. The number and nature of dissolved molecules in the cytosol result in very unique solvent properties of the intracellular water which ultimately also facilitate polymerisation of biomolecules [35,36]. One can differentiate between water of hydration (see Box 3 'Water of hydration'), which is strongly absorbed to proteins and other cellular compounds, and water that has the physical and chemical properties of bulk water [37]. In crystallised proteins, for example, about 40% of the crystal weight comes from water, a mixture of water of hydration and bulk water [37]. The two forms of water have very different properties. Water of hydration has a higher heat capacity, is less mobile and more ordered than bulk water, such that they differ in

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Box 2. What is water activity?

Water activity is a measure of the 'effective concentration' of water in a system, also referred to as 'bulk water'. When bulk water molecules bind to ions, to surfaces, or are trapped otherwise, the overall water activity of a system decreases.

A simple means to obtain the water activity is to measure the water vapour pressure of an aqueous solution. Fundamental thermodynamic relations show:

$$\frac{p_w}{p_w^*} = a_w = f_w \cdot x_w$$

where p_w is the water vapour pressure of the aqueous solution.

p_w^* is the vapour pressure of pure water at the same external pressure and temperature as the solution

X_w is the mole fraction of water in the solution, and

f_w is the dimensionless activity coefficient.

Water activity is particularly affected by strong electrostatic interaction of the partial charges of water with ions from dissolved salts. The strong interaction leads to comparable low water activity. In case of a sufficiently diluted, 'ideal' solution, $f_w \approx 1$ and:

$$\frac{p_w}{p_w^*} = x_w = 1 - x_2$$

$$x_2 = 1 - \frac{p_w}{p_w^*}$$

$$x_2 = \frac{(p_w^* - p_w)}{p_w^*}$$

According to this relation the relative lowering of the vapour pressure of the solvent is equal to the mole fraction x_2 of the solved compound, for example the salt in an aqueous salt solution (Raoult's empirical law from 1890) [40].

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their abilities to dissolve different compounds [37]. For enzyme activity, water of hydration is essential as it stabilises tertiary and quaternary conformations via hydrogen bonding, charge–dipole interactions and hydrophobic (entropic) effects. In short: water of hydration keeps enzymes intact for catalysis [38,39].

Salts can also exert immense influence on water's characteristics as a solvent [45,46]. Sodium (Na^+),

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Box 3. Water of hydration

The term water of hydration is used in chemistry to designate water in the crystal structure of a metal complex or salt which is not directly bound to the cation. Often the crystal properties are lost when this water is removed by heating. In a biological context, the importance of water of hydration is obvious. Proteins crystallise with up to 50% water in their lattice, much more than inorganic salts [37,41]. Often their enzymatic function is reduced and their structure changed if that water is removed. Most water in biological cells is water of hydration [37]. In this paper, we use the concept of water of hydration in a broader sense than just water of crystallisation to take effects in biological systems into account.

   Water of hydration is considered here as all water that is not bound to the fluctuating network of bulk water (pure liquid water) but to other species in the aqueous phase. Often water of hydration is stronger and more orderly bound than bulk water [42] and therefore exhibits a substantially different Gibbs free energy [43]. Water of hydration can be bound in the first or second solvation shell of the cation or anion of a solved salt, bound to the charged side chain(s) of amino acids in a protein, ordered to hydrophobic molecules or chemical groups, bound to a chain of water molecules in a membrane protein channel conducting protons or bound to a solid surface [41]. All these interactions can be classified according to their Gibbs free binding energy which can be quite large. A high vacuum in a reactor, for example, can only be obtained by heating its walls well above 100 °C for many hours during pumping (a process known as bake-out) to release the tightly attached monolayers of water [44].

potassium (K^+), magnesium (Mg^{2+}), phosphate (PO_4^{3-}) and chloride (Cl^-) contribute to protein and enzyme folding, structure and specificity by creating in situ microenvironments where certain ions are more abundant than in the bulk [36,47]. These microenvironments regulate water activity via electrostatic interaction. Hydrophilic surfaces are associated with a higher concentration of water whereas hydrophobic surfaces cause water to migrate to other sites. This complex interplay helps to increase heat stability of the macromolecules in their aqueous surrounding [48–50]. The resulting intracellular water dynamics—also called biological water activity—enable polymerisation

and energy conservation as enzymes control water-mediated interactions. Among these interactions are hydrogenations [51,52], condensations [53,54] and hydrolysis. The latter is central for energy metabolism [55,56], because endergonic reactions can be coupled to an energy-releasing hydrolytic reaction, ATP hydrolysis in particular, thus facilitating endergonic reactions in metabolism. Amino acid polymerisation, for example, is endergonic and does not take place spontaneously in pure aqueous (abiotic) systems [57]. Salt-induced or wet–dry cycle-driven peptide formation [58,59], hydrothermal synthesis [60] or polymerisation in the adsorbed phase, for example on mineral surfaces [61,62], have been introduced as possible mechanisms to promote peptide formation in an abiotic context [57].

   In cells however, amino acid polymerisation requires the transfer of AMP from ATP to activate the amino acid for polymerisation via tRNAs [63–65]. The transfer releases pyrophosphate (PP_i) which is subsequently hydrolysed to make the activation irreversible under physiological conditions ($\Delta G_0' = -19.3$ kJ·mol^{-1}) [65]. A similar mechanism is employed in nucleotide condensation, where pyrophosphate is released when a phosphodiester bond between two nucleotides is formed [66]. Pyrophosphate is immediately hydrolysed into two free phosphate groups by pyrophosphatases [65], enzymes that use Mg^{2+} ions to promote controlled hydrolysis (shown in Fig. 1) [67]. Mg^{2+} ions also assist with the assembly of nucleic acids by positioning the nucleotides in the correct conformation [68]; another Mg^{2+} then activates the hydroxyl group at the 3′ end of the primer nucleotide, promoting the formation of the ensuing phosphodiester bond [69].

   The utility of ATP in biology resides its ability to be hydrolysed at a phosphoanhydride bond, thus generating less energy-rich ADP and inorganic PO_4^{3-} (P_i). The energy released by the reaction from ATP to ADP and P_i is 30.5 kJ·mol^{-1} and 45.6 kJ·mol^{-1} for the reaction of ATP to AMP and PP_i [65]. The continuous cycle of hydrolysis and condensation reactions of ATP, ADP, AMP, PP_i, and P_i molecules allows energy harnessing from macromolecules in metabolism via energy coupling reactions [70,71]. In addition to ATP, there are many other molecules in biology with the ability to transduce chemical energy via energy coupling reactions such as NAD, acyl thioesters, aminoacyl esters and ribosyl moieties [72–75].

   A number of reactions in the cell involve controlled hydrolysis during the degradation of lipids, nucleic acids and proteins. The enzymes responsible for these reactions are hydrolases, which represent, with about one third of all known enzymes, the largest enzyme
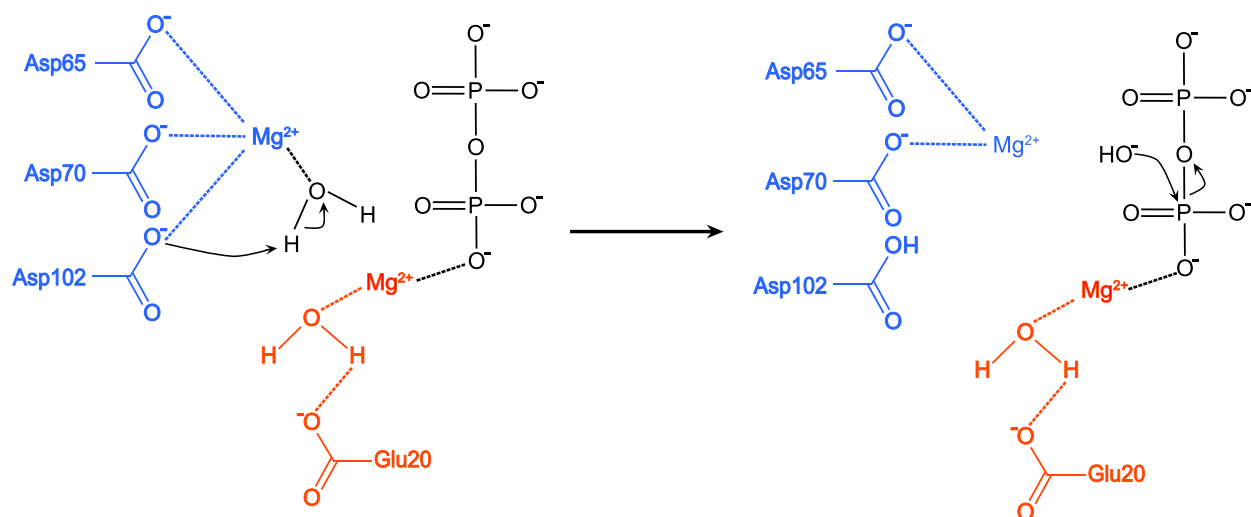
Fig. 1. Catalytic mechanism of Mg^{2+}- or Mn^{2+}-containing hydrolases (pyrophosphatase-type hydrolases). The metal complex formed with the carboxyl groups of acidic amino acids and water molecules can activate both substrate (site shown in red) and water (site forming OH^-, shown in blue). Adapted from [67].

class [67]. Subgroups of hydrolases employ divalent metal cofactors like Mg^{2+}, manganese (Mn^{2+}), cobalt (Co^{2+}) and zinc (Zn^{2+}) [67,76–78], all of which can also be relevant in a prebiotic context [79–82]. Four hydrolases are considered particularly ancient, all of them employing metal cofactors, three of them function without nucleotide-derived cofactors like ATP or NAD [83], pointing to a conserved and possibly primordial mechanism. Among the most ancient hydrolases are thioester hydrolases (e.g. acetyl-CoA hydrolase) which operate with Mg^{2+} or Mn^{2+} in their active centres [78,83].

The molecular mechanisms of catalysis in hydrolases are quite well understood [67], although not known in detail for every subgroup of the enzyme family yet. Fig. 1 depicts the mechanism of a hydrolase active site containing Mg^{2+} or Mn^{2+}, showing that acidic amino acids are crucial for positioning the ions such that they produce OH^- from water to perform targeted hydrolysis. As described earlier, the hydrolysis of pyrophosphate enables the polymerisation of amino acids and nucleotides. Notably, Mg^{2+} can also catalyse hydrolysis on its own, without the protective environment of the enzymatic active site [84]. In abiotic systems, where there is no enzymatic activity, the overall availability of salts and other charged molecules can regulate rates of hydrolysis and condensation.

Hydrolysis is also a central aspect of carbon metabolism [85–87]. One example is the participation of water in all known CO_2 fixation pathways, including the acetyl-CoA pathway, which allows acetogens and methanogens to grow from H_2 and CO_2 [88]. Genomic reconstructions indicate that the last universal common ancestor (LUCA) followed a similar route, using the acetyl-CoA pathway for carbon and energy metabolism [89]. As already stated, recent studies show that formate, acetate and pyruvate form overnight from H_2 and CO_2 in water using mineral catalysts alone under hydrothermal conditions [22]. Similar abiotic routes have recently been shown for intermediates and products of the reverse citric acid cycle [24,90]. The gap between such hydrothermal conditions and the chemistry of real life as it is manifested in modern autotrophs is narrowing in this respect. Thus, there might also be connection between the biochemistry in autotrophs and the geochemistry in serpentinising systems when it comes to hydrolysis.

In hydrogenotrophic methanogenesis, the first step towards the production of methane is the reduction of CO_2 to formyl-methanofuran (formyl-MFR) using ferredoxin as the electron donor. This reaction is catalysed by formyl-MFR dehydrogenase. In the enzymatic mechanism, the CO_2 molecule is funnelled through a hydrophobic channel towards a catalytic chamber with a tungsten active centre, into which electrons are drawn via a long [4Fe-4S] cluster chain [91]. In the catalytic chamber, CO_2 is reduced to formate, a hydrophilic molecule. Formate (or formic acid) diffuses from the active site via a hydrophilic tunnel to a zinc active centre where it is conjugated with MFR as a carbamate [91]. This is a redox reaction involving electrons from ferredoxin; water is generated as secondary

product during carbamate formation. That is, an interplay of hydrophobic and hydrophilic surfaces in cooperation with metallic catalysts promotes controlled water–substrate interactions at the initial CO_2 fixation step. The carbamate is reduced to a MFR-bound formyl-group. A stepwise reduction to a MFR-bound methyl-group follows, before the group is transferred to coenzyme M. After a last reduction step, the methyl-group is released as CH_4 [88].

It is common knowledge that enzymes tend to exclude water from the active site and can readily catalyse reactions against the water activity of the cytosol. The question of what environmental conditions could have promoted a prebiotic route from CO_2 towards organics and life is more challenging.

The hydrolysis conundrum in origin of life research

Water is indispensable for life as we know it to emerge. But at the same time, water elimination (condensation, polymerisation) is one of the most common reactions in metabolism underpinning the synthesis of cells [53,54]. The reverse reaction, water addition, is hydrolysis and can be chemically destructive to many essential biomolecules. Through hydrolysis, water literally works against the synthesis and accumulation of polymers at life's emergence.

Although enzymatically mediated hydrolysis can break any type of known carbon bond [92], modern biomolecules are protected from random hydrolysis by their structured spatial arrangement of hydrophobic/hydrophilic surfaces and by interactions with inorganic ions and other organic molecules [37]. They contribute to an environment in which water participates in the biochemical processes in a very controlled and targeted manner. Of course, modern biomolecules have undergone permanent selection during evolution to remove those that are unstable, insoluble or toxic in the cellular environment. At life's origins, however, during the phase of prebiotic chemistry before there were genes and encoded proteins, there were no enzyme pockets, no complex proteins, no ordered membranes or enzymatically elaborated energy metabolism to manage the constant chemical pressure of reactions involving water. Hence, the primordial polymerisation of simple molecules into the precursors of proteins and nucleic acids via the removal of water, a prerequisite for life, took place without the help of the biological mechanisms that modulate water activity in a living cell. Nevertheless, we will see below that serpentinisation itself generates inorganic mechanisms that modulate water homeostasis.

At face value, the origin of life and fully aqueous chemistry do not really work together well, especially if high temperatures are required for chemical reactions to take place [93,94]. But at the same time, the cytosol of a cell is also not an environment of fully aqueous chemistry. So, is there something wrong with the basic concept of hydrothermal origins, or is there something wrong with the premise that hydrothermal settings generally involve fully aqueous chemistry? There are specific physicochemical sites within serpentinising systems that harbour and even maintain low water activity (see Box 2) at rather moderate temperatures (100–200 °C). Such systems provide a spectrum of conditions as are required to get from C_1 compounds to complex organics [93,94]. As in cells, free water in the right geochemical environment could be bound as water of hydration with the help of salts and other polar molecules, decreasing the water activity and thus promoting the synthesis of larger molecules [37,93]. Another possible way to promote hydrolysis-sensitive reactions are mineral surfaces [22,27,95–104]. Mineral surfaces provide diversity of catalytic environments, enabling reactions at the solid–liquid phase boundary under aqueous conditions [22,23,101,104–106]. A combination of highly catalytically active minerals and low water activities (provided by high salt conditions or other means) could enable a variety of possible chemical pathways.

Modern high salt and high temperature environments present extreme settings for life. As such harsh conditions were likely prevalent on the early Earth—especially inside its crust—at the time when life emerged [15,107], biologists have long suspected that modern extremophiles might hold clues about the biology of the first cells [108,109]. Found in Earth's most life-defying places, extremophiles could provide valuable insights into the transition from nonlife to life. Haloarchaea, for example, can survive in salt crystals for very long periods of time, possibly over geological time periods, although how long exactly is open to speculation [40,110]. In order to counteract the osmotic pressure of the saline environment, some halophiles transfer K^+ ions into the cell [40]. Most other halophiles, however, choose a more energy-intensive route to deal with high salt concentrations by synthesising osmolytes like sugars, glycerol or amino acid derivatives. With this strategy, only organisms with very effective metabolic rates (high levels of ATP synthesis) can survive under very saline conditions [111].

Salt can have interesting effects on the three-dimensional structure of proteins under abiotic conditions. It has been shown, for example, that homochiral leucine-lysine (Leu-Lys) polypeptides fold as random coils in

pure water [112]. But in salt they form thermostable and hydrolysis stable bilayers of beta-sheets with the hydrophilic side chains of Lys (positively charged amino group) pointing outwards into the salt solution and the hydrophobic side chains of Leu (isobutyl group) inwards [112]. The structure of larger proteins (140 amino acids) is also influenced by high salt concentrations (up to 2 M). Acidic amino acid chains are stabilised by the salt cations, and beta-sheets fold so as to point outwards. With simple hydrophobic amino acids as Leu, Ile (isoleucine) and Val (valine) pointing inside, a hydrophobic core/pocket can be formed, where possible polymerisation reactions would be protected from the surroundings [113,114]. Such studies show how high salt settings—and thus environments with low water activity—can provide dynamic physico-chemical conditions that naturally modulate structures for prebiotic chemistry while keeping the risk of hydrolysis lower than in purely aqueous environments. These conditions arise via the operation of simple ionic forces.

Various mechanisms have been proposed to solve or circumvent the hydrolysis problem at the origin of life. The most commonly encountered of these evokes the existence of wet–dry cycles [59,97,106,115,116], which entail alternating periods of hydrolysis (high water activity) and condensation (no water activity) in order to achieve polymerisation. Although such cycles deliver promising results for nucleotide synthesis under laboratory conditions [115], it is important to not only constrain an environment for origins by water avoidance, but also by considerations of carbon or energy availability, the main prerequisites for microbial life. An underexplored alternative to wet–dry cycles might be solid phase–aqueous phase interfaces in geochemical systems where water activity is constantly low. This was proposed recently for hydrothermal sedimentary layers where pore spaces between volcanic particles can be filled with silica gels [117], which leads to less free water in the geochemical system. Of course, water activity is a prominent variable in efforts to detect life beyond the confines of Earth, as such there are many implications of water activity for astrobiology (discussed in Box 4).

In environments with very low water activity, hydrolysis might even require catalysis to occur. As mentioned above, divalent ions such as Mn^{2+} and Mg^{2+} can promote hydrolysis both in the active centre of hydrolases and under abiotic conditions (without enzymes). So it is possible that such ions, which are quite abundant in hydrothermal vent/serpentinising settings, could provide exactly the rate of hydrolysis needed if the average water activity in a system is

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Box 4. Implications of water activity for astrobiology

Water activity bears directly upon theories and experiments dealing with the origin of life. The search for life on other planets or moons is guided by the search for water [118]. When it comes to finding evidence for ongoing rock–water interactions in appreciable magnitude, the moons of Saturn Enceladus and Titan have been in the focus of research recently. The Cassini mission has delivered spectrometric evidence for the existence of serpentinisation on Enceladus [119]. Its rocky interior is covered by several kilometres of liquid water that are in turn covered by several kilometres of ice that form the surface [120,121]. The water is kept in the liquid state because of the gravitation of Saturn, which constantly kneads the small moon of roughly 500 km diameter so as to generate heat [120,121]. Enceladus has geysers at its South pole that spew liquid water hundreds of kilometres into space [120,121].

During the Cassini mission, H_2 was measured in the geyser plumes of Enceladus [122]. The presence of H_2, the detection of silicate nanoparticles and models suggesting an alkaline pH of Enceladus' ocean is interpreted as evidence for ongoing serpentinisation under the moon's ice crust [120,122,123]. The plumes also contain organic compounds. These could be either fragments of polyaromatic hydrocarbons, that is, breakdown products of carbon brought to Enceladus by meteorites [124,125] or products of *de novo* organic synthesis fuelled by serpentinisation [126]. Although the exact source of the organic compounds in the plumes is still not clear, rock–water interactions seem to be taking place on Enceladus, meaning that there is nothing special about the process that would limit its occurrence to Earth. If we entertain the possibility that life might have evolved in serpentinising systems, rather than exclude the possibility *a priori* [127], the implications for astrobiology are far reaching. Sunlight would have little to no role in origins and that, in turn, would expand the habitable zone in our solar system and in newly characterised solar systems, to regions where sunlight provides no energy. The chemical energy of serpentinisation would require only water-reducing rocks and CO_2 to unfold.

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small enough. At the same time, various salt ions can actually help decrease the water activity by binding water (hydration). Thus, a complex interplay of salts, ions, minerals, gels, clays and water, not to mention organic compounds themselves should these be

present, figure into the water conundrum at the origin of life.

Serpentinising systems and water activity

Natural environments of low water activity need not to be restricted to terrestrial surfaces, they can and do exist in serpentinising hydrothermal systems and thus may have been germane to the geochemical setting in which life arose. Of course, low water activity is not the only requirement that such a setting has to meet. Nonequilibrium conditions, carbon (cells are roughly 50% carbon by dry weight) [128], nitrogen (cells are 10% nitrogen by dry weight) [129], as well as sulfur and phosphorus, but perhaps most importantly a continuous source of energy was required at origins. Serpentinising systems combine carbon, energy and electrons in the form of CO_2 and H_2 in environments replete with low water activity [130–133]. Serpentinisation is a widespread phenomenon in today's oceanic crust [134,135]. Ultramafic, iron silicate containing rocks react with seawater, release H_2, and are transformed into serpentine group minerals ('serpentinite', $Mg_3Si_2O_5(OH)_4$) in the process—hence the name serpentinisation. One of the best investigated serpentinisation sites is the Lost City hydrothermal field, discovered near the mid-Atlantic ridge in 2001 [8]. At this geological site, Earth mantle rocks containing an iron-magnesium silicate mineral group called olivine are, due to magma upwelling, exposed at the seafloor where they react with seawater to produce H_2 [136].

Modern serpentinising systems harbour temperature, pH and redox gradients [94] and provide carbon (mostly in the form of CO_2/carbonates), sulphur (H_2S and sulphide minerals), and in lesser or trace amounts also nitrogen and phosphate [137]. A unique feature of serpentinising systems is the continuous production of hydrogen (H_2) from water, providing the surroundings with a constant supply of electrons, a very low midpoint potential [138], and a chemical energy source [139–141]. H_2 production entails also a constant renewal of iron-containing minerals that can function as catalysts for prebiotic reactions [98,99,142,143], among them magnetite (Fe_3O_4), a direct product of serpentinisation, iron sulphides like pyrite (FeS_2) or greigite (Fe_3S_4) [19,21,144], and even native metal compounds such as awaruite (Ni_3Fe) [19]. Prebiotic reactions catalysed by such minerals supposedly started with the energy-releasing reaction of CO_2 and H_2 [22] that today is still utilised by acetogens and methanogens for growth [11,88,133,145–149].

What about water activity in serpentinising systems? It decreases as vast amounts of sea water are consumed in the interactions between seawater and olivine and salinity in the rock pores increases [150]. The ultramafic minerals participating in the process sequester seawater, resulting in mineral hydration (hydroxide formation) [151]. A recent *in situ* serpentinisation experiment (at 280 °C and 500 bar) provided important mechanistic insights into the interactions between rock and water and into the influence of salinity on free water in mineral pores and thus on water activity [15]. Via Raman spectroscopy and microscopy, Lamadrid et al. [15] monitored the concentration of salts and minerals in the micropores of olivine. The formation of serpentine minerals, brucite ($Mg(OH)_2$), magnetite (Fe_3O_4) and H_2, consumes water. As the water content in the pores decreases, the concentrations of salts and minerals increase during serpentinisation. Ultimately, the pores are filled with a highly concentrated, 'crowded' hydrothermal fluid with low water activity. What Lamadrid et al. [15] describe is a situation that comes surprisingly close to what is observed in intracellular fluids. In the geochemical microsites they observe [15], serpentinisation stops as soon as the water activity gets too low (meaning the salinity gets too high) and new seawater with lower salinity has to diffuse into the system to restart serpentinisation. The rock volume increases during serpentinisation [139], leading to open fractures and seawater migration into the (micro)cracks which allows serpentinisation to continue. This scenario of fluctuating water activity resembles wet–dry cycles described above. Lamadrid et al. [15] report a kind of self-regulating system that hones in on low water activity with the exergonic reaction (H_2 production) ceasing when water activity becomes too low, resuming only when water is added. Chemically, water availability during serpentinisation at very small scales of micron sized inorganic compartments resembles ion homeostasis in modern cells at several levels.

Within inorganic pores of ultramafic rocks, a combination of mineral surface catalysis, low water activity and continuous supply of energy and carbon (in addition to nitrogen and other nutrients), could, in principle, lead to complex biomolecules. Such a sustained source of specific 'food' and energy is required for the emergence of autocatalytic networks, metabolism-like chemical reaction systems that are simpler than metabolism in modern cells as they support themselves without enzymes [78]. Such reaction systems are thought to be intermediates in the transition from non-living to living systems [152,153]. From the geochemical standpoint, there is every reason to think that

serpentinisation was a very common reaction on early Earth [11,16,154,155]. The necessary ingredients are seawater and ultramafic rocks which the early Earth had in virtually unlimited supply [156]. Radioactive isotope dating of the carbonate structures and sediments in Lost City show an age of at least 30 000 years for that serpentinising system. There is enough mineral in the massif below Lost City to drive serpentinisation for further hundreds of thousands, if not millions, of years [139]. Rocks from former serpentinising systems preserved at depth beneath the Mid-Atlantic Ridge, where Lost City resides, revealed abiotically synthesised organic compounds including amino acids and more complex molecules just recently [157–159]. Such findings emphasise the potential of prebiotic synthesis serpentinising systems offer. This ultimately means that serpentinising systems such as alkaline hydrothermal vents can provide low water activity. Many misconceptions about vents are seated in water activity and hydrolysis, misconceptions that presume chemistry in free aqueous solution at hydrothermal vents. [127,160,161].

To date, the catalytic and organic synthetic potential of serpentinising systems, in combination with low water activity, has not been exhausted in sufficient detail in laboratory experiments. The effluents of active sites such as the Lost City hydrothermal field are regularly monitored, mainly showing simple carbonic acids like formate, methane and possibly acetate as abiotic products of the redox reactions occurring inside the porous crust [137,141,148,162]. Direct *in situ* observation of the reactions within serpentinising systems in submarine crust poses immense technical challenges. But the rewards of direct observations could be equally great, as current findings suggest that serpentinising systems catalyse biomimetic reactions [157–159]. A problem is, however, that vents are densely inhabited by microbes and possibly bear sources of biologically derived organic matter, such that controlled laboratory experiments in sterile systems [22–24] are needed to complement *in situ* chemical sampling studies.

Conclusions and perspectives

Avoiding constant hydrolysis while retaining access to hydrolysing chemical steps is pivotal for the emergence of prebiotic chemical networks [163]. So far, most prebiotic CO_2 fixation experiments are performed in aqueous solution which can block heterogeneous mineral catalysts. From industrial processes, it is known that water poisons mineral catalysts through hydroxyl formation on their surfaces, blocking the prospective

catalysis sites [164]. This would also explain why the yields of all aqueous CO_2/H_2 experiments are usually very low in comparison with those of industrial gas-phase chemical processes, such as Fischer–Tropsch and Haber–Bosch synthesis [22,23,151,164,165]. On the other hand, studies with water vapour in hydrogenation processes have shown that H_2O, although lowering the output of industrially relevant products including methane and larger hydrocarbons, increases the percentage of C_1–C_4 'oxygenates'—oxidised carbon compounds—possibly including organic acids central in metabolism [166]. Thus, limited water poisoning might be a manageable problem and possibly beneficial as long as water activity is generally low.

Hydrolysis is often viewed as a destructive force in prebiotic chemistry [93]. But in the context of the mechanistic details of small-scale geochemical reactions within serpentinising systems, hydrolysis becomes a surprising and potentially powerful force that could foster self-organisation in prebiotic chemistry (Fig. 2). There are two possible roles. First, in an environment with a constantly low water activity, hydrolysis is impaired, transforming it from an omnipresent problem into an essential reaction that can be mediated by divalent ions as found in modern hydrolases [67,84]. Second, under medium (not high) water activity conditions, hydrolysis can be a selective driving force towards complex molecules. Under simulated hydrothermal (high water activity) conditions, mineral catalysts, reductants and CO_2 can generate reduced carbon compounds including 2-oxoacids [22,23] overnight, in the presence of activated nitrogen amino acids also readily form [24,25]. In metabolism, amino acids are the precursors of nitrogenous heterocyclic compounds (cofactors and nucleobases), the starting material and end products of protometabolic autocatalytic networks [31,78,153]. With high salt concentrations and low water activity inside the pores of serpentinising rocks (medium to low water activity), peptides could form from amino acids, even at higher temperatures [114]. Peptides then become even more resilient against hydrolysis once they reach lengths that permit the formation of alpha-helices and beta-sheets [167]. Under conditions of sustained peptide synthesis and sustained peptide hydrolysis, a selection process sets in through which hydrolysis-resistant peptides can accumulate [168,169], but not by virtue of faster synthesis, rather by virtue of their slower hydrolysis ('survival of the sturdy').

In this way, low water activity could foster synthesis of random peptides, while hydrolytic removal of the most labile among them would enrich for nonrandom structures within the realm of randomly synthesised
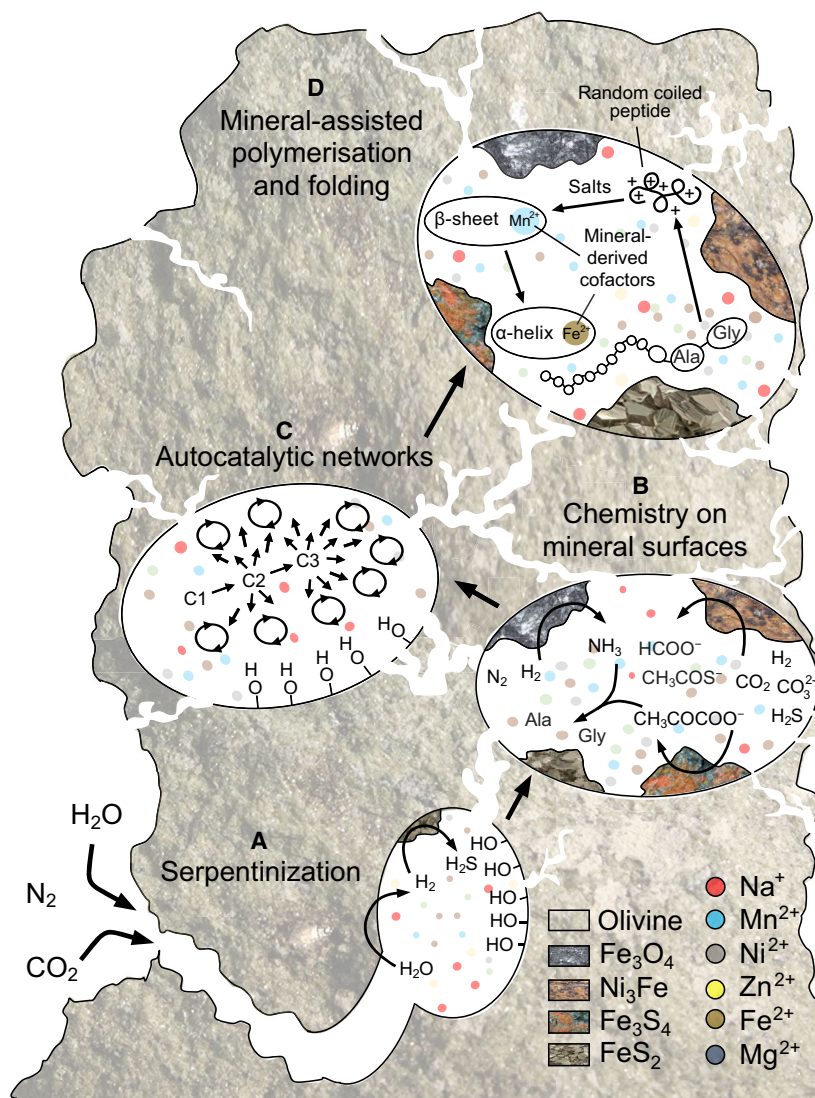
Fig. 2. Serpentinising systems, water activities and origins. The figure schematically depicts hydrated pores (rock-bound water, hydroxyl groups), embedded in the olivine matrix of serpentinising systems. (A) *Serpentinisation*. H_2 is synthesised via interactions between water and olivine (for details see). Catalytically active minerals including magnetite (Fe_3O_4), iron sulphides (FeS_2, Fe_3S_4) and Ni, Fe-alloys (Ni_3Fe) are constantly produced, whereas Fe_3O_4 arise from serpentinisation, and sulphides and alloys as reaction products of H_2, H_2S and Fe^{2+} or Ni^{2+} ions. (B) *Chemistry on mineral surfaces*. With the help of such minerals as catalysts, N_2 can be hydrogenated to ammonia [26–28], and CO_2 reduced to carbon compounds like α-ketocarboxylic acids [22–24]. The latter can react with activated ammonia to amino acids [24,25]. Theoretically, also thioesters could be synthesised at this stage, although this is debatable [172]. (C) *Autocatalytic networks*. The reduction products could react to a variety of other N-containing carbon compounds like cofactors or nucleobases, especially in low water activity (high salt) conditions [29,30]. Such complex monomers would fuel autocatalytic protometabolic networks [31,78,153]. (D) *Mineral-assisted polymerisation and folding*. Due to high salt concentrations and low water activity inside the pores, polymers such as polypeptides can form from amino acids [113,114]. Most folded proteins could achieve the necessary structure precision for their catalytic function without nucleic acids as templates, merely directed by water activity and salt concentration [112]. They could concentrate substrates in their protected interior. Here, controlled hydrolysis (e.g. via trapped Mg^{2+} or Mn^{2+} ions as shown in Fig. 1) and condensation reactions through mineral-derived cofactors could occur. Also targeted CO_2 fixation would be possible in such protein pockets, using amino acids with nucleophilic side chains and incorporated transition metals such as Fe^{2+} or Ni^{2+} [22,23,88,173]. The micropores in ancient serpentinising hydrothermal fields could be the earliest precursors of biological cells. All reactions described in this figure could subsequently happen in the same micropore, but pores at different physicochemical conditions may be needed for some of the stages to evolve. So chromatographic effects (separation of products while migrating between two different pores) should be considered.

variants, possibly without genetic instruction. Such peptides would necessarily harbour specific structural surfaces, hence the equivalent of multiple active sites [170], and could in turn favour some kinetically controlled reactions over others, thereby accelerating molecular self-organisation. Prebiotic peptide synthesis requires a sustained source of amino acids and energy [171], in addition to suitable surfaces, to catalyse polymerisation [57,97]. Serpentinising systems could provide both over geological time scales, with survival of the sturdiest enriching for nonrandom structures with catalytic properties of their own, as catalytically active peptides that currently serve as elements of autocatalytic networks preserved in microbial metabolism [78].

In summary, we have described how highly saline, serpentinising, porous rock environments could circumvent and perhaps even modulate uncontrolled hydrolysis in a manner analogous—similar but unrelated—to modern metabolism (Fig. 2). Whether these processes could also be homologous, that is, similar by virtue of common ancestry, is an open question for further study.

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Author contributions

MP wrote the manuscript with contributions from AV on hydrolysis in biology, contributions from KK on hydrolysis in a chemical context and serpentinisation and contribution from WFM on the origin of life context. KK researched and drafted Boxes 1–3 and formulated, together with MP and WFM, the scheme depicted in Fig. 2. MP adapted Fig. 1, AV has drawn Fig. 2 with input from KK, WFM and MP. AV and WFM drafted Box 4. WFM edited and contributed throughout the manuscript.

References

1 Westall F and Brack A (2018) The importance of water for life. *Space Sci Rev* **214**, 1–23.

2 Speight JG (2018) Hydrolysis. In Reaction mechanisms in environmental engineering, pp. 203–229. CD & W Inc., Laramie, Wyoming, United States.

3 Sousa FL, Hordijk W, Steel M and Martin WF (2015) Autocatalytic sets in *E. coli* metabolism. *J Syst Chem* **6**, 4.

4 Ereciflska M and Wilson DF (1982) Regulation of cellular energy metabolism. *J Membr Biol* **70**, 1–14.

5 Lever MA, Rogers KL, Lloyd KG, Overmann J, Schink B, Thauer RK, Hoehler TM and Jørgensen BB (2015) Life under extreme energy limitation: a synthesis of laboratory- and field-based investigations. *FEMS Microbiol Rev* **39**, 688–728.

6 Tashiro T, Ishida A, Hori M, Igisu M, Koike M, Méjean P, Takahata N, Sano Y and Komiya T (2017) Early trace of life from 3.95 Ga sedimentary rocks in Labrador, Canada. *Nature* **549**, 516–518.

7 Ueno Y, Yamada K, Yoshida N, Maruyama S and Isozaki Y (2006) Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature* **440**, 516–519.

8 Kelley DS, Karson JA, Blackman DK, Früh-Green GL, Butterfield DA, Lilley MD, Olson EJ, Schrenk MO, Roe KK, Lebon GT *et al.* (2001) An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. *Nature* **412**, 145–149.

9 Früh-Green GL, Orcutt BN, Green SL, Cotterill C, Morgan S, Akizawa N, Bayrakci G, Behrmann J-H, Boschi C, Brazelton WJ *et al.* (2017) Northern sites. *Proc Int Ocean Discov Progr* **357**.

10 Klein F, Grozeva NG and Seewald JS (2019) Abiotic methane synthesis and serpentinization in olivine-hosted fluid inclusions. *Proc Natl Acad Sci* **116**, 17666–17672.

11 McCollom TM and Seewald JS (2013) Serpentinites, hydrogen, and life. *Elements* **9**, 129–134.

12 Reeves EP and Fiebig J. (2020) Abiotic synthesis of methane and organic compounds in Earth's lithosphere. *Elements* **16**, 25–31.

13 Kasting JF and Holm NG (1992) What determines the volume of the oceans? *Earth Planet Sci Lett* **109**, 507–515.

14 Langmuir CH and Broecker W (2012) How to build a habitable planet. Princeton University Press, NJ.

15 Lamadrid HM, Rimstidt JD, Schwarzenbach EM, Klein F, Ulrich S, Dolocan A and Bodnar RJ (2017) Effect of water activity on rates of serpentinization of olivine. *Nat Commun* **8**, 16107.

16 Sleep NH, Meibom A, Fridriksson T, Coleman RG and Bird DK (2004) H_2-rich fluids from serpentinization: geochemical and biotic implications. *Proc Natl Acad Sci USA* **101**, 12818–12823.

17 Klein F, Bach W and McCollom TM (2013) Compositional controls on hydrogen generation during serpentinization of ultramafic rocks. *Lithos* **178**, 55–69.

18 McCollom TM and Bach W (2009) Thermodynamic constraints on hydrogen generation during serpentinization of ultramafic rocks. *Geochim Cosmochim Acta* **73**, 856–875.

19 Klein F and Bach W (2009) Fe-Ni-Co-O-S phase relations in peridotite-seawater interactions. *J Petrol* **50**, 37–59.

20 Foustoukos DI, Bizimis M, Frisby C and Shirey SB (2015) Redox controls on Ni-Fe-PGE mineralization and Re/Os fractionation during serpentinization of abyssal peridotite. *Geochim Cosmochim Acta* **150**, 11–25.

21 Findlay AJ, Estes ER, Iii GWL, Gartman A, Yücel M, Kamyshny AK and Luther GW III, Jr (2019) Iron and sulfide nanoparticle formation and transport in nascent hydrothermal vent plumes. *Nat Commun* **10**, 1597.

22 Preiner M, Igarashi K, Muchowska K, Yu M, Varma SJ, Kleinermanns K, Nobu MK, Kamagata Y, Tüysüz H, Moran J *et al.* (2020) A hydrogen-dependent geochemical analogue of primordial carbon and energy metabolism. *Nat Ecol Evol* **4**, 534–542.

23 Varma SJ, Muchowska KB, Chatelain P and Moran J (2018) Native iron reduces CO_2 to intermediates and endproducts of the acetyl-CoA pathway. *Nat Ecol Evol* **2**, 1019–1024.

24 Muchowska KB, Varma SJ and Moran J (2019) Synthesis and breakdown of universal metabolic precursors promoted by iron. *Nature* **569**, 104–107.

25 Barge LM, Flores E, Baum MM, Vandervelde DG and Russell MJ (2019) Redox and pH gradients drive amino acid synthesis in iron oxyhydroxide mineral systems. *Proc Natl Acad Sci USA* **116**, 4828–4833.

26 Schoonen MAA and Xu Y (2003) Nitrogen reduction under hydrothermal vent conditions: implications for the prebiotic synthesis of C-H-O-N compounds. *Astrobiology* **1**, 133–142.

27 Kandemir T, Schuster ME, Senyshyn A, Behrens M and Schlögl R (2013) The Haber-Bosch process revisited: on the real structure and stability of "ammonia iron" under working conditions. *Angew Chemie – Int Ed* **52**, 12723–12726.

28 Dörr M, Käßbohrer J, Grunert R, Kreisel G, Brand WA, Werner RA, Geilmann H, Apfel C, Robl C and Weigand W (2003) A possible prebiotic formation of ammonia from dinitrogen on iron sulfide surfaces. *Angew Chemie – Int Ed* **42**, 1540–1543.

29 Saladino R, Crestini C, Pino S, Costanzo G and Di Mauro E (2012) Formamide and the origin of life. *Phys Life Rev* **9**, 84–104.

30 Heinz VB, Ried W and Dose K (1979) Thermische Erzeugung von Pteridinen und Flavinen aus Aminosauregemischen. *Angew Chemie* **91**, 510–511.

31 Preiner M, Xavier JC, Vieira N, Kleinermanns K, Allen JF and Martin WF (2019) Catalysts, autocatalysis and the origin of metabolism. *Interface Focus* **9**, 20190072.

32 Delgado F, Cermak F, Hecht NC, Son VC and Li S (2013) Intracellular water exchange for measuring the dry mass, water mass and changes in chemical composition of living cells. *PLoS One* **8**, 67590.

33 Minton AP, Colclasure GC and Parker JC (1992) Model for the role of macromolecular crowding in regulation of cellular volume. *Proc Natl Acad Sci USA* **89**, 10504–10506.

34 Ishihama Y, Schmidt T, Rappsilber J, Mann M, Hartl FU, Kerner MJ and Frishman D (2008) Protein abundance profiling of the *Escherichia coli* cytosol. *BMC Genom* **9**, 102.

35 Groen J, Foschepoth D, te Brinke E, Boersma AJ, Imamura H, Heus HA and Huck WTS (2015) Associative interactions in crowded solutions of biopolymers counteract depletion effects. *J Anal Appl Pyrolysis* **137**, 13041–13048.

36 Sarkar M, Smith AE, Pielak GJ and Baldwin RL (2013) Impact of reconstituted cytosol on protein stability. *Proc Natl Acad Sci USA* **110**, 19342–19347.

37 Fulton AB (1982) How crowded is the cytoplasm? *Cell* **30**, 345–347.

38 Rupley JA, Yang P-H and Tollin G (1980) Thermodynamic and related studies of water interacting with proteins. *Water Polym* **127**, 111–132.

39 Pal SK and Zewail AH (2004) Dynamics of water in biological recognition. *Chem Rev* **104**, 2099–2123.

40 Grant WD, Danson MJ, Scott DJ, Halling PJ, Engberts JBFN, Ho MW and Berendsen HJC (2004) Life at low water activity. *Philos Trans R Soc B Biol Sci* **359**, 1249–1267.

41 Blake CCF, Pulford WCA, Artymiuk PJ and Huber R (1983) X-ray studies of water in crystals of lysozyme. *J Mol Biol* **167**, 693–723.

42 Smith JD, Smith JD, Cappa CD, Wilson KR, Messer BM, Cohen RC and Saykally RJ (2013) Energetics of hydrogen bond network rearrangements in liquid water. *Science* **306**, 851–853.

43 Hribar B, Southall NT, Vlachy V and Dill KA (2002) How ions affect the structure of water. *J Am Chem Soc* **124**, 12302–12311.

44 Rozanov LM and Hablanian MH (2002) Vacuum Technique London; Taylor and Francis Taylor, New York.

45 Cossins BP, Jacobson MP and Guallar V (2011) A new view of the bacterial cytosol environment. *PLoS Comput Biol* **7**, 1002066.

46 Spitzer J (2011) From water and ions to crowded biomacromolecules: *in vivo* structuring of a prokaryotic cell. *Microbiol Mol Biol Rev* **75**, 491–506.

47 Spitzer J and Poolman B (2013) How crowded is the prokaryotic cytoplasm? *FEBS Lett* **587**, 2094–2098.

48 Gi M (2012) Enzyme activity in the crowded milieu. *PLoS One* **7**, 39418.

49 Politou A and Temussi PA (2015) Revisiting a dogma: the effect of volume exclusion in molecular crowding. *Curr Opin Struct Biol* **30**, 1–6.

50 Spitzer JJ and Poolman B (2005) Electrochemical structure of the crowded cytoplasm. *Trends Biochem Sci* **30**, 536–541.

51 Joó F, Balogh N, Horváth LI, Filep G, Horváth I and Vígh L (1991) Complex hydrogenation/oxidation reactions of the water-soluble hydrogenation catalyst palladium di (sodium alizarinmonosulfonate) and details of homogeneous hydrogenation of lipids in isolated biomembranes and living cells. *Anal Biochem* **194**, 34–40.

52 Curtis W and Ordal EJ (1954) Hydrogenase and hydrogen metabolism in Micrococcus aerogenes. *J Bacteriol* **68**, 351–361.

53 Vagelos R (1964) Lipid metabolism. *Annu Rev Biochem* **33**, 139–172.

54 Calvin M (1974) Biopolymers: origin, chemistry, and biology. *Angew Chemie – Int Ed* **13**, 121–131.

55 Davies JM, Poole RJ and Sanders D (1993) The computed free energy change of hydrolysis of inorganic pyrophosphate and ATP: apparent significance. for inorganic-pyrophosphate-driven reactions of intermediary metabolism. *Biochim Biophys Acta – Bioenerg* **1141**, 29–36.

56 Akola J and Jones RO (2003) ATP hydrolysis in water - a density functional study. *J Phys Chem B* **107**, 11774–11783.

57 Lambert JF (2008) Adsorption and polymerization of amino acids on mineral surfaces: a review. *Orig Life Evol Biosph* **38**, 211–242.

58 Rode BM (1999) Peptides and the origin of life. *Peptides* **20**, 773–786.

59 Forsythe JG, Yu S, Mamajanov I, Grover MA, Krishnamurthy R, Fernández FM and Hud NV (2015) Ester-mediated amide bond formation driven by wet–dry cycles: a possible path to polypeptides on the prebiotic Earth. *Angew Chemie – Int Ed* **54**, 9871–9875.

60 Imai EI, Honda H, Hatori K, Brack A and Matsuno K (1999) Elongation of oligopeptides in a simulated submarine hydrothermal system. *Science* **283**, 831–833.

61 Le Son H, Suwannachot Y, Bujdak J and Rode BM (1998) Salt-induced peptide formation from amino acids in the presence of clays and related catalysts. *Inorganica Chim Acta* **272**, 89–94.

62 Zaia DAM (2004) A review of adsorption of amino acids on minerals: was it important for origin of life? *Amino Acids* **27**, 113–118.

63 Berg P and Ofengand EJ (1958) An enzymatic mechanism for linking amino acids to RNA. *Proc Natl Acad Sci USA* **44**, 78–86.

64 Ofengand EJ, Dieckmann M and Berg P (1961) The enzymic synthesis of amino acyl derivatives of ribonucleic acid. *J Biol Chem* **236**, 1741–1747.

65 Berg JM, Tymoczko JL, Gatto GJJ and Stryer L (2015) Biochemistry, 8th. W. H. Freeman and Company, New York.

66 Kottur J and Nair DT (2018) Pyrophosphate hydrolysis is an intrinsic and critical step of the DNA synthesis reaction. *Nucleic Acids Res* **46**, 5875.

67 Varfolomeev SD and Gurevich KG (2001) Enzyme active sites: Bioinformatics, architecture, and mechanisms of action. *Russ Chem Bull* **50**, 1709–1717.

68 Yao NY and O'Donnell ME (2016) Evolution of replication machines. *Crit Rev Biochem Mol Biol* **51**, 135–149.

69 Deutscher MP and Kornberg A (1969) Enzymatic synthesis of deoxyribonucleic acid. *J Biol Chem* **244**, 3019–3028.

70 Jensen PR and Michelsen O (1992) Carbon and energy metabolism of *atp* mutants of *Escherichia coli*. *J Bacteriol* **174**, 7635–7641.

71 Müller M, Mentel M, Van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu R-Y, Van DerGiezen M, Tielens AGM and Martin WF (2012) Biochemistry and evolution of anaerobic energymetabolism in eukaryotes. *Microbiol Mol Biol Rev* **76**, 444–495.

72 Oppenheimer NJ (1994) NAD hydrolysis: Chemical and enzymatic mechanisms. *Mol Cell Biochem* **138**, 245–251.

73 Spencer AK, Greenspan AD and Cronan JE Jr. (1978) Thioesterases I and II of *Escherichia coli*. Hydrolysis of native acyl-acyl carrier protein thioesters. *J Biol Chem* **253**, 5922–5926.

74 Wolfenden R (1963) Themechanism of hydrolysis of amino acyl RNA. *Biochim Biophys Acta* **47**, 353.

75 Lee HC and Aarhus R (1993) Wide distribution of an enzyme that catalyzes the hydrolysis of cyclic ADP-ribose. *Biochim. Biophys Acta – Protein Struct Mol Enzymol* **1164**, 68–74.

76 Varfolomeev SD (2004) Catalytic centres of enzymes: structural paradoxes, the phenomenon of structural unity and new reactions. *Mendeleev Commun* **14**, 185–188.

77 Rack JGM, Ariza A, Drown BS *et al.* (2018) (ADP-ribosyl)hydrolases: structural basis for differential substrate recognition and inhibition. *Cell Chem Biol* **25**, 1533–1546.e12.

78 Xavier JC, Hordijk W, Kauffman SA, Steel M and Martin WF (2020) Autocatalytic chemical networks at the origin of metabolism. *Proc Royal Soc B Biol Sci* **287**.

79 Holm NG (2012) The significance of Mg in prebiotic geochemistry. *Geobiology* **10**, 269–279.

80 Belmonte L and Mansy SS (2016) Metal catalysts and the origin of life. *Elements* **12**, 413–418.

81 Soares ARM, Anderson DR, Chandrashaker V and Lindsey JS (2013) Catalytic diversification upon metal scavenging in a prebiotic model for formation of tetrapyrrole macrocycles. *New J Chem* **37**, 2716.

82 Schoonen M, Smirnov A and Cohn C (2004) A perspective on the role of minerals in prebiotic synthesis. *AMBIO A J Hum Environ* **33**, 539–551.

83 Goldman AD, Baross JA and Samudrala R (2012) The enzymatic and metabolic capabilities of early life. *PLoS One* **7**, e39912.

84 Williams NH (2000) Magnesium ion catalyzed ATP hydrolysis. *J Am Chem Soc* **122**, 12023–12024.

85 Prince RC, Amande TJ and McGenity TJ (2010) Prokaryotic hydrocarbon degraders. In Handbook of hydrocarbon and lipid microbiology (Timmis, KN, ed), pp. 1669–1692. Springer International Publishing, Berlin, Heidelberg.

86 Weeks A, Lund L and Raushel FM (2006) Tunneling of intermediates in enzyme-catalyzed reactions. *Curr Opin Chem Biol* **10**, 465–472.

87 Ragsdale SW (2008) Enzymology of the Wood-Ljungdahl pathway of acetogenesis. *Ann N Y Acad Sci* **1125**, 129–36.

88 Fuchs G (2011) Alternative pathways of carbon dioxide fixation: Insights into the early evolution of life? *Annu Rev Microbiol* **65**, 631–658.

89 Weiss MC, Sousa FL, Mrnjavac N, Neukirchen S, Roettger M, Nelson-Sathi S and Martin WF (2016) The physiology and habitat of the last universal common ancestor. *Nat Microbiol* **1**, 16116.

90 Muchowska KB, Varma SJ, Chevallot-Beroux E, Lethuillier-Karl L, Li G and Moran J (2017) Metals promote sequences of the reverse Krebs cycle. *Nat Ecol Evol* **1**, 1716–1721.

91 Wagner T, Ermler U and Shima S (2016) The methanogenic CO_2 reducing-and-fixing enzyme is bifunctional and contains 46 [4Fe-4S] clusters. *Science* **354**, 114–117.

92 Timm DE, Mueller HA, Bhanumoorthy P, Harp JM and Bunick GJ (1999) Crystal structure and mechanism of a carbon–carbon bond hydrolase. *Structure* **7**, 1023–1033.

93 Pace NR (1991) Origin of life-facing up to the physical setting. *Cell* **65**, 531–533.

94 Baross JA and Hoffman SE (1985) Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. *Orig Life Evol Biosph* **15**, 327–345.

95 Wächtershäuser G (1988) Before enzymes and templates: Theory of surface metabolism. *Microbiol Rev* **52**, 452–484.

96 Bernal JD (2002) The physical basis of life. *Proc Phys Soc Sect A* **62**, 537–558.

97 Erastova V, Degiacomi MT, Fraser DG and Greenwell HC. Mineral surface chemistry control for origin of prebiotic peptides. *Nat Commun* 1–9.

98 Vincent L, Berg M, Krismer M, Saghafi ST, Cosby J, Sankari T, Vetsigian K, Ii HJC and Baum DA (2019) Chemical ecosystem selection on mineral surfaces reveals long-term dynamics consistent with the spontaneous emergence of mutual catalysis. *Life* **9**, 80.

99 Hazen RM and Sverjensky DA (2010) Mineral surfaces, geochemical complexities, and the origins of life. *Cold Spring Harb Perspect Biol* **2**, a002162.

100 McCollom TM and Seewald JS (2003) Experimental study of the hydrothermal reactivity of organic acids and acid anions: II. Acetic acid, acetate, and valeric acid. *Geochim Cosmochim Acta* **67**, 3645–3664.

101 Chen QW and Bahnemann DW (2000) Reduction of carbon dioxide by magnetite: Implications for the primordial synthesis of organic molecules [11]. *J Am Chem Soc* **122**, 970–971.

102 Santos-Carballal D, Roldan A, Dzade NY and De LeeuwNH (2018) Reactivity of CO_2 on the surfaces of magnetite (Fe_3O_4), greigite (Fe_3S_4) and mackinawite (FeS). *Philos Trans R Soc A* **376**, 20170065.

103 Roldan A, Hollingsworth N, Roffey A, Islam HU, Goodall JBM, Catlow CRA, Darr JA, Bras W, Sankar G, Holt KB *et al.* (2015) Bio-inspired CO_2 conversion by iron sulfide catalysts under sustainable conditions. *Chem Commun* **51**, 7501–7504.

104 Guan G, Kida T, Ma T, Kimura K, Abe E and Yoshida A (2003) Reduction of aqueous CO_2 at ambient temperature using zero-valent iron-based composites. *Green Chem* **5**, 630.

105 Huber C and Wächtershäuser G (1997) Activated acetic acid by carbon fixation on (Fe, Ni)S under primordial conditions. *Science* **276**, 245–248.

106 Fontecilla-Camps JC (2019) Geochemical continuity and catalyst/cofactor replacement in the emergence and evolution of life. *Angew Chemie – Int Ed* **58**, 42–48.

107 Wiegel J and Adams MWW (eds.) (1998) Thermophiles: The Keys to Molecular Evolution and the Origin of Life. Taylor & Francis, London.

108 Singh RP, Buttar HK, Kaur R and Manchanda G (2020) The multifaceted life of microbes: survival in varied environments. In Microbial Versatility in Varied Environments (Singh RP, Manchanda G, Maurya IK and Wei Y, eds), pp. 3–12. Springer Singapore, Singapore.

109 Stetter KO (2006) Hyperthermophiles in the history of life. *Philos Trans R Soc B* **361**, 1837–1843.

110 Roedder E (1984) The fluids in salt. *Am Mineral* **69**, 413–439.

111 Oren A (2011) Thermodynamic limits to microbial life at high. *Environ Microbiol* **13**, 1908–1923.

112 Barbier B and Brack A (1992) Conformation-controlled hydrolysis of polyribonucleotides by sequential basic polypeptides. *J Am Chem Soc* **114**, 3511–3515.

113 Longo LM, Lee J and Blaber M (2013) Simplified protein design biased for prebiotic amino acids yields a foldable, halophilic protein. *Proc Natl Acad Sci USA* **110**, 2135–2139.

114 Longo LM and Blaber M (2014) Prebiotic protein design supports a halophile origin of foldable proteins. *Front Microbiol* **4**, 2013–2015.

115 Becker S, Schneider C, Dejmek M, Carell T, Okamura H and Crisp A (2018) Wet–dry cycles enable the parallel origin of canonical and non-canonical nucleosides by continuous synthesis. *Nat Commun* **9**, 163.

116 Tucker MC, Srinivasan V, Ross PN and Weber AZ. (2013) Performance and cycling of the iron-ion/hydrogen redox flow cell with various catholyte salts. *J Appl Electrochem* **43**, 637–644.

117 Westall F, Hickman-Lewis K, Hinman N, Gautret P, Campbell KA, Bréhéret JG, Foucher F, Hubert A, Sorieul S, Dass AV *et al.* (2018) A hydrothermal-sedimentary context for the origin of life. *Astrobiology* **18**, 259–293.

118 Landis GA. (2001) Martian water: are there extant halobacteria on mars? *Astrobiology* **1**, 161–164.

119 Matson DL, Castillo JC, Lunine J and Johnson TV (2007) Enceladus' plume: Compositional evidence for a hot interior. *Icarus* **187**, 569–573.

120 Hsu H-W, Postberg F, Sekine Y, Shibuya T, Kempf S, Horányi M, Juhász A, Altobelli N, Suzuki K, Masaki Y *et al.* (2015) Ongoing hydrothermal activities within Enceladus. *Nature* **519**, 207–210.

121 Shibuya T, Postberg F, Hsu H-W, Suzuki K, Masaki Y, Kuwatani T, Mori M, Hong PK, Yoshizaki M, Tachibana S *et al.* (2015) High-temperature water-rock interactions and hydrothermal environments in the chondrite-like core of Enceladus. *Nat Commun* **6**, 1–8.

122 Waite JH, Glein CR, Perryman RS, Teolis BD, Magee BA, Miller G, Grimes J, Perry ME, Miller KE, Bouquet A *et al.* (2017) Cassini finds molecular hydrogen in the Enceladus plume: Evidence for hydrothermal processes. *Science* **356**, 155–159.

123 Glein CR, Baross JA and Waite JH (2015) The pH of Enceladus' ocean. *Geochim Cosmochim Acta* **162**, 202–219.

124 Postberg F, Khawaja N, Abel B, Choblet G, Glein CR, Gudipati MS, Henderson BL, Hsu H-W, Kempf S, Klenner F *et al.* (2018) Macromolecular organic compounds from the depths of Enceladus. *Nature* **558**, 564–568.

125 Alexander CMOD, Cody GD, De Gregorio BT, Nittler LR and Stroud RM (2017) The nature, origin and modification of insoluble organic matter in chondrites, the major source of Earth's C and N. *Chem Erde* **77**, 227–256.

126 Khawaja N, Postberg F, Hillier J, Klenner F, Kempf S, Reviol R, Zou Z and Srama R (2019) Low-mass nitrogen-, oxygen-bearing, and aromatic compounds in Enceladean ice grains. *Mon Not R Astron Soc* **489**, 5231–5243.

127 Deamer D and Damer B (2017) Can life begin on Enceladus? A perspective from hydrothermal chemistry. *Astrobiology* **17**, 834–839.

128 Bratbak G and Dundas I (1984) Bacterial dry matter content and biomass estimations. *Appl Environ Microbiol* **48**, 755–757.

129 Vrede K, Heldal M, Norland S and Bratbak G (2002) Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. *Appl Environ Microbiol* **68**, 2965–2971.

130 Holm NG (1992) Why are hydrothermal systems proposed as plausible environments for the origin of life? In Marine Hydrothermal Systems and the Origin of Life (Holm NG, ed.), pp. 5–14. Springer, Dordrecht.

131 Nisbet E and Sleep NH (2001) The habitat and nature of early life. *Nature* **409**, 1083–1091.

132 Frost RB and Beard JS (2007) On silica activity and serpentinization. *J Petrol* **48**, 1351–1368.

133 Martin WF and Russell MJ (2007) On the origin of biochemistry at an alkaline hydrothermal vent. *Philos Trans R Soc B Biol Sci* **362**, 1887–1925.

134 Früh-Green GL, Connolly JAD, Plas A, Kelley DS and Grobety B. (2004) Serpentinization of oceanic peridotites: Implications for geochemical cycles and biological activity. In The Subseafloor Biosphere at Mid-Ocean Ridges Geophysical Monograph Series 144 (Wilcock WSD, Delong EF, Kelley DS, Baross JA and Craig CS eds), pp. 119–136. American Geophysical Union 10.1029/144GM08.

135 Holm NG, Oze C, Mousis O, Waite JH and Guilbert-Lepoutre A (2015) Serpentinization and the formation of H_2 and CH_4 on celestial bodies (planets, moons, comets). *Astrobiology* **15**, 587–600.

136 Schrenk MO, Kelley DS, Bolton SA and Baross JA (2004) Low archaeal diversity linked to subseafloor geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. *Environ Microbiol* **6**, 1086–1095.

137 Lang SQ and Brazelton WJ (2020) Habitability of the marine serpentinite subsurface: a case study of the Lost City hydrothermal field. *Philos Trans R Soc A* **378**, 20180429.

138 Boyd ES, Amenabar MJ, Poudel S, Templeton AS and Boyd ES (2020) Bioenergetic constraints on the origin of autotrophic metabolism. *Philos Trans R Soc A* **378**, 20190151.

139 Kelley DS, Karson JA, Früh-Green GL, Yoerger DR, Shank TM, Butterfield DA, Hayes JM, Schrenk MO, Olson EJ, Proskurowski G *et al.* (2005) A serpentinite-hosted ecosystem: the Lost City Hydrothermal Field. *Science* **307**, 1428–1434.

140 Proskurowski G, Lilley MD, Seewald JS, Fru h-Green Gl, Olson EJ, Lupton JE, Sylva SP and Kelley DS (2008) Abiogenic hydrocarbon production at Lost City hydrothermal field. *Science* **319**, 319–607.

141 Lang SQ, Butterfield DA, Schulte M, Kelley DS and Lilley MD (2010) Elevated concentrations of formate, acetate and dissolved organic carbon found at the Lost City hydrothermal field. *Geochim Cosmochim Acta* **74**, 941–952.

142 Huber C, Eisenreich W and Wächtershäuser G (2010) Synthesis of α-amino and α-hydroxy acids under volcanic conditions: implications for the origin of life. *Tetrahedron Lett* **51**, 1069–1071.

143 Camprubi E, Jordan SF, Vasiliadou R and Lane N (2017) Iron catalysis at the origin of life. *IUBMB Life* **69**, 373–381.

144 Russell MJ and Hall AJ (1997) The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *J. Geol Soc London* **154**, 377–402.

145 Shock EL and Helgeson HC (1988) Calculation of the thermodynamic and transport properties of aqueous species at high pressures and temperatures: Correlation algorithms for ionic species and equation of state predictions to 5 kb and 1000°C. *Geochim Cosmochim Acta* **52**, 2009–2036.

146 Shock EL (1990) Geochemical constraints on the origin of organic compounds in hydrothermal systems. *Orig Life Evol Biosph* **20**, 331–367.

147 Konn C, Charlou JL, Holm NG and Mousis O (2015) The production of methane, hydrogen, and organic compounds in ultramafic-hosted hydrothermal vents of the Mid-Atlantic Ridge. *Astrobiology* **15**, 381–399.

148 Schrenk MO, Brazelton WJ and Lang SQ (2013) Serpentinization, carbon, and deep life. *Rev Mineral Geochemistry* **75**, 575–606.

149 Martin W, Baross J, Kelley D and Russell MJ (2008) Hydrothermal vents and the origin of life. *Nat Rev Microbiol* **6**, 805–814.

150 Preiner M, Xavier J, Sousa F, Zimorski V, Neubeck A, Lang S, Greenwell H, Kleinermanns K, Tüysüz H, McCollom T *et al.* (2018) Serpentinization: connecting geochemistry, ancient metabolism and industrial hydrogenation. *Life* **8**, 41.

151 Miller HM, Mayhew LE, Ellison ET, Kelemen P, Kubo M and Templeton AS (2017) Low temperature hydrogen production during experimental hydration of partially-serpentinized dunite. *Geochim Cosmochim Acta* **209**, 161–183.

152 Kauffman SA (1986) Autocatalytic sets of proteins. *J Theor Biol* **119**, 1–24.

153 Steel M, Hordijk W and Xavier JC (2019) Autocatalytic networks in biology: Structural theory and algorithms. *J R Soc Interface* **16**, 20180808.

154 Russell MJ, Hall AJ and Martin WF (2010) Serpentinization as a source of energy at the origin of life. *Geobiology* **8**, 355–371.

155 Etiope G and Sherwood Lollar B (2013) Abiotic methane on Earth. *Rev Geophys* **51**, 276–299.

156 Zahnle KJ, Arndt N, Cockell C, Halliday A, Nisbet E, Selsis F and Sleep NH (2007) Emergence of a habitable planet. *Space Sci Rev* **129**, 35–78.

157 Ménez B, Pisapia C, Andreani M, Jamme F, Vanbellingen QP, Brunelle A, Richard L, Dumas P and Réfrégiers M *et al.* (2018) Abiotic synthesis of amino acids in the recesses of the oceanic lithosphere. *Nature* **564**, 59–63.

158 Sforna MC, Brunelli D, Pisapia C, Pasini V, Malferrari D and Ménez B (2018) Abiotic formation of condensed carbonaceous matter in the hydrating oceanic crust. *Nat Commun* **9**, 5049.

159 Andreani M and Ménez B (2019) New perspectives on abiotic organic synthesis and processing during hydrothermal alteration of the oceanic lithosphere. In Deep Carbon (Orcutt BN, Daniel I and Dasgupta R, eds), pp. 447–479. Cambridge University Press, Cambridge.

160 Benner SA, Kim H-J and Carrigan MA (2012) Asphalt, water, and the prebiotic synthesis of ribose, ribonucleosides, and RNA. *Acc Chem Res* **45**, 2025–2034.

161 Chandru K, Gilbert A, Butch C, Aono M and Cleaves HJ (2016) The abiotic chemistry of thiolated acetate derivatives and the origin of life. *Sci Rep* **6**, 1–11.

162 Charlou JL, Donval JP, Fouquet Y, Jean-Baptiste P and Holm N (2002) Geochemistry of high H_2 and CH_4 vent fluids issuing from ultramafic rocks at the Rainbow hydrothermal field (36°14′N, MAR). *Chem Geol* **191**, 345–359.

163 Xavier JC, Hordijk W, Kauffman SA, Steel M and Martin WF. (2019) Autocatalytic chemical networks preceded proteins and RNA in evolution. *BioRxiv* [PREPRINT].

164 Porosoff MD, Yan B and Chen JG (2016) Catalytic reduction of CO_2 by H_2 for synthesis of CO, methanol and hydrocarbons: Challenges and opportunities. *Energy Environ Sci* **9**, 62–73.

165 Leigh GJ (2004) Haber– Bosch and other industrial processes. In Catalysts for nitrogen fixation: nitrogenases, relevant chemical models, and commercial processes (Smith BE, Richards RL and Newton WE, eds), pp. 33–54. Dordrecht: Springer.

166 Satterfield CN, Hanlon RT, Tung SE, Zou Z mln and Papaefthymiou GC (1986) Effect of water on the iron-catalyzed Fischer-Tropsch synthesis. *Ind Eng Chem Prod Res Dev* **25**, 407–414.

167 Brack A (1987) Selective emergence and survival of early polypeptides in water. *Orig Life* **17**, 367–379.

168 Wolfenden R (2011) Benchmark reaction rates, the stability of biological molecules in water, and the evolution of catalytic power in enzymes. *Annu Rev Biochem* **80**, 645–667.

169 Radzicka A and Wolfenden R (1996) Rates of uncatalyzed peptide bond hydrolysis in neutral

solution and the transition state affinities of proteases. *J Am Chem Soc* **118**, 6105–6109.

170 Tokuriki N and Tawfik DS (2009) Protein dynamism and evolvability. *Science* **324**, 203–207.

171 Danger G, Plasson R and Pascal R (2012) Pathways for the formation and evolution of peptides in prebiotic environments. *Chem Soc Rev* **41**: 5416–5429.

172 Reeves EP, Mcdermott JM and Seewald JS (2014) The origin of methanethiol in midocean ridge hydrothermal fluids. *Proc Natl Acad Sci U S A* **111**, 5474–5479.

173 Ragsdale SW and Pierce E (2008) Acetogenesis and the wood-Ljungdahl pathway of CO_2 fixation. *Biochim Biophys Acta* **1784**, 1873–1898.