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UNDERGRADUATE DISSERTATION

A SHORT PEPTIDE CAPABLE OF SELF-REPLICATING WHILE ADSORBED ON MINERAL SURFACES

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Literature review.

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ABSTRACT

A single small peptide (from 5 to 9 residues long) is one of the finest candidates for being the first replicator that appeared on the primitive Earth and ultimately led to the emergence of life. However, it is not recognized as so, and replication-first theories tend to automatically disregard very small peptides as possible replicators. Their small size makes them seem unable to efficiently store and replicate information, but this might just be a false impression. In this dissertation, a hypothetical self-replication mechanism that can be carried out by a small peptide is presented and it shows that only a slight selectivity and catalytic activity are needed for replication to take place, even if many errors are made along the self-replication process. The fundamental role of minerals is also underlined as they could catalyze certain chemical reactions and provide the appropriate conditions that allow this small peptide to self-replicate.

Keywords

Origin of life, Peptide, Self-replication, Replication-first, Abiogenesis.

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1. Introduction

On the search for the origin of life one of the most important challenges yet to overcome is finding the first replicator, a molecule with the ability to self-replicate and evolve in a hypothetical prebiotic scenario that can ultimately lead to the emergence of complex life forms. At the same time, this first replicator has to be simple enough to have been randomly synthesized by the previously existing chemicals.

However, even if the majority view among scientists is that the spontaneous appearance of a replicator led to the emergence of life (Dyson, 1999), it is a hypothesis that poses some serious difficulties (Shapiro, 2000; Shapiro, 2007; Kun et al., 2015) and is viewed with skepticism by a significant number of scientists. They advocate for a metabolism-first approach where "replication (and therefore a genetic apparatus) could not have arisen until more or less complicated molecules needed for the replication process had accumulated, with a metabolism based either on simple prebiotic organic molecules or on inorganic carbon sources such as CO2" (Anet, 2004). These metabolism-first approaches are not endorsed in this dissertation because very consistent arguments against them exist (Anet, 2004; Orgel, 2008; Vasas et al., 2010).

On the other hand, there are many experimental efforts that demonstrate the plausibility of a replication-first scenario: several self-replicating nucleic acids (Robertson and Joyce, 2014), nucleic acid analogues (Plöger and von Kiedrowski, 2014), peptides (Lee et al., 1996) and other kinds of molecules (Clixby and Twyman, 2016) have been observed and synthesized in the recent years. Unfortunately, none of them has been considered by the scientific community to be a firm candidate that can explain the rise of life on Earth. This lack of consensus exists due to the fact that open-ended evolution has never been accomplished by these self-replicating systems (Duim and Otto, 2017). The concept of open-ended evolution is explained in Duim and Otto's paper and it refers to a chemical system that "can diversify and increase in complexity and invent new functions indefinitely".

So, what should be done to accelerate the process of finding this first replicator? Extensive empirical work, although expensive, is indispensable in a field where possibilities are infinite and experimental efforts scarce. Worse still, the few experimental efforts that

exist suffer from a too-rational approach as they only test small groups of molecules and often aim for the synthesis of replicators with limited evolvability. Due to these circumstances the main scope of this dissertation is to encourage big experimental screening arrays for the identification of self-replicating molecules, specifically oligopeptides. It is proposed that oligopeptides should be studied because they are excellent candidates for being the first replicator: the simplicity and the great variety of amino acids that exists means that oligopeptides are far more versatile than other oligomers (e.g. oligoribonucleotides). On top of that, thanks to their small size, their chance of being randomly synthesized is high, they need less resources to replicate, they have a lower error threshold of replication (Eigen, 1971; Biebricher and Eigen, 2005) and a lower chance of being hydrolyzed, oxidized or degraded by other means.

The only downside of such tiny molecules is that it is impossible to imagine them self-replicating by themselves in a dilute aqueous solution. If we were to think, for example, about a dissolved heptapeptide, we would suddenly realize that it would just start "floating around", rapidly and constantly interchanging between an "enormous number of quasi-isoenergetic conformations" (Toniolo and Temussi, 2016). Thus, it could be easily deduced that, if there is any chance for the heptapeptide to self-replicate it must be while it is adsorbed on a certain solid surface which allows it to stabilize specific conformations (Bertrand and Brack, 2000) or even adopt new ones (Sivasankar and Vasudevan, 2004). Natural occurring minerals, a central part of numerous abiogenesis theories (Hazen, 2005), would be this solid surface we are talking about, and their function might not be limited to just stabilizing a specific conformation. They can also promote the concentration of chemical compounds and orientate the molecules that are adsorbed onto them, changing the reactivity and selectivity of the organic reactions that occur between these molecules (Westall et al., 2018). Therefore, all of these events combined might have allowed a small peptide to catalyze its own synthesis finally resulting in the rise of life on Earth.

2. Objectives

The main scope of this dissertation is to encourage big experimental screening arrays for the identification of self-replicating molecules, specifically oligopeptides. This is done by reviewing the current literature about the origin of life to justify the importance that is given to small peptides and minerals and by presenting a hypothetical mechanism by which they would be able to replicate.

3. Methodology

The research in this dissertation was conducted using mostly two databases: Scopus and Google scholar. Web of Science was not used because of its poor search results in the origin of life field when compared to Scopus. Keywords such as “peptide”, “replication-first”, “origin of life”, “abiogenesis” and many more were searched for in both databases in order to take advantage of their strengths (Falagas et al., 2007) and avoid biased search results.

From these search results the most relevant papers, books and PhD theses were chosen, with special attention being placed in the prestige of their authors and publishers. Furthermore, Scopus’ “View all citing documents” tool and Google Scholar’s “Cited by” tool were used when possible to check the state of the art of the chosen documents.

Finally, all these documents were downloaded and classified using Mendeley, a reference management software. Only one of them, the book *Chemical Evolution* (Calvin, 1969), was not available in digital format, so a written copy was borrowed from the US library CRAI Antonio de Ulloa.

The original figures were drawn with ChemDraw (version 16) and with Adobe Photoshop CS6 software. For the figures that were taken from other sources and required it, copyright permission was obtained by using the Copyright Clearance Center’s web page: https://s100.copyright.com.

4. Results and discussion

4.1 The RNA world and the role of peptides

Initially, when the most famous hypothesis about the origin of life, the RNA-world, was given its name (Gilbert, 1986) and probably due to the enthusiasm generated by the recent discovery of the existence of ribozymes (Kruger et al., 1982), proteins and peptides were forgotten for a moment and an RNA molecule was quickly proposed to be the origin of this RNA-world. Gilbert himself, in the same paper where the RNA-world term is used for the first time, states: “The first stage of evolution proceeds, then, by RNA molecules performing the catalytic activities necessary to assemble themselves from a nucleotide soup”. Nevertheless, because of the problems that the *de novo* appearance of an RNA molecule on the prebiotic Earth poses (Bernhardt, 2012), the idea of an RNA-world became more flexible with common use. Its actual meaning was described by Orgel and Joyce (Joyce and Orgel, 1993) (the more recent edition of the article, written by Robertson and Joyce, is cited here):

“The RNA World means different things to different investigators, so it would be futile to attempt a restrictive definition. All RNA World hypotheses include three basic assumptions: (1) At some time in the evolution of life, genetic continuity was assured by the replication of RNA; (2) Watson-Crick base-pairing was the key to replication; (3) genetically encoded proteins were not involved as catalysts”. (Robertson and Joyce, 2012).

That means that any self-replicating molecule/system could have also preceded it: artificial genetic systems similar to DNA/RNA such as peptide nucleic acids, threose nucleic acids, glycerol-derived-nucleic acid analogues and pyranosyl-RNA (Joyce, 2002), compositional genomes (Segre et al., 2000), small molecules (Clixby and Twyman, 2016), the famous self-replicating clays (Cairns-Smith, 1982) and of course proteins/peptides.

Protein first approaches are way older than the concept of the RNA world, and they have been present since the beginning (Oparin, 1924; Haldane, 1929; Bernal, 1947) of the modern discussion about the origin of life. As time went by, the vague references about

the structure and properties of these proteins became more and more accurate, eventually leading to a wide variety of hypotheses and experiments which also include peptides and proteinoids.

Proteinoids (Fox and Harada, 1958) are aggregates formed by heating a mixture of amino acids. They are not actually proteins because the bonds between the amino acids are not just peptide bonds; a high degree of cross-linking exists. They are easily formed in prebiotic conditions and they have demonstrated a certain degree of catalytic activity (Quirk, 2009), but probably because of their chaotic structure and their difficulties for self-replicating they are not a hot topic in the debate on the origin of life.

In contrast, peptides have received much more attention. The first self-replicating peptide ever observed (Lee et al., 1996) was artificially made (to date, no naturally occurring self-replicating peptide is known). It consisted of an α-helix made of 32 amino acids which was able to catalyze its own synthesis by promoting the formation of a peptide bond between two previously activated peptides of 15 and 17 amino acids. This was possible because the α-helix acted as a template and stabilized the two peptides next to each other. When they reacted, the newly formed α-helix, identical to its "mother", was set free and prepared for carrying out a new reaction (Figure 1).

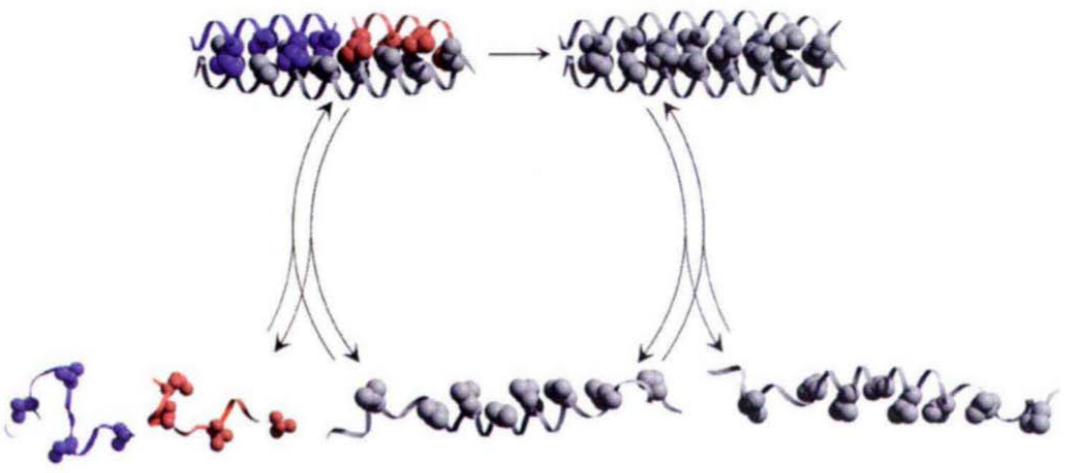

Figure 1. Illustration of the self-replicating α-helix described above. Reprinted by permission from Springer Customer Service Centre GmbH: (Lee et al., 1996), Copyright © 1996, Springer Nature.

A similar type of self-replication was developed later using shorter peptides, of 12 residues, that formed β-sheets instead of an α-helix (Takahashi and Mihara, 2004; Rubinov et al., 2009). Their amphiphilic structure made them to join together in β-sheet aggregates. The authors hypothesized that, in the edges of the β-sheet or in areas of imperfect packing, the self-replicating peptide could couple with two smaller peptides and catalyze the formation of the peptide bond (Figure 2).

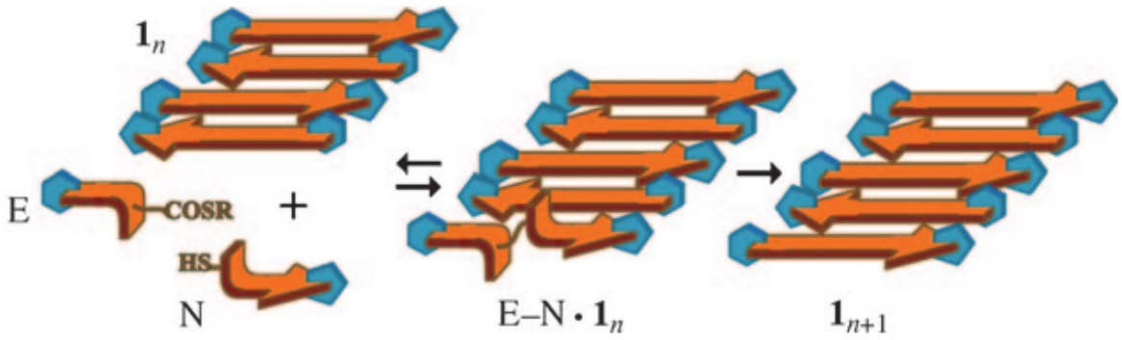

Figure 2. The antiparallel β-sheet 1n serves as a template for the ligation of peptides E and N. As a result the β-sheet 1n+1 is formed, ready to catalyze again the ligation between E and N. Reprinted by permission from John Wiley and Sons: (Rubinov et al., 2009), Copyright © 2009, John Wiley & Sons, Inc.

The experiments described above provide us with interesting insights about the origins of life. That said, we are still a long way off a convincing answer. An ideal first replicator must be capable of undergoing open-ended evolution and, preferably, should be able to catalyze its own synthesis from small and common building blocks (amino acids or dipeptides, if we are talking about a small self-replicating peptide). That is not the case though in the experiments described above, where the replicator is only able to use a very specific kind of large and complex building blocks for creating more copies of itself.

Then, why is not a simple self-replicating peptide capable of using small and simple building blocks being designed? Well, that would be the answer to all of our problems, but it has been proven to be very difficult because self-replicating peptides that use large and complex building blocks is all that has been accomplished by following a rational molecular design process. So, maybe we should contemplate a less-rational approach: for example, screening large arrays of very small peptides might help us to find these simple

self-replicating systems that can explain the origin of life.

Only metabolism-first approaches, such as Kauffman's work on autocatalytic sets of proteins, have studied this possibility and suggested that small peptides can self-organize in autocatalytic cycles and that these cycles might be able to self-replicate (Kauffman, 1986). However, a cooperative network of different types of peptides is needed for replication to occur and none of the peptides alone are able to synthesize copies of themselves, so it greatly differs from our proposal of one unique peptide being able to self-replicate. Besides that, these kind of approaches have received some strong criticism (Anet, 2004; Orgel, 2008).

Apart from metabolism-first approaches, the short peptides we propose that should be studied (from 5 to 9 residues long) have always been assumed to be unable to self-replicate by themselves. A clear example of the "discrimination" that small peptides suffer in replication-first hypotheses can be found while reading Lambert's review about the adsorption and polymerization of amino acids on mineral surfaces (Lambert, 2008). When he writes about the estimates that have been made regarding the length of a hypothetical self-replicating protein/peptide, he cites two papers to give us a general picture:

"Widely divergent estimates have been put forward, depending on the initial assumptions: thus, Hoyle and Wickramasinghe (quoted in Shapiro 1986) required the random assembly of a set of 2,000 proteins having an average length of 200 AAs, comparable in complexity to a small bacterium (…).
At the other extreme, Trifonov (2006) supposed that the formation of a peptide as small as 20 AAs was enough to start chemical evolution and went on to calculate that "a small truckload of chemicals" (370 kg of an amino acids mixture) was enough to achieve this".

If a 20 residues long peptide being able to start chemical evolution is viewed as an "extreme" estimate, as the shortest peptide that could ever be imagined accomplishing this task, then peptides made of only 5-9 residues are completely dismissed and they should not even be thought about. And sadly, that is what has been done so far. However, is there a solid scientific basis behind these estimates, or are they just comfortable limits that we have arbitrarily drawn? Unfortunately, the latter seems to be the right answer.

The only major objection against 5-9 residues long peptides being the first replicator is that there are no hints about how they could self-replicate by using simple building blocks; there are no known natural or artificial systems which behave in a similar way. However, that is not a good enough reason for casting them aside because they are very attractive for fulfilling the role of a first replicator and there might exist a plausible mechanism that allows them to self-replicate.

4.2 A hypothetical mechanism for peptide self-replication

To find such a mechanism we have to go back to 1969, when Melvin Calvin imagined in his book *Chemical Evolution* a peptide (P1) that is able to catalyze the synthesis of a specific polypeptide (P2), without a template. This is possible by following two "simple" steps: (1) P1 consecutively catalyzes the condensation of free amino acids (or dipeptides, tripeptides, etc., it is not specified) in one terminus or residue. (2) P1 cleaves from itself (with the help of a metal complex that allows for a stereospecific and amino acid specific peptide bond hydrolysis) the new polypeptide that is formed, and is available to start again with the first step (Calvin, 1969).

It must be pointed out that P1 is able to specify the correct amino acid that needs to be condensed so, instead of a random peptide, P2 is formed. This is what Calvin calls "growing-end control", and he cites two examples where it has been documented, one "artificial" and the other "natural". The artificial one can be found in a paper written by Steinman and Cole, where they explain that "with all other factors being equal, the probability of union of individual amino acids with one another is determined by the size of the side chains" (Steinman and Cole 1967). That means that the reaction of different amino acids in aqueous solution is not random, it has a certain degree of selectivity (for example, even at the same relative abundance, it is much more probable for glycine to react with alanine than with phenylalanine).

The natural example is the bacterial synthesis of the pentapeptide (UDP-GNAc-Lactic-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala) (Ito and Strominger, 1960), that does not need a templating mechanism. It is an example of "growing-end control" because the amino acid

that needs to be added to the growing peptide is chosen depending on the residue in the end. The enzyme that catalyzes this reaction will only attach D-glutamic acid to the L-alanine residue, then it will only attach L-lysine to the D-glutamic acid residue, etc. Nowadays hundreds of enzymes like this have been identified: they belong to the large family of non-ribosomal peptide synthetases (Süssmuth and Mainz, 2017). A more recent discussion about their implications in the role of the origin of life can be found in this review (Poole, 2011).

However, this bacterial synthesis only serves as the proof that growing-end control can be extremely precise. We cannot forget that the hypothetical method for producing a specific peptide that we are looking for must occur in a prebiotic scenario, so there is no place for something as complex as an enzyme. Calvin himself acknowledges that a non-enzymatic growing-end control mechanism based only in the partial selectivity intrinsic to the condensation reaction of the different amino acids would not be very accurate. To that end he proposed other theoretical methods to overcome this problem (Bjornson, 1970).

These theoretical methods are not useful for us because what we want to do is to adapt the mechanism imagined by Calvin to a small self-replicating peptide. To do this, it could be theorized that: (1) the sequence of P1 can be functional while being 5-9 amino acid long, and (2) the sequence of P2 is the same as the sequence of P1 (Figure 3). In this scenario accuracy is not a big problem, because as long as P1 produces two copies of itself during its whole existence its net-replication rate will be positive and exponential growth will occur.

To illustrate how a small self-replicating peptide like the one in Figure 3 could function, even if a great number of errors are made along the replicating process, we can perform a small thought experiment. Imagine a prebiotic 7-residue long peptide which is able to catalyze, as explained above, the condensation of free amino acids into one of its terminus and then cleave the newly formed 7-residue long peptide. Then imagine that the only free amino acids available are those that could have existed in the prebiotic Earth (there is no consensus about which were these amino acids (Zaia et al., 2008), but Longo and Blaber have proposed a prebiotic set of 10 α-amino acids (Longo and Blaber, 2012), a number that we can arbitrarily raise to 12 for our calculations, owing to the lack of consensus).

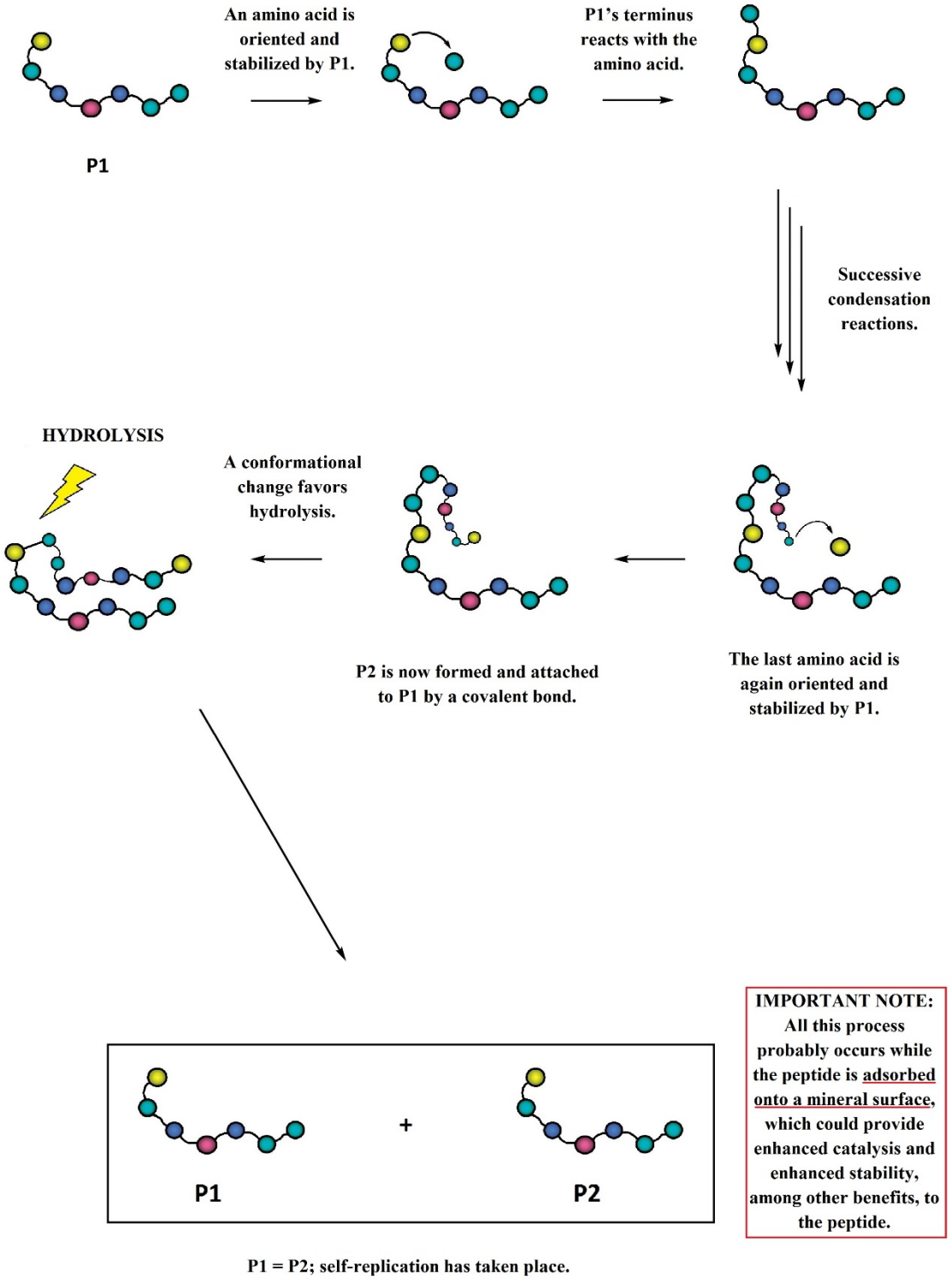

Figure 3. A hypothetical mechanism by which small peptides might be able to self-replicate. Each colored ball represents an amino acid.

The number of possible combinations for this 7 amino acid long peptide that can be made of 12 different amino acids is $12^7 = 3.58 \cdot 10^7$. If the half-life of the peptide bond ranges between 350 and 600 years at 25 ºC and neutral pH (Radzicka and Wolfenden, 1996), then the half-life of a peptide made of 7 amino acids (and thus 6 peptide bonds) should be:

$$Peptide\ half-life: \frac{350\ years \cdot 1\ peptide\ bond}{6\ peptide\ bonds} = 58\ years$$

However, it turns out that for small peptides with a free N-terminus the main cause of their degradation is not peptide bond hydrolysis, but a much faster reaction: the successive formation of diketopiperazines in their N-terminus (Figure 4) (Steinberg and Bada, 1983). The half-life of this reaction can be calculated from its estimated rate constant at 25 ºC ($8 \cdot 10^{-8}\ s^{-1}$) (Radzicka and Wolfenden, 1996):

$$Half-life: \frac{\ln\ (2)}{8 \cdot 10^{-8}\ s^{-1}} = 8.66 \cdot 10^6\ seconds;$$

$$; 8.66 \cdot 10^6\ seconds \cdot \frac{1\ minute}{60\ seconds} \cdot \frac{1\ hour}{60\ minutes} \cdot \frac{1\ day}{24\ hours} = 100\ days$$

When a diketopiperazine is formed two residues are cleaved from the N-terminus, so according to these calculations a 7 amino acid long peptide would be degraded into a 5 amino acid long peptide in "only" 100 days. Fortunately, thanks to the self-replication process, our peptide is constantly growing on its N-terminus so its half-life would be much longer. In addition, a very simple and plausible mechanism that makes it very resistant (more than 10 years) to decomposition by formation of diketopiperazines might exist. This protective mechanism will be explained in the section **"Possible solutions to two challenging fields: thermodynamics and diketopiperazine formation".**

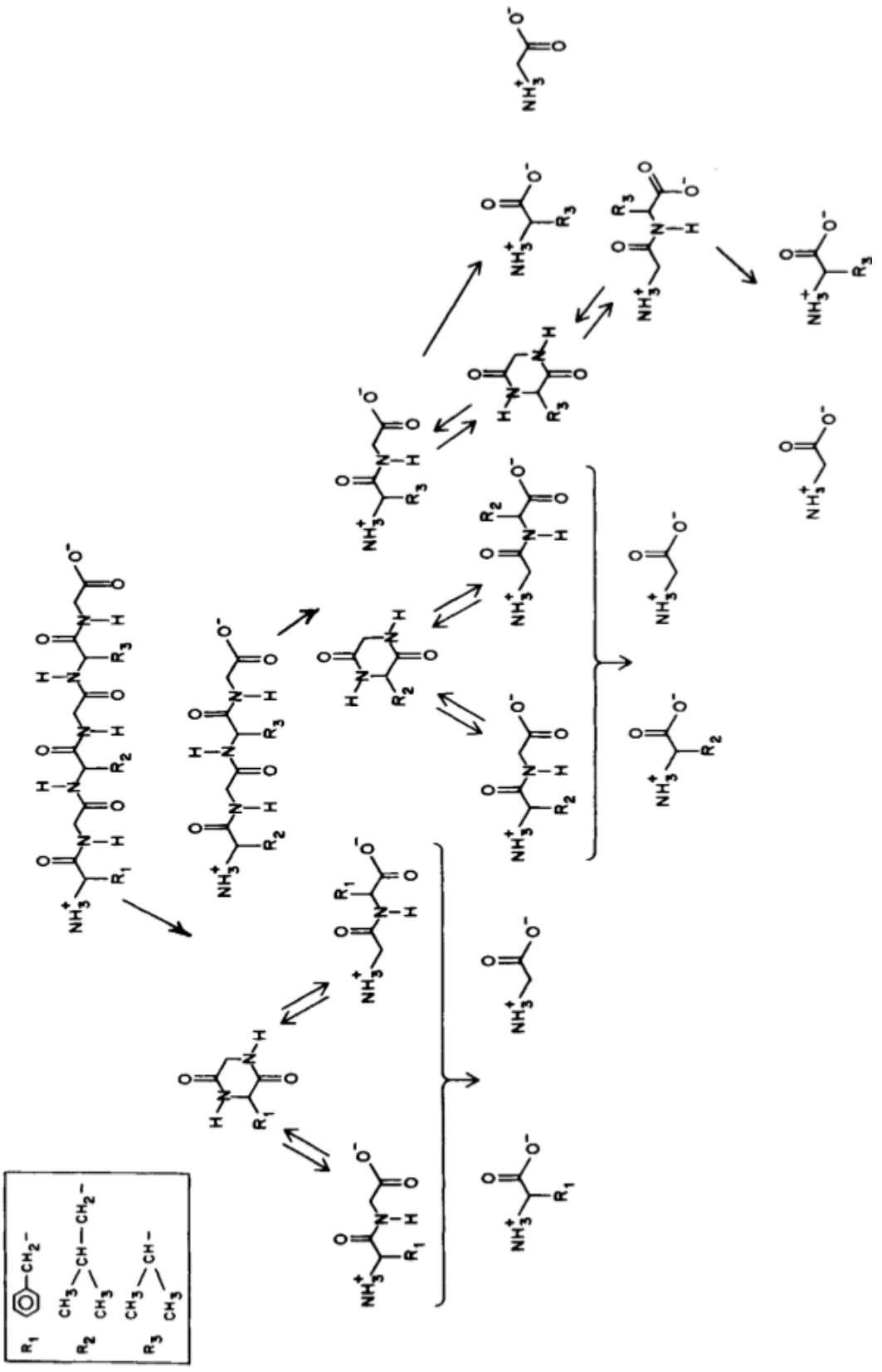

Figure 4. Hydrolysis due to diketopiperazine formation of the hexapeptide Phe-Gly-Leu-Gly-Val-Gly at pH 7.26 and 100ºC. Reprinted by permission from (Steinberg and Bada, 1983), Copyright © 1983, American Chemical Society.

From now on it will be assumed that the half-life of the self-replicating peptide is 10 years. Remember that it is a 7 amino acid long peptide that can be made of 12 different amino acids ($12^7 = 3.58 \cdot 10^7$), thus, if the addition of each amino acid is random and the concentration of each amino acid is the same, it would need to produce $3.58 \cdot 10^7$ peptides in 10 years to generate its own sequence (replicate) only once. These conditions have not been observed in experiments that simulate the primitive Earth environment, since Gly and Ala seem to give a much higher yield (even from different simulated sources of amino acids, such as hydrothermal vents and UV radiation) than the rest of amino acids (Zaia et al., 2008). It could be easily imagined then, that 3 out of the 7 residues of our self-replicating peptide are Gly residues. Also, because of the higher concentration of Gly in the solution, it would have a higher chance of being condensed into the growing peptide than the other free amino acids. For simplicity, it could be stated that Gly has a 50% chance (0.5) of being added to the new peptide, and the 50% left belongs to the other 11 amino acids (0.5/11) (the number 22 is used in the denominator so the numerator will be a whole number):

$$Odds\ for\ Gly\ being\ added\ to\ the\ new\ peptide = 0.5 = \frac{11}{22}$$

$$Odds\ for\ the\ other\ 11\ amino\ acids\ of\ being\ added\ to\ the\ new\ peptide = \frac{0.5}{11} = \frac{1}{22}$$

$$Odds\ of\ the\ peptide\ being\ replicated = \frac{11}{22} \cdot \frac{11}{22} \cdot \frac{11}{22} \cdot \frac{1}{22} \cdot \frac{1}{22} \cdot \frac{1}{22} \cdot \frac{1}{22} = \frac{1}{1.87 \cdot 10^6}$$

That means that out of every $1.87 \cdot 10^6$ peptides synthesized our peptide will create a copy of itself. Until now that is the result that would be obtained if the effect of concentration was taken into account and the free amino acids randomly reacted with our peptide. It can, however, get even lower if we theorize that our peptide has a low selectivity for adding the correct amino acid (besides the intrinsic partial selectivity of the condensation reaction between amino acids (Steinman and Cole, 1967; Weber and Orgel, 1979) which, for simplicity, will not be considered). To represent this low selectivity we have arbitrarily raised the chance of adding the correct amino acid from 1/22 to 6/22, while leaving intact the 11/22 chance of adding a correct Gly residue:

$$Odds\ of\ the\ peptide\ being\ replicated = \frac{11}{22} \cdot \frac{11}{22} \cdot \frac{11}{22} \cdot \frac{6}{22} \cdot \frac{6}{22} \cdot \frac{6}{22} \cdot \frac{6}{22} = \frac{1}{1446}$$

Therefore, by accounting for concentration and selectivity effects the number of one replication every $3.58 \cdot 10^7$ peptides produced has been lowered to one replication every 1446 peptides produced (more favorable and yet realistic scenarios can be imagined where this number is even lower). This means if we hypothesize that our peptide, which has a half-life of 10 years (3650 days), could produce one peptide per day, it would replicate once every 1446 days; producing more than two copies of itself before its degradation and thus beginning a process of exponential self-replication.

But is the production of one peptide per day a reasonable rate? Well, this implies the formation of 7 peptide bonds and the hydrolysis of 1 to separate the newly formed peptide from its maker. It could therefore be said that peptide bond formation, knowing the estimate of its uncatalyzed rate constant at 25 ºC (~ $3 \cdot 10^{-4}$ M^{-1} s^{-1}) (Weber and Orgel, 1979), would be fast enough, even with a low amino acid concentration, to allow the formation of 7 peptide bonds per day. On the other hand, the hydrolysis of 1 peptide bond would be too slow, as can be deduced from its low uncatalyzed rate constant at 25 ºC (~ $6.3 \cdot 10^{-11}$ s^{-1}) (half life = ~ 350 years) (Radzicka and Wolfenden, 1996). Fortunately, many well-documented metal catalysts which can raise the rate constant up to $6.4 \cdot 10^{-5}$ s^{-1} (half-life = 3 hours) at 50 ºC exist for this reaction (Wezynfeld et al., 2016). From this it could be deduced that one peptide per day is a reasonable rate.

However, it would be pointless spending time considering the rate of peptide production per day because at this point a lot has already been speculated. The range of possible variables in the prebiotic Earth are enormous, and the results can differ depending on which variables are taken into account. These calculations were only made to show that, for small oligomers, very little selectivity is required for producing a specific sequence, even if a huge amount of errors are made along the way. This is not an easy-to-realize idea because the number of errors *seems* very high for self-replication (and information transfer, and evolution) to occur, but this is not true.

It also needs to be noted that currently, there are no known small peptides which are able to catalyze the peptide bond formation/hydrolysis reaction. The peptide Ser-His was believed for a few years to be capable of carrying out this task (Li et al., 2000), but a recent article has reevaluated its presumed catalytic activity and concluded that "previously reported results cannot be interpreted as evidence that Ser-His or any other short His- containing peptide is competent to catalyze amide hydrolysis" (Macdonald et al., 2016). We acknowledge that the catalysis of this reaction by 5 to 9 amino acid long peptides in solution is not a very likely event, and that is the exact reason that we emphasize the importance of the role of minerals so much. It is very difficult for a small peptide alone to catalyze any kind of reaction because they lack the preorganized environment found in enzymes which is responsible for the major part of their catalytic power:

"In water the free energy of moving from the ground state to the transition state is larger than in the enzyme since the water dipoles have to reorganize and pay for the reorganization energy, whereas in the enzyme the polar groups or the catalytic groups are preorganized in the correct direction and thus do not have to pay large preorganization energy. The difference between the large reorganization energy in water and the small reorganization of the protein groups is the reason for catalysis" (Jindal and Warshel, 2017).

Organization of water molecules is also commonly seen on mineral surfaces (Perry IV et al., 2007; Khatib et al., 2016), where they have a reduced orientational and translational entropy (Wang et al., 2006). This could mimic, in a basic way, the preorganized environment found in enzymes and allow a small peptide to catalyze (at least at a low rate) the peptide bond formation reaction. It does not stop here; there are many different ways in which minerals could help small peptides to self-replicate, but they will be discussed later.

4.3 Why small peptides and minerals?

The mechanism of self-replication described above (Figure 3) could also be, theoretically, carried out by an oligoribonucleotide (also known as a small RNA strand) instead of an oligopeptide.

The preference for small peptides rather than for oligoribonucleotides lies mostly in structural reasons. Because of their simplicity, amino acids are very easy to synthesize from inorganic compounds, so there are a great deal of very plausible ways in which they could have been formed on the prebiotic Earth: by electric discharges, as shown by the Miller experiment (Miller, 1953) and more modern experiments that were carried out after our understanding of the composition of the early atmosphere changed (Plankensteiner et al., 2006; Cleaves et al., 2008), by proton irradiation (Kobayashi et al., 1990), in submarine hydrothermal vents (Marshall, 1994), by the Strecker reaction (Strecker, 1850) and many more reviewed in (Brack, 2007; Ruiz-Mirazo et al., 2014).

However there is also more direct evidence that amino acids were present in the prebiotic Earth. They have been found in various meteorites (Cobb and Pudritz, 2014), specifically carbonaceous chondrites, a type of stony meteorites formed in the early Solar System which have a well-preserved composition. The Murchinson meteorite is the most studied of this group and it has been shown to contain a great variety of amino acids (Figure 5).

Therefore it is accepted that, in one way or another, amino acids were widely available in the prebiotic Earth. On top of that, since they are the building blocks that peptides are made of, the synthesis of peptides was also possible. There are numerous processes by which this synthesis could have been carried out:

“One may cite salt-induced peptide formation (SIPF) (Rode et al. 1991; Eder and Rode 1994;Rode 1999), hydrothermal synthesis (Imai et al. 1999), impact polymerization (Lyons and Vasavada 1999; Blank et al. 2001), polymerization by coupling to metaphosphate hydrolysis (Yamagata and Inomata 1998), and polymerization in the adsorbed phase (...)—several solutions may be combined in one scenario, e.g. surface polymerization+SIPF (Le Son et al. 1998)”. (Lambert, 2008).

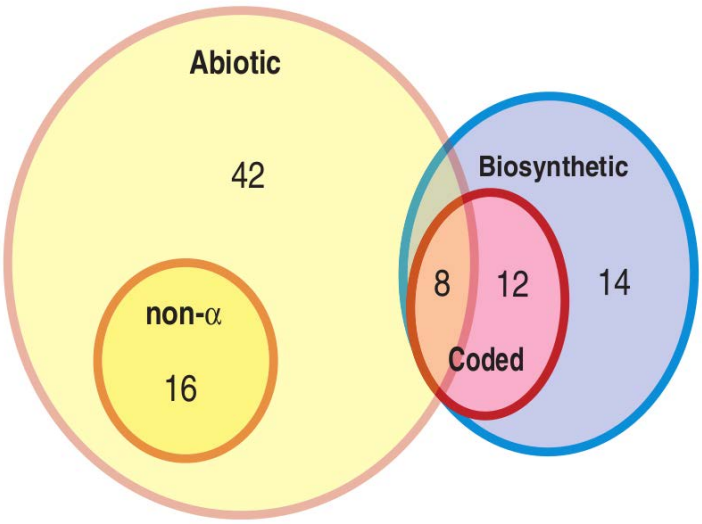

Figure 5. "Venn diagram showing the number of amino acids represented in different categories of chemical space. Abiotic refers to the 66 amino acids reported in the Murchison meteorite (including 8 members of the standard alphabet). Non-a refers to the 16 amino acids reported from the Murchison meteorite that have longer carbon ''backbones'' than those used in genetic coding. Coded refers to the 20 amino acids used within the standard genetic code. Biosynthetic refers to the additional 12 members of the standard alphabet and a further 14 amino acids that are produced as intermediates in their production". (Philip and Freeland 2011). Color images available online at www.liebertonline.com/ast.

Even though the real importance that each process had is difficult to infer (mainly because of the many uncertainties about the prebiotic Earth's nature), it is reasonable to believe that small peptides were produced on a regular basis by at least one of them. The rate of formation of each peptide and the maximum length that could be obtained with each process is also a difficult guess, but linear sequences made of up to 8 amino acids could certainly be formed (Gulik et al., 2009).

On the other hand, because of their more complex structure, the prebiotic synthesis of oligoribonucleotides is much less plausible than the prebiotic synthesis of peptides. Its low feasibility has even earned it the name of "The Prebiotic Chemist's Nightmare", and it is believed by many scientists that "the *de novo* appearance of oligonucleotides on the primitive earth would have been a near miracle" (Joyce and Orgel, 1993). In addition, nucleosides are less versatile than amino acids as basic building blocks because of their bigger size and less variable structure (Figure 6). We should not forget that the molecule

that gave rise to life on Earth is a minimal self-replicating molecular machine that was randomly built from scratch, so chances are that it was built with the most simple and versatile building blocks (amino acids).

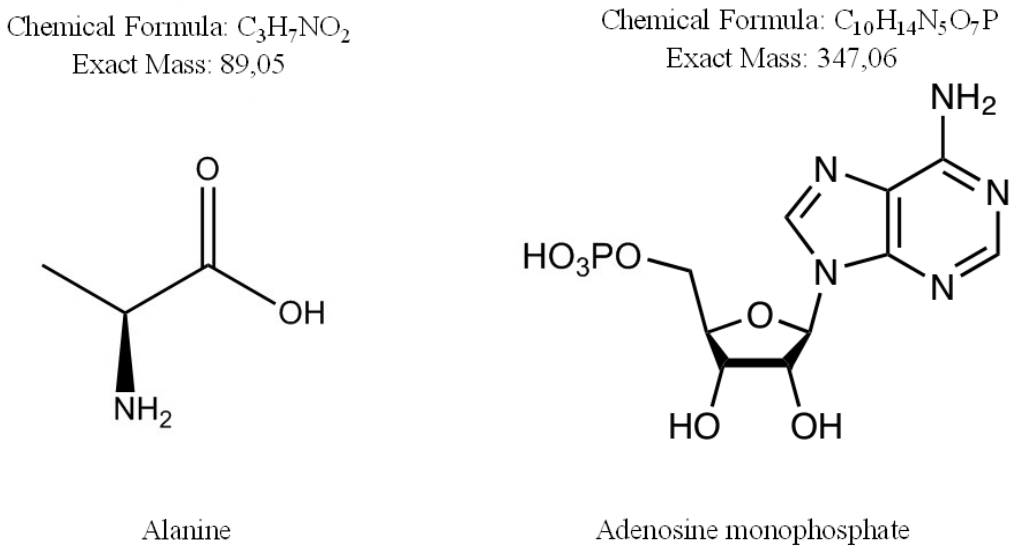

Figure 6. The structure of an amino acid (alanine), the monomer for peptides, compared to a nucleoside monophosphate (adenosine monophosphate), the monomer for oligoribonucleotides. Notice the difference in size and number of chemical bonds. The exact masses in the figure were calculated by ChemDraw 16 and are expressed in daltons.

In any case, even if a peptides-first scenario for the origin of life is defended in this dissertation, the arguments in favor of this scenario are far from conclusive, and the debate about the nature of the first replicator is (and will be, probably for a long time) still open. What is not open to debate, though, is the important role of minerals in the origin of life, especially if our hypothesis is that peptides as short as 5 to 9 amino acids long could have been this first replicator. Some of the most significant functions that they could have had are listed below:

-Preorganization of water molecules: as explained earlier, “in water the free energy of moving from the ground state to the transition state is larger than in the enzyme since the water dipoles have to reorganize“ (Jindal and Warshel, 2017). That is the main reason why it is so difficult for small peptides to act as catalysts; however, some minerals might be able to organize water molecules on their surfaces in a fashion that helps small peptides

to act as catalysts.

-Polymerization During Adsorption (Lambert, 2008): peptide bond formation is a condensation reaction, which requires the elimination of a water molecule. In aqueous solution and standard conditions the Gibbs energy is positive for this reaction (Stojanoski and Zdravkovski, 1993; Bruce Martin, 1998), so hydrolysis is favored over the formation of the peptide bond. This problem is of great importance, because a self-replicating peptide cannot create new copies of itself if the thermodynamics of the reaction do not favor the formation of new peptide bonds. Here is where minerals can come into play: if the free energy of adsorption of the product (a peptide) onto the mineral is more negative than that of the reactants (two amino acids, or a peptide and an amino acid, or two peptides) it can change the sign of the overall value of the free energy of the reaction and favor peptide bond formation over hydrolysis.

-Thermal and chemical stability: minerals can protect biomolecules from being degraded at high temperatures, by reaction with other chemical species (Ricardo et al., 2004; Hazen and Sverjensky, 2010) or by radiation (Bernal, 1949).

-Catalysis: minerals can catalyze different chemical reactions (Basiuk and Sainz-Rojas, 2001; Cleaves et al., 2012). They can also adsorb metal ions (Gupta and Bhattacharyya, 2011), which are able to act as catalysts too (Belmonte and Mansy, 2016).

-Concentration (Westall et al., 2018) and selection of prebiotic chemicals: “selection” of certain chemical species means that some are more strongly adsorbed than others onto the mineral surface. This effect has been observed not only in nucleobases (Sowerby et al., 2001), but also in chiral molecules such as D- and L-amino acids (Hazen et al., 2001). This enantioselective adsorption might even explain the origin of biochemical homochirality.

4.4 The two greatest adversaries of small self-replicating peptides: thermodynamics of peptide bond formation and diketopiperazine formation.

It is obvious that any self-replicating peptide, regardless of its size, must be able to create new peptide bonds. However, before doing this a major problem needs to be solved: the unfavorable thermodynamics of peptide bond formation. As we have already explained, in aqueous solution and standard conditions the Gibbs energy is positive for this reaction (Stojanoski and Zdravkovski, 1993; Bruce Martin, 1998), so hydrolysis is favored over the formation of the peptide bond. Then, for a self-replicating peptide to function properly, the right thermodynamic conditions for peptide bond formation need to be procured.

The most popular way to do this is by activating the carboxylic acids of the free amino acids or peptides by using coupling agents that decrease the Gibbs energy of the reaction of peptide bond formation. Their popularity comes from their widespread use in organic chemistry; one can easily find dozens of reviews about the numerous coupling agents that are used to promote amide bond formation (to cite a few: (Valeur and Bradley, 2009; El-Faham, 2011; Dunetz et al., 2016)). Regarding the topic of the origin of life, prebiotically relevant coupling agents are reviewed here (Danger et al., 2012). It is also interesting to note that all the artificially made self-replicating peptides that were previously mentioned in this dissertation (Lee et al., 1996; Takahashi and Mihara, 2004; Rubinov et al., 2009) use the native chemical ligation method to synthesize new peptide bonds (Dawson et al., 1994), where the terminal carboxylic acid of a peptide is activated by transforming it into a thioester.

But peptide bond formation can also be promoted without using coupling agents. Increasing the temperature tends to favor peptide bond formation (Shock, 1992), and there are some reports about the formation of peptides from unactivated amino acids in the presence of minerals and at temperatures as low as 50 – 55 ºC (Basiuk and Sainz-Rojas, 2001; Iqubal et al., 2017). Wetting-drying cycles, a process where, continuously, water is evaporated and then the remaining solution is rehydrated again (Erastova et al., 2017), and polymerization during adsorption (already explained in page 18) can also favor peptide bond formation.

Hence, the first problem is solved: some prebiotic coupling agents that activated the free amino acids in the primitive Earth, or maybe certain conditions such as an increased temperature, might have provided the right thermodynamic conditions that allowed a self-replicating peptide to catalyze its own formation. Yet, even if this peptide was able to self-replicate, there is a relatively fast degradation process that could threaten its existence: diketopiperazine formation.

As we have explained earlier, a nucleophilic attack by the free N-terminus of a peptide to its own structure causes an intramolecular aminolysis (Ryakhovsky and Ivanov, 2012), and the **two** N-terminal residues are cleaved from the peptide and released as a diketopiperazine (Figure 4). This reaction has a half-life of ~ 100 days at 25 ºC (Radzicka and Wolfenden, 1996), so a normal 7 residue long peptide will be degraded into a 5 residue long peptide in only 100 days. However, if the 7 residue long peptide is a self-replicating peptide that is constantly growing in its N-terminus (Figure 3), it will be degraded only if the diketopiperazine is formed while there are zero or just one additional amino acid in its N-terminus (the self-replicating peptide will be degraded into a non-functional 5 amino acid long peptide in the former case, and into a non-functional 6 amino acid long peptide in the latter). On the other hand, if there are two or more additional amino acids and a diketopiperazine is formed, they will be cleaved from the structure, but the self-replication process would still function and new amino acids would be condensed in the N-terminus (Figure 7).

Nevertheless, even though slower, the degradation process would still break down the self-replicating peptide in a few months. For example, we can suppose that this 7 amino acid long self-replicating peptide produces another 7 amino acid long peptide each day (remember that this implies the formation of 7 peptide bonds and the hydrolysis of 1 peptide bond). Then, based on its metal-catalyzed rate constant at 50 ºC ($6.4 \cdot 10^{-5}$ s^{-1}) (Wezynfeld et al., 2016) the hydrolysis of 1 peptide bond will occur every ~ 3 hours. There are 21 hours left in a day where 7 peptide bonds need to be created, so the condensation of one amino acid in the N-terminus would need to occur at least every 21/7 = 3 hours. Based on the estimated uncatalyzed rate constant of peptide bond formation at 50 ºC (~ 10^{-3} M^{-1} s^{-1}) (Sievers et al., 2004) it can be confirmed that this will happen at concentrations (for both the peptide and the free amino acids) as low as 0.01 M.

Therefore, each day there will be 3 hours where no additional amino acids have been condensed in the N-terminus of our self-replicating peptide, and another 3 hours where only one additional amino acid has been condensed in its N-terminus (thus the peptide will be susceptible to degradation only 3 + 3 = 6 hours per day).

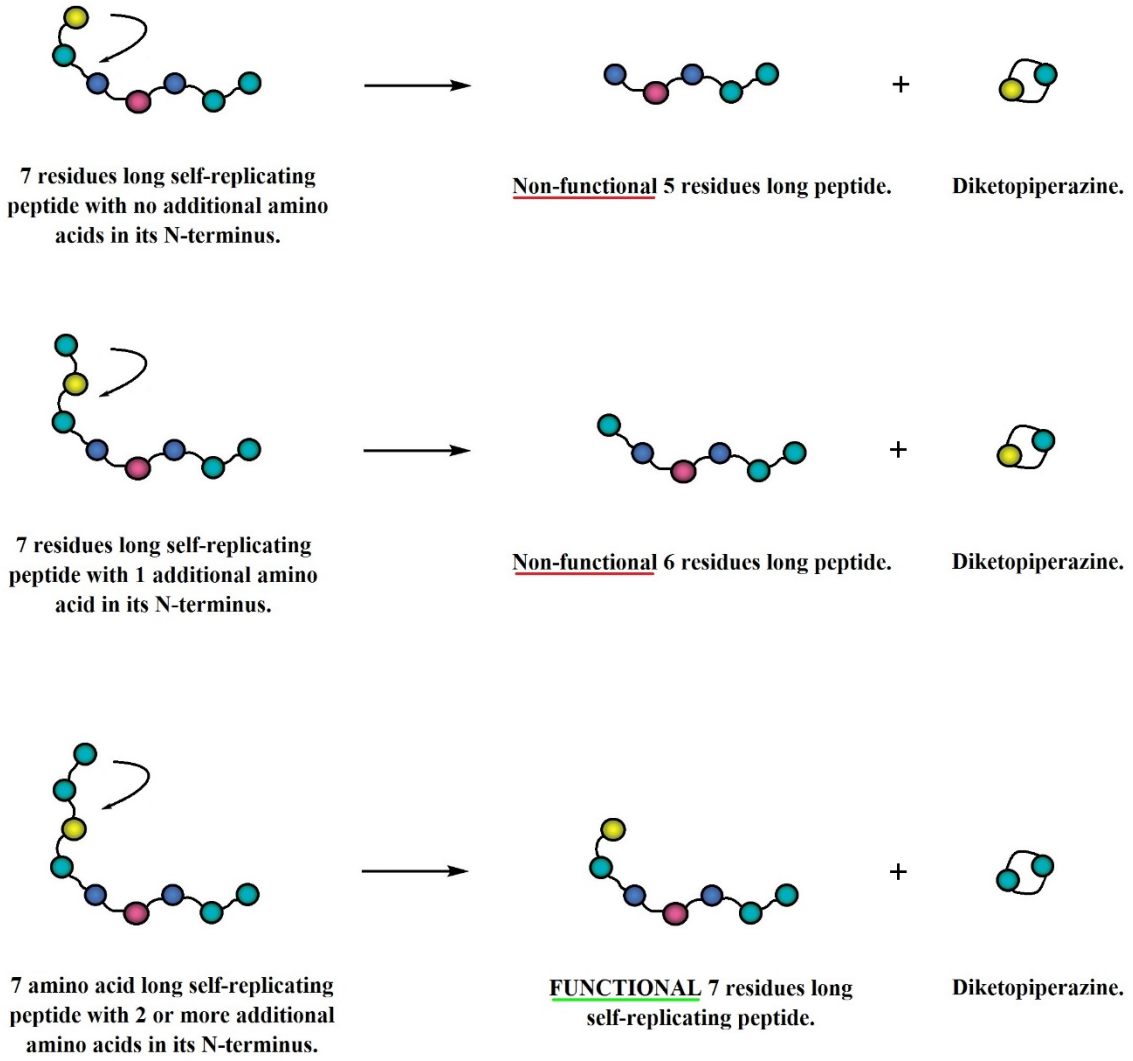

Figure 7. If there are two or more additional amino acids in the N-terminus, diketopiperazine formation will not affect the self-replicating structure. The peptide will still function and it will keep adding new amino acids into its N-terminus.

Being susceptible to degradation for only 6 hours per day raises the half-life of our self-replicating peptide from 100 to 400 days. But the resistance to diketopiperazine degradation could still be greatly improved if the self-replicating peptide was able to

produce some sort of “protective cap” on its N-terminus. At a first glance, this might sound like an overly difficult process to be carried out by a small peptide, but if this peptide is able to condense free amino acids into its N-terminus, then building a protective cap is a very easy task for it. Having a protective cap would mean that, when the 7 amino acid long peptide is synthesized it would consecutively catalyze the condensation of free amino acids into its N-terminus as explained in Figure 3, but instead of condensing 7 amino acids and then hydrolyzing the newly formed 7 amino acid long peptide from its structure, it would condense 9 amino acids into its N-terminus and then hydrolyze a 7 amino acid long peptide from its structure (Figure 8).

With this simple mechanism, our peptide is almost immune to degradation by diketopiperazine formation. Just as it was calculated earlier, the condensation of one amino acid into the N-terminus occurs every 3 hours, so if one peptide is produced every day, there will be 3 hours per day where the protective cap has no additional amino acids in its N-terminus, and another 3 hours per day where it has only one additional amino acid in its N-terminus (thus the protective cap will be susceptible to degradation for 3 + 3 = 6 hours per day). Then, if the half-life for diketopiperazine formation is 100 days, the protective cap would be degraded once every 400 days. But if the protective cap is degraded, the self-replicating peptide can produce another one by condensing two free amino acids into its N-terminus, so in 6 hours the peptide would be protected again against diketopiperazine formation. Therefore, a self-replicating peptide with a protective cap would be susceptible to degradation for only 6 hours every 400 days, which gives it a half-life of 438 years. With such a long half-life it can be affirmed that the self-replicating peptide would be virtually immune to degradation by diketopiperazine formation.

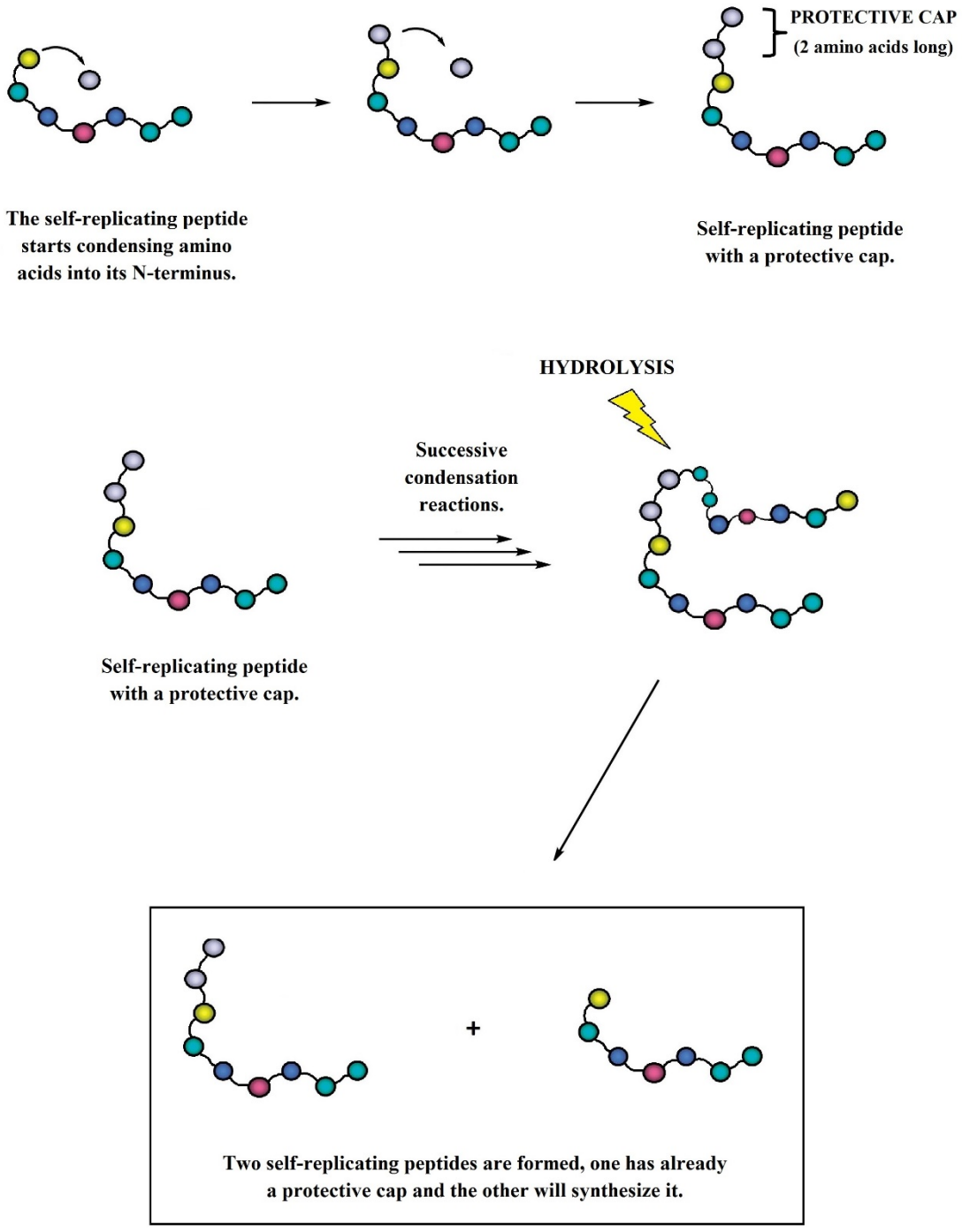

Figure 8. A self-replicating peptide might be able to protect itself from degradation by diketopiperazine formation by synthesizing a 2 amino acid long cap before starting to produce new peptides.

5. Conclusion

In the first instance it is not very intuitive to think about a short peptide (from 5 to 9 residues long) as a potential self-replicating entity that was responsible for the origin of life. It has never been demonstrated that a peptide so small can catalyze peptide bond formation, a necessary requirement for the synthesis of new peptides. Moreover, even if a peptide was capable of catalyzing this reaction two things would still be required for it to be considered a self-replicating molecule: the ability to connect the free amino acids in the right order so a copy of itself is formed, and the ability to hydrolyze one specific peptide bond so the newly formed peptide is set free. Of course, all of this has to happen while the right thermodynamic conditions that favor peptide bond formation are met and before the fast degradation of the peptide due to diketopiperazine formation in its N-terminus occurs.

However, the plausibility of small peptides as possible self-replicating molecules can be appreciated when one realizes that these problems can be solved with relative ease: a self-replicating peptide which is constantly growing in its N-terminus is resistant to degradation by diketopiperazine formation; there are many ways for making the thermodynamic conditions favorable to peptide bond formation; metals can catalyze peptide bond hydrolysis; if given enough time, only a little selectivity is needed for forming a few copies of a small peptide; and that while it is true that there are no known peptides which can catalyze the peptide bond formation, the catalytic properties of small peptides in the presence of minerals are poorly studied (minerals might change the catalytic properties of peptides by allowing them to adopt new conformations or by providing them with a specific microenvironment).

On top of that, other hypothetical first replicators such as bigger peptides/proteins, RNA, or an RNA-protein combined hybrid come with more problems than small peptides. Bigger peptides/proteins face the same problems as smaller peptides, and because of their bigger size they need a much more robust replication system. The formation of RNA is also thermodynamically unfavorable, and the hydrolysis of each phosphodiester bond occurs with a half-life of only 4 years (Thompson et al., 1995). Furthermore, the use of activating agents in phosphodiester bond formation “leads to backbones with mixed 3′,5′ and 2′,5′ linkages, misincorporated (i.e., non-Watson-Crick pairing) bases, and truncation

products resulting from strand cyclization" (Bean et al., 2009).

Therefore, in view of the plausibility of a small self-replicating peptide as a hypothetical first replicator, more experimental work is needed to either prove or disprove this hypothesis. For example, screening large arrays of small peptides in the presence of different minerals and free amino acids at different temperatures and pHs while looking for the formation of new peptide bonds would be a nice starting point.

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