

# Modulation of Reactivity in the Cavity of Liposomes Promotes the Formation of Peptide Bonds

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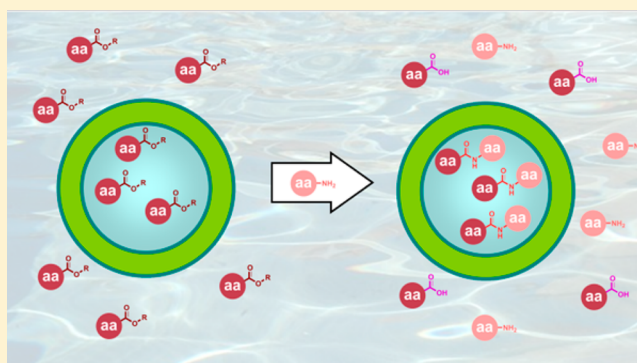
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## S Supporting Information

**ABSTRACT:** In living cells, reactions take place in membrane-bound compartments, often in response to changes in the environment. Learning how the reactions are influenced by this compartmentalization will help us gain an optimal understanding of living organisms at the molecular level and, at the same time, will offer vital clues on the behavior of simple compartmentalized systems, such as prebiotic precursors of cells and cell-inspired artificial systems. In this work we show that a reactive building block (an activated amino acid derivative) trapped in the cavity of a liposome is protected against hydrolysis and reacts nearly quantitatively with another building block, which is membrane-permeable and free in solution, to form the dipeptide. By contrast, when the activated amino acid is found outside the liposome, hydrolysis is the prevalent reaction, showing that the cavity of the liposomes promotes the formation of peptide bonds. We attribute this result to the large lipid concentration in small compartments from the point of view of a membrane-impermeable molecule. Based on this result, we show how the outcome of the reaction can be predicted as a function of the size of the compartment. The implications of these results on the behavior of biomolecules in cell compartments, abiogenesis, and the design of artificial cell-inspired systems are considered.



## INTRODUCTION

In living cells, most reactions take place within compartments bound by a lipid membrane. Clearly, the compartmentalization can have various effects on the reactions. For instance, compartmentalization can lead to larger local concentration of reactants, speeding up reactions, or may prevent or slow down reactions between reactants found in different compartments. Moreover, the lipid membrane itself can enhance the reaction between hydrophobic molecules that dissolve preferentially within the membrane.<sup>1,2</sup> Some of the effects in compartmentalized reactivity are less obvious, however, and, when researchers are looking at a living cell, may remain hidden by the complex regulatory processes typical of biomolecular systems. These additional effects can be revealed by the study of simpler compartmentalized systems in the form of lipid vesicles. For example, studies have shown that compartmentalization assists some enzymatic reactions by preventing parasitic reactions that slow them down.<sup>3,4</sup> Also, some enzymatic reactions that lead to the formation of multimeric enzyme assemblies are favored by compartmentalization.<sup>5</sup> These are important effects that are difficult to observe and quantify when studying the cell, but become all too apparent in simpler systems. The implications are that, whatever the relative importance that they may have within complex biological

systems, they will dictate, to a large extent, the behavior of these simpler systems. In other words, it is important to be able to quantify and predict these effects in order to (i) improve our ability to design cell-inspired materials (such as nano- and microreactors for sensing and drug delivery,<sup>6–10</sup> or the bottom-up design and assembly of minimal cells with tailored functionality<sup>11–18</sup>) and (ii) gain a better understanding of abiogenesis,<sup>11,19–22</sup> a process that involved the chemical evolution of non-living matter to cell ancestors (termed proto-cells) and then to living cells. Clearly, the formation of proto-cells is nature's version of the assembly of a minimal cell, and, as such, understanding it should improve our ability to create cell-like *de novo* systems.

Current research in the effects of compartmentalization focuses on enzymatic reactions.<sup>4</sup> In the context of the assembly of nanoreactors and artificial minimal cells, the effects of compartmentalization on enzyme kinetics are extremely important. The effects of compartmentalization in non-enzymatic reactions may also be important, in particular for the assembly of very simple functional systems with truly bottom-up design, i.e., in the absence of any complex

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biomolecules. Also, the fate of non-enzymatic reactions in compartmentalized systems is particularly important in the context of abiogenesis (i.e., the assembly of the initial proto-cells), especially if we focus our attention on the initial steps of proto-cell evolution, where enzymes as we know them would not have been present.

In the current understanding of proto-cell formation, it is widely accepted that it involved the assembly of lipid vesicles (formed of fatty acids and alcohols) that would entrap short oligonucleotides within their cavity.<sup>23,24</sup> The formation of these oligonucleotide-containing vesicles is attributed to the catalytic action of some minerals' surfaces.<sup>25,26</sup> Oligonucleotides are membrane-impermeable, while nucleotides can permeate fatty acid membranes. This simple fact—the differential permeability of the lipid membrane—is enough to start a process of growth and division that may lead to a natural selection process benefiting oligonucleotide-containing vesicles over empty ones, thus starting the long road toward cellular life.<sup>27–31</sup> It is believed that the minimum size of RNA with ribozyme polymerase functionality requires in excess of 200 oligonucleotides.<sup>32</sup> Therefore, in the initial steps of abiogenesis, a small trapped oligonucleotide would have been unable to catalyze its own self-replication with enzymatic efficiency, but could still promote the synthesis of complementary strands by a simple template effect.<sup>29,27</sup> The implications are that, in the absence of enzymatic catalysis, any modulation of reactivity brought about by the compartmentalization would have a clear impact on the evolution of the system. For example, any role that compartmentalization may play in the synthesis of oligonucleotides or other biopolymers, such as oligopeptides, will be a central role in the behavior of the system.

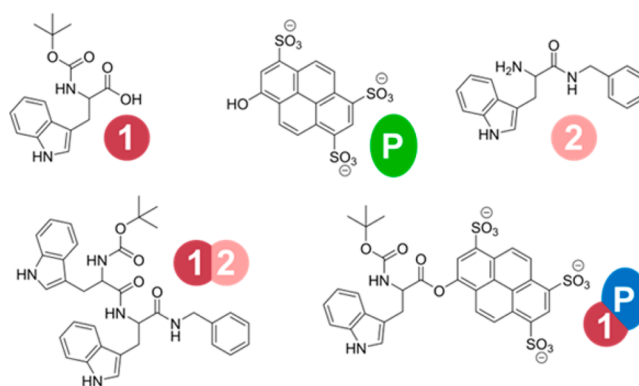
Lipid membranes are known to catalyze condensation reactions of amino acids<sup>1,2</sup> and have been seen to enhance this type of reaction when the catalysts are simple oligopeptides.<sup>33</sup> This effect is attributed to the preferential association of hydrophobic amino acids with the membrane (leading to an increase in local concentration) coupled with the fact that, on the surface of the membrane, the concentration of water is lower than that within the bulk solution, favoring condensation reactions such as peptide bond formation (or phosphodiester bond formation in nucleic acids). This ability is, however, displayed on the surface of the membrane, and it is unclear how it may affect the composition or behavior of chemical species within the cavity of the proto-cell, unless all reactants are, perchance, found within the proto-cell and are both membrane-impermeable. In such a case, however, reactions in the compartment of the proto-cell would be dictated solely by their initial composition. It is clear that evolution requires a system that is separated from the environment by the membrane which defines the compartment and allows the transit of some molecules, thus making it responsive to changes in the chemical environment. This ability is displayed by oligonucleotide-containing fatty acid vesicles, in which the trapped oligonucleotide is impermeable yet individual nucleotides can cross the membrane.<sup>28</sup> In this context, it becomes essential to understand how the reactivity of cavity-confined molecules versus molecules that can cross the compartment membrane is modulated by the compartment.

Here we use a model system to investigate the modulation of reactivity within the cavity of lipid-bound compartments, in the form of lipid vesicles. The model reactants are suitably modified amino acids, one of which is membrane-impermeable (to ensure that the reaction takes place either outside or in the

cavity of the liposome) and the other membrane-permeable. We demonstrate that, while the lipid membrane is shown to catalyze the condensation reaction to some extent in all cases, the residence within the liposomal cavity of one of the building blocks leads to an almost quantitative dipeptide formation. The results allow us to simulate the observed effect with the size of the compartment and to describe a plausible sequence of events that may have led to the assembly of the first vesicle-trapped genetic oligomers.

## RESULTS AND DISCUSSION

Tryptophan derivatives **1P** and **2** and dipeptide **12** (Figure 1) were synthesized using standard peptide chemistry methods



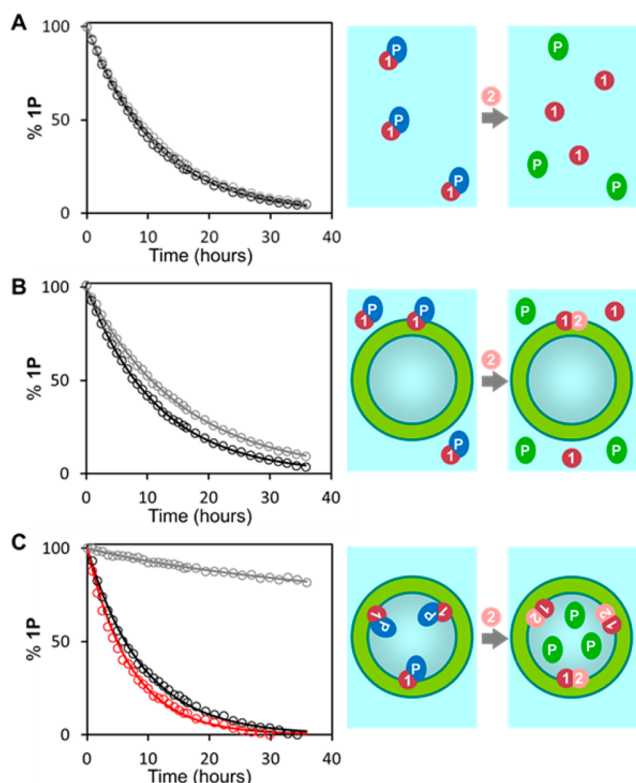
**Figure 1.** Chemical compounds used in this work. The naming and their cartoon representation is also shown.

and purified by preparative HPLC (see the [Supporting Information](#) for details of the experimental methods). These amino acid derivatives were chosen for convenience: they are simpler and easier to handle than, for example, nucleotide derivatives. Also, the presence of the pyranine moiety in **1P** allows the straightforward monitoring of reactions of peptide bond formation and hydrolysis using optical spectroscopic methods. Their behavior can, however, be extrapolated to other systems where condensation reactions compete with hydrolysis, such as phosphodiester bond formation leading to nucleic acid oligomerization.

**1P** is very water-soluble and undergoes hydrolysis in buffered aqueous solutions. The rate of hydrolysis was determined by following changes in the UV–vis spectrum of **1P** over time readily attributed to the release of the pyranine moiety, **P**, and fitting them to a first-order kinetics equation of the form

$$A = \epsilon_P[\mathbf{1P}]_0 - \Delta\epsilon[\mathbf{1P}]_0 e^{-k_{\text{app}}t} \quad (1)$$

where  $A$  is the absorbance at the wavelength under study,  $\epsilon_P$  the molar extinction coefficient of **P** at the same wavelength,  $\Delta\epsilon$  the difference in molar extinction coefficient between **1P** and **P**,  $[\mathbf{1P}]_0$  the initial concentration of **1P**, and  $k_{\text{app}}$  the apparent rate constant of reaction. In this experiment,  $k_{\text{app}}$  is the constant of hydrolysis in the bulk solvent,  $k_{\text{hb}}$ . Analysis of the UV data showed that the half-time of hydrolysis in the experimental conditions is around 8 h (Figure 2A, Table 1). Tryptophan **1P** and **2** may, in principle, react with each other to form dipeptide **12**. When **2** was added to a solution of **1P** in water, the rate of **1P** consumption, followed by UV spectroscopy, was similar to that seen in the absence of **2**, with the half-time of reaction being the same within the error (Figure 2A, Table 1). This result suggests that, in the presence



**Figure 2.** (A) Changes in relative concentration of **IP** over time derived from the UV data in the absence (gray circles) and presence (black circles) of **2**. The gray and black lines represent the best fit to a single-exponential decay. The panel to the right shows a cartoon representation of the products of reaction in the presence of **2**. (B) Same as (A) but in the presence of vesicles. (C) Same as (A) but for samples with **IP** confined in the cavity of liposomes. The red circles and trace correspond to a sample with twice the concentration of **2**. In all experiments, the concentration of **IP** was  $30 \mu\text{M}$ , that of lipids  $500 \mu\text{M}$ , and that of **2** either  $300 \mu\text{M}$  or  $600 \mu\text{M}$  (see Supporting Information and Supplementary Table 1 for further sample details).

of **2**, the hydrolysis of **IP** is still the main reaction taking place in the experimental conditions (Figure 2A). HPLC analysis after complete reaction of **IP** confirmed that, both in the absence and presence of **2**, the only detectable amino acid derivative produced in the reaction is *boc*-tryptophan **1** (Supplementary Figure 4, Table 1).

**IP** is an amphiphilic molecule that can insert into lipid membranes using the side chain of the tryptophan moiety as a lipid anchor. We evaluated the binding affinity of **IP** for lipid membranes of vesicles composed of egg yolk phosphatidylcholine (EYPC) by means of a UV titration method. The dissociation constant derived from the titration data,  $K_{\text{IP-L}}$ , is

$0.7 \text{ mM}$  (see Supporting Information and Supplementary Figure 1 for details). In the presence of EYPC liposomes, the rate of hydrolysis of **IP** is somewhat reduced, as shown by the analysis of the changes in the UV-vis spectrum (Figure 2B, Table 1). This result is consistent with the fact that a part of **IP** is anchored in the lipid membrane, where it is protected from hydrolysis to some extent. The apparent rate constant is therefore a combination of the rate constant on the bulk,  $k_{\text{hb}}$ , and the rate constant when anchored in the lipid membrane,  $k_{\text{hl}}$ :

$$k_{\text{app}} = x_{\text{IP-L}}k_{\text{hl}} + (1 - x_{\text{IP-L}})k_{\text{hb}} \quad (2)$$

where  $x_{\text{IP-L}}$  is the fraction of **IP** bound to the membrane, which, in the presence of an excess of lipids, may be assumed to remain constant during the course of the reaction (see Supporting Information for details).

The value displayed in Table 1 for this reaction is  $k_{\text{app}}$ , obtained from the fitting of the data. This value is consistent (within the error) with the value obtained by using the values of  $k_{\text{hb}}$  (calculated in the experiment in the absence of liposomes) and  $k_{\text{hl}}$  (calculated in the experiment with confined **IP**, see below). Tryptophan derivative **2** is also an amphiphilic molecule that is able to insert into lipid membranes. The dissociation constant, derived from fluorescence spectroscopy titration methods, for membranes of EYPC vesicles,  $K_{2-L}$ , is  $1.3 \text{ mM}$  (see Supporting Information and Supplementary Figure 1 for details). When **2** was added to **IP** in the presence of EYPC liposomes, a small but clear acceleration of the rate of **IP** reaction was observed (Figure 2B, Table 1). HPLC analysis of the products showed the presence of the product of hydrolysis **1** together with **12**, in approximately a 7:3 ratio (Table 1, Supplementary Figure 4). In the experimental conditions, fractions of both **IP** and **2** are found associated with the liposomal membrane. Clearly, in the experimental conditions, the lipid membrane acts as a simple catalyzer, bringing the reagents together and thus enhancing the formation of **12** (Figure 2B), a phenomenon that has been described in both liposomal and micellar systems.<sup>1,2</sup> The apparent rate constant, shown in Table 1, is in these conditions a function of the rate constants of hydrolysis in the bulk and in the membrane ( $k_{\text{hb}}$  and  $k_{\text{hl}}$ ) and that of coupling with **2**,  $k_{12}$ :

$$k_{\text{app}} = x_{\text{IP-L}}k_{\text{hl}} + x_{\text{IP-L}}k_{12} + (1 - x_{\text{IP-L}})k_{\text{hb}} \quad (3)$$

where  $k_{12}$  is a pseudo-first-order kinetic constant that depends on  $K_{2-L}$  and the concentration of **2**. We can use this constant, given the fact that **2** is always in large excess relative to **IP**. The value of  $k_{12}$  can be derived by using the known values of  $k_{\text{hb}}$  and  $k_{\text{hl}}$  (see Supplementary Table 2).

**IP** does not permeate through the EYPC vesicular membrane in a time scale of days (see Supporting Information

**Table 1. Kinetic Parameters and Outcome of **IP** Reactions**

	without <b>2</b>			with <b>2</b>			
	no vesicles	<b>IP</b> outside vesicles	<b>IP</b> inside vesicles	no vesicles	<b>IP</b> outside vesicles	<b>IP</b> inside vesicles	<b>IP</b> inside vesicles <sup>c</sup>
$k \text{ (h}^{-1}\text{)}^a$	0.084	0.065	0.0034	0.089	0.087	0.11	0.14
$t_{1/2} \text{ (h)}^a$	8.2	11	200	7.8	8	6.2	4.8
% of <b>1</b> <sup>b</sup>	>99	>99	>99	>99	68	23	18
% of <b>12</b> <sup>b</sup>	nd	nd	nd	nd	32	77	82

<sup>a</sup>The error in  $k$  and  $t_{1/2}$  is on the order of 20%. <sup>b</sup>The percentages of **1** and **12** were estimated at the end point of the reaction (after 48 h) by HPLC. The error is on the order of 5%. See Supporting Information for the experimental details. <sup>c</sup>Used twice the concentration of **2**. See Supplementary Table 1 for sample details. nd = not determined.

and Supplementary Figure 2). Vesicles loaded with **1P** were produced by suspending the solid lipids in a buffer containing dissolved **1P**. Most non-trapped **1P** was removed by gel permeation. Fluorescence quenching experiments and analysis of the UV spectra showed that around 85% of unreacted **1P** (75% of the total **1P** used to prepare the experiment) was found inside the vesicles at the beginning of the experiment (see Supporting Information and Supplementary Figure 2). The average concentration of **1P** inside the liposomes was estimated from the concentration of lipids, the average area per EYPC molecule, and the average size of liposomes (see Supporting Information for details). The concentration was estimated to be around 14.5 mM, which is 4.8 times larger than the initial concentration of **1P** in the buffer used to prepare the vesicles. This increase in concentration is attributed to the amphiphilic nature of **1P** and its inability to cross the membrane once the vesicle is formed. Thus, upon formation of the vesicles, a fraction of **1P** incorporates into the membrane. The fraction that ends up in the inner leaflet remains trapped, leading to an increase in the concentration of **1P** in the cavity of the liposome in relation to the original concentration of **1P** in pure buffer. For samples containing liposome-confined **1P** (but no **2**), changes in the UV spectra did not fit well to a single-exponential function (typical of a simple first-order kinetic reaction) but were instead fitted to a double-exponential of the form

$$A = \varepsilon_{\text{P}}[\mathbf{1P}]_0 + x_{\text{1P}}[-\Delta\varepsilon[\mathbf{1P}]_0 e^{-k_{\text{app}}t}] + (1 - x_{\text{1P}}) [-\Delta\varepsilon[\mathbf{1P}]_0 e^{-k_{\text{app}}t}] \quad (4)$$

The need of a double-exponential to fit the data is consistent with the presence of two separate populations of **1P** reacting at different rates. The fraction confined in the vesicles is  $x_{\text{1Pc}}$  (estimated to be around 85% of the total unreacted **1P** at the beginning of the experiment), with an apparent rate of reaction of the confined fraction,  $k_{\text{app-c}}$ . Since in excess of 99% of confined **1P** is associated with the membrane (see Supporting Information for details), it follows that  $k_{\text{app-c}}$  is the rate of hydrolysis of **1P** associated with the membrane,  $k_{\text{hl}}$ . On the other hand, the apparent rate constant for the fraction found outside the liposomes,  $k_{\text{app}}$ , is a function of the hydrolysis of the membrane-bound and free **1P** (see eq 2). In the fitting of the data, we use the value of  $k_{\text{app}}$  obtained from the experiment with **1P** outside the liposome as a known parameter, and  $k_{\text{app-c}}$  (that is,  $k_{\text{hl}}$ ) is obtained as a parameter of the fitting. For this experiment, the value reported in Table 1 is  $k_{\text{app-c}}$ . The rate constant of hydrolysis of liposome-confined **1P** is very small and leads to a reaction half-time of 200 h (Table 1).

For samples with liposome-confined **1P**, the addition of **2** led to an acceleration of the rate of between 30- and 40-fold (depending on the concentration of **2** added), with an observed half-time of approximately 5–6 h (Figure 2C, Table 1, Supplementary Table 2). In these experiments there were also two separate populations of **1P**, where the apparent rate constant for confined **1P** is

$$k_{\text{app-c}} = k_{\text{hl}} + k_{\text{12}} \quad (5)$$

The UV data, however, fit reasonably well to a single exponential. This fact is attributed to a similar rate of reaction of both populations. Clearly, whatever reduction in the rate of hydrolysis afforded by the membrane for liposome-confined **1P** was offset by reaction of **1P** with **2** inside the liposome, making  $k_{\text{app-c}}$  and  $k_{\text{app}}$  too similar to be differentiated. Analysis by HPLC

of the products confirmed that **12** was the main product of reaction in these conditions, at around 80% of **12** against 20% of product of hydrolysis **1** (Table 1, Supplementary Figure 4). Since 25% of total **1P** used was found outside the liposomes and the hydrolysis outside the vesicles is around 25 times faster than that inside the vesicles, we attribute the presence of the product of hydrolysis to the hydrolysis of **1P** outside the cavity of the liposomes, which is its main reaction when found outside the liposome. Conversely, liposome-confined **1P** reacted almost quantitatively with **2** to produce **12** in the experimental conditions. The near-quantitative formation of **12** inside the liposome is readily attributed to the combination of (i) the protection against hydrolysis of **1P** afforded by the lipid membrane at the high local concentration inside the vesicle and (ii) the ability of the membrane to bring both reagents, **1P** and **2**, together.

It is clear that, in the presence of liposomes, the yield of dipeptide **12** depends, among other factors, on the extent to which **1P** and **2** are bound to the membrane of the liposomes. On the one hand, the association of **1P** with the membrane decreases the rate of hydrolysis. On the other hand, the association of both **1P** and **2** brings the reagents together, favoring the formation of **12**. In the presence of an excess of **2**, the yield of **12**, expressed as the mole fraction  $x_{\text{12}}$ , can be written as

$$x_{\text{12}} = x_{\text{1P-L}}x_{\text{1P-L}}^{\text{12}} \quad (6)$$

where  $x_{\text{1P-L}}$  is the fraction of **1P** associated with the membrane and  $x_{\text{1P-L}}^{\text{12}}$  the fraction of **1P** associated with the membrane that undergoes reaction with **2**.  $x_{\text{1P-L}}$  depends, among other factors, on the concentration of lipids. For molecules confined in the cavity of the liposome, the concentration of lipids depends on the radius  $r$  of the liposome.  $x_{\text{1P-L}}$  can therefore be written as a function of the radius of the liposome as follows:

$$x_{\text{1P-L}} = \frac{c}{rK_{\text{1P-L}} + c} \quad (7)$$

where  $c$  is a confinement factor that depends on the area per lipid molecule (see Supporting Information for details). On the other hand,  $x_{\text{1P-L}}^{\text{12}}$  can be written as

$$x_{\text{1P-L}}^{\text{12}} = \frac{k_{\text{s}}x_{\text{2-L}}}{k_{\text{hl}} + k_{\text{s}}x_{\text{2-L}}} \quad (8)$$

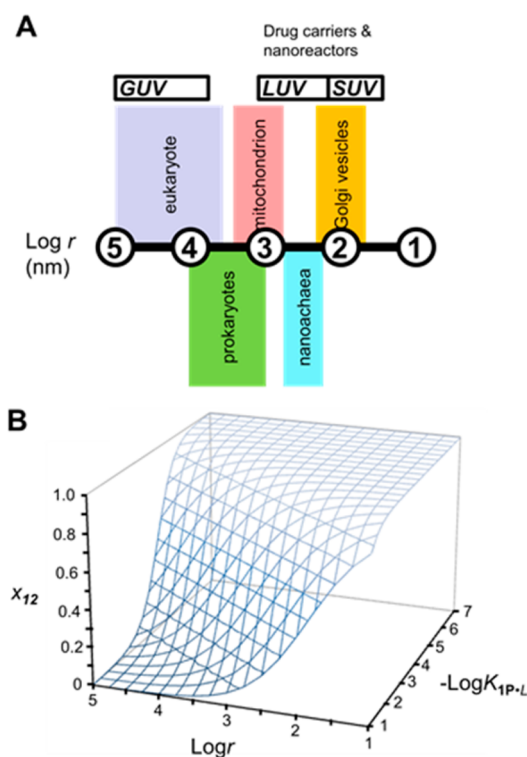
where  $k_{\text{s}}$  is the intrinsic second-order rate constant for the coupling reaction, while the in-membrane concentration, expressed as mole fraction of membrane-associated **2**,  $x_{\text{2-L}}$ , can be calculated from the values of total concentration of **2** and lipid, and the affinity constant  $K_{\text{2-L}}$ .

Combining eqs 6–8, we have

$$x_{\text{12}} = \frac{c}{rK_{\text{1P-L}} + c} \frac{k_{\text{s}}x_{\text{2-L}}}{k_{\text{hl}} + k_{\text{s}}x_{\text{2-L}}} \quad (9)$$

which allows one to estimate the yield of dipeptide **12** as a function of all the relevant parameters—in particular, as a function of the size of the compartment and the affinity of the confined building block for the membrane (Figure 3).

These experiments show that the confinement of a reactive molecule inside a liposome has a strong impact on the reactions it undergoes. Reactions of hydrolysis may be drastically slowed down, to a much larger extent than when found in the presence of lipid vesicles, but not confined within the aqueous cavity.<sup>33</sup> This effect is readily attributed to the larger concentration of



**Figure 3.** (A) Graphical representation of the typical sizes of choice of membrane-bound compartments. GUV, LUV, and SUV stand for giant, large, and small unilamellar vesicle, respectively. Drug carriers and nanoreactors are typically in the size range of LUV and SUV. (B) Yield of the product of coupling as a function of the size of the compartment and the dissociation constant from the membrane of the component confined (**1P**). The simulation has been generated using eq 9, with values of  $k_s = 0.85 \text{ M}^{-1} \text{ h}^{-1}$ ,  $[L] = 0.5 \text{ mM}$ ,  $[2] = 0.3 \text{ mM}$ , and  $K_{2-L} = 1.3 \text{ mM}$ .

lipids that a membrane-impermeable molecule experiences when confined in a small liposome cavity. For example, using non-encapsulated reagents, it can be seen that the suppression of hydrolysis is enhanced with increasing lipid concentration (Supplementary Figure 5 and Supplementary Table 3). Since the concentration of lipids in the cavity is inversely proportional to the radius of the lipid vesicle, it is clear that the stabilization against hydrolysis reactions increases as the compartment shrinks. A reactive molecule in the cavity remains therefore available to react with a membrane-permeable molecule to yield dimers. The overall effect can be approximately emulated in non-encapsulated systems, using large concentrations of lipid (Supplementary Figure 6). In general, eq 9 allows for the quantification of the ability of a lipid-bound compartment to inhibit hydrolysis and promote oligomerization reactions. While enzymatic reactions may not be directly affected by the compartmentalization factor described in eq 9, they may be affected indirectly. For example, depending on the size of the compartment, an enzyme will interact to a larger extent with the membrane, which may modify its catalytic ability. The same reasoning applies to the availability of the substrates. It is therefore conceivable that biomolecular processes are regulated, to some extent, by the size of the compartment in which they take place. It is, however, in the absence of enzymes where the presence of lipids has a clear and measurable effect on the outcome of the reactions.<sup>33</sup> Thus, eq 9 can assist in the design of more efficient nanoreactors with applications as drug carriers,

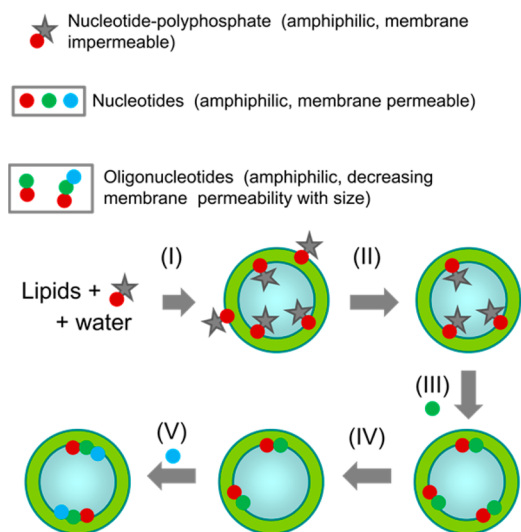
for example, where the correct choice of reagents may lead to a better control of the release of a confined drug.

It is in abiogenesis where the implications of these findings are the clearest. Abiogenesis is a phenomenon that, among other chemical changes,<sup>34–38</sup> requires the oligomerization of simple building blocks into more complex, functional macromolecules. Any process that assists oligomerization and inhibits hydrolysis is therefore an important candidate to have played a role in abiogenesis.<sup>25,26,33,39</sup> The candidacy becomes even clearer when the promotion of oligomerization and inhibition of hydrolysis take place in such a way that yields readily made compartments, or proto-cells, enriched with the oligomeric molecules trapped inside. In the context of abiogenesis, the phenomenon described here will lead to the accumulation of oligomers in the cavity in the presence of (i) a starter monomer that is an amphiphile as well as membrane-impermeable and (ii) additional membrane-permeable, amphiphilic building blocks. In our experiment, the starter monomer is **1P**, which has been chosen for convenience: the pyranine moiety prevents membrane permeation and is a convenient molecular probe; tryptophan is suitably hydrophobic. In prebiotic settings, this role could be fulfilled by a nucleotide derivative bearing an oligophosphate chain as a membrane-impermeable moiety. The rest of the components may include any suitable sugar (including ribose) and a nitrogen base. Interestingly, it has been shown that decanoic acid, a candidate for prebiotic membrane lipids, interacts with the bases and sugar found in RNA.<sup>40</sup> A plausible starting point toward a compartment enriched in polymers would begin with the accumulation of simple lipids (such as fatty acids and fatty alcohols) together with the starter polyphosphate nucleotide. There are a number of natural processes that could bring these components together, including the formation of eutectic ice phases upon water freezing, or simply by evaporation. Upon re-dilution (by, for example, melting of the ice), vesicles would spontaneously form, incorporating the amphiphilic membrane-impermeable starter monomer. Further dilution (as the ice fully melts) would result in the dissociation of the monomer sitting in the outer leaflet of the vesicles, leading to its hydrolysis. By contrast, the fraction located in the inner leaflet would be protected against hydrolysis. The other monomers present in the medium (for example, membrane-permeable monophosphate nucleotides) would associate with the membrane and readily react with the trapped starter, especially if they feature activated versions of the phosphate group (Figure 4).

The reaction may result in the loss of the membrane-impermeable oligophosphate moiety. Nevertheless, larger oligomers are likely to be less membrane-permeable, and, as they extend, the likelihood of them remaining in the cavity for an extended period increases, allowing for further growth of the polymer located in the cavity. The scenario depicted is a plausible sequence of events that would lead to the formation of primordial oligonucleotides from very simple building blocks. A similar mechanism can be depicted for the formation of other functional oligomeric molecules, such as oligopeptides, a possibility that is consistent with a scenario where peptides and oligonucleotides evolve independently in the first instance, being only integrated in the same metabolic network at a later stage.

## CONCLUDING REMARKS

In summary, the phenomenon described here is an emergent property of chemical systems<sup>41,42</sup> featuring small membrane-



**Figure 4.** A plausible scenario for the initial stages of proto-cell evolution. A mixture of lipids and an amphiphilic nucleotide polyphosphate is hydrated (I). Following formation of the vesicles, the nucleotide–polyphosphate associated with the outer membrane is diluted, leading to dissociation from the membrane (II). In the presence of membrane-permeable nucleotides, the formation of dinucleotides is catalyzed by the inner leaflet of the membrane (III). Some dinucleotide may be lost by permeation (IV). Slow permeation of larger molecules may, however, enable the extension of the nucleotide in the inner compartment leading to increasingly less permeable oligomers that would remain trapped (V).

bound compartments. In addition to the clear implications that it has for abiogenesis (or rather, because of these implications), this phenomenon can also inform the development of synthetic biology approaches that relate to the bottom-up design of artificial, cell-inspired devices. These devices may have applications ranging from chemical synthesis (as an alternative methodology for the synthesis of peptides or oligonucleotides) to biosensing and as drug delivery vehicles. The plausibility of these approaches, together with further implications of these results in prebiotic research, is currently being explored in our laboratories.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b06207.

Experimental methods (including synthesis and characterization of **1P**, **2** and **12**, sample preparation, titration experiments, kinetic experiments, and HPLC characterization of the products of reaction), detailed derivation of the equations, and supplementary figures and tables (PDF)

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### Notes

The authors declare no competing financial interest.

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