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Opinion

Catalysis before Enzymes: Thiol-Rich Peptides as Molecular Diversity Providers on the Early Earth

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Abstract: The multiplicity of simple molecules available on the primitive Earth probably made possible the development of extremely diverse prebiotic chemistry. The importance of thiols is widely recognized in the community studying the origin of life. De Duve's "thioester world" has been considered a major contribution in this regard, where thioester bonds have high energies and thus can contribute to several chemical reactions. Herein, we propose specific models of thiols that exhibit unique activities toward several chemical reactions. Thanks to aminothiol and aminonitrile behaviors, we were able to obtain thiol-rich peptides with interesting catalytic activities leading to the formation of structurally diverse molecules. In a broader context, such chemistry could be introduced into systems chemistry scenarios in which it would be associated with the chemistry of nucleic acids or their precursors, as well as that of fatty acids.

Keywords: evolution of enzymes; origin of life; prebiotic catalysis; cysteine; thioesters

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1. Introduction

Life is extremely diverse. However, at the microscopic level, the organization of each cell is relatively similar, and it is necessary to go down to the molecular level to find another considerable variety, even if it is very close from one cell to another. If it is so, it is likely that this diversity has been present since the very first cells on Earth, ca. 4 billion years ago. LUCA, the Last Universal Common Ancestor, was surely not much less complex than today's cells [1]. If LUCA was so complex, if it contained so many different molecules, it was thanks to the chemistry that had preceded it, what we call "prebiotic chemistry", which provided these thousands of molecules.

In our team, we have developed methods to achieve the synthesis of many molecular objects, often in very complex mixtures, under plausible prebiotic conditions. It is this chemistry that we summarize here, convinced that without the diversity achieved by prebiotic chemistry, LUCA, and therefore no cell or organism, would have been born on Earth. Life exists primarily thanks to the multiplicity of possibilities offered by organic chemistry.

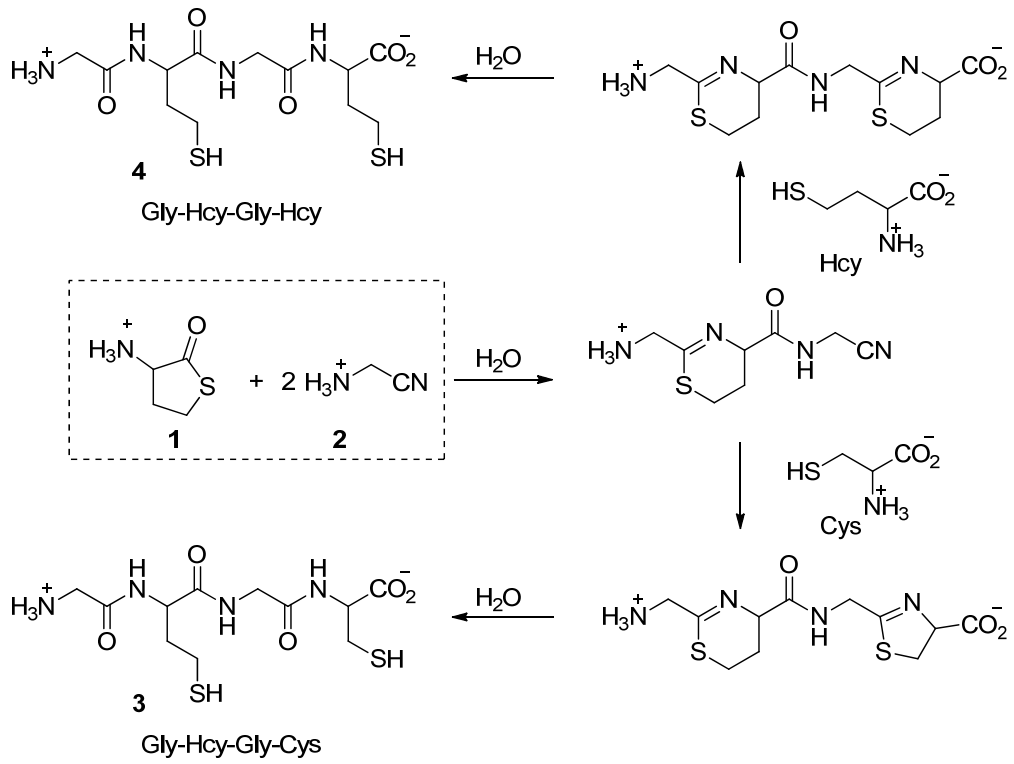
Among the diverse molecules available, some reasonably had the ability to accelerate particular reactions that intertwined into a primitive network of metabolic reactions [2,3]. The major role of these biocatalyst ancestors would have been to ensure continuity and energy release in molecular mixtures far from equilibrium [4]. The structure of the very first biocatalysts remains a matter of debate (enzymes, ribozymes) [1,5–7], the surest thing being that it was defined by the geochemical factors of the primitive environment. Besides, regardless of the energy source that life chose to begin its journey with, redox processes were a big part of it [8].

The enzymes that mobilize electrons transfer from one molecule to another are oxidoreductases [9]. Looking around in today's biology, especially that of microorganisms—the oldest and most widespread living being category on the planet—many oxidoreductases

are proteins rich in disulfide bridges and cooperate with cofactors to achieve the catalytic reactions [10,11]. In a simpler version compatible with the primitive environment, peptides rich in cysteine with the assistance of metal could catalyze redox primitive processes and transfer electrons. In this paper, we propose examples of diverse pre-enzyme redox catalysts that allow increasing diversity from simple molecules.

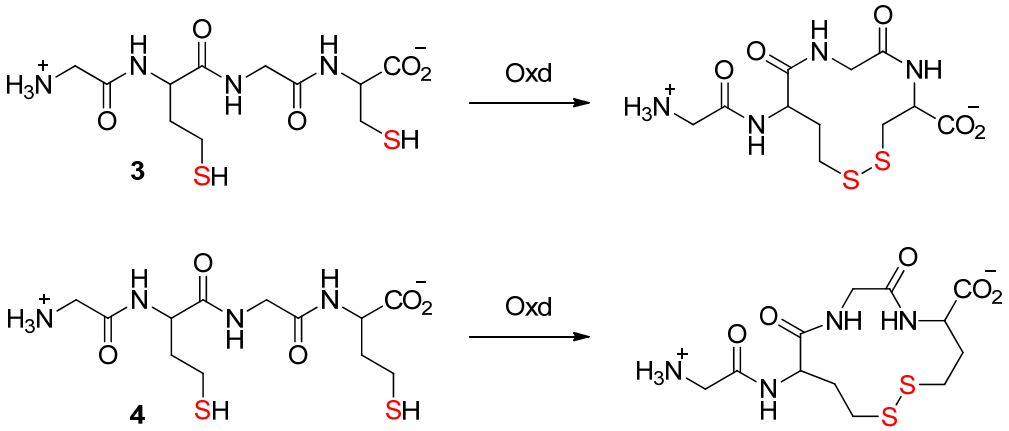
2. Thiols in the Making of Small Peptides and Nitrogen Heterocycles

If today's biological catalytic pathways make abundant use of thiols [12], this is probably because life has been keeping in its memory the first catalyst used for its primitive metabolism [13]. The objective of our team was to synthesize long peptide chains in prebiotic environmental conditions. In this work, we underlined the important role of sulfur [14,15]. This has been first seen in the ability of homocysteine thiolactone **1** to react with aminonitriles (for example, aminoacetonitrile **2**) to produce short chains of homocysteine containing peptides [16,17]. Further reactions with cysteine or homocysteine amino acids were shown to give bis-thiol tetrapeptides, Gly-Hcy-Gly-Cys **3** and Gly-Hcy-Gly-Hcy **4**, respectively (Scheme 1) [17].



Scheme 1. Formation of tetrapeptides, Gly-Hcy-Gly-Hcy **3** and Gly-Hcy-Gly-Cys **4**, in a prebiotic mixture in water, starting from Hcy-thiolactone **1** and aminoacetonitrile **2**. (Gly: glycine, Hcy: homocysteine, Cys: cysteine).

Because of the high reactivity of the thiol group, we strongly believe that this group must have played a vital role in catalyzing several pre-biochemical reactions, such as redox reactions. We observed the formation of the oxidized form of thiols, disulfides, in our mixtures [18] (Scheme 2). Electrons can be transferred and stored thanks to the disulfide bond. This phenomenon might have been of importance at the beginning of life on Earth, when electron storage mechanisms were limited [19].

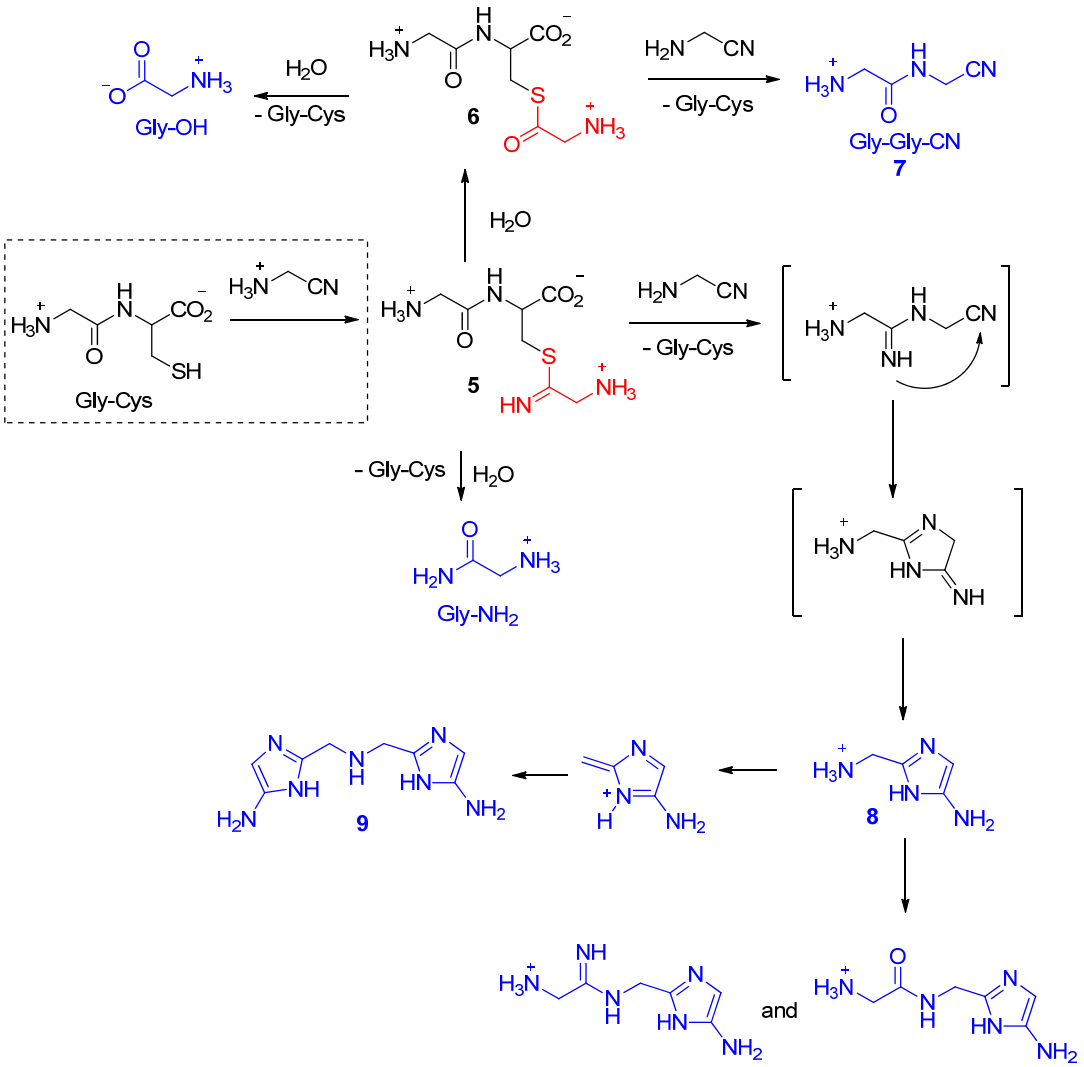


Scheme 2. Disulfide formation in the prebiotic mixture obtained from Hcy-thiolactone and aminoacetonitrile.

In addition, thiols can be easily activated to obtain the high-energy thioester bond, which in turn can participate in many reactions, such as the formation of new bonds and the transformation of functional groups [20]. Our findings agree with the "thioester world" proposed by Christian de Duve and supported by several scientists [21]. We propose a specific scenario in which thiol-rich molecules acted as the first enzymes on the primitive Earth.

In order to test the behavior of our thiols toward the formation of thioesters, we mixed Gly-Cys with an excess of aminoacetonitrile in water at 45 °C (Scheme 3). In this condition, we observed the formation of cysteine thioester derivatives **5** and **6**, which led to glycine amino acid Gly-OH, and glycine amide Gly-NH_2. We also noticed that the generated thioester underwent further reaction with aminoacetonitrile to form a new peptide bond with the formation of the amidonitrile Gly-Gly-CN **7** (Scheme 3). Similar results were obtained in the case of Gly-Hcy. Moreover, we found that the obtained thioester could react with aminoacetonitrile to give an amidine structure that is found to cyclize readily and give an imidazole ring, 2-(aminomethyl)-1H-imidazol-5-amine **8**. Its formation was followed by other reactions that led to more complex aromatic structures, such as bis-imidazole rings **9** (Scheme 3). It is noticeable that Gly-Cys is released in all these processes; it is not consumed, so it acts as a catalyst.

What is fascinating about these results is the appearance of such aromatic and active structures in our primitive soup. Indeed, such imidazole structures can be related to adenine and guanine nucleic bases that are known in today's biochemistry. This is evidence that thiol-containing peptides can lead to the emergence of simple but essential components that might have participated in the formation of pre-RNA units at the very beginning of life.



Scheme 3. Proposed pathways in the reaction of excess aminoacetonitrile with Gly-Cys, to obtain Gly-OH, Gly-NH_2, Gly-Gly-CN, and several imidazole derivatives.

3. Thiols in the Making of Polypeptides

Besides small molecules, longer peptide chains have also been formed using aminothiol-nitrile precursors [18]. The proposed pathway leads to the synthesis of linear and cyclic cysteine containing peptides through repeated formation and hydrolysis of thiazoline moieties. For example (Figure 1), Cys-Val-CN **10** polymerized easily in water. Thiazolines were formed and some of them underwent hydrolysis. The MALDI mass spectrum of the obtained mixture is shown in Figure 1. It demonstrates the formation of polypeptides of up to 60 residues. For each number of residues, thiazolines were hydrolyzed in part, which explains the shape of the observed groups of the peak.

As our simple thiols showed a high reactivity toward the formation of new peptide bonds, nitrogen aromatic rings, and disulfide bonds, then we believe that the obtained oligo- and polypeptides would show similar catalytic behavior. We found these macromolecules to contain both reduced (thiol) and oxidized (disulfide) forms and to be stable in primitive conditions for a long period of time. The high content of thiols would make such peptides very efficient primitive machines for redox catalysis. For instance, Figure 2a shows a cyclic peptide that contains 24 cysteine units, which was obtained from the cysteine nitrile monomer, Cys-CN, in aqueous solution at pH 6 and at room temperature [22]. Such cyclic structures would also have the ability to complex with several metal ions, which could give these molecules the power to select the best metals for the following steps in biochemistry.

As illustrated in Figure 2b, we present our prebiotic peptide models as the earliest enzymes on primitive Earth, where they were able, for instance, to perform redox reactions, to produce fatty acid precursors, and to activate many other important reactions related to CO_2 fixation.

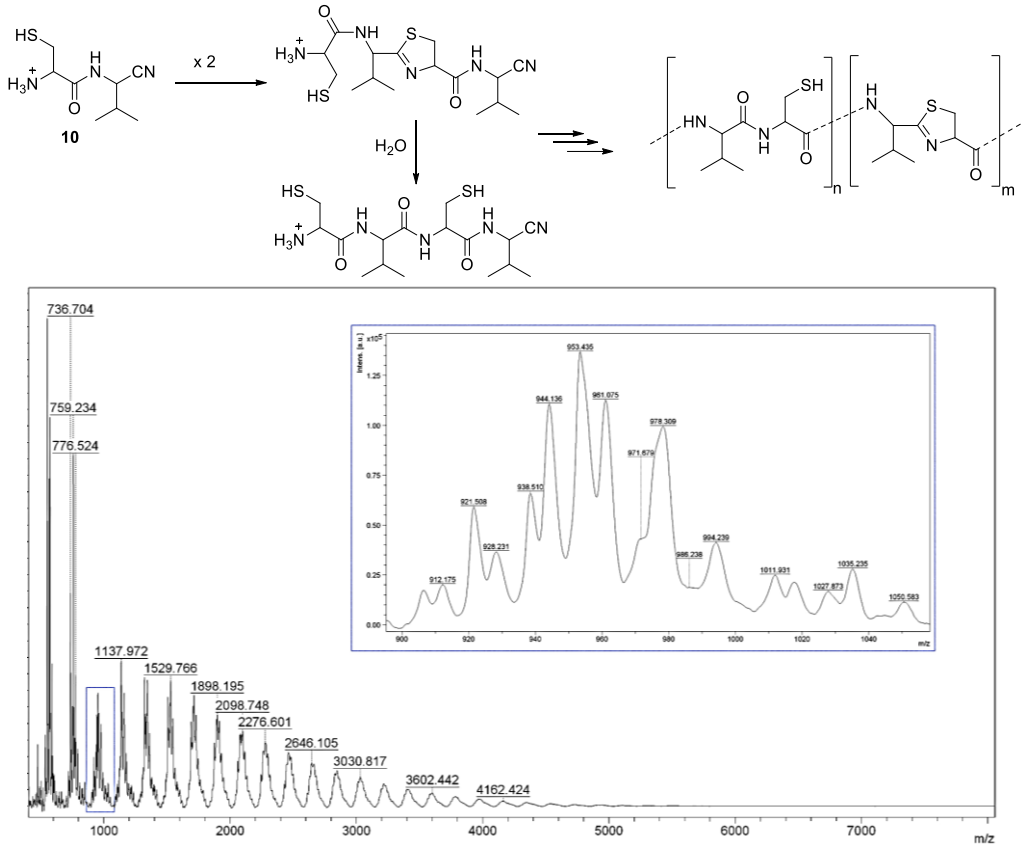


Figure 1. Peptides obtained from aminothiol-containing amidonitriles: polymerization scheme for Cys-Val-CN **10** and MALDI mass spectrum of the obtained mixture with enlargement of the zone corresponding to $(Cys\text{-}Val)_5$. (Cys: cysteine, Val: valine).

The formation of peptide bonds, achieved in our chemistry, is one of the most important chemical transformations in the field of prebiotic chemistry. Peptides play an important catalytic role in the formation of diverse biotic molecules and biopolymers. The proposed chemistry accepts any type of amino acid or peptide to yield more diverse, thus more realistic, molecules. Even though high cysteine nitrile concentration in a primitive pool cannot be regarded as a possible scenario, we confirmed the stability of cysteine nitrile in acidic conditions over a long period of time. Moreover, once formed, cysteine and homocysteine can react with any possible aminonitrile that may be present in water. We believe that, together with other proposed prebiotic catalytic pathways [3,23,24], catalysis resulting from a thiol-rich peptide world have had its place in the catalysis processes progressing toward the modern catalytic activities.

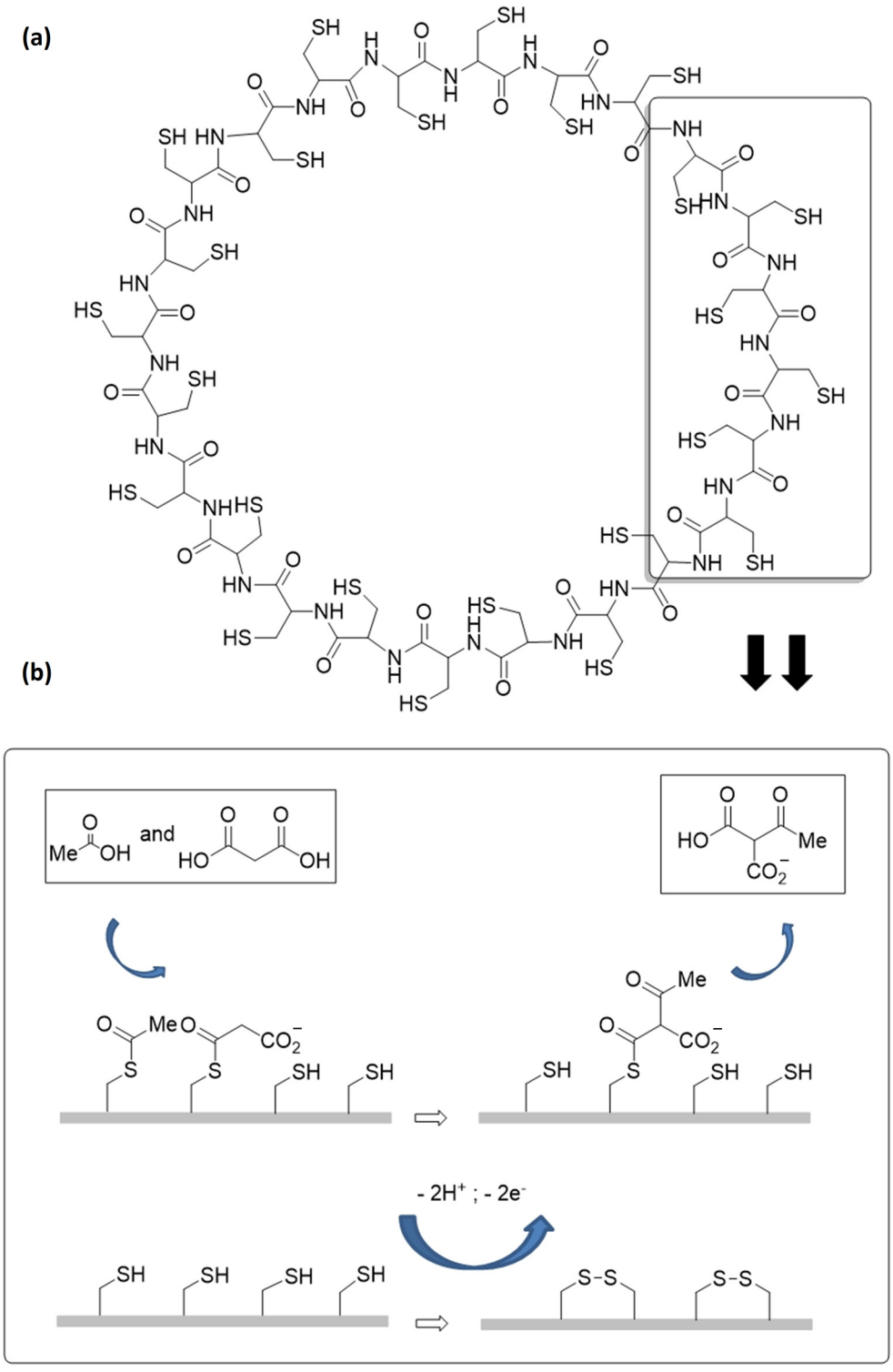


Figure 2. (**a**) Example of a detected polycysteine, starting from Cys-CN; (**b**) proposed catalytic pathways of thiol-rich peptides in primitive environment.

4. Conclusions

To conclude, we have obtained a wide range of thiol-containing peptide models that are interesting model catalytic structures for many important reactions, which were required to construct the basic components of life. We found that these peptides are able to form active thioester bonds that trigger the building of longer peptide chains en route to proteins. In addition, we have demonstrated that dipeptides such as Gly-Cys and

Gly-Hcy are able to promote the formation of heterocyclic molecules (nitrogenous bases), which share common structural features with the nucleic bases that we know in today's biochemistry and are essential to obtain RNA and DNA. This remarkable catalytic behavior might have had a crucial role to advance chemical evolution processes and might have played the role of pre-enzymes in the primitive Earth.

The chemistry we propose provides access to both peptides, some relatively long and some with catalytic properties, and nitrogen heterocycles. It would be interesting to extend it further so that it includes molecules closer to nucleic acids, in a "systems chemistry" model [25,26], towards increasingly complex mixtures. To approximate biochemistry, it should be conducted in cell-like structures such as fatty acid vesicles [27,28]. Polythiols would constitute a good part of the catalytic machinery in these vesicles and the fatty acid membrane would maintain a local high concentration. The question of how information and selection would emerge from such a disordered mixture to make an admittedly complex, but homeostatic mixture, remains unanswered. We can certainly imagine information carried by the already formed peptides, which would favor their own copy. However, it would probably be more efficient to polymerize nucleotides or nucleotide-like molecules to generate, in the long term, an operating mode for storing and copying information [29].

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