Life did not originate from amyloids and ATP

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Recent proposals for how life might have begun from ordinary, simple chemicals on the early earth do not withstand scientific scrutiny. One proposal is the amyloid world hypothesis, coupled with the idea that ATP could form prebiotically. Both ideas have little hard scientific evidence. ATP does not form readily in or near hydrothermal vents. All three phosphate group additions to a nucleoside are very endothermic, ensuring that the steady-state concentration of the nucleoside triphosphate, such as ATP, would have to be vanishingly low. ATP would have a very short lifetime near hydrothermal vents. The proposed pH gradients to accomplish ATP synthesis would have required ~1-nm-thin mineral membranes, which would not have been strong enough to form stable, hermetical regions separating oceanic and vent water having very different temperatures and pHs. The theoretical embedded inorganic nanomachines needed to extract useful energy remain pure speculation. Amyloids do not possess true ATPase properties, being unable to position ATP and target substrates in transition-state-like orientations. Furthermore, the concept of a LUCA (Last Universal Common Ancestor) that 'bubbled off' overlooks that the alkaline interior would have soon neutralized the proton gradient that drove the hypothetical energy-producing machines.

Dr Maury, Emeritus Professor from the Department of Medicine, University of Helsinki, Finland, has had a long and distinguished career centred around amyloids, amyloidosis, and fibrillogenesis research from a medical perspective.[1] He has since chosen to become a leading proponent of the amyloid world hypothesis. This is a shift away from his expertise in medicinal chemistry.

In an influential review article on the amyloid world theory, Maury claimed that prebiotic amyloids produced a series of biology-like new functions including self-replication, catalytic activities, information transfer, chiroselective addition of amino acids, and error-correcting information-processing system.[2]

Other amyloid world proponents such as Rout *et al.* have observed correctly that forming highly ordered amyloid aggregates is a simple physical process analogous to crystallization.[3]

None of the OoL experiments associated with the amyloid world hypothesis have any resemblance to realistic prebiotic conditions.[4–6] Critically, the high concentration of single-sequence peptides would not have been present in aqueous conditions.[7,8]

There is a current trend for the mass media, including YouTubers, to provide simplistic and severely flawed versions of the chemistry involved. Technical publications are skimmed over, exaggerated claims extracted (especially from the abstracts), and then a plausible-sounding narrative is offered with some technical terms to provide a veneer of credibility.

For example, one supporter of the amyloid world theory is a YouTuber who reviewed Maury's paper.[2] Remarkably, six commonly circulated errors were presented in a single paragraph.

> "[1] And ATP is another naturally occurring energy source near hydrothermal vents. [2] So, we know that amyloids have ATPase activity they can utilize the energy of ATP to do work. [3] And once you develop this interaction between amyloids and ATP it's only a matter of time until you start having refinements into the information storing system. [4] The stringing of ATP and different triphosphate nucleotides together into DNA and RNA, [5] the incorporation of lipids which are again found naturally at hydrothermal vents and which spontaneously formed bilayer membranes; [6] as well as the creation of protein enzymes to do specific functions [labels 1–6 added]."[9]

These claims, labelled 1 to 6, have been encountered before and will now be examined. We will not address the many other chemical errors in the above lecture.[10–12]

The most serious misconception is that just a "matter of time" explains what materialists cannot explain using sound scientific principles—the source of new information.

Error 1—ATP does not form naturally near hydrothermal vents

Although the claim is often encountered that ATP is produced under prebiotic conditions, Chu and Zhang pointed out, in 2023, that

> "Although the de novo synthesis of ATP has not been reported, the origin of its components, including ribose, adenine, and the triphosphate group has been extensively studied."[13]

Later in this paper, Chu and Zhang speculate about prebiotic conditions that might produce ATP. Most of the experimental conditions proposed are not realistic in terms of temperatures, concentrations of reactants, or the need for purification. Not even one experiment was reported. This type of speculation in the literature is far from supporting a claim that "ATP is another naturally occurring energy source near hydrothermal vents."[9]

Fictitious pH gradient and machines in hydrothermal vents

Allegedly, ATP was readily created in pH gradients in hydrothermal vents.

> "But back at the prebiotic Earth proton gradients were formed naturally at hydrothermal vents which are key and integral in forming ATP in the first place."[14]

This claim, presented as a self-evident fact, was based on someone else's unfounded speculations. In the early 1990s, Russell *et al.* proposed a clever source of energy to drive chemical reactions to produce ATP, which is an endothermic (i.e., thermodynamically unfavourable) reaction. This required a *hypothetical* pH gradient separating extremely hot alkaline fluid generated in hydrothermal vents from colder acidic oceanic water.[15]

Meanwhile, three groups, headed by M.J. Russell, W. Martin, and N. Lane, have integrated this notion in their hypothesis of a non-organic, mineral autotrophy origin of life. The key papers have been referenced and discussed in a decidedly unflattering review article by Jackson, who observed:[16]

> "… these have been extensively cited but not critically reviewed in the literature."

Nevertheless, these vacuous speculations evolved into 'scientifically proven facts' in the mass media.

The highest proton motive force would result from the greatest difference in pH. These are usually optimistically assumed to be pH = 6 in the ocean and pH = 10 in the hydrothermal vent side.[16] If the entire ΔpH of 4 could be used effectively, this would provide a maximum energy of $24\ kJ\ mol^{-1}$.[16] This energy is claimed to have driven the reduction of CO_2 by H_2 to form $HCOO^-$ (which could generally unhelpfully decompose to form carbon monoxide, CO).

To prevent water having different pH values from mixing, a stable inorganic membrane was proposed. Various very complex precipitate membranes have been hypothesized, consisting of partly composed of iron sulphides doped with Ni, Co, and Mo, as well as ferrous/ferric oxyhydroxides, dosed with Ni and Mo.[17] Russell *et al.* also proposed metal-layered hydroxides such as the hydrotalcite pyroaurite $[\sim Mg_6Fe_2(CO_3)(OH)_{16}\cdot 4(H_2O)]$ and fougèrite variants like $\sim[(Fe^{II},Mg)_2Fe^{III}(OH^-)_5\cdot CO_3^{2-}]$.[17] Other candidate precipitates include montmorillonites, smectites, and other cationic clays.

There is no evidence that any of the kinds of membrane systems did, or could have, existed, possessing steep pH gradients, neither at the extensively studied Lost City hydrothermal field, located 20 km west of the Mid-Atlantic Ridge nor anywhere else.[16]

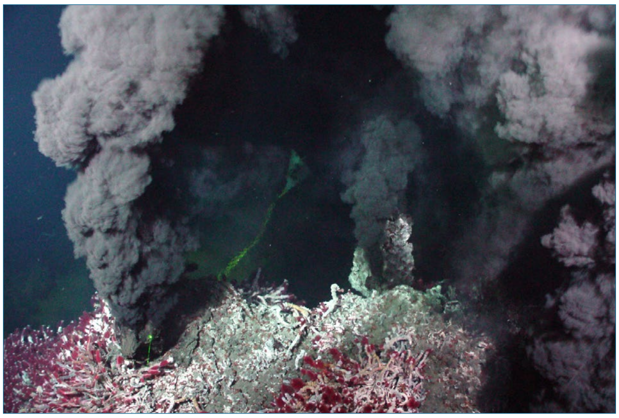

Figure 1. Black smoker at Sully Vent in the Main Endeavour Vent Field in the northeast Pacific Ocean

Image: National Oceanic and Atmospheric Administration (NOAA) NOAA

Russell *et al.* pointed out that a *simple* inorganic proton electrochemical gradient machine would have been needed to replace an extraordinarily sophisticated rotary mechanism such as ATP synthetase.[17] This machine must traverse the membrane, being in contact in some manner with both the source of protons (the cold acidic ocean at lower pH) and the hot alkaline hydrothermal vent side. The hypothetical machine must play the role of cellular H^+-ATP synthase (which is about 10 nm in diameter) and the H^+-pyrophosphate synthase. The inner membranes of mitochondria in which the huge ATP synthase complexes are embedded are only about 5–6 nanometres (nm) thick.

Since the hypothetical much simpler embedded prebiotic machine would have had to be very small, the membrane must not have been much thicker at the location of proton influx. Otherwise no H^+ transfer would have been possible. Consequently, the required membrane would have had to be exceedingly thin, no wider than about 1 nm, and therefore very fragile.

Photographs (like figure 1) and YouTube videos illustrate the explosive force of high-pressure hydrothermal vents at around 400°C, offering a reality check for how plausible it would be to form delicate 1-nm-thick layers of soluble minerals hermetically sealed from the surrounding ocean water. Random precipitates would not have been conveniently limited to 1 nm layers, possessing some kind of mineral machine.

Thicker membranes would have been conceptually possible if there were channels through which protons could flow, and the machines would be fortuitously placed along the open paths. However, this would have decreased the electrical potential difference, realistically even entirely.

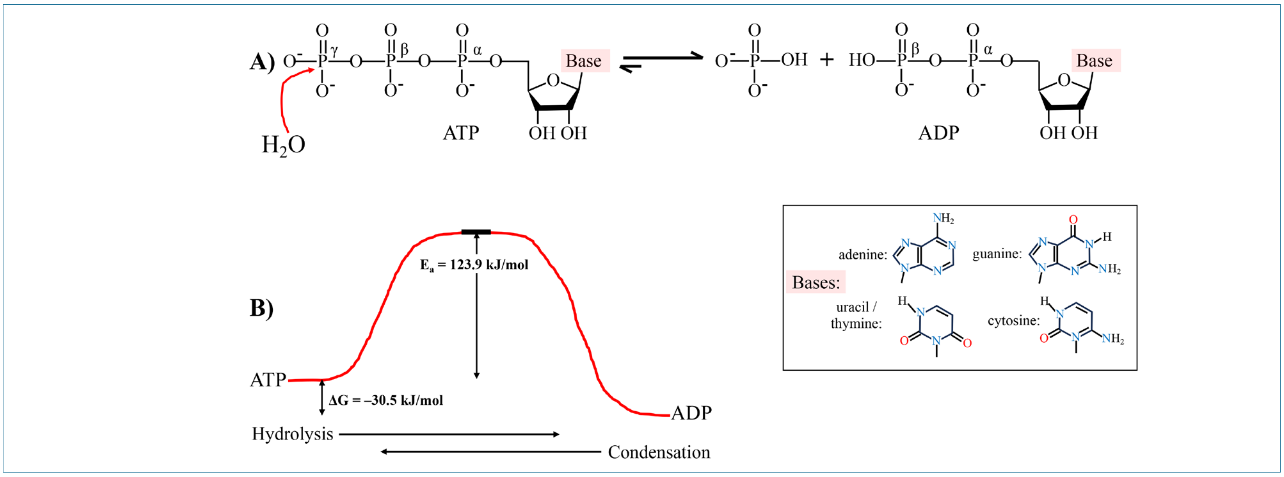

Figure 2. Triphosphate nucleotides hydrolyze when dissolved in water. A) Hydrolysis of the γ-phosphate group. Adenine in the inset is the base used by ATP. The four bases shown are used by RNA. B) Energy of activation, E_a, is high for both hydrolysis and formation of ATP, but the hydrolysis reaction is very exothermic. ΔG values are reported under standard conditions (25°C, 1 bar, 1 mol/L, pH = 7). ATP = adenosine triphosphate, ADP = adenosine diphosphate.

Note that the theoretical maximum energy extractable by a perfect machine, 24 kJ mol^{-1}, is already near the minimum to be of any chemical value.[16]

Speculations on the composition of the theoretical machines have centred recently on Fe(Ni)S and silicate structures. However, vents at Lost City are dominated by forms of calcium carbonate and magnesium hydroxide, unsuitable as membranes. Furthermore, silicate was present as only a trace component within the chimney materials at Lost City, with no sulphide whatsoever.[16]

Jackson accurately described the narratives on how these fictitious proton transporters were claimed could have worked as

> "… splendidly imaginative, but not in the least supported by observations in the laboratory."[16]

In all the decades since these kinds of fictitious inorganic membranes were first proposed, no details of their hypothetical properties and functioning have been put forward.[16]

Instead of ATP being a "naturally occurring energy source near hydrothermal vents", we see instead that production of ATP from non-biotic sources near hydrothermal vents has not been demonstrated at all; only speculative models exist; and no evidence for a mineral membrane exists. Worse, Jackson has pointed out that *not even a laboratory proof-of-concept* has been offered. To deserve any scientific credibility:

> "A direct demonstration that a model, laboratory-synthesized, non-protein, molecular machine can utilize a ΔpH established across a laboratory-synthesized, inorganic membrane is needed."[16]

The possibility of a steady supply of concentrated ATP forming near hydrovents to support pre-RNA life will be disproven next, based on chemical principles.

ATP does not form in high concentrations near hydrothermal vents

ATP is a high-energy molecule used to fuel countless cellular processes. Hydrolysis of each phosphate is very exothermic. In the case of the end phosphate, –28.0 to –33.5 kJ/mol would be released under standard conditions, as shown in figure 2.[18–21]

Increasing the temperature to overcome the high energy of activation barriers shown in figure 2B would accelerate addition of each of the three phosphate groups to adenosine. This is because providing reactants like ADP and inorganic phosphate (P_i) with a higher kinetic energy would increase their frequency of collision and the proportion able to overcome the activation energy barrier.

However, the three condensation/hydrolyzation processes are reversible, so that increasing temperatures would accelerate both the forward and reverse reactions:

$$\text{Adenosine} \underset{H_2O}{\overset{P_i}{\rightleftharpoons}} \text{AMP} \underset{H_2O}{\overset{P_i}{\rightleftharpoons}} \text{ADP} \underset{H_2O}{\overset{P_i}{\rightleftharpoons}} \text{ATP} \tag{1}$$

where ATP means adenosine triphosphate, ADP adenosine diphosphate, AMP adenosine monophosphate, and P_i represents the inorganic phosphate group. Once ATP forms, the energy of activation of hydrolysis is high enough for it to remain intact for some time *at low temperatures*.

During a sequential addition of phosphate groups under prebiotic conditions, as shown in scheme (1), AMP would first need to build up, then ADP, and finally ATP. At equilibrium,

$$k_{con}[ADP]_{eq}[P_i]_{eq} = k_{hyd}[ATP]_{eq} \tag{2}$$

where k_{con} is the rate constant of condensation, and k_{hyd} of hydrolysis. As usual in chemistry, the concentration of H_2O (55.5 M) will be considered unchanged and k_{hyd} already incorporates this.

The highest concentration of ATP beginning with adenosine according to scheme (1) would result at the system's equilibrium point. It cannot be higher according to the definition of equilibrium expressed by eqn (2). The equilibrium concentration of [ATP] is what matters, not how fast the equilibrating forward and reverse reactions occur.

Finding the equilibration concentration of ATP at 350°C (623 K)

Assume that all the ATP present at equilibrium would be equilibrating with only ADP; i.e.,

$[ADP]_{eq} + [P_i]_{eq} \leftrightarrows [ATP]_{eq}$. The rationale for this approximation being reasonable is given in Appendix 1. The equilibrium constant is

$$K_1 = [ATP]_{eq} / [ADP]_{eq}[P_i]_{eq} \qquad (3)$$

K_1 can be found using the van 't Hoff equation

$$\Delta G° = –RT \times \ln(K_1) \qquad (4)$$

where ΔG° is the Gibbs free energy change under standard conditions, R is the gas constant (8.314 J/mol·K), and T is the temperature in Kelvin. Fortunately, ΔG° values are available from literature sources and eqn (4) can be expressed as

$$K_{eq} = e^{(–\Delta G°/(RT))} \qquad (5)$$

Using ΔG = –30,500 J/mol[19,20,21] leads to

$$K_1 = \approx 2.8 \text{ x } 10^{-3} \qquad (6)$$

at 350°C, since $K_1 = e^{(–(30{,}500 \text{ J/mol}) / (8.314 \text{ J/(mol·K) x } 623 \text{ K}))}$. The units of the equilibrium constants were excluded in this paper to simplify the notation.

Rearranging (3) leads to

$$[ATP]_{eq} = K_1 \times [ADP]_{eq} [P_i]_{eq} \qquad (7)$$

Hence, the values for $[P_i]_{eq}$ and $[ADP]_{eq}$ are needed to solve this equation.

Concentration of prebiotic inorganic phosphate

The average phosphate concentration in oceans is approximately 70–72 µg/L.[22] Not all these phosphates are in the P_i form which is needed to form ATP, so setting $[P_i]$ = 70 µg/L is generous. Using a molecular weight of P_i = 94.97 g/mol leads to an average molarity in ocean water of

$$[P_i] \approx 7.4 \times 10^{-7} \text{ M}. \qquad (8)$$

The current concentration of phosphates measured is higher than would have been present billions of years ago, since they are being transported off continents mostly via river runoff. Near hydrothermal vents phosphates are adsorbed onto iron oxyhydroxides, removing them from solution, and remobilization within the sediments is limited afterwards.[23] These considerations imply that the effective prebiotic $[P_i]$ would not have been higher than given in (8).[23]

Concentration of prebiotic [ADP]

Prebiotic ADP would have formed via the very unfavourable equilibrium AMP + $P_i \leftrightarrows$ ADP + H_2O. The equilibrium constant is

$$K_2 = [ADP]_{eq} / [AMP]_{eq}[P_i]_{eq} \qquad (9)$$

Using ΔG = +30.5 kJ/mol[24] in (5) leads to

$$K_2 \approx 2.8 \times 10^{-3} \qquad (10)$$

$$[ADP]_{eq} = K_2[AMP]_{eq}[P_i]_{eq} \qquad (11)$$

The concentration of $[AMP]_{eq}$ is now needed to solve this equation.

Concentration of prebiotic [AMP]

Prebiotic AMP would have formed via the very unfavourable equilibrium adenosine + $P_i \leftrightarrows$ AMP + H_2O. The equilibrium constant is

$$K_3 = [AMP]_{eq} / [adenosine]_{eq}[P_i]_{eq} \qquad (12)$$

Using ΔG = +14.2 kJ/mol[24] in (5) leads to

$$K_3 \approx 6.5 \times 10^{-2} \qquad (13)$$

at 350°C. Rearranging (12) leads to

$$[AMP]_{eq} = K_3[adenosine]_{eq}[P_i]_{eq} \qquad (14)$$

The $[ATP]_{eq}$ in eqn (7) can now be expressed using $[ADP]_{eq}$ from eqn (11) and $[AMP]_{eq}$ from (14):

$$[ATP]_{eq} = K_1 \times K_2 \times K_3[adenosine]_{eq}[P_i]^3_{eq} \qquad (15)$$

Since $K_1 = 2.8 \text{ x } 10^{-3}$ from (6), $K_2 = 2.8 \times 10^{-3}$ from (10), $K_3 = 6.5 \times 10^{-2}$ from (13), and $[P_i] = 7.4 \times 10^{-7}$ M from (8), this leads to

$$[ATP]_{eq} \approx 2.1 \text{ x } 10^{-25} \text{ x } [adenosine]_{eq} \qquad (16)$$

at 350°C. The equilibrium constant of [adenosine] will determine how much [ATP] would be produced. But key life-relevant chemicals like adenosine are rapidly destroyed at such high temperatures. As Levy and Miller pointed out:[25]

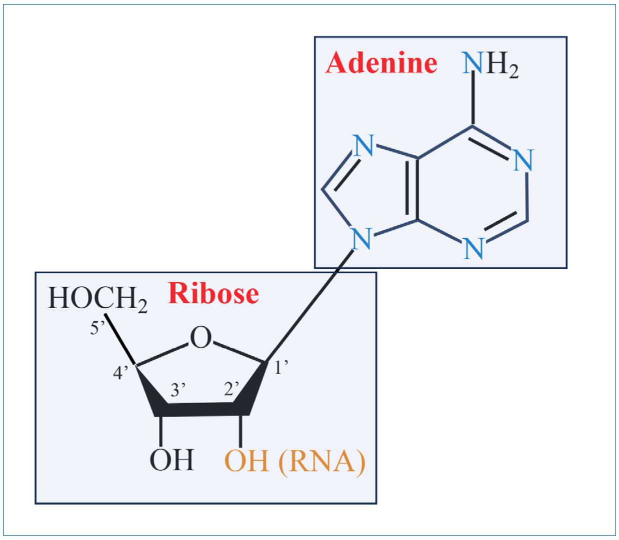

Figure 3. Chemical structure of adenosine. When a hydroxyl group is attached at the 2'-position, this is a ribose, used by RNA. When the 2'- group is absent, this is a deoxyribose, used by DNA.

> "Previous studies have shown that a major problem with an origin of life between 250°–350°C is the stability of the presumed components of the first organisms, where the half-lives for decomposition are at most a few minutes."

One of these components is ribose, a precursor of adenosine, which has a half-life of only 73 min at pH 7.0 and 100°C and 44 years at pH 7.0 and 0°C.[26] At higher pH, the half-lives are shortened significantly.

In fact, the half-life of adenosine, shown in figure 3, is less than 15 seconds at 350°C,[25] assuming any could have been formed. Shapiro pointed out that adenine synthesis requires unrealistically high HCN concentrations of at least 0.01 M to obtain any at all. However, HCN forms many other similar chemicals, and isomers instead of adenine.[27]

From eqn (16), the molar $[ATP]_{eq}$ is the product of $[adenosine]_{eq} \approx 0 \times 2.1 \times 10^{-25}$. Clearly, no relevant amount of ATP would have formed at hydrothermal temperatures of around 350°C.

Lower temperature scenarios to form ATP

Since adenosine is thermally unstable, perhaps ATP may have formed somewhere *near* a prebiotic hydrothermal vent but at much less than 350°C. Lower temperatures, though, would *decrease* the values of K_1, K_2, and K_3, as summarized in table 1.

To illustrate, eqn (15) was solved using equilibrium constants at other temperatures summarized in table 1. Since at 350°C the half-life of adenosine is less than 15 seconds, the temperature range 25–100°C would be worth considering. Using the K_1, K_2, and K_3, and $[P_i] = 7.4 \times 10^{-7}$ M, we can predict:

$$[ATP]_{eq} \text{ (at 25°C)} = 2.7 \times 10^{-32} \, [adenosine]_{eq} \qquad (17)$$

$$[ATP]_{eq} \text{ (at 100°C)} = 1.2 \times 10^{-29} \, [adenosine]_{eq} \qquad (18)$$

Suppose a high $[adenosine]_{eq} = 10^{-3}$ M could have been produced prebiotically at 25°C, and a factor 1,000 less at

Table 1. Half-life of adenosine and equilibrium constants for ATP precursors at different temperatures. Equilibrium constants calculated using $K = e^{(-(\Delta G \text{ J/mol}) / (8.314 \text{ J/(mol·K)} \times T \text{ (K)})}$.

		Equilibrium constants				
°C	Half-life Adenosine [a]	$ADP + P_i \leftrightharpoons ATP + H_2O$ ($\Delta G \approx +30.5$) [b] $\underline{K_1}$	$AMP + P_i \leftrightharpoons ADP + H_2O$ ($\Delta G \approx +30.5$) [c] $\underline{K_2}$	$Adenosine + P_i \leftrightharpoons AMP + H_2O$ ($\Delta G \approx +14.2$) [d] $\underline{K_3}$	$AMP + PP_i \leftrightharpoons ATP + H_2O$ ($\Delta G \approx +46$) [e] $\underline{K_4}$	$2 P_i \leftrightharpoons PP_i + H_2$ ($\Delta G \approx +29.3$) [f] $\underline{K_5}$
25	10,000 yr.	4.50×10^{-6}	4.50×10^{-6}	3.24×10^{-3}	8.64×10^{-9}	7.31×10^{-6}
100	1 yr.	5.35×10^{-5}	5.35×10^{-5}	1.03×10^{-2}	3.61×10^{-7}	7.88×10^{-5}
250	< 35 min.	8.99×10^{-4}	8.99×10^{-4}	3.82×10^{-2}	2.54×10^{-5}	1.18×10^{-3}
350	<15 sec.	2.77×10^{-3}	2.77×10^{-3}	6.45×10^{-2}	1.39×10^{-4}	3.49×10^{-3}

[a] From Levy and Miller[25]
[b] +30.5 kJ/mol from several refs.[19–21] 32 kJ/mol according to Wimmer *et al.*[28]
[c] +30.5 kJ/mol from vrchemistry[24]
[d] +14.2 kJ/mol from vrchemistry[24]
[e] +46 kJ/mol from Wimmer *et al*[28]
[f] +29.3 kJ/mol from Kotter and Nair[29]

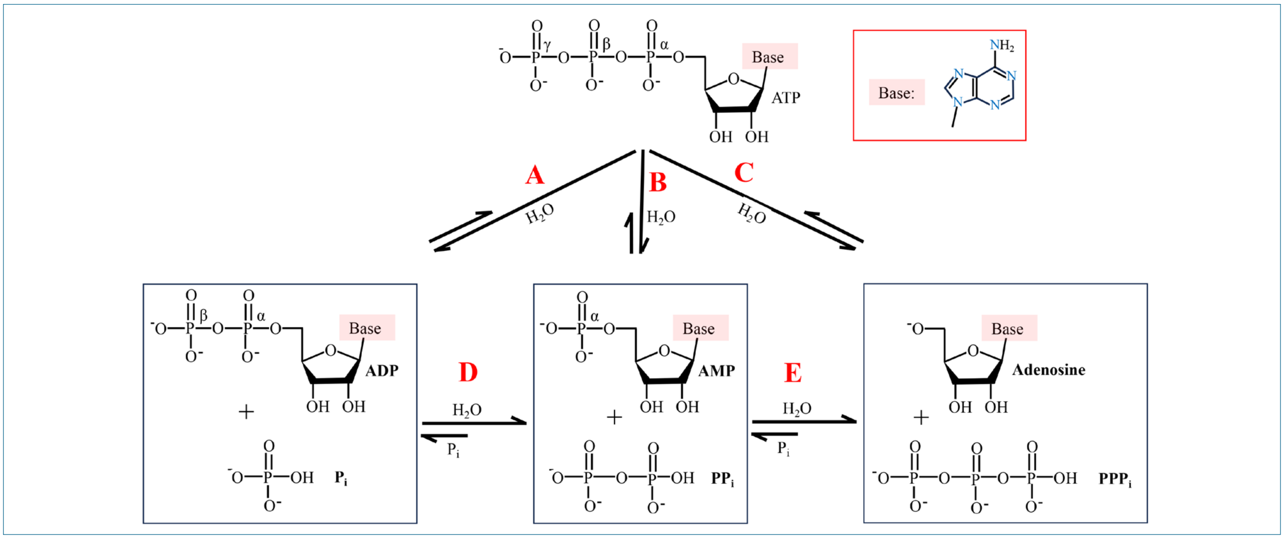

Figure 4. Equilibrium reactions involved in the formation and hydrolysis of ATP

100°C. Then in this temperature range $[ATP]_{eq}$ would have been $< 1 \times 10^{-35}$ M. From Avogradro's number a $[ATP]_{eq}$ = 1 M would have contained about 6×10^{23} ATP molecules. Therefore, based on the collection of optimistic assumptions made, in the temperature range 25–100°C, less than 1 ATP molecule would have been present per 10^{11} litres of water.

Other potential pathways to form ATP

Figure 4 shows the various equilibrating chemicals which are involved in forming and hydrolyzing ATP. It is important to understand that the pathways A, B, and C which connect ATP all strongly favour hydrolysis of ATP thermodynamically. The overall *synergetic outcome* of having multiple hydrolysis options is to increase the concentration of P_i and the hydrolyzed precursors to ATP at equilibrium.

Pathway A $\leftrightarrows$ D $\leftrightarrows$ E was analyzed in the discussion above. Would an alternative involving already condensed P_i be more feasible? No, since the reaction 2 $P_i \leftrightarrows PP_i$ also has $\Delta G \approx +29.3$ kJ, as shown in the last column of table 1;[28] the same as for addition of a P_i to a phosphate which was already bonded to an ATP precursor.[19–21,25] Furthermore, column 6 of table 1 shows that the reaction between AMP + PPi to form ATP has the most unfavourable ΔG (+46 kJ/mol) of all the equilibria options.

ATP could not have accumulated in a concentration of any relevance for OoL speculations, since it can readily hydrolyze, as could its precursors. This is elaborated on in Appendix 2, which considers the scenario of ATP forming elsewhere through unknown processes, which then migrates to the vicinity of a hydrothermal vent. The final equilibrium state is the same whether the reaction is initiated with an excess of reactants or products.

A prebiotic catalyst would not change the equilibrium constants. So the conclusion is inescapable that ATP concentrations of any relevance for OoL purposes would not have existed in the vicinity of hydrothermal vents.

Prebiotic pH gradient membranes won't form

A large difference in pH between alkaline vent fluids and acidic ocean water has been proposed to power addition of three phosphate groups to adenosine. This would have required a hypothetical membrane to separate the high from lower proton-containing water. We will now revisit this second mistake.

What would have prevented mixing of very high-pressure-and-temperature hydrothermal vent water with icy ocean water? The membrane would have been less than 1 nm thick to permit the conceptual molecular motor to traverse it. For water with a high pH difference to have formed, a *hermetical membrane* would have had to form virtually *instantaneously before any mixing occurred.*

A membrane, formed through slow precipitation in less turbulent lower temperature alkaline water, would also have provided sufficient time for the protons to mix.

The region impermeable to sea water would have had to replenish ATP, which has a half-life on the order of minutes or days, depending on the temperature. That implies that the complex adenosine molecule, which is also unstable (in addition to its precursor ribose; see figure 3), would have had to be replenished continuously. Recall that the concentration of adenosine would have had to be much higher than that of ATP due to the unfavourable equilibrium constants. A high concentration of phosphate would also have been indispensable. Clearly, these requirements would not have been fulfilled and maintained for millions of years naturally.

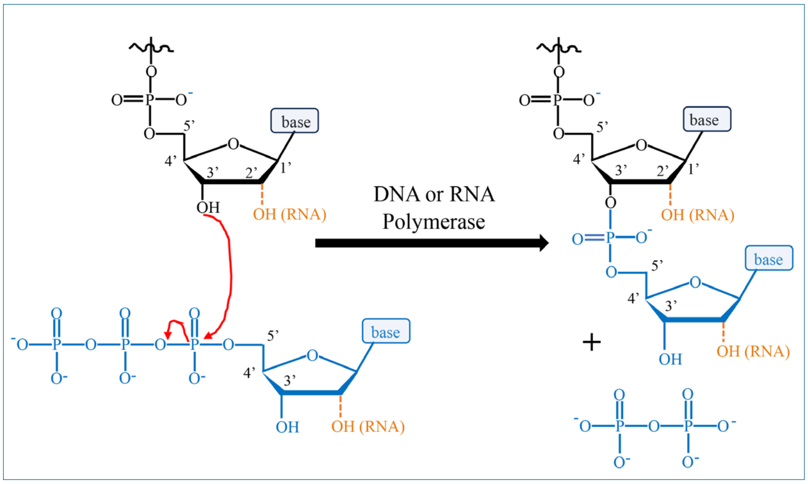

Figure 5. DNA and RNA polymerases, which consist of multiple enzymes, are indispensable to form long DNA and RNA chains. The –OH group on the 3’ carbon of ribose or deoxyribose displaces PP$_i$ in an exothermic (thermodynamically favourable) reaction.

We explored the mathematical aspects of ATP hydrolysis and formation further in Appendix 1.

Error 2—Random ATP hydrolysis won’t produce useful work

We saw, above, that hydrolysis of ATP would have prevented ATP from building up. The claim that amyloids act as ATPases (i.e., accelerators of hydrolysis) would have only made matters worse. Many substances could have catalyzed hydrolysis of ATP, but this does not make them enzymes known as ATPases!

Enzymes function by structuring reactants to achieve an energetically favourable transition site. By definition, highly customized ATPase enzymes must do much more than merely hydrolyze ATP. They must form distinct three-dimensional structures able to position ATP and reactant together to achieve the geometry of the transition state. This lowers the E_a necessary to hydrolyze a useful chemical reaction at the right time.

Motors don’t work by dousing them with a high-energy fuel and setting them on fire. Useful work is not produced by random dissipation of energy. If anything, hydrolysis of multiple ATPs might even occasionally break the amino acid bonds that form amyloids.

Error 3—Time does not automatically refine information-storing systems

We have addressed the relevant meaning of *information* in the context of biology elsewhere.[30,31] An important example of information is the language encoded in DNA which specifies the sequence of proteins, usually many thousands of them in the same organism.

Maury misused the multiple meanings of the word information to create a ‘play on words’, claiming that

> “Information transfer on the early Earth for about 4,000 million years ago occurred, according to the amyloid hypothesis, by means of a β-sheet peptide-based prion-like amyloid system in which *environmentally derived information encrypted* in the β-sheet zipper structure was *transmitted* by a templated conformational *self-replication* mechanism to ‘daughter’ amyloid entities. Recognition was mediated by amino acid side chain complementarity and *coding* by the β-sheet zipper structure [emphases added].”[2]

‘Environmentally derived information’, like any deterministic response to environmental influences (e.g., expansion of a gas upon heating), is not how instructions are ‘encrypted’ by genomes and has no relevance to the topic of biological information. The fundamental characteristic of coded information systems is that the symbols must be freely arrangeable in order to provide a variety of unrelated instructions.[32,33]

An important reason that time does not automatically generate goal-oriented information is based on the Second Law of Thermodynamics: there is a natural tendency in nature toward maximum entropy, which would corrupt the physical carriers on which information is encoded.

DNA cannot ‘self-replicate’ by physical interactions with another strand. Furthermore, this would leave unanswered where the extra identical copies, which were then moulded by the pre-existing ones, came from. This is a critical consideration that also applies to amyloids, which require a large number of identical copies to be physically templated by pre-existing ones in an alleged form of ‘self-replication’.

Different β-sheet zipper structures found in amyloids could form and unform under the influence of temperature fluctuations and hydrolysis. But this has no more relevance to how biological information is coded than random changes during the manufacture of silicon chips would determine what is encoded on them using computer programs.

Maury’s misuse of the fundamental entity of biological information allowed free reign to mental imagery by others. One explained online that Maury’s paper demonstrated that then “*refinements of the RNA and DNA information storing system is inevitable*”.[9] Reviewing the online video confirmed that coded information in the form of instructions based on nucleotide sequences was meant, and that these instructions had been self-created. No explanation was offered as to why such refinement would be inevitable. Under cold conditions, amyloids would freeze solid; under very hot conditions, they would disintegrate. This is irrelevant to refining the DNA and RNA information-storing system. Such physical-chemical behaviour does not address the source of biological information in any manner.

Error 4—Triphosphate nucleotides don't produce DNA and RNA in water

As mentioned above, bonding two P_i groups has an unfavourable $\Delta G \approx + 29.3$ kJ, and linking triphosphates would require two such reactions. An insignificant amount could have been produced. But more importantly, DNA and RNA are not produced by mixing triphosphate nucleotides, neither in free nature nor in cells. The relevant part of the reaction, which involves the 3'–OH group, is shown in figure 5 and occurs with the help of the DNA polymerase enzymes.[34] DNA and RNA are not produced in water by "stringing of ATP and different triphosphate nucleotides together".[9]

Error 5— Amyloids do not spontaneously form bilayer membranes

Research on designing organic amphipathic molecules to form liposomes, micelles, and bilayer sheets has been going on for about a century. OoL chemists use well-known principles to design long hydrocarbon chains with polar ionic ends which they know will form bilayers. The claim in OoL literature that these carefully designed laboratory experiments represent "plausible prebiotic conditions" is not reasonable, though.[35,36]

Typically, dyes are added to the surfactant solutions, and researchers show that for a few hours they are separated between the two layers. This has virtually nothing to do with any life-relevant membrane requirements, which must selectively pump the correct substance inside and selectively pump out the waste products and deleterious substances.

In the above quote, supposedly after amyloids and ATP interact, there follows:

> "… the incorporation of lipids which are again found naturally at hydrothermal vents and which spontaneously formed bilayer membranes."[9]

This is a non-sequitur. What lipids are incorporated into what? Jackson had something to say about hypothetical membranes. In his review articles, Jackson critiqued the notion that mineral membranes preceded biological membranes.[16,37] Some amyloid world hypothesis proponents have suggested that following the phase of hydrothermal vent chemistry a LUCA (Last Universal Common Ancestor) transferred to a new environment. The vague evolutionist imagery involves a LUCA-engulfed membrane that 'bubbled off'.[38] No mention is even made of an amphipathic bilayer.

Jackson explained the dilemma. The alkaline solution in a tiny bubbled-off volume would become increasingly pH-neutral as protons continued to flow in. Therefore, the ΔpH-dependent molecular machines in the new organism would have quickly fizzled out.[36]

A potential solution would have required a second, new kind of proton pump to *remove* protons to re-establish a proton gradient. To operate, it would have needed to be linked to a new energy source, such as some kind of internal inorganic redox reactions. In the immense volume of the hydrothermal vent, this second machine would not have been needed, so how and why should this now suddenly become available?[16] Natural processes know no teleology. Complex new chemical equipment does not arise naturally for the convenience of a future biological life.

Error 6—Protein enzymes performing specific functions don't automatically arise

In the lecture above, the claim, "it's only a matter of time" included several allegedly inevitable processes, including "the creation of protein enzymes to do specific functions".[9]

Protein enzymes having the necessary amino acid sequences to perform specific functions aren't created simply because amyloids are present. It is well known that the vast majority of random sequence peptides won't lead to reliably folded proteins having useful functions.[39,40]

These six are serious errors (several more will not be addressed here but some are mentioned in the endnotes).[10–12,14]

The intention here is not so much to draw attention to technical errors being disseminated[9] but to discourage ***the speculation rampant in much of OoL research****. Perhaps the origin of life can be explained by* ***experimental*** *results using simple chemicals that might have been present on the early earth under conditions that could have reasonably existed then and there. But this will only be accomplished by* ***disciplined scientific work rather than pure speculation or an appeal to the passage of time****.* Today, a great many are not convinced that a reasonable alternative to a supernatural creation has been provided.

Appendix 1. Concentration of ATP precursors when multiple equilibria exist

The equilibrium concentration of ATP was calculated above at different temperatures using equilibrium constants derived from the Gibbs free energy of the various reactions under standard conditions. A complication arises when the same chemical partakes in multiple equilibrating reactions, such as AMP in this example:

$$\textbf{Adenosine} \underset{H_2O}{\overset{P_i}{\rightleftharpoons}} \textbf{AMP} \underset{H_2O}{\overset{P_i}{\rightleftharpoons}} \textbf{ADP}$$

For the condensation of AMP and adenosine, the equilibrium constant is $[AMP]_{eq}$ / $[adenosine]_{eq}[P_i]_{eq}$, which is K_3 in eqn (12). At equilibrium

$$k_{con1}[adenosine]_{eq}[P_i]_{eq} = k_{hyd1}[AMP]_{eq} \qquad (19)$$

However, at equilibrium, AMP must also satisfy the second equilibrium step:

$$k_{con2}[AMP]_{eq}[P_i]_{eq} = k_{hyd2}[ADP]_{e} \qquad (20)$$

What is the shared $[AMP]_{eq}$ for the last two equations? This can be understood by considering how the equilibrium state was reached. Initially, some initial concentration of $[adenosine]_i$ would have been slowly consumed as it condensed with P_i to form AMP. Absent any other reactions, AMP and adenosine would have reached equilibrium concentrations, where $[adenosine]_i - [AMP]_{eq} = [adenosine]_{eq}$. Since ΔG^0 is so high, $[adenosine]_i >> [AMP]_{eq}$ and therefore to a close approximation $[adenosine]_i \approx [adenosine]_{eq}$. In addition to the thermodynamic aspects, eqn (19) shows that condensation requires adenosine to react with P_i, which has a very low concentration, whereas AMP reacts with water, which has a concentration of about 55.5 M.

As [AMP] begins to build up before equilibrium is reached, some now reacts with P_i to form ADP. Condensation is once again thermodynamically unfavourable; in addition, the reaction depends on P_i, which is found in very low concentration, compared to water for the reverse hydrolysis process (20). As a consequence, very little of the available [AMP] is consumed, and $[AMP]_{eq} >> [ADP]_{eq}$. Therefore, it is acceptable to treat this second equilibrium involvement of AMP as hardly affecting the concentration compared to what would have existed had only AMP ⇆ adenonine + P_i been present.

The slight decrease in AMP is replenished by a corresponding amount of adenosine → AMP. Ultimately, any ADP formed followed by ATP must decrease adenosine by the same amount.

In conclusion, K_3 can be used, as shown in eqn (14), to estimate the 'true' $[AMP]_{eq}$ to a very close approximation; K_2 to find $[ADP]_{eq}$, as shown in eqn (11); and K_1 to find $[ATP]_{eq}$, as shown in eqn (7).

Appendix 2. Inability of ATP to build up during exponential hydrolysis

In the main text, the prebiotic scenario to produce ATP was that it built up slowly by adding individual P_i groups. Since ATP hydrolyzes relentlessly over time, especially at high temperatures, in a relatively short time the equilibrium concentration will have been reached *no matter how it was formed*. This must be taken into account when evaluating experiments where ATP was synthesized under prebiotically irrelevant conditions. For OoL purposes, this ATP would at some point need to be in an aqueous solution to have any relevance.

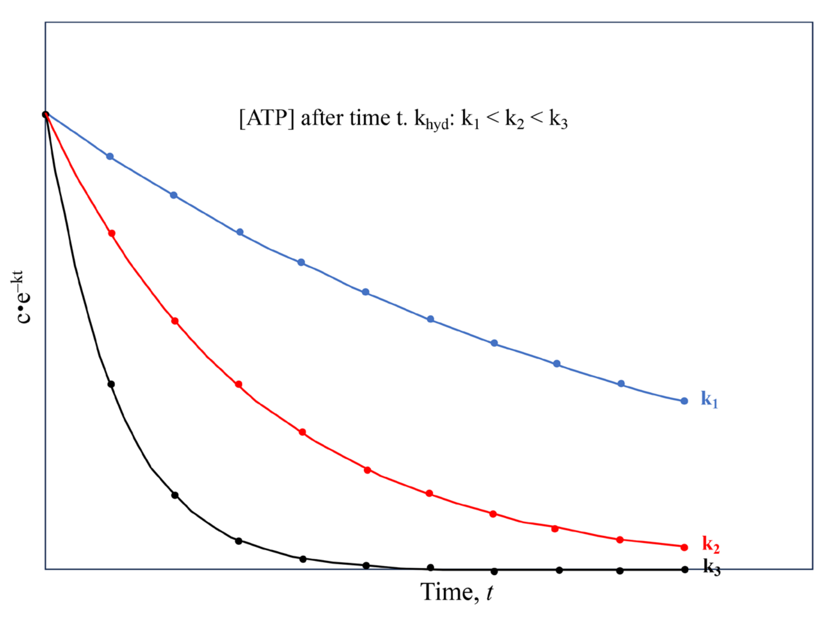

Figure 6. ATP remaining after decomposition by hydrolysis decreases at an exponential rate. Higher values for k_{hyd} result in faster disappearance of ATP. Here a constant influx of new ATP is assumed at a constant rate, *c*, and $k_1 = 0.1$; $k_2 = 0.3$; $k_3 = 0.9$ for illustration purposes.

An example is the experiments reported by Cheng *et al.,* using pre-synthesized sodium trimetaphosphate salt mixed in high concentration with adenosine and a catalyzing ion under multiple rounds of wet–dry cycles.[41]

The hydrolysis of ATP's triphosphate nucleotides is a first-order reaction when ATP is surrounded by water molecules and follows an exponential decay rate law:

$$[\mathrm{ATP}]_t = [\mathrm{ATP}]_0 e^{-kt} \qquad (21)$$

which can be expressed as

$$\ln([\mathrm{ATP}]_t \,/\, [\mathrm{ATP}]_0) = -kt \qquad (22)$$

where $[\mathrm{ATP}]_0$ is the concentration at a point in time; $[\mathrm{ATP}]_t$ is the concentration after an interval of duration *t*; and k can be solved as the slope of a linear fit of ATP concentrations taken over a range of time intervals.

Hydrolysis of ATP has been studied in unbuffered solutions of 0.1 mol/L of ATP with pH values of 3, 5, and 7.[42] Rate constants of hydrolysis were found to increase about an order of magnitude per 20°C increase in temperature. The rate constants for hydrolysis at 120°C were 4.34×10^{-3} s^{-1} at pH 3 and 2.91×10^{-3} s^{-1} at pH 7. These correspond to ATP *half-lives of only a few minutes*.

Studies of ATP hydrolysis using pure and tap water with a pH of 7 led to a half-life of 4–5 days at 24°C, and 8–10 days at 4°C.[43] Impurities accelerated the rate of hydrolysis; i.e., it was slower in pure milli-Q water.

As mentioned above, forming triphosphate nucleotides is strongly disfavoured both kinetically and thermodynamically. The Gibb's free energy, ΔG for the hydrolysis of one mole of ATP into ADP and P_i is about −30.5 kJ/mol, (−7.3 kcal/mol) under standard conditions. In addition, the energy of activation for the phosphate groups are each about E_a = 123.9 kJ/mol (29.6 kcal/mol). It is worth recalling that according to Jackson the theoretically maximum energy extractable by a *perfect pH gradient machine* would have been only 24 kJ mol^{-1}, based on an electromotive force caused by ΔpH = 4.[16]

Suppose that in a contained area around a hydrothermal vent ATP was being produced at a constant rate, *c*, per time unit. After a short interval, *t*, the amount produced would have been be *c·dt*. But simultaneously the ATP just produced would also be hydrolyzing at a rate of $(c{\cdot}dt)e^{-k\cdot t}$. The next interval of duration, *t*, a fresh amount of ATP would have formed, $(c{\cdot}dt)e^{-kt}$, during which time the formerly produced ATP would have continued to hydrolyze, with $(c{\cdot}dt)e^{-k\cdot 2t}$ of it remaining after 2 intervals of duration *dt*. This is repeated for as many dt intervals as we wish. The total amount remaining after many intervals is the area under the integral, as shown in figure 6, where the rightmost region was recently produced, and in the longer period region, the ATP which was produced long before which has not yet hydrolyzed. The integral can be expressed as:

$$\int_{t=0}^{t=t} ce^{-kt}dt = -\frac{c}{k}\, e^{-kt} \qquad (23)$$

Evaluation between t = t and t = 0, using large values of t leads to

$$-\frac{c}{k}\left(e^{-kt} - e^{0}\right) = -\frac{c}{k}(0-1) = \frac{c}{k} \qquad (24)$$

Slower hydrolysis rate constants (such as found at lower temperatures) lead to longer survival of ATP, as shown in the top curve in figure 6.

Any value for k can be used in (24), depending on the temperature, pH, etc. Assuming as an example that $t_{½}$ = 1 day leads to a steady state of [ATP] which converges to 1.4·*c*.

All three phosphate groups can hydrolyze, and the analysis here indicates that the concentration of ATP which could have accumulated would have been slightly above the constant rate of daily production of ATP. It was shown above that the addition of each of the P_i is thermodynamically unfavourable, and $[P_i]$ would have been very low. The daily rate of production of new ATP, *c·dt*, would be very small.

Should, at some point, an influx of ATP have occurred, it would have temporarily increased the concentration generated by *c·dt* during that time period. However, the exponential rate of hydrolysis would apply to the new combined concentration, reducing half of it during the same half-life as before. Concurrently, fresh ATP would be added at the rate of *c·dt*. Therefore, after a few half-lives, the effect of the original influx of ATP would have no discernible effect on [ATP].

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11. Ref. 9, starting at 13:20: "All of the building blocks that go into the formation of amyloids have all been shown to be naturally occurring". This is not true. The key building blocks were homochiral peptide templates, which do not form naturally. In addition, these were almost always built from amino acids not believed to have been available prebiotically.
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