Peptides at vesicle and mineral prebiotic interfaces

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Abstract

The origin of life likely involved a complex interplay between organic molecules and mineral surfaces, yet the molecular details of these interactions remain poorly understood. Over recent decades, considerable research has focused on the individual roles of key biomolecules—such as RNA, lipids, and proteins—in early abiogenesis. However, this reductionist view offers only a partial picture; the emergence of life likely involved networks of molecular interactions that collectively shaped early functional assemblies. In this study, we examine the potential of peptides—arguably one of the most abundant early polymers—to interact with mineral surfaces and lipid vesicles, prebiotic interfaces and compartments. Focusing on peptide compositions derived from prebiotically plausible amino acids, we demonstrate that early peptides could bind to mineral surfaces (such as fluorapatite studied here) via negatively charged residues and contribute to the release of bioavailable phosphate from geological reservoirs. Nevertheless, the recruitment of amino acids from the modern alphabet was likely adaptive for favourable interactions with lipid vesicles, leading to enhanced vesicle growth and stability. Our findings suggest that while early random peptides could contribute to functionality at geochemical interfaces, the evolving complexity of the peptide/protein alphabet was likely a key factor in the evolution of protocells.

Introduction

Mineral pores, coacervates, and vesicles are believed to have been among the earliest compartmentalization structures on Earth, supporting prebiotic chemical processes essential for the emergence of life by influencing key processes such as molecular assembly, catalysis, and assisting stabilization [1–9]. While extensive research has explored the physicochemical properties of these possible early life compartments, significantly less is known about their interactions with peptides - arguably the most abundant and functionally versatile polymers in prebiotic environments. This is especially evident in the context of mineral pores and fatty acid vesicles, and recent studies that have begun to elucidate the roles of peptides within model complex coacervate systems [10–13].

Multiple studies have demonstrated prebiotic feasibility of up to ~15-mer peptide synthesis under a range of conditions [14–19]. Selected di-/tri- peptides are capable of a range of catalytic activities, such as proteolytic, nucleolytic and esterase activities [20–22]. Moreover, specific short peptides have been shown to bind decanoic acid vesicles and increase vesicle stability to metal ions and high salt concentrations [22,23]. Although these studies imply that peptides were significant contributors to protocell evolution—whether as stabilizers, catalysts, or active participants in primitive metabolic networks—their possible role at prebiotic interfaces has not been explored [23]. Before the emergence of more advanced biosynthetic pathways and full evolution of the canonical amino acid alphabet, only some amino acids were likely abundant on early Earth. Approximately half of the current canonical alphabet has been identified in plausible prebiotic environments, inferred from atmospheric discharge experiments, meteorite/asteroid analyses, and hydrothermal vent studies [24–26]. Additionally, a plethora of non-canonical amino acids have been observed in prebiotic conditions and could have been incorporated into an early statistical collection of peptides, before the onset of templated synthesis and selection of the life's amino acid alphabet. Compared with alternatives from the prebiotic pool of amino acids, the "early" set of canonical residues shows exceptional distribution of volume and hydrophobicity, emulating the patterns found in the full canonical alphabet [27]. Furthermore, peptides composed of this "early" amino acid set have greater secondary structure propensity than other tested prebiotically available alternatives and would better support protein folding, despite the absence of basic residues [28–30]. These early-alphabet peptides were shown or hypothesized to be capable of metal and organic cofactor binding, interaction with RNA, and potentially serve as functional hubs, bridging the gap between abiotic and enzyme catalysis [31–34].

Beyond catalysis, mineral surfaces can act as adsorption scaffolds and can prevent the "diluting out" problem of origin-of-life scenarios [3,6,35], offering a primitive form of compartmentalization. Peptides frequently utilise acidic residues such as Asp and Glu for specific binding to minerals, suggesting that early canonical peptides would have the propensity to adsorb onto mineral surfaces [36–40].

Fatty acid membranes and prebiotic peptides are assumed to have coexisted in the same environments on the early Earth. Membrane forming molecules such as single-chain amphiphiles would have been widely available for vesicle formation [41], particularly in hydrothermal systems where Fischer-Tropsch Type syntheses readily produce a range of relevant SCAs [42]. In addition, fractions of Murchison meteorite were shown to self-assemble into vesicles [43]. Yet what shaped the

co-evolution of membranes and peptides is not yet well known. Specific peptides were shown to influence vesicle growth, morphology, and stability. Hydrophobic and amphiphilic peptides integrate into lipid membranes and can promote vesicle growth and protect against disruptive effects of NaCl and divalent cations [44–49]. Individual hydrophobic amino acids like valine, isoleucine, and leucine stabilize membranes and induce greater lamellarity (amount of membrane per vesicle) [22,44], while less hydrophobic amino acids, such as glycine and alanine, show weaker interactions [45,49]. Positively charged amino acids like lysine and arginine can enhance membrane stability and are crucial for membrane association and penetration [48]. Overall, most individual amino acids exhibit some degree of interaction with fatty acid membranes [45,46,50,51].

Despite their potential significance, the interactions between peptides, vesicles, and minerals in prebiotic context remain understudied. This reflects a long-standing divide in origin-of-life research, where compartmentalization, peptide chemistry, and mineral catalysis have often been investigated separately rather than as interconnected processes [52]. Most existing studies thus far have focused on the impact of individual, shorter peptides, leaving the role of the representative prebiotic sequence space unexplored. Here we synthesize 8-mer peptide libraries representing different prebiotically plausible compositions and explore their propensity to interact with minerals and vesicles.

Results and discussion

Peptide library amino acid composition affects the extent and nature of peptide-vesicle and peptide-mineral interaction

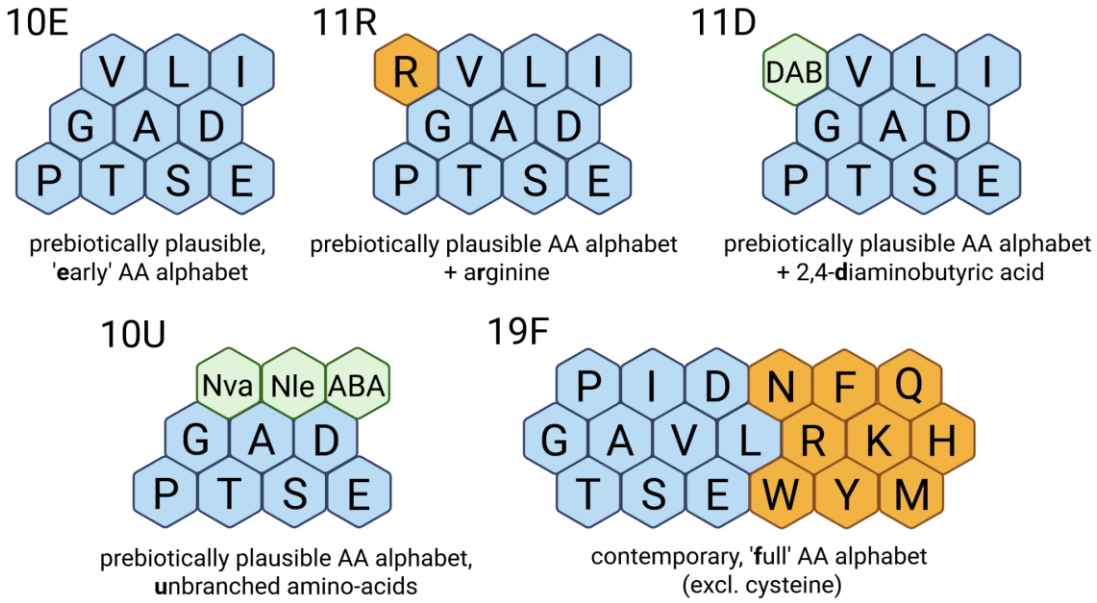

Figure 1. Amino acid composition of the analyzed random peptide libraries. All libraries were composed of 8-mers with entirely random sequences. Blue hexagons denote prebiotically available amino acids [25]; orange hexagons denote "late" amino acids that require biosynthesis; green hexagons denote prebiotically available amino acids that were not incorporated into the genetic code (non-canonical amino acids).

Representing prebiotically plausible lengths, we synthesised five different 8-mer peptide libraries (Figure 1). Their amino acid content is equimolar, and amino acid repertoire of the libraries resembles either the contemporary amino acid alphabet ("19F", with the exclusion of cysteine due to the Fmoc-based peptide synthesis restrictions) or prebiotically plausible[25] amino acid alphabets, previously studied for their biophysical properties[29]: specifically, the "10E" library consists of ten early canonical AAs; the "10U" library includes unbranched prebiotically available analogues replacing the branched residues in 10E; and the "11D" library expands 10E by adding diaminobutyric acid (DAB), a prebiotically available basic amino acid [53]. Finally, the "11R" library includes arginine to assess the potential functional impact of a contemporary positively charged residue on prebiotic evolution (Supplementary Figure 1). Although not typically detected in meteorites and atmospheric discharge experiments, plausibility of arginine prebiotic synthesis has been proposed from the cyanosulfidic protometabolism [54]. The hydrophobicity and secondary structure propensity of the libraries was previously described [29]. When arranged by hydrophobicity, highest to lowest, it follows 19F > 11R > 11D > 10U > 10E, and 11R > 11D > 19F > 10E = 10U when arranged by the average charge (from positive to negative).

We tested how peptide libraries of different amino acid compositions interact with mineral and vesicle surfaces. N-decanoic acid and n-decanol (DA:DOH) vesicles were selected as a prebiotically plausible vesicle system, as reported in previous studies [22,44]. The mineral fluorapatite was selected due to its abundance and prebiotic availability, as well as being a debated, yet potentially abundant source of phosphate in prebiotic chemistry [55,56]. Fluorapatite is more stable than hydroxyapatite and is commonly present in geological contexts on the Earth and Mars [57,58]. Peptide interactions were studied by incubation with DA:DOH vesicles or fluorapatite, followed by filtration for vesicle study, and centrifugation and release of bound peptides for fluorapatite. Resulting batches were then submitted to amino acid analysis.

Every peptide library screened exhibited minor binding propensity towards fluorapatite, with approximately 1-5% of peptides within the library bound under the studied conditions. We find a several-fold increase in binding of more negatively charged peptide libraries (10E and 10U) compared to more positively charged ones (19F, 11R, 11D), suggesting an importance of negatively charged amino acid residues for mineral binding. Moreover, across all studied libraries, aspartate and glutamate residues were enriched in the peptides bound to fluorapatite (Supplementary figure 2), consistent with their known affinity for apatite through coordination to calcium ions [59,60]. This enrichment happened even in libraries 19F and 11R, where negative charge was less prominent due to the presence of positively charged residues and due to the potential cross-binding of basic residues with the acidic ones within the library, blocking peptides from mineral interaction.

A previously reported zero charge point of fluorapatite is at pH of 6.8 [61]. Our experiment was conducted at pH 5.5, in which the mineral would have a net positive charge. This might also explain the higher binding of negatively charged peptide libraries like 10E. The fluorapatite used in our experiments was sintered at a temperature similar to that on prebiotic Earth (700 ℃), suggesting similar peptide-binding capacity of fluorapatite [62].

Next, we evaluated the binding propensity of peptide libraries to DA:DOH vesicles. We first confirmed the presence of vesicles using transmission electron microscopy and fluorescent imaging

(Supplementary figure 3). Upon incubation with vesicles, peptide concentrations varied depending on amino acid composition (Figure 2, C). Amicon Ultra-4 3K centrifugal filters have been used to separate the vesicle-bound and unbound peptides as reported in previous studies [22,63]. Nevertheless, we observed considerable binding of peptides to the filter itself and therefore only relative comparisons between the different libraries could be analyzed with confidence. As such, we find that 10E and 10U libraries have ~80 % higher peptide concentration in the unbound phase than libraries with positively charged amino acids (19F, 11R and 11D), suggesting that positively charged amino acids play a role in peptide-vesicle binding association, in agreement with previous reports on basic amino acid behaviour [15,44,48,64]. Interestingly, the 11D library (containing DAB) displayed a similar degree of vesicle binding as the 11R library (containing arginine), suggesting that DAB may modulate peptide binding to fatty acid vesicles, despite its shorter side chain compared to Arg. However, in the search of specific residues that would modulate binding to vesicles, we do not find substantial changes in amino acid distribution of peptides filtered from vesicle solution (Supplementary figure 4).

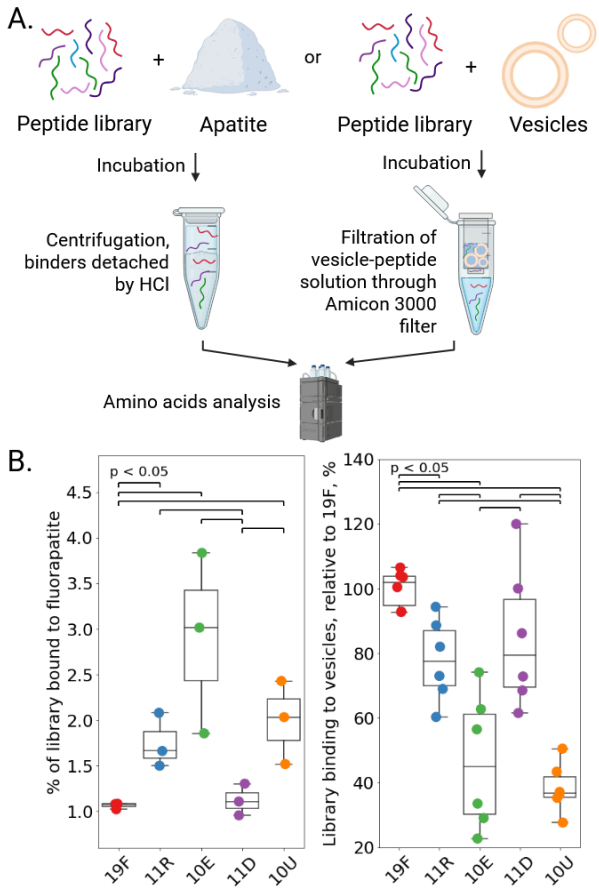

Figure 2. Peptide libraries association with prebiotic surfaces. A. Outline of experimental pipeline for establishing maximum adsorption of peptide libraries to fluorapatite and DA:DOH vesicles. Since it is not feasible to centrifuge fatty acids down and alcohols interfere with absorbance at 220 nm, HPLC was used to establish reduction in relative peptide concentration for both setups. B. Drop in peptide concentration upon incubation with fluorapatite (left) or DA:DOH vesicles (right), as derived from amino acid analysis.

Peptide library binding enables phosphate release from apatite

Given the binding of prebiotically plausible peptides to fluorapatite, we asked whether it could impact phosphate release from the mineral. We employed the molybdenum blue assay (Figure 3, A) and calibrated phosphate release against a standard curve (Supplementary figure 5). We find that all screened libraries induced phosphate release from the mineral, increasing the efficiency up to two-fold compared to the control (Figure 3, B). We do not find unequivocal effects of different amino acid compositions on the phosphate release. Every peptide library had Asp and Glu in their composition, and we suggest that Asp and Glu coordinating with calcium ions shifted the equilibrium towards the phosphate release. This finding aligns with previous reports on calcium removal mediating phosphate release from apatites [65]. Binding of each of the peptide libraries induces phosphate release at the acidic pH of 5.5, which is higher than the previously reported cutoff of pH 4 of efficient phosphate dissolution [65], and thus we hypothesize that peptides – particularly those rich in Glu and Asp – could have helped to shift the equilibrium towards phosphate release in a pH range closer to neutral. The extent to which specific binding to phosphate is possible by short, prebiotic peptides remains unclear [66].

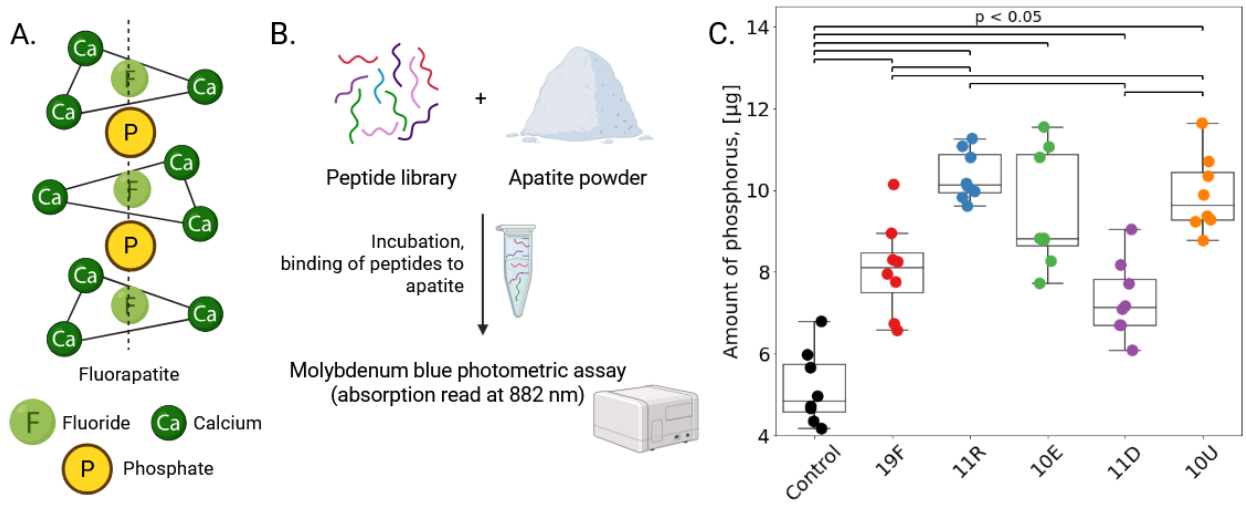

Figure 3. A. Partial schematics of fluorapatite $Ca_{10}(PO_4)_6F_2$ structure [60,67]. B. Outline of experimental workflow for establishing the amount of phosphate released from fluorapatite upon different peptide library presence conditions. We used molybdenum blue photometric assay[68] in order to establish the amount of phosphorus in the sample through phosphate measurement. C. Phosphate release from fluorapatite, as measured after the addition of each peptide library under different pH and temperature. Error bars - standard deviations from 8-plicates.

Peptide libraries affect vesicle stability

We explored whether there is composition-dependent impact of peptide libraries on prebiotically plausible vesicles. The Hadean Ocean, in which life is hypothesized to have emerged [69], probably had a significant salt content [70]. Both NaCl and divalent cations are necessary for life, yet both were reported to collapse and flocculate (aggregate) prebiotically plausible vesicles, although it should be noted that this effect would be likely alleviated in prebiotic environment by the intrinsically high heterogeneity of amphiphilic membrane constituents [22,63,71]. Previous reports indicate protective effects of some (but not all) amino acids and di- and tri-peptides against salt-induced flocculation[44], yet, to the best of our knowledge, no research on longer and more compositionally diverse peptides

was conducted in a context of prebiotic evolution. We therefore measured changes in vesicle abundance and stability in the presence of peptide libraries with and without addition of NaCl and $MgCl_2$ by spinning-disc fluorescent microscopy and dynamic light scattering (DLS). This was accompanied by measurements of optical density at 490 nm (OD_{490}), which was reported to correlate with the level of multilamellarity and flocculation [22,63].

As such, we observe that the effects of peptide libraries on vesicle stability are composition-dependent and emerge under disruptive conditions. Vesicle morphology, OD_{490}, and average particle size remained largely unaffected in control conditions, regardless of peptide library presence (Supplementary table 1). Nevertheless, we find a number of peptide library-dependent morphological and physicochemical changes in the samples upon addition of NaCl and $MgCl_2$.

We find a large increase in OD_{490} (Figure 4, A) upon addition of $MgCl_2$, if peptide libraries were present in the sample. This effect can partially be explained by the formation of punctate structures[22] and/or multilamellar vesicles, depending on the peptide library used (Figure 4, B, images M-R). The destabilization of vesicles in the presence of both peptide libraries and divalent cations may originate from increased osmolarity or disruption of hydrogen bonding networks. These effects seem to be negated by the late canonical amino acids present in the 19F peptide library. We hypothesise a potential benefit for their incorporation into life's repertoire, assuming that more complex vesicle systems would face the issues of salt-induced instability.

Upon addition of NaCl to peptide-vesicle samples (with the exception of the 19F library), we observe formation of filamentous structures that resemble growing and budding [72] fatty acid membranes (Supplementary Figure 6). These filamentous structures are especially pronounced in presence of 11R, 11D and 10U libraries (Figure 4, B, images I, K and L). Curiously, these libraries also show the greatest disruption in vesicle formation upon addition of $MgCl_2$ (Figure 4, B, images O, P and R). The peptide library effects are then ambivalent: while induction of vesicle growth and budding in the presence of NaCl might be more beneficial than flocculation, collapse of vesicles into punctate structures in the presence of $MgCl_2$ indicates potential loss of compartmentalisation.

Amino acid composition of peptides seems to be the driving force for the above-mentioned effects. We suggest that positively charged and unbranched amino acids enhance peptide interaction with fatty acid bilayer when compared to other peptide libraries. In the case of the 10U library, this might be possibly due to the longer chain length of unbranched residues like norvaline and norleucine facilitating fatty acid packing and/or membrane penetration, as well as higher peptide flexibility being potentially beneficial for fatty acid/peptide alignment. We suggest that life could have benefited from transiently employing non-canonical amino acids (ncAAs) in peptide structures to balance vesicle flocculation, growth and stability. Interestingly, it aligns with previous reports on ncAAs such as DAB or ornithine being beneficial for peptide-RNA coacervation, which is suggested to have played a role in prebiotic evolution as an auxiliary compartmentalisation solution [73–75].

The effects of arginine incorporation into library composition are more pronounced than that of DAB, a prebiotically available positively charged AA. It aligns with previous studies showing the uniqueness of guanidinium group, such as "top" and "bottom" face asymmetry of the guanidium

group and its functionality as a multiple hydrogen bond donor [64,76]. This further solidifies arginine's position as a unique amino acid of evolutionary importance.

The 19F library, despite not showing definitive protective effects against flocculation, seems to preserve vesicle integrity in the presence of $MgCl_2$, as indicated by microscopically observable membranes (Figure 4, B, images G and M). Its low solubility [29] and the inclusion of aromatic and "late" basic amino acids might be driving the library's effects. These results suggest a potential evolutionary benefit to incorporate late cAAs in order to support membrane integrity.

To test whether the effects of the libraries could be attributed to altered membrane formation propensity, we measured critical vesicle concentration (CVC), using pyrene fluorescence in vesicle-peptide dilutions. All libraries caused only a modest increase in CVC from ~1 mM to ~1.2-1.4 mM of DA:DOH. We find, then, that the changes in vesicle stability cannot be solely explained by an increase in CVC.

Put together, our findings suggest potentially ambivalent effects of longer peptides and expand on previous reports, where individual amino acids and short peptides typically had neutral or stabilizing effects on prebiotic vesicles [22,45,50,64]. We propose that the longer peptide length (8-mers) in our study could allow for secondary structure formation (≥4 residues for alpha-helices, ≥5 residues for beta-strands [77,78]), and these structures could be integrating into the fatty acid membranes with disruptive effects. Longer, hydrophobic peptides might arguably integrate into the bilayer, consistent with a prior simulation showing disruptive effects of Leu-Leu dimers [44].

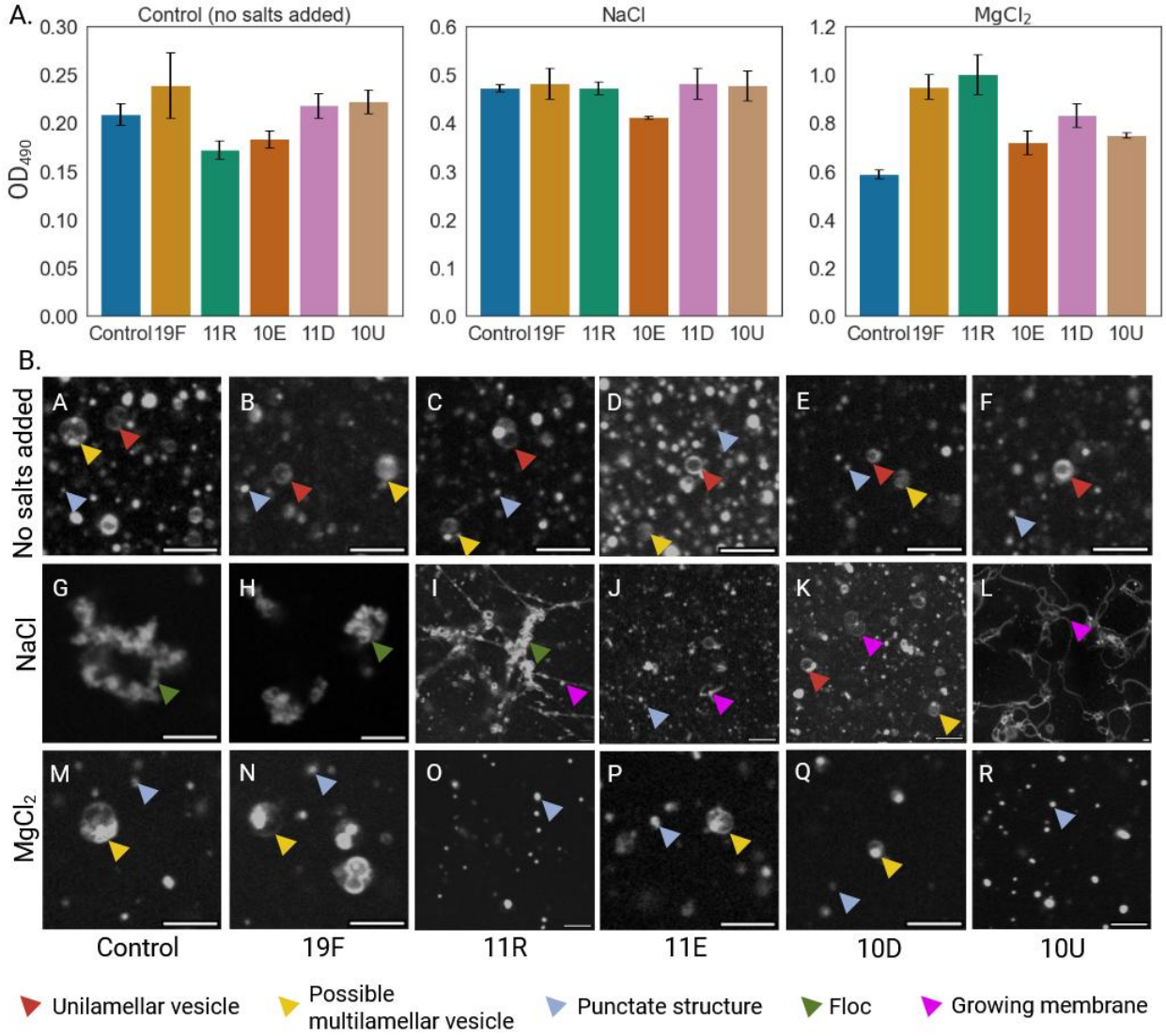

Figure 4. A. Optical density at 490 nm for vesicles-peptide library samples ("19F", "11R", "10E", "11D", and "10U", peptide libraries added to free fatty acids before vesicle formation) under three conditions: no salts added, NaCl (363 mM final) and $MgCl_2$ (8.9 mM final). Error bars represent the standard deviation from triplicates. B. Microscopic images of DA:DOH (1:1) vesicles under different conditions. Vesicular flocculation can be observed upon addition of NaCl, as well as reduction in the number of visible particles upon addition of $MgCl_2$. Staining with Rhodamine 6G. Scale bar: 5 μm; Red arrow - unilamellar vesicle; yellow arrow - possible multilamellar vesicle; blue arrow - punctate structure; green arrow - flocs, pink arrow - growing membranes.

Addition of $MgCl_2$ or NaCl enhances peptide library co-localisation with DA:DOH vesicles

Peptide co-localisation with prebiotic vesicles is poorly understood [44]. Surface charge is of importance to the vesicle-peptide interactions and can mediate peptide's binding to membrane surfaces [79]. We used ζ-potential measurements to assess the correlation between the vesicle behaviour and their relative charge. We find that 38.5 mM DA:DOH vesicles have a ζ -potential of -61.5±4.2 mV; in presence of 363 mM NaCl and 8.9 mM $MgCl_2$, the ζ -potential drops to -17.6±9.2 mV and -31±0.4 mV, respectively (Supplementary table 1). This likely contributes to vesicle flocculation: since vesicles' surface charge is negated by the presence of ions, they are subject to much less

repulsion, and, as such, tend to flocculate. Addition of peptide libraries only slightly reduces the ζ -potential (with the greatest reduction observed for 10U library, at -49.9±0.9 mV), so we argue that ζ -potential might not be a decisive factor mediating peptide libraries effects on vesicles, but that the reduction of ζ -potential in the presence of salt and divalent cations might ease peptide's association with fatty acids.

To visualize peptide-vesicle co-localisation, we labeled peptide libraries with canonical amino acid alphabet (19F, 11R, and 10E) with TAMRA and imaged them using spinning-disc microscopy alongside DiD-stained vesicles. We observed that even peptide libraries with weak vesicle association in prior filtration assays exhibited marked co-localization with vesicle membranes upon the addition of NaCl and Mg^{2+} (Figure 5), possibly due to the above-mentioned reduction in vesicles' ζ-potential. We propose that the neutralisation of repelling forces on both membranes and peptides by NaCl and Mg^{2+} increases co-localisation of peptides and fatty acids, and that Mg^{2+} can also act as a "bridging" agent between these molecules. We argue that this co-localisation of peptide libraries indicates potential association, binding or even membrane integration, as recently reported by molecular dynamics simulation [44].

Notably, we find visual co-localisation of peptide libraries with DA:DOH membranes if peptide libraries were added to free fatty acids prior vesicle formation; when added to pre-made vesicles, no observable co-localisation occurred between DA:DOH vesicles and 10E and 10R libraries. We suggest that peptide libraries could integrate into the forming membranes and potentially modulate membrane formation, as indicated by the relative changes in OD_{490} in earlier experiments. In contrast, peptides added to vesicle post-assembly could likely be repelled by the negative surface charge of vesicles.

Although DA:DOH vesicles were reported to allow the passage of molecules through the membrane [80], we did not find substantial accumulation of fluorescently labelled peptides in intravesicular lumen by microscopy if peptides were added to pre-made vesicles (Figure 5). We hypothesise that, due to their composition, at least 19F and 11R libraries could potentially permeate through the fatty acid membranes, but, due to the short time period in which the measurements were conducted upon the formation of vesicles (~5-10 minutes), the membrane permeability should not have been substantial enough to observe.

Overall, our results suggest that peptide localisation relative to fatty acids is of high importance to the emergent effects. Given that peptides, free fatty acids, and self-assembled vesicles could conceivably coexist in the same prebiotic environment [42,81], we argue that there could have been a mix of both integration and surface binding of peptides into early organic interfaces.

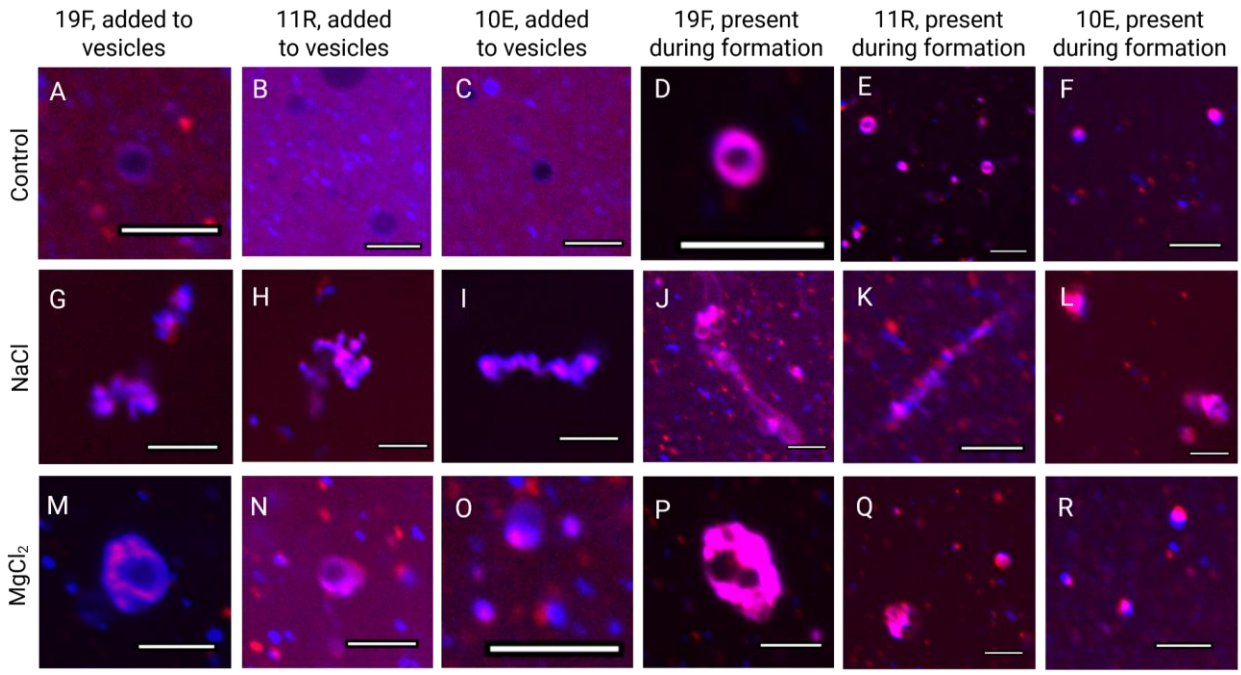

Figure 5. Fluorescence microscopy images of vesicles with peptide libraries either added to already formed vesicles (columns A-C, G-I, M-O) or present during vesicle formation (columns D-F, J-L, P-R) under three conditions: Control (top row), 363 mM NaCl (middle row), and 9.1 mM $MgCl_2$ (bottom row). Peptide libraries (19F, 11R, and 10E) are stained with TAMRA (red channel), while lipids are stained with DiD (blue channel). Addition of NaCl (middle row) induces vesicle aggregation and peptide co-localisation with the membranes (columns G-I), with similar, yet less pronounced effects upon addition of $MgCl_2$ (columns M-O). Peptide libraries present in the solution before vesicle formation (columns D-F, J-L, P-R) consistently exhibit more stable co-localisation with vesicular membranes across conditions. Scale bars represent 5 µm.

Limitations of the study

The distribution of amino acids within peptide libraries studied is equimolar, that is, flat and not representing amino acid distribution of contemporary or prebiotically generated peptides. As such, we focus on amino acid composition as the primary library quality, even if it evades the inquiry into the effects of amino acid distribution as a result.

Libraries used in our study are a uniform mix of unique peptides. Individual sequences with exceptional, emergent properties were thus not accessible to the experiments conducted in this work. This, then, explains why the effects of the studied libraries on prebiotic interfaces are partially in line with the properties of individual constituent amino acids.

Both mineral surfaces and fatty acid vesicles are complex systems that require additional caution when approaching methodologically. As such, data on fluorapatite binding might be dependent on the particle size, surface area and amount of mineral used. Relatively low binding of libraries, alongside a potential heterogeneity of the mineral used, impedes high resolution of binding propensity of peptides. Fluorapatite used in our experiment is a pure mineral, lacking impurities that might influence its physicochemical properties in an actual prebiotic scenario. Lastly, our experimental setup lacked geologically relevant flow chambers or similar setups, which would greatly impact the mineral properties, in line with previous reports [65].

We also find that minor changes in the pH of the final solution and the timing of sample shaking lead to observable changes in vesicle stability (Supplementary Figure 7). As such, quantitative

description of vesicle stability and morphology should be approached with caution due to the great noise brought by the sample handling. Nevertheless, since the same vesicle stock solution was used for every condition screened, we can rely on comparative analysis of the peptide libraries' impact on DA:DOH vesicles.

OD_{490} was previously reported to be a correlate of multilamellarity and flocculation [22], and our results suggest that oil droplets might further impact this measurement. As such, OD_{490} proves to be a rather indirect indicator of vesicular stability in the context of our study, and it required further analyses such as microscopical description, DLS, and zeta-potential study.

Lastly, our study exclusively explored only a single variant of prebiotically plausible vesicles and minerals as models for potential prebiotic interfaces. We do not exclude that there is possibility for a great variety in the peptide-interface interactions depending on the model mineral or membrane forming molecule(s) used.

Conclusions

As plausible predecessors of proteins, peptides likely helped to shape the path between the chemical networks provided by geochemistry and life's biochemistry as we know it. Besides their structural and catalytic potential, the interaction of peptides with other prebiotically abundant species could have driven the chemical evolution of the amino acid alphabet towards its biological repertoire. In this study, we explored the effects of peptide compositions on their propensity to interact with vesicles and mineral surfaces using vast peptide libraries.

Based on the results presented by our study, we suggest that early peptides interacted favourably with mineral surfaces, even if compositionally limited to only prebiotically available amino acids. Yet it appears that there was a need for the expansion of the amino acid alphabet in order to favour lipid-peptide interactions and improve fatty acid membrane stability. In conclusion, our results imply that the evolution of the peptide/protein alphabet was an important factor *en route* to protocell emergence.

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Authorship confirmation/contribution statement

Ivan Cherepashuk: investigation, validation, formal analysis, writing - original draft, review & editing, visualisation (lead); conceptualisation, methodology, funding acquisition (equal).

Mikhail Makarov: conceptualisation, investigation, methodology, funding acquisition (equal).

Radko Soucek: investigation, resources, methodology (equal); writing - original draft, review & editing (equal).

Robin Krystufek: resources, investigation (supporting).

Romana Hadravova: resources, investigation (supporting).

Valerio Guido Giacobelli: supervision, conceptualisation, project administration, writing - original draft, review & editing, visualisation (supporting).

Kosuke Fujishima: supervision, conceptualisation, project administration, funding acquisition, writing - review & editing (equal).

Sean F. Jordan: supervision, conceptualisation, methodology, writing - review & editing (equal).

Liam M. Longo: supervision, conceptualisation, methodology, writing - review & editing (equal).

Klara Hlouchova: supervision, conceptualisation, project administration, funding acquisition, writing - review & editing (lead).

Authors' disclosure statement

The authors declare that there are no conflicts of interest related to this work.

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