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Mineral surface chemistry control for origin of prebiotic peptides

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Some seventy years ago, John Desmond Bernal proposed a role for clays in the origin of life. While much research has since been dedicated to the study of silicate clays, layered double hydroxides, believed to be common on the early Earth, have received only limited attention. Here we examine the role that layered hydroxides could have played in prebiotic peptide formation. We demonstrate how these minerals can concentrate, align and act as adsorption templates for amino acids, and during wetting—drying cycles, promote peptide bond formation. This enables us to propose a testable mechanism for the growth of peptides at layered double hydroxide interfaces in an early Earth environment. Our results provide insights into the potential role of mineral surfaces in mimicking aspects of biochemical reaction pathways.

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The emergence of life, the point of transition from organic geochemistry to biochemistry, is one of the enduring unresolved questions in science. Though even defining life is still a matter of debate, the abilities to metabolize and replicate with inheritable mutations are essential criteria for Darwinian evolution. Life as we know it today, is dependent on the fidelity of information transfer through sequential polymeric systems, i.e. nucleic acids and peptides, interconverting structure into information, and information into structure.

Following Wöhler's abiological synthesis of urea, many hypotheses have been proposed for the origin of life through molecular evolution, in which simple organic molecules, whether they be amino acids, carbohydrates, or nucleosides, are initially formed from simple abiotic reactants. This synthesis of simple starting components is followed by the subsequent polymerization of organic monomers into biomolecules of increasing complexity and function. It is the latter step into which we seek to add insight by this present study. A number of challenges arise when attempting to understand how proto-biological monomers can form oligomers, which in time may become both functional and capable of Darwinian evolution. In living systems, biological catalysts and enzymes fill this role working through a variety of mechanisms based on the active sites and tertiary structures formed by proteins.

The idea of using hydrated mineral surfaces to replace biological catalysts on a pre-biological Earth dates to the 1940s, following Oparin and Bernal[1, 2]. Such surfaces offer sites at which simple monomers can concentrate from dilute solutions. Some of these surfaces may have very high enthalpies of rehydration, providing a driving force for condensation reactions. As such, hydroxides, silicates, carbonates, and borates have all been studied as reagents capable of aiding the polymerization of prebiotic monomers[3]. The internal surface of minerals, whether pores in three-dimensional systems or interlayer regions in two-dimensional systems, also offers a safe haven for nascent biopolymers from the effects of UV radiation[4].

Amino acids provide an interesting starting point for studying mineral—biomolecule interactions[5]. Protein—mineral interactions are prevalent in many biomineralization pathways, as well as are of interest to early Earth chemistry. Unlike nucleic acids for which a plausible prebiotic synthesis route is still being developed[6], the presence of amino acids on the Hadean Earth is considered very likely. Amino acids have been found in meteorites and other cosmic bodies[7, 8], which indicates a simple chemical synthesis process in the interstellar medium. Experimental evidence is also available for the abiotic synthesis of amino acids, under a variety of potential early Earth environmental conditions[9–12]. The charge of amino acids is pH-dependent and, hence, allows their association with different minerals in different environments.

A potential drawback of the mineral catalyzed synthesis of biopolymers is that polymers, with multiple points of attachment to a mineral surface, can be inherently hard to remove; as Lambert[3] noted, "If the initial steps of life really occurred on surfaces, how then did life escape surfaces at a later stage?"; thus, a full transition to biopolymers would require a significant change in external conditions to remove the polymer (or dissolve the mineral). An additional hurdle is that, whereas many negatively charged biomolecules exist, there are relatively few mineral surfaces with net permanent positive charge. The most common hydrated aluminosilicate minerals all carry net negative layer charges owing to permanent isomorphous substitutions. In order to act as catalytic templates for negatively charged biomolecules (i.e. nucleic acids or proteins at pH > 7), charge inversion is required through, for example, bridging cations.

One of the challenges in studying early Earth biomolecule evolution pathways is thus, to identify plausible environments and conditions for the occurrence of pre-biotic chemistry. Following the seminal work of Russell and Martin, low to mid-temperature alkaline hydrothermal systems have been shown to be suitable[13, 14]. Present-day analogs such as, the lost city hydrothermal vent field (LCHF) have also attracted much research interest since their discovery in 2000[15]. The LCHF is believed to have been active for 30,000 years. More recently, Price has been investigating the Strytan Hydrothermal Field, a Lost City-like Hydrothermal Vent in shallow waters. These groups of vents have high pH of 9–11, and temperatures of 70–150 °C. The vents are powered by exothermic serpentinization reactions. Owing to their likely ancient existence on Earth, their exothermic nature and presence of layered ordered inorganic materials, these vents could be a plausible location for life's origin.

Many studies have been carried out on silicate clays, to study their potential role in the formation of protobiomolecules[3, 16–19]. In contrast, layered double hydroxides (LDH), which also exist in hydrothermal vents and were common in early Earth[20], have attracted only limited attention[21, 22]. LDH materials are mixed brucite like clays with positive layer charges, created by the substitution of 2+ with 3+ metal ions. These positive charge sites give rise to ion exchange properties, allowing LDHs to concentrate amino acids, and act as templates for polymerization, as well as protecting reaction products.

Characterization of the complexes between small monomers and hydrated mineral surfaces is difficult, especially when the interactions occur in internal pores or between the layers of clay or clay like minerals. Addressing this area has shown the combination of methods in computational and experimental chemistry, with the former providing atomic and molecular level insight of mineral—organic interactions. Molecular dynamics simulations have notably been employed by Coveney and co-workers to study the interactions of nucleic acids with aluminosilicates and LDHs[23, 24]. Whereas nucleic acid—surface interactions have been studied in some detail, there remains a remarkable paucity of simulation data for peptide—LDH interactions.

We carry out a large scale computational modeling study of interactions between amino acids and LDHs under reducing early Earth conditions. Our LDH layers have the composition of $[Mg_3Al(OH)_8]^+$, while interacting with amino acids and peptides are deprotonated at pH 9.5. For this study, we chose a variety of amino acids (alanine, aspartate, leucine, lysine, histidine, and tyrosine), their mixtures, di- and hexa- peptides, and a randomly created 24-amino-acid-long peptides from the amino acid distributions mimicking naturally occurring ones. Analysis of diffusion, adsorption and arrangement of amino acids onto the LDH surface, as well as their concentration dependence and trends in a wetting—drying cycle, reveals possible mechanisms for peptide formation.

Results

Intercalation of amino acids affects LDH layer dynamics. Irrespective of the identity of the amino acid, the LDH interlayer dehydrates with the same trend, indicating that the basal *d*-spacing is proportional to the number of atoms (organic load, Fig. 1) present in the interlayer, rather than the charge on amino acids (Supplementary Fig. 2a). Moreover the distance between two adjacent LDH layers is not constant across an interlayer, because the LDH layers display undulations (Supplementary Fig. 2b) caused by an aggregation of amino acids that locally bridge the layers, thus reducing the local *d*-spacing. The water expelled from these regions leads to swelling of neighboring areas. This

peristaltic-like phenomenon is particularly apparent in the case of leucine and tyrosine at 15 waters per amino acid (W/AA). Leucine has a large and strongly hydrophobic side chain, which allows leucine molecules that are adsorbed onto opposing LDH layer faces to interact, thus pulling the layers together. Where no such interaction is possible, the expelled water aggregates, and then leucine molecules rearrange, pointing their hydrophilic C-terminals towards the accumulated interlayer water to shield their hydrophobic side-chains. In the case of tyrosine, undulations arise from an interaction between an OH group of the side-chain of one amino acid, and a C-terminal of the other. This is due to the competition between the hydroxyl groups of the LDH and tyrosine. At 10 W/AA both tyrosine and leucine side-chains are long enough to interlock with the opposing ones, making the *d*-spacing constant with low-amplitude undulations. In the case of aspartate, at 20 W/AA (i.e. 10 W/Al) some amino acids are able to bridge two layers via a mediating molecule of water. This again brings the layers together, creating larger undulations. At 15 W/AA nearly all aspartate molecules are able to bridge across to the opposing layer either via bridging water or directly, creating evenly layered LDHs.

Amino acids and peptides adsorb on LDHs via their C-termini. All systems, except lysine, show a constant increase in amino acid adsorption on the LDH surface (Fig. 2a) upon dehydration. At hydration greater than 7 W/AA, approximately 75% of amino acids are adsorbed, with nearly all amino acids adsorbed at lower hydrations. At higher hydration levels aspartate shows preferential adsorption via its backbone than its side-chain (by a factor of 1.3). This is because the carbon in the C-terminal has a lower positive partial charge (0.34) than the carbon in the side-chain carboxylate (0.62). Notably, some aspartate molecules adsorb via both the side-chain and the backbone. Below 10 W/AA both backbone and side-chain carboxylates are fully adsorbed. Zwitterionic lysine shows a steady increase of adsorption from 30% at the highest hydration towards 90% at no interlayer water. Here the side-chain is strongly positive, and therefore interacts with the carboxylate of the backbone of neighboring molecules, reducing the adsorption on the LDH surface. In the case of peptides, shorter chains show higher adsorption than longer ones (Fig. 2b). In the case of aspartate, there is still a preference for adsorption via the backbone than by a side-chain factor of 1.3. Importantly, simulations of peptide mixtures (MIX3 and MIX4) indicate that further increase in chain length does not lead to a substantial decrease in their adsorption.

Arrangement of adsorbed species is templated by LDH structure. In order to detect possible templating effects of the LDH on the arrangement of adsorbed amino acids, we analyzed the radial distribution function (RDF) of amino acids' C-terminal oxygen atoms with respect to LDH's aluminum (Supplementary Fig. 3). In both the cases of pure systems and amino acid mixtures (MIX1 and MIX2), peaks matching the distribution of aluminum atoms are observed, indicating that the arrangement of amino acids' C-termini onto the LDH surface is templated by LDH charging sites.

To gain further insights into the arrangement of amino acids, we analyzed their orientation with respect to the surface. At high levels of hydration, both amino acids and peptides mostly adsorb by their C-termini. Upon dehydration, backbones uniformly arrange, so that C-termini oxygen atoms either adsorb on the same surface or bridge between two adjacent ones (Supplementary Fig. 4a, b). These observations indicate that dehydration enforces specific alignment on adsorbed species.

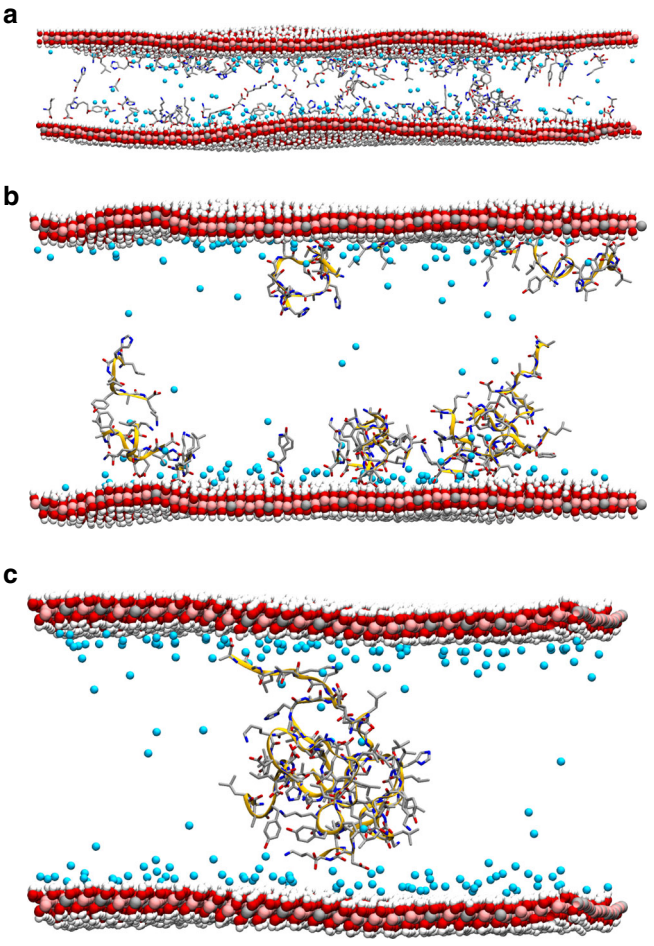

Fig. 1 Amino acids and peptides intercalated in hydrated LDH. Example of modeled systems, showing how natural mixtures of **a** amino acids, **b** short, and **c** long peptides adsorb onto the LDH interlayers via their C-terminal. Colors are as follows: Mg, pink spheres; Al, gray spheres; Cl, blue spheres; O, red; H, white; N, blue; and backbone is represented with a yellow spline. For clarity water and H atoms on the amino acids are not shown

LDHs promote amino acid polymerization. Deprotonated amino acids hold a strongly negative charge on the carboxyl side that prevents nucleophilic attack by the amino group. Our quantum mechanical calculations show that amino acid adsorption onto the LDH surface allows redistribution of charges (from −0.41e to −0.23e on oxygen and 0.07e to 0.18e on carbon), thus activating the carboxylic group for subsequent peptide bond formation (charges given in Supplementary Table 2 and Supplementary Fig. 7).

When concentrated on the LDH surface, amino acids are prone to co-alignment. Figure 3b highlights close contacts between C- and N-termini. Upon dehydration C-termini are observed to interact with the LDH in multiple ways, all providing an accessible site for potential polymerization (Fig. 3c). We quantified the amount of these sites as a function of hydration, obtaining a count of reactive pairs. While hydrated systems (over 7 W/AA) only feature a small number of pairs (below 5%, Fig. 3a) dehydration leads to their rapid increase. In the case of tyrosine and lysine this phenomenon is only modest, because of competitive interactions between C-termini and NH_3^+ (lysine) or OH (tyrosine) groups of the side-chains. In the case of leucine, histidine, and alanine, the increase is more significant with up to 25% of amino acids coordinating in a potentially reactive arrangement upon dehydration. Alanine shows the highest

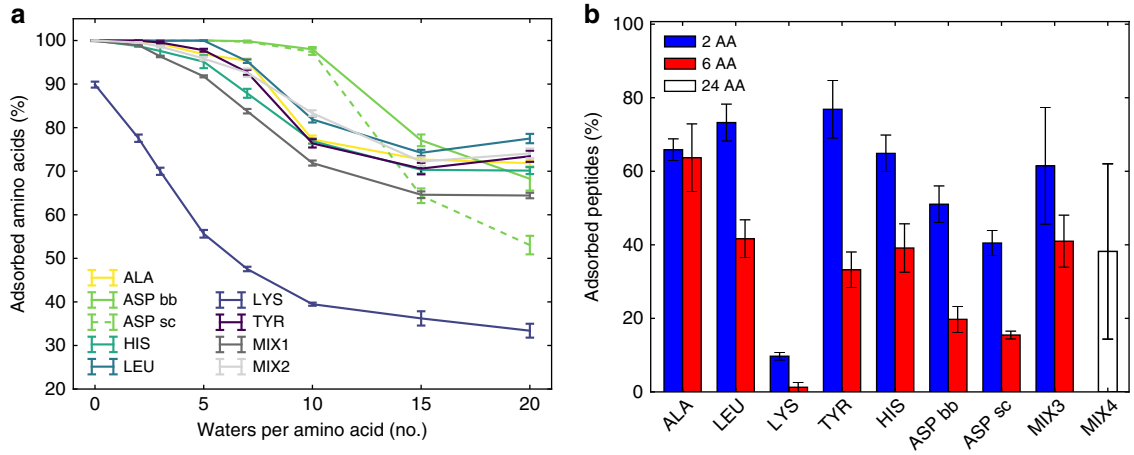

Fig. 2 Amino acids and peptides adsorbed onto LDH. **a** Percentage of adsorbed amino acids as a function of their hydration (number of water molecules per amino acid). **b** Percentage of adsorbed di-peptides, hexa-peptides, and 24-mers in a fully hydrated system. Error bars represent standard deviation. With the exception of lysine, both single amino acids and peptides adsorb onto the LDH surface (distance <2.5 Å). Aspartate can adsorb both via its backbone (labeled ASP bb) and side-chain (ASP sc). Though less adsorbed than di-peptides, hexa-peptides, and 24-mers still feature significant adsorption rates, indicating that chain length has only a minor effect on peptide adsorption

increase in reactive pairs, as its small side-chain does not hinder its backbone co-alignment. In the case of aspartate, the highest number of reactive pairs occurs at 2 W/AA, but, unlike other amino acids, no further increase is observed upon full dehydration because of its lower concentration per unit volume (due to its double charge). Aspartate can form α- or β-peptides and in this work we report the sum of two. Importantly, mixed amino acids behave similarly to pure systems, and at 40% load (MIX2) the amount of reactive pairs is still comparable to that of a fully saturated system (MIX1). This indicates that the process of reactive pair formation is mostly independent from both amino acids' nature and saturation of LDH interlayers.

Peptide bond formation on LDHs is energetically favorable. All of the systems readily rehydrate when exposed to water (Supplementary Fig. 5), as their hydration energy is always smaller than that of a reference SPC water system (−33.25 kJ mol^{-1}). Such behavior is only slightly dependent on the nature of the intercalated amino acids. The more the systems dehydrate, the higher the energy gain. The formation of a peptide bond releases a molecule of water, thus contributing to the rehydration of the interlayer. We note that peptide bond formation is endergonic with a free energy change of 10–20 kJ mol^{-1} [25]. This is comparable to that of the system's rehydration, which provides a driving force for the polymerization reaction.

Adsorbed amino acids and peptides diffuse on LDH surface. Despite being adsorbed, amino acids/peptides are never fully immobilized at the LDH surface (Fig. 4a). Their diffusion velocity on the LDH surface is small (5–10 Å ns^{-1}) for dehydrated systems (below 5 W/AA), where the amino acids are confined between two layers. In contrast, at higher hydrations, amino acids move rapidly (20–30 Å ns^{-1}), exploring the surface of the layer. Remarkably, the mobility of the 40% loaded mixture (MIX2) is only slightly smaller than that of a fully loaded system (MIX1). By studying the amino acids' trajectories on the LDH surface, we observed that their diffusion favors specific directions. Autocorrelation analysis demonstrates that amino acid diffusion follows a six-fold symmetry, templated by the LDH (Supplementary Fig. 6). Diffusion along preferential axes further increases the likelihood of an encounter between two amino acids.

Generally, upon a first binding event, all amino acids spend the majority of time bound to the surface and occasionally desorb (Fig. 4c). Even lysine, which is observed not to be strongly adsorbing, follows this trend upon binding. Importantly, since peptide chains adsorb via their C-termini, peptides mimic the behavior of amino acids; with no strong correlation between chain length, diffusion velocity, and residence time observed for a nascent protein chain (Fig. 4b, d).

Discussion

In this work we have explored the conceptual challenges associated with the formation of proto-biopolymers on mineral surfaces using the example of oligopeptides and amino acids at LDH surfaces. All of the amino acids tested were observed to be strongly adsorbed on the positively charged LDH surface, with the negative C-terminal oxygens forming H-bonds with the hydroxide groups at the LDH interface. It was notable that, when adsorbed, the molecules were mobile, exploring the full surface of the clay with preferential movement along the six-fold symmetry of the mineral. Amino acids diffused on the surface with a velocity inversely proportional to the crowding at the surface, and occasionally desorbed.

LDH dehydration may arise from wetting—drying cycles, heat, conversion of the water to hydrogen during serpentinization reaction, or as a result of inflow of highly saline water as described in the salt induced peptide formation (SIPF) theory[26]. Dehydration decreases LDH *d*-spacing, consequently increasing amino acid crowding. Even though *d*-spacing is proportional to the number of intercalated atoms, layer undulations are dependent on the nature of the intercalated amino acids. For instance, when amino acids bridge two layers, large static fluctuations are observed. Templated adsorption and partial dehydration create favorable arrangements and environments for the formation of peptide bonds. Remarkably, templating is not dependent on the concentration of amino acids in the interlayer, but rather on the amount of water per unit area of LDH surface.

As with single amino acids, di-, hexa- and 24-mer peptides remain attached by their negative C-terminals, while the backbone desorbs from the surface. This allows the preservation of peptide mobility independently of its length or system concentration. When the peptide sequence features aspartate, the carboxyl side-chain can also adsorb onto the LDH, contributing

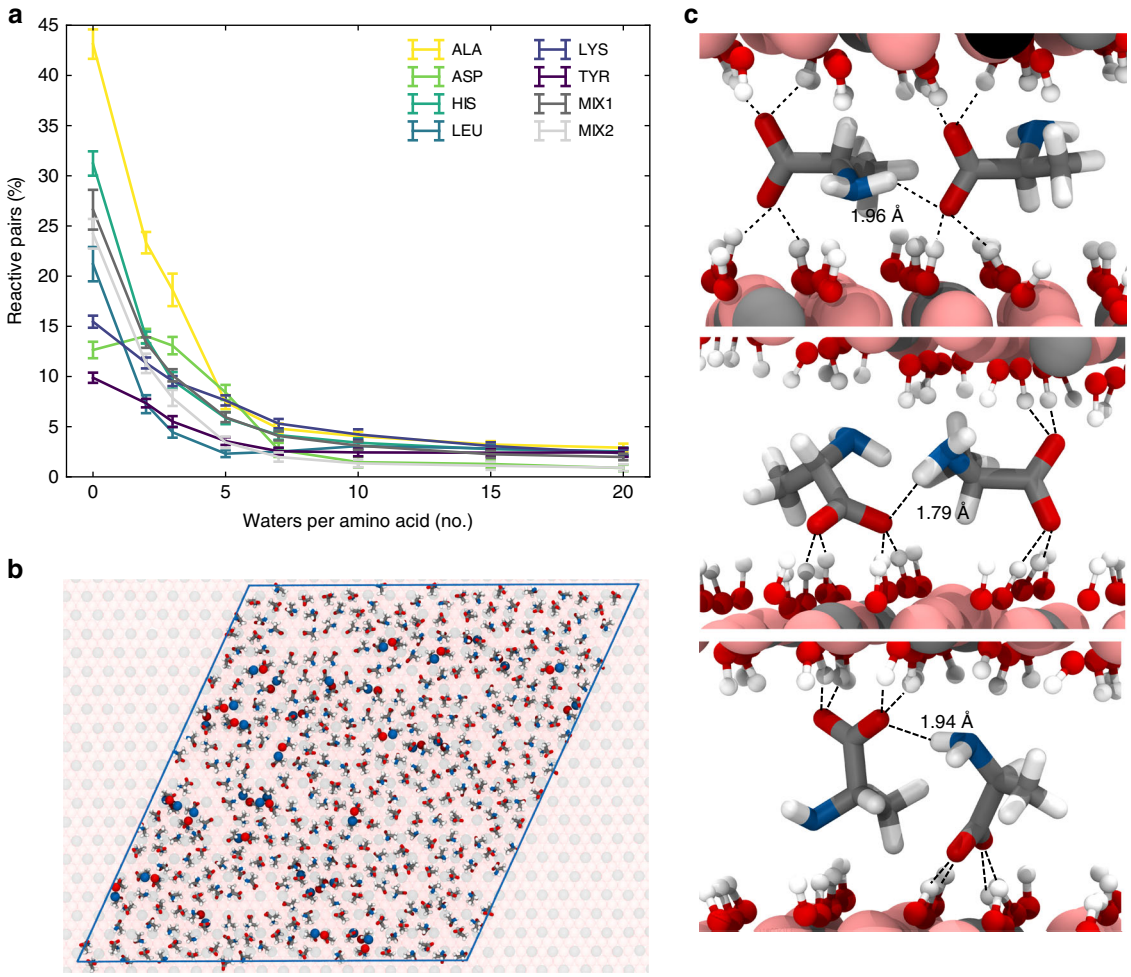

Fig. 3 Dehydration promotes formation of reactive pairs. **a** Percentage of amino acids' reactive pairs as a function of their hydration (number of water molecules per amino acids). Error bars represent standard deviation. For all amino acids, the percentage of reactive pairs in the system increases upon dehydration. **b** Top view of amino acids adsorbed and co-aligned onto the LDH surface, forming potential reactive pairs (shown spheres, where the backbone nitrogen is colored in blue, and most proximal backbone oxygen in red). **c** Reactive pairs show a range of different possible orientation, involving one or both adjacent LDH layers. Colors are as follows: Mg, pink spheres; Al, gray spheres; Cl, blue spheres; O, red; H, white; N, blue; and backbone is represented with yellow spline. For clarity water and H-atoms on the amino acids are not shown

to the stabilization of the peptide on the surface. Importantly, the model of a realistic system of 24-mers at low concentration features the same adsorption and dynamics of short peptides.

These results suggest a detailed mechanism of peptide formation, as shown in Fig. 4a. At high pH, amino acids adsorb onto the positive LDH layers via their negative C-termini. Partial dehydration creates an energy demand in the system, making condensation reactions increasingly favorable. Formation of a peptide bond leads to the loss of charged group. This, in turn, facilitates the introduction of a new amino acid to an adjacent site, where it then can react with the peptide's C-terminus. The growing peptide chain remains tethered at the LDH surface via the C-terminus of the latest amino acid added.

Our model suggests that, unlike previous observations[27, 28], the formation of a long and biologically relevant peptide is feasible through multiple rehydration cycles and is a slow and controlled process. Figure 5b shows the model of amino acids' polymerization kinetic process (Methods section and Supplementary Fig. 8). The model indicates that in order to form a significant amount of 10-mers (the length of chignolin, the shortest protein), more than 10 rehydration cycles must occur, while the system remains coupled to an infinite amino acid solution bath. When accounting for the timescale of the emergence of life on the planet, such a process would seem hardly unfeasible.

The LDH-amino acid/peptide coupling can be thought of as a mineral active site, where bond-making between polymers may be facilitated, but the polymer then has no permanent association. Such a mechanism is unlike that previously observed both for nucleic acids adsorbed on LDH[23, 24] and amino acids on clay surfaces[3], while having a strong resemblance to ribosome-catalyzed peptide bond formation[7]. In our simulations we observe that at high concentrations hydrophobic amino acids aggregate. This indicates that, within the clay layers, peptide chains should be able to undergo hydrophobic collapse, an essential mechanism of protein folding. Selectivity dependent on amino acid composition and ordering of peptides may occur due to the slight difference of amino acid affinity to the surface, as well as their relative concentration in the solution bath. In summary, our results outline a testable mechanism for prebiotic peptide formation assisted by LDHs under early Earth conditions.

Methods

Molecular models set-up. This study considers the layered double hydroxide (LDH) of stoichiometry $Mg_3Al(OH)_8$ with one positive charge per unit cell (Supplementary Fig. 1). The LDH layer thickness is 5.3 Å. All intercalated amino

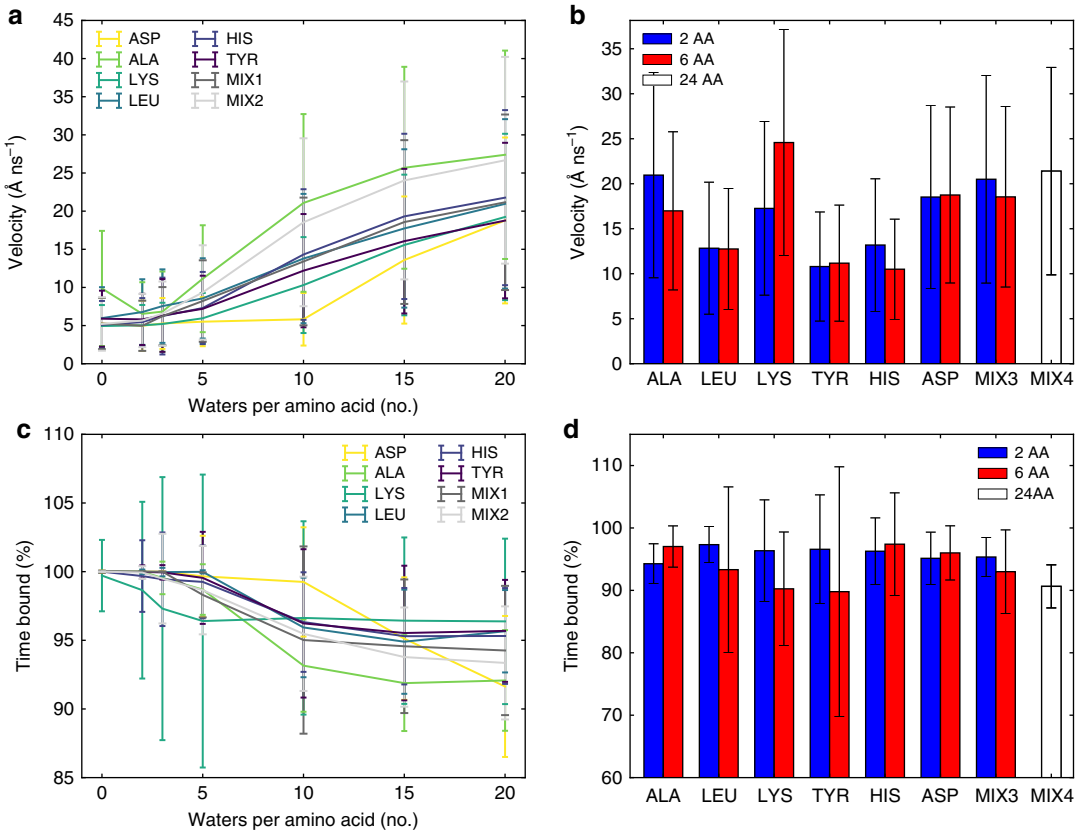

Fig. 4 Adsorbed amino acids and peptides remain mobile. **a** Velocities of adsorbed amino acids as a function of their hydration (number of water molecules per amino acid). **b** Velocities of adsorbed di-peptides, hexa-peptides, and 24-mers in a fully hydrated system. **c** Time bound of adsorbed amino acids after a first adsorption event as a function of their hydration (number of water molecules per amino acid). **d** Time bound of adsorbed amino acids after a first adsorption event of adsorbed di-peptides, hexa-peptides, and 24-mers in a fully hydrated system. Error bars represent standard deviation. When adsorbed, amino acids and peptides remain mobile upon the LDH surface. Even upon full dehydration, drift on the LDH plane can be observed for all amino acids. Upon a first adsorption event, both amino acids and peptides remain in contact with the LDH surface for majority of the time. Nevertheless, short desorption events (in average ~5% of the time) are still observed

acids' and peptides are deprotonated according to their pKa values to represent pH 9.5. Each group having a pKa lower than 9.5 was deprotonated. Amino acids quantity and charge, as well as the nature and quantity of charge balancing ions used to neutralize each simulation box, are reported in Supplementary Table 1. We considered a range of systems so as to investigate the processes that lie behind peptide formation in the context of the origins of life. The data presented first models a wetting—drying cycle by intercalating a single type of amino acid (ALA, ASP, LEU, LYS, HIS, and TYR) or a mixture (fully counterbalancing layer charge, MIX1, 40% amino acids, and 60% Cl^-, MIX2) within the hydrated interlayer (20 water per amino acid for all cases but MIX2, where it is 20 water per anion). The water is then removed in a stepwise manner (20, 15, 10, 7, 5, 3, 2, and 0 water).

The behavior of short peptides on the surface of the hydrated LDH was also studied. In this case, a single type of di- (2ALA, 2ASP, 2LEU, 2LYS, 2HIS, 2TYR) or hexa- (6ALA, 6ASP, 6LEU, 6LYS, 6HIS, 6TYR) peptide was intercalated between layers of hydrated LDH. Additionally, realistic mixtures of amino acids on LDH were studied. We created random mixtures based on the percentages of amino acids present in Nature today[29], using the Assemble![30] software. One (MIX3) was built from di- and hexa- peptides at 40% charge balance to LDH, while another (MIX4) was created from three 24-amino-acid-long peptides. Full details of system size and composition are given in Supplementary Table 1.

Molecular dynamics simulation protocol. The layered double hydroxide mineral in the simulations was modeled using the ClayFF force field[31]. The force field is specifically parameterized to model clay-like minerals, and the charges were adjusted to create a net +1 charge, as described in our earlier paper[21]. The CHARMM27 force field[32] was used to model the amino acids. ClayFF has been already tested and used with CHARMM force field[33]. Both force fields are parameterized for use along SPC water.

Molecular dynamics simulations were performed with GROMACS 4.6.7[34]. Each simulation was first energy-minimized using the steepest descents algorithm, with convergence when the maximum force on any atom was less than 100 kJ mol^{-1} nm^{-1}. Then the systems were equilibrated for 0.5 ns in NPT ensemble with velocity-rescale Berendsen thermostat at 300 K, temperature coupling constant set to 0.1 ps, and a semi-isotropic Berendsen barostat at 1 bar, with a pressure coupling constant of 1 ps. The minimization and equilibration simulations were run with real-space particle-mesh-Ewald (PME) electrostatics and a van der Waals cutoff of 1.2 nm. Production runs of 10, 20, or 50 ns (Supplementary Table 1) were then performed. The simulations were run with PME electrostatics and a van der Waals cutoff of 1.4 nm in NPT ensemble, with the same parameters as in the equilibration step illustrated above. For stepwise dehydration simulations, the water was removed after the equilibration phase. The resulting systems were then equilibrated again, prior to the production runs.

Layer thickness and undulation. For every equilibrated frame in every dehydration simulation (Supplementary Table 1), all LDH metal atoms coordinates were extracted. In order to assign these coordinates to one of the five simulated clay layers, we exploited the DBSCAN clustering algorithm. As such, layers have been defined as collections of metal atoms being apart by a maximum of 5 Å. Adjacent layers have been identified according to their mean position along the *z*-axis. *d*-spacings were calculated by collecting measurements between every pair of adjacent layers (Supplementary Fig. 2a). To obtain information about local *d*-spacing fluctuations, a 5 × 5 Å sliding window, moved with 1 Å steps on the *xy*-plane, was applied. Local *d*-spacing was calculated as the distance between the mean *z*-axis values of atoms inside the window in two adjacent layers. Mean and standard deviation were calculated for all the collected measures.

To calculate layer undulations (Supplementary Fig. 2b), we translated the center of every layer to the origin, accumulated all metal atoms coordinates, and then calculated the standard deviation of the resulting data sets along the *z*-axis. The standard deviation of the *d*-spacing shows the local differences in the thickness of the interlayer. When the standard deviation is small, the layer undulations are correlated; when the standard deviation is large, the layer undulations are de- or anti- correlated.

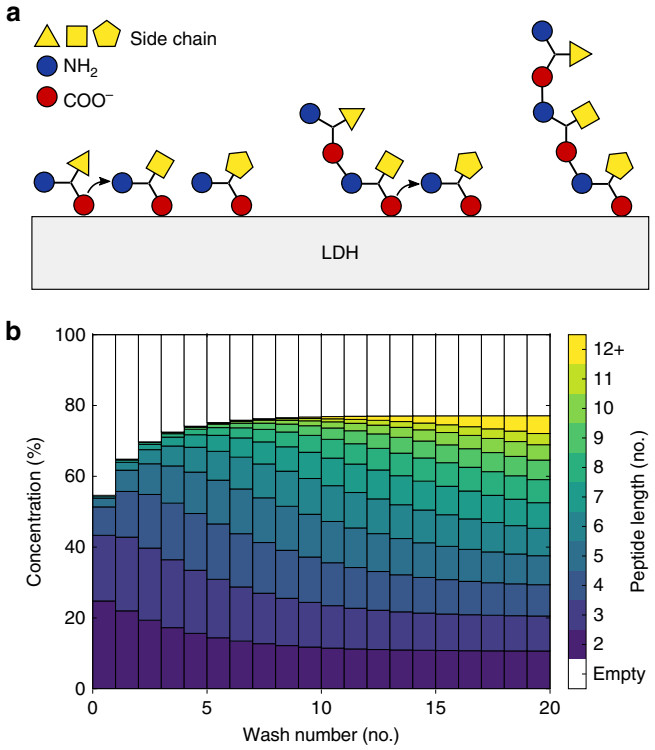

Fig. 5 Proposed mechanism for LDH supported peptide bond formation. **a** Upon dehydration, the N- and C-termini of adsorbed amino acids co-align, allowing the formation of a peptide bond. The newly formed di-peptide remains tethered via C-terminus only. The bond formation leads to the loss of charge, facilitating introduction of a new amino acid. The N-terminal of amino acid is then able to form a bond with the C-terminal of di-peptide, thus triggering further peptide growth. **b** A kinetic model of peptide growth upon multiple dehydrations—rehydration cycles. After a single dehydration, only dimers to hexamers are observed. Subsequent washing cycles lead to the formation of longer chains

Amino acid adsorption. The percentages of adsorbed amino acids were calculated for all dehydration studies and reported as a function of the hydration of the systems (Fig. 2a). For peptide systems (di-peptide, hexapeptide, and mixtures of peptides) the adsorption percentages were also calculated (Fig. 2b). The amino acid/peptide was considered to be adsorbed when a C-terminal oxygen (OT1 and OT2) forms an H-bond with the LDH surface. We adopted a distance cutoff of 2.5 Å, corresponding to the distance of the first hydration layer of LDH. Aspartate can also adsorb via its side-chain (OD1 and OD2), which is reported separately. In the case of di- and hexa- peptides there are twice or six times, respectively, more side-chains than in the backbone and so their adsorbed percentages are scaled accordingly.

Radial distribution function. For all the dehydration studies, we computed the radial distribution function (RDF) and the C-terminal atoms of the amino acids using LDH aluminum as reference (Supplementary Fig. 3). The RDF allows us to describe the templating effect of LDH on the arrangement of the amino acids. For comparison, the RDF of aluminum atoms against themselves was also calculated.

Vectorial analysis. We calculated the alignment of every amino acid adsorbed with respect to the *xy*-plane of the LDH surface. A vector was assigned between C and N in the backbone, and its elevation as described in spherical coordinates (Θ angle) collected. Histograms of elevations were generated with 1° bin size. Angles of 0° identify vectors perpendicular to the plane, and 90° those parallel to the plane (Supplementary Fig. 4).

Reactive pair count. For dehydration studies, the likelihood of amino acids co-aligning to form a peptide was calculated (Fig. 3). A reactive pair may be defined as two adsorbed amino acids with their respective C- and N- termini at less than 4 Å distance from one another. In the case of aspartate, alignments that can lead to cyclic reaction were excluded, and the total count of reactive pairs that could lead to either alpha- or beta- peptides is presented.

Hydration energy. The energies associated with dehydration of the LDH-amino acid interlayer were calculated as detailed in Wang et al.[35] and are based on the definition of hydration energy introduced in early clay-swelling studies[36],

$$\Delta U_{\mathrm{H}} = \frac{\langle U(N)\rangle - \langle U(0)\rangle}{N}, \quad (1)$$

where $\langle U(N)\rangle$ is the average potential energy of the equilibrium system with N-water molecules and $\langle U(0)\rangle$ is the energy of the fully dehydrated system. The hydration energy can then be compared to the average energy of bulk water (−33.25 kJ mol^{-1}) and, if lower, the layers will be prone to rehydrate, i.e. swell (Supplementary Fig. 5).

Diffusion analysis. For every simulation, the position of the C-terminal carbon is tracked for every equilibrated frame. A carbon was considered as adsorbed if any of its bound oxygens (OT1 and OT2) were within 2.5 Å from the LDH surface. The percentage of time that every carbon spends adsorbed on the surface was calculated after the first adsorption event (Fig. 4c, d). For every carbon adsorbed in two consecutive simulation frames, the diffusion velocity and direction were calculated (Fig. 4a, b). In this situation, we considered diffusion that takes place on the *xy*-plane only, and its direction was expressed in polar coordinates. All calculated diffusion directions were collected and their distribution represented as histogram. To highlight whether any favorite diffusion axes were present, the direction of the distribution's autocorrelation was calculated (Supplementary Fig. 6). In the case of LDH, the magnesium atoms are hexagonally arranged (i.e. they feature three symmetry axes). If amino acids preferentially diffuse along these symmetry axes, this would be revealed on the autocorrelation plot as six periodic peaks, while random or no diffusion would lead to a flat auto-correlation plot.

Visualization. All the snapshots were produced with VMD 1.9.1[37] and graphs were produced with Matplotlib[38].

Quantum calculations. In order to calculate the change of partial charges of amino acids upon adsorption on LDH, we have set up five systems—the pure LDH surface of four unit cells, single alanine molecule in vacuum, single alanine adsorbed on the four-unit-cell surface of LDH, four alanine molecules in vacuum and four alanine molecules on an LDH surface. The calculations were performed with CASTEP[39], using norm-conserving planewave pseudopotential and the generalized gradient approximation of Perdew Burke and Ernzerhof[40]. The systems were geometry optimized using density mixing, and the total atomic energy was calculated using the Broyden—Fletcher—Goldfarb—Shanno algorithm; van der Waals forces were applied via the Grimme 06[41]. The following convergence criteria were used for all models: electronic energy tolerance of 1×10^{-6} eV, energy change 5×10^{-6} eV per atom, maximum displacement of 5×10^{-4} Å, and maximum force of 3×10^{-2} eV Å^{-1}. After geometry optimization, the charge density of the models was analyzed using Hirshfield population analysis.

Kinetic model. We developed a kinetic model to describe the process of peptide formation assisted by the LDH interlayer. Negative amino acids and peptides readily adsorb onto the positive LDH surface. Adsorption occurs via the negative C-terminal by activating [reducing the negative charge on] the C-atom for nucleophilic attack (Supplementary Fig. 7):

$$\mathrm{X}_1 + ^* \rightarrow \mathrm{X}_1^*, \quad (2)$$

where X_1 is the amino acid, * is a surface site and X_1* is adsorbed/activated amino acid. Upon sufficient dehydration, amino acid can react with another adsorbed amino acid to form a di-peptide X_2, or, with an adsorbed peptide X_n and extend it by one monomer unit X_{n+1}. The formation of a peptide creates a surface site vacancy:

$$\mathrm{X}_1^* + \mathrm{X}_n^* \rightarrow \mathrm{X}_{n+1}^* + ^*, \text{ where } n = 2, 3, \ldots \quad (3)$$

Upon rehydration, the formed peptides can desorb, creating another surface site vacancy:

$$\mathrm{X}_n^* \rightarrow \mathrm{X}_n + ^* . \quad (4)$$

Since all of the reactions occur only with surface activated species, we omit * for clarity. For all the reactions we assume the same rate constant $k = 1$, and, therefore, it is omitted in the further equations. The following rate equations are identified. The concentration of amino acid X_1 will decrease for each peptide bond formation:

$$\frac{\mathrm{d}[\mathrm{X}_1]}{\mathrm{d}t} = -\sum_{i=2}^{n} [\mathrm{X}_1][\mathrm{X}_n]. \quad (5)$$

The concentration of peptide X_n is dependent both on its formation from X_{n-1} and its use in the further polymerization towards X_{n+1} and therefore can be expressed as:

$$\frac{d[X_n]}{dt} = [X_1][X_{n-1}] - [X_1][X_n]; \forall\, n>1 \quad (6)$$

For the first step, we assume that the LDH layer is fully populated only by amino acids $[X_1] = 100$ and therefore $[X_n] = 0$ for all $n > 1$. Concentrations will converge (i.e. reactions will stop) when $[X_1] = 0$. Upon convergence, we allow the adsorbed species to desorb at 5% to model the species release upon LDH rehydration (Fig. 4). Upon rehydration, LDH vacant sites can be repopulated by the amino acids, bringing the total surface population to 100. We refer to such surface repopulation and peptide release as a wetting step. The process between each wetting step, including of dehydration, peptide formation, rehydration, peptide desorption, and surface repopulation, is referred as a wetting—drying cycle. We track species concentrations after each cycle's convergence, as well as the quantities released from the clay. In Fig. 5a, the concentration after convergence of 20 cycles is presented; a zoom into only 3 cycles is shown in Supplementary Fig. 8a. Supplementary Fig. 8b shows 50 cycles, and Supplementary Fig. 8c shows the cumulant of all species released from LDH. Supplementary Fig. 8d shows cumulative species concentration upon each cycle convergence. The software is developed in Python and the integration is performed with scipy integrate module.

Data availability. All other data are available from the authors upon reasonable request.

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Author contributions

V.E. and M.T.D. performed the simulations and analyzed the data. V.E., M.T.D., D.G.F., and H.C.G. designed the research and wrote the manuscript.

Additional information

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Competing interests: The authors declare no competing financial interests.

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Supplementary Table 1: Summary of the systems modelled, their composition and simulation times

**Realistic mix*: relative amino acid concentration matching naturally occurring one[1]. MIX3: 10 dimeric and 15 hexameric different random chains produced with *Assemble!*[2] and replicated in the box. These had amino acid concentration: ALA 26.0%, ASP 18.3%, HIS 7.7%, LYS 15.4%, LEU 22.1%, TYR 10.6%. MIX4: three 24 amino acid-long chains were randomly created with *Assemble!*[2], total concentration of amino acids: ALA 19.4%, ASP 9.7%, HIS 2.8%, LYS 16.7%, LEU 36.1%, TYR 6.9%.

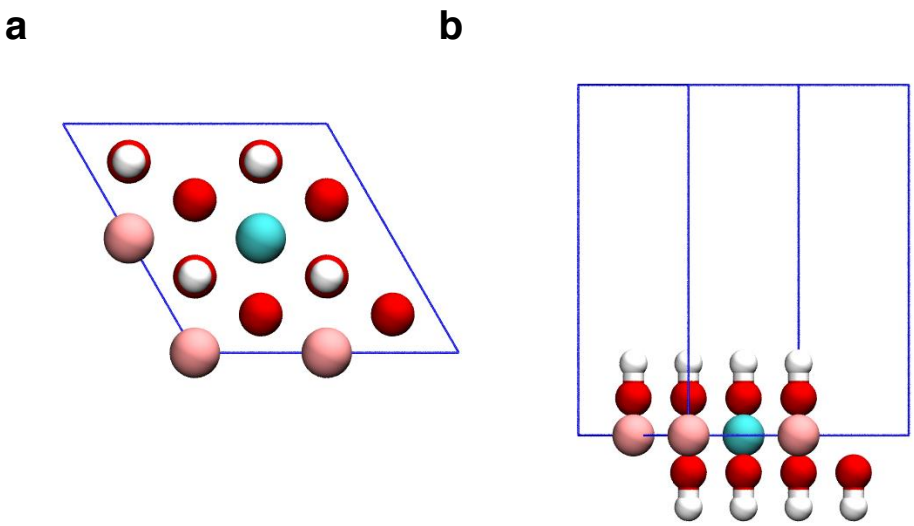
<table>
<tr><th>System name</th><th>LDH, no of unit cells, x y z, total</th><th># of amino acids</th><th>Charge per amino acid/peptide</th><th>Counterbalancing ions</th><th># of water</th><th>Volume, x y z, nm</th><th>Simulation time</th><th>Description</th></tr>
<tr><td>ASP</td><td rowspan="8">15x24x5
total 1800</td><td>180 per layer, total 900</td><td>-2</td><td></td><td rowspan="5">Stepwise: 20, 15, 10, 7, 5, 3, 2 and 0 per AA</td><td rowspan="7">From ~11x11x12 to ~ 11x11x4</td><td rowspan="7">10 ns per dehydration step</td><td rowspan="5">Stepwise dehydration</td></tr>
<tr><td>LYS</td><td>360 per layer, 1800 total</td><td>0</td><td>1800 Cl^-</td></tr>
<tr><td>ALA</td><td rowspan="3">360 per layer 1800 total</td><td rowspan="3">-1</td><td rowspan="3"></td></tr>
<tr><td>LEU</td></tr>
<tr><td>HIS</td></tr>
<tr><td>TYR</td><td rowspan="2">60 AA of each type per layer, 1800 total</td><td rowspan="2">As above</td><td rowspan="2"></td></tr>
<tr><td>MIX1</td></tr>
<tr><td>MIX2</td><td>24 AA of each type per layer, 720 total</td><td>As above</td><td>1080 Cl^-</td><td>From 20 to 0 per anion</td><td>Dehydration, 40% amino acid charge balancing load</td></tr>
<tr><td>2ASP</td><td rowspan="6">10x18x1
total 180</td><td>90</td><td>-3</td><td>90 Na^+</td><td rowspan="6">~20 waters per AA
~7000 total</td><td rowspan="6">~7x8x5</td><td rowspan="6">20 ns</td><td rowspan="6">Dipeptide in the hydrated interlayer</td></tr>
<tr><td>2LYS</td><td>180</td><td>+1</td><td>320 Cl^-</td></tr>
<tr><td>2ALA</td><td rowspan="4">180</td><td rowspan="4">-1</td><td></td></tr>
<tr><td>2LEU</td><td></td></tr>
<tr><td>2HIS</td><td></td></tr>
<tr><td>2TYR</td><td></td></tr>
</table>

Supplementary Table 1, contd.

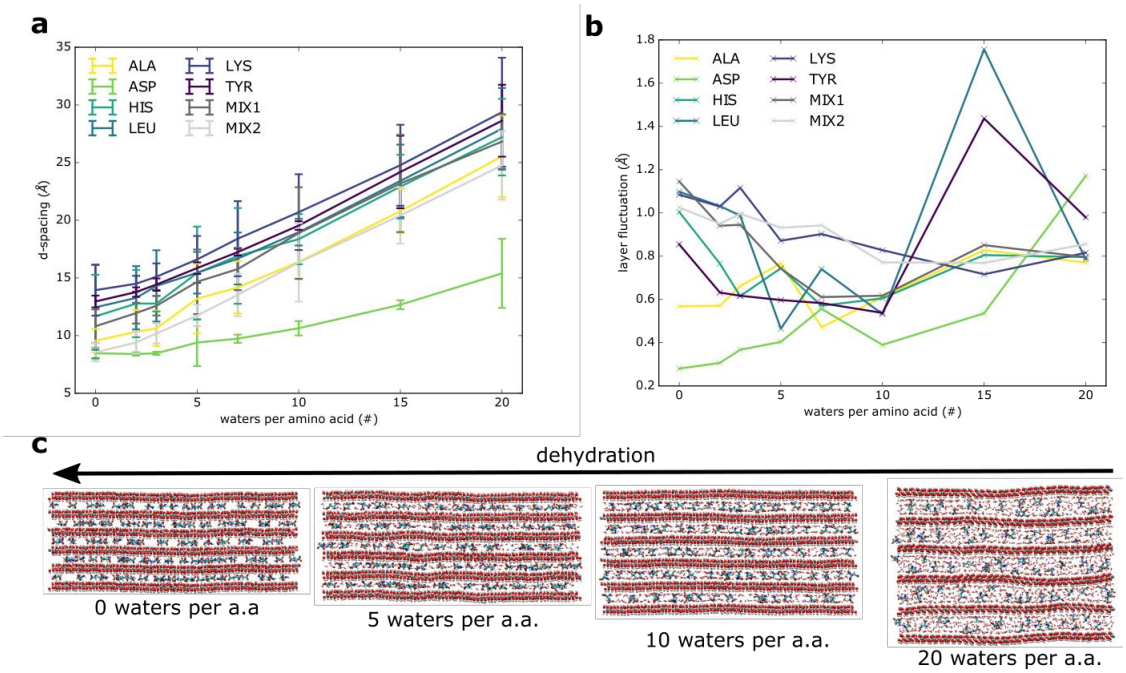
System name	LDH, no of unit cells, *x y z, total*	# of amino acids	Charge per amino acid/peptide	Counterbalancing ions	# of water	Volume, *x y z*, nm	Simulation time	Description
6ASP	10x18x1	90	-7	450 Na^+	~20 waters per AA ~20000 total	~7x8x11	20 ns	Hexapeptide in the hydrated interlayer
6LYS		90	+5	630 Cl^-				
6ALA		180	-1					
6LEU								
6HIS								
6TYR								
MIX3	10x18x1	15 dimer 15 hexamer see below	See composition below	149 Cl^-	~60 water per AA ~8000 total	~7x8x5	50 ns	Realistic mix* of di- and hexa- peptides at 40% of charge balancing load
MIX4	10x18x1	3 x 24mer See below	See composition below	182 Cl^-	~100 water per AA ~7200 total	~7x8x4	50 ns	Dilute realistic mix* of three 24mer peptides

Supplementary Table 2
Calculated atomic charges of C-terminal atoms of alanine in vacuum and adsorbed onto the LDH surface with Hirshfeld population analysis.

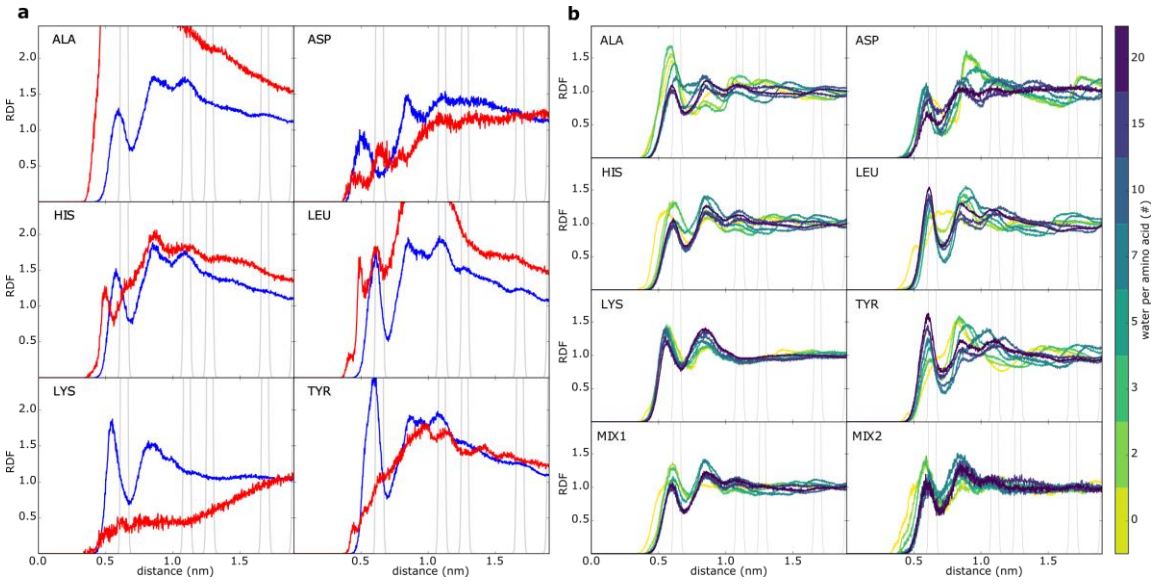
System	Atomic charge, e	
	C	**O**
1 ALA vacuum	0.06	-0.4, -0.42
4 ALA vacuum	0.09, 0.07, 0.08, 0.07	-0.4, -0.4, -0.4, -0.43, -0.35, -0.4, -0.42, -0.42
Average	**0.074 ± 0.011**	**-0.405 ± 0.022**
1 ALA adsorbed on LDH	0.19	-0.22, -0.23
4 ALA adsorbed on LDH	0.17, 0.16, 0.18, 0.18	-0.23, -0.26, -0.28, -0.21, -0.23, -0.21, -0.24, -0.22
Average	**0.176 ± 0.011**	**-0.233 ± 0.022**



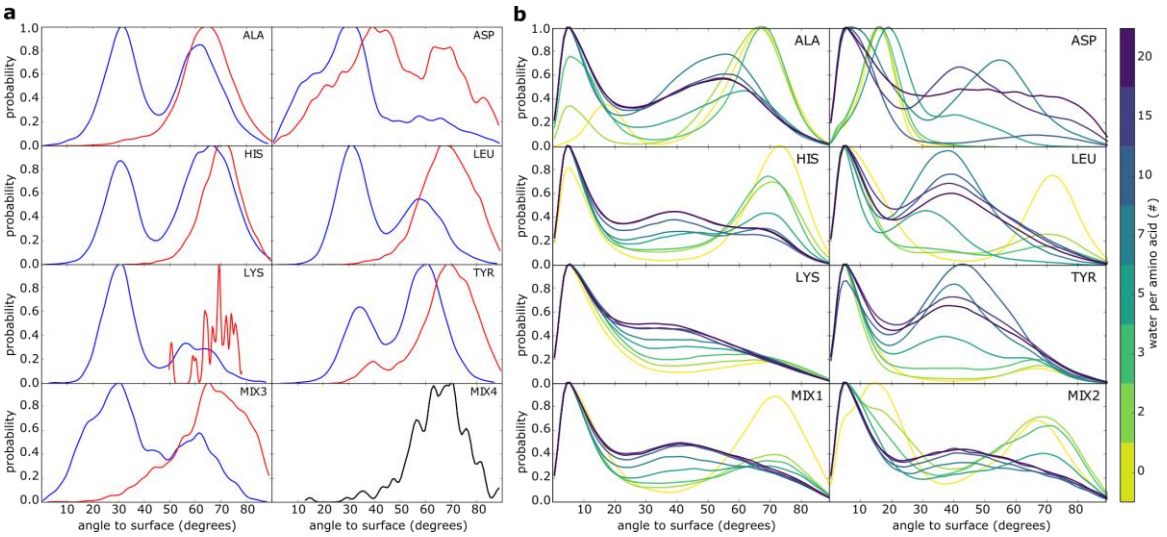
Supplementary Figure 1: Unit cell of LDH
Layered double hydroxide unit cell **(a)** top and **(b)** side view. Colours are: Al – cyan, Mg – pink, O – red and H – white.



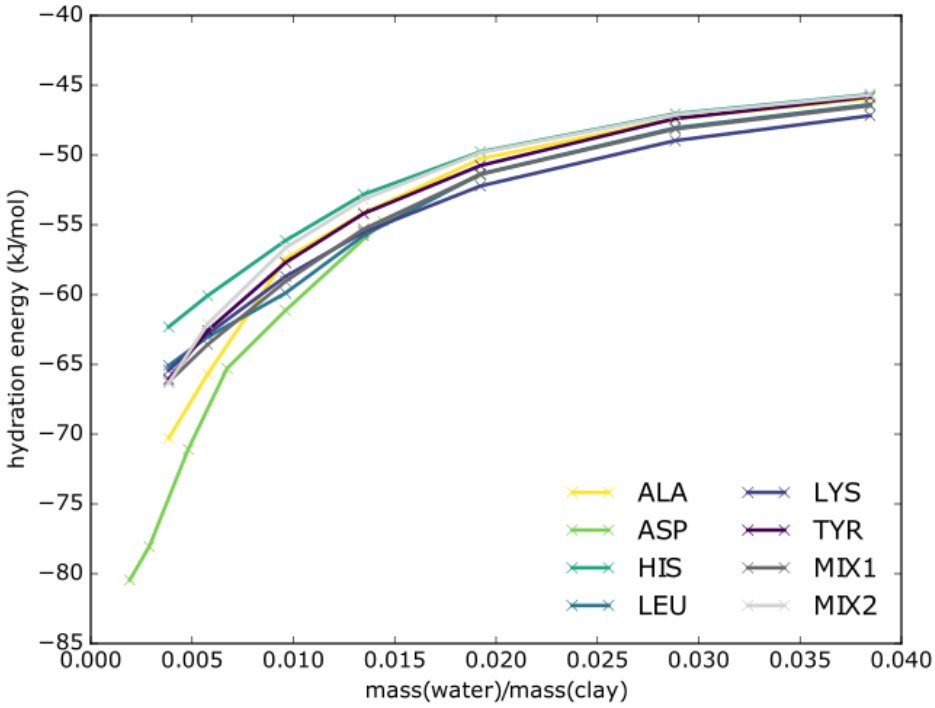
Supplementary Figure 2: *d*-spacing and layer undulations
(a) LDH interlayer *d*-spacing as a function of hydration (waters per amino acid), with different intercalated with amino acids. Note: ASP is doubly charged, so is present in half the amount with respect to other amino acids, MIX2 has 40% of amino acid load with 60% of Cl^- counterbalancing ions). **(b)** LDH layer undulations as a function of hydration (waters per amino acid). **(c)** snapshots of the dehydration of ASP-intercalated LDH.



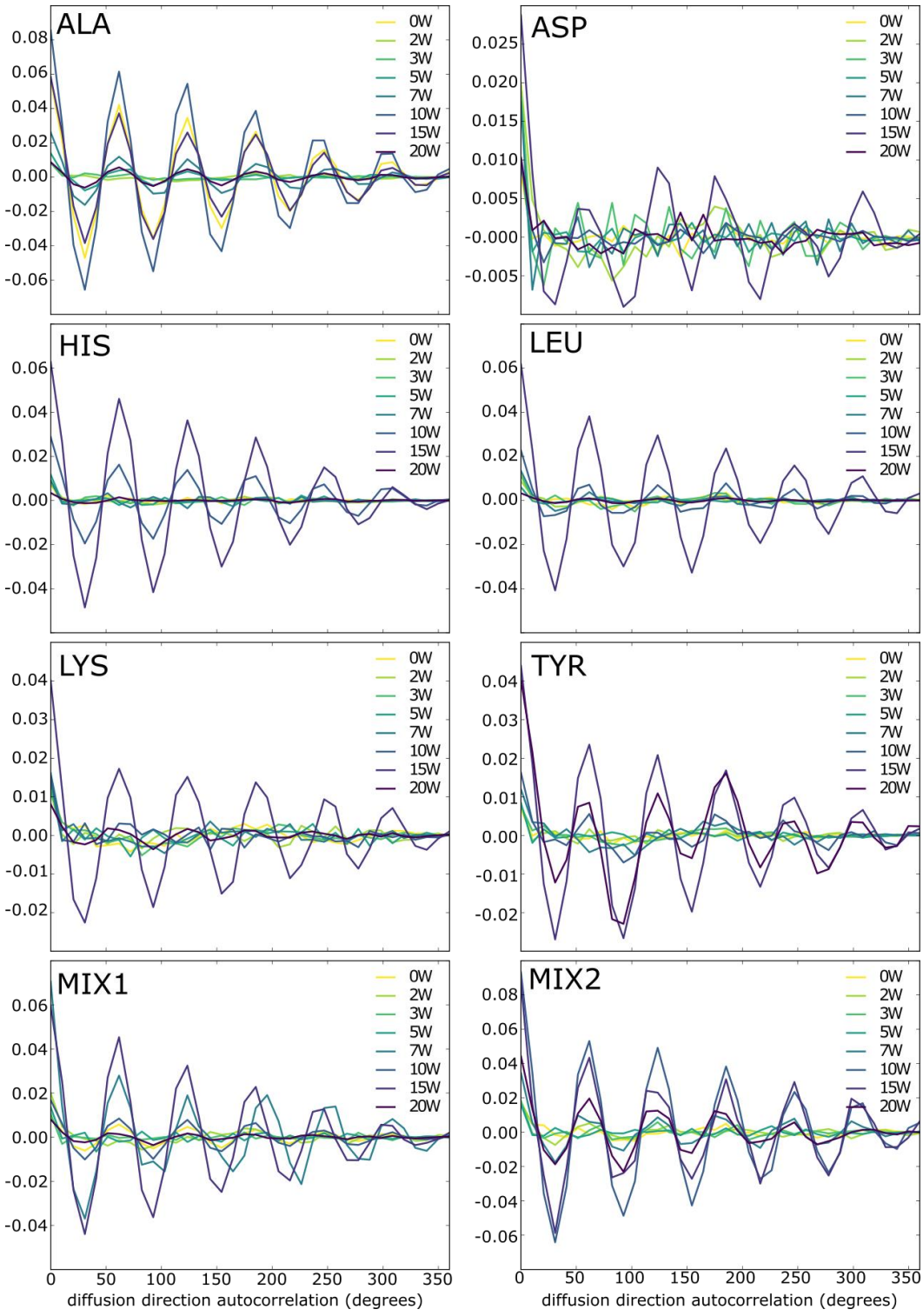
Supplementary Figure 3: Radial Distribution Function of amino acids
We report the Radial Distribution Function (RDF) of amino acids and peptides C-termini with respect of LDH aluminium atoms. **(a)** RDF of homo-peptides. Blue – dimer, red – hexamer. **(b)** RDF of amino acids, with colour gradient as a function of hydration. In grey, the distribution of aluminium atoms is indicated. A clear first shell is present (0.64 nm), indicating that the C-terminal of amino acids arrange with the same repetition of LDH unit cells. The second peak (0.8 nm) corresponds to C-termini across the LDH layer. The following peaks (1.11 nm and 1.28 nm) correspond to C-termini arranged on the diagonal unit cell and their neighbouring ones. The LDH templating effect on amino acids arrangement is observed for all adsorbed amino acids. Aspartate features a slightly different pattern, as one aspartate holds a 2– charge and therefore is shared between two unit cells of LDH, often interacting *via* both its carboxylic groups. Amino acid mixtures (MIX1 and MIX2) show the same behaviour as the pure systems.



Supplementary Figure 4: Alignment of adsorbed species with respect to LDH
A vector is assigned between C and N atoms of the adsorbed amino acid's backbone, and its elevation (in spherical coordinates) reported. A 0 degrees elevation corresponds to a backbone perpendicular to the LDH surface, a 90 degrees to a backbone parallel to it. **(a)** Alignment of homo-peptides (top three rows) and hetero-peptides (bottom row) with respect to the surface. Dimers, hexamers and 24mers are shown in blue, red and black, respectively. The C-termini of the shortest peptides align like those of single hydrated amino acids; while in the case of longer peptides (including 24mer, with an exception of hexa-aspartate) C-termini align near-perpendicularly to the surface. Polyaspartates can co-adsorb with their side-chains and so remain parallel to the surface. **(b)** Alignment of single amino acids, as a function of their hydration level. At high levels of hydration, amino acids mainly adsorb by their C-terminals. Although still mobile, the backbones show preferential alignments: either planar, or at 40 degree to the surface. Upon dehydration, backbones arrange more uniformly, either perpendicular or parallel to the surface. This is because C-termini have two oxygens, able either to adsorb to the same LDH surface, or to bridge to the opposing one. Due to the specific adsorption behaviour of lysine and aspartate, the alignments to the surface do not follow the general trend. Tyrosine favours a planar alignment upon dehydration, due to π-stacking of the aromatic rings of the side chains.

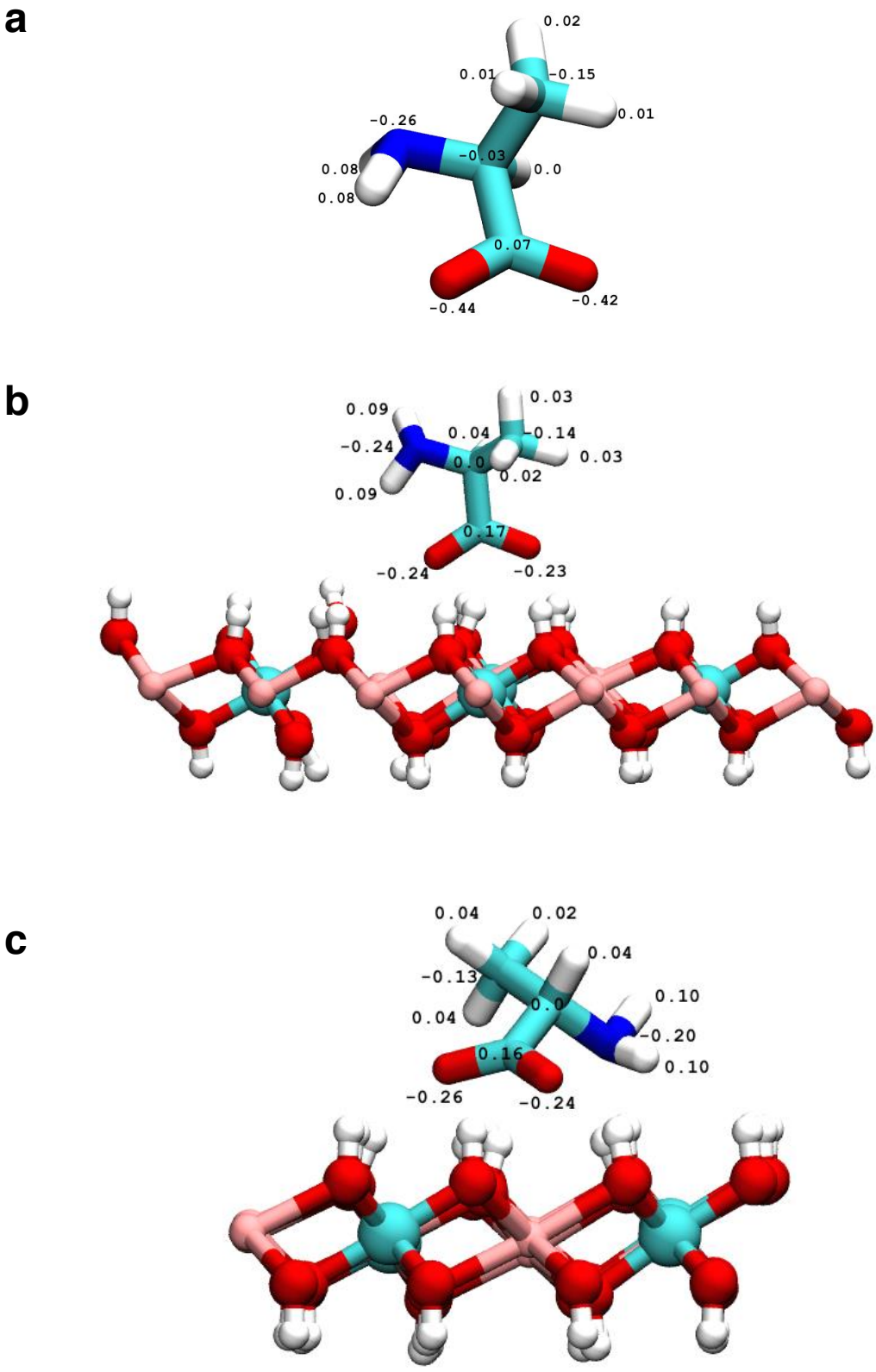


Supplementary Figure 5: Hydration energy
The energies associated with dehydration of the LDH-amino acid interlayer, as a function of intercalated amino acid composition. The hydration energy of a pure water system is -33.25 kJ mol-1. All of the systems are below this value, and therefore will rehydrate. Notably, the more dehydrated is the system; the more rehydration is energetically favourable.

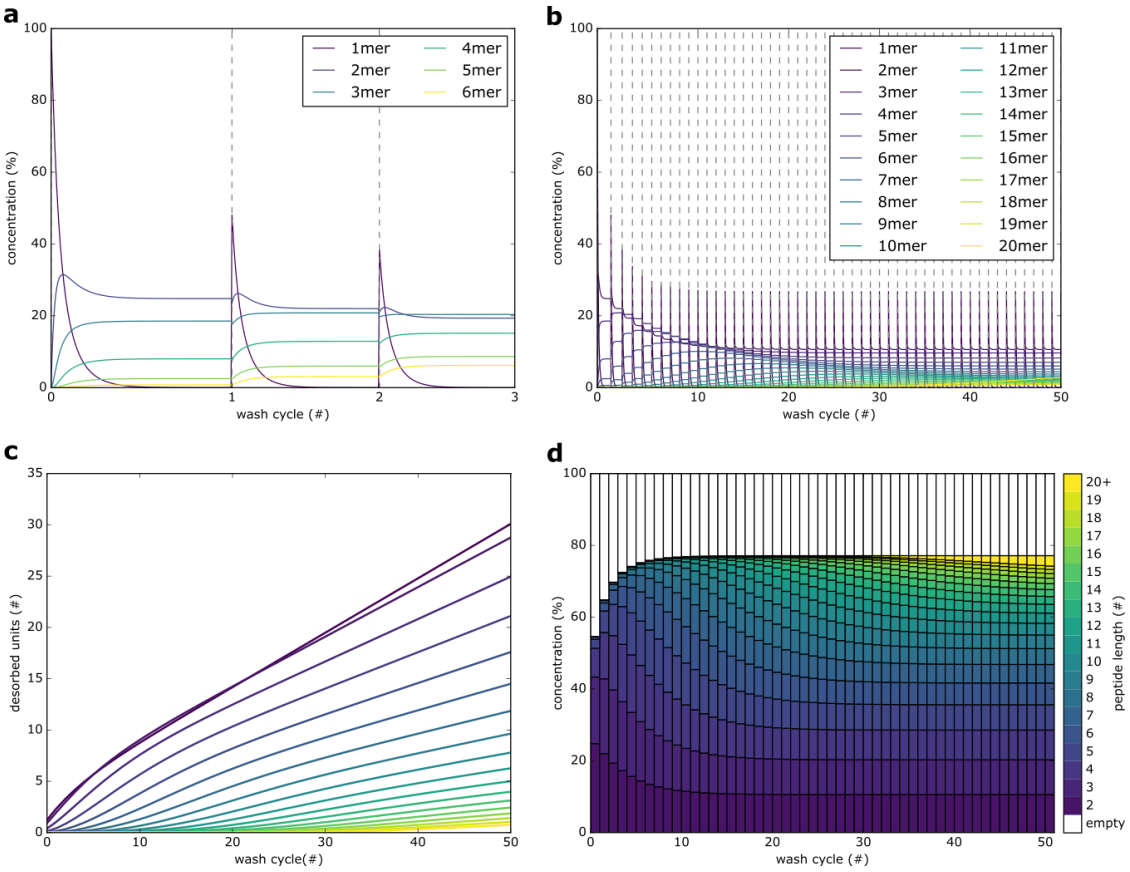


Supplementary Figure 6. Autocorrelation of amino acid diffusion direction on LDH surface

We describe the diffusion of each amino acid adsorbed on the LDH surface as a two dimensional vector, i.e. a direction on the *xy*-plane. In each system, six distinct peaks are observed, with higher intensities observed in more hydrated systems. This indicates that amino acids are more mobile in hydrated systems, with a strong hexagonal movement preference templated by LDH metal ions arrangement (see Supplementary Fig. 1).



Supplementary Figure 7: Partial charges on alanine
(a) Atomic charges calculated on alanine in vacuum. **(b)** Atomic charges of an alanine adsorbed on the LDH surface via its C-terminal **(c)** Atomic charges an alanine adsorbed via both its C- and N-terminal.



Supplementary Figure 8: Kinetic model of amino acids polymerisation on LDH
(a) Peptide concentration in an LDH interlayer per wash cycle. Three wash cycles are shown. At the beginning of each cycle, the system is saturated with monomers that subsequently react with existing multimeric species, until being totally consumed. At the end of each cycle, 5% of each species is removed from the system, to simulate species release upon LDH rehydration. **(b)** After a 50 wash cycles, higher order oligomers become detectable. **(c)** Cumulant of all species released from LDH, per wash cycle. After a short equilibration phase, linear trends can be observed for each specie, showing that LDH undergoing dehydration-rehydration cycles should produce a steady amount of peptides of different stoichiometries. **(d)** Species concentration upon each cycle convergence. Upon convergence, LDH is always >20% empty, and is thus capable of adsorbing new monomeric amino acids during the next rehydration phase.

Supplementary References

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

This is a very interesting modeling study of amino acids condensation on a specific mineral support, namely hydrotalcite. Although the surface condensation of biomolecules has been studied often, we still lack a fundamental understanding that would allow to make sense of disconnected experimental data. The present paper raises some crucial questions and goes some way to answer them. I believe it can be of interest to a general audience, beyond specialists in prebiotic chemistry.

A first original contribution is the evidence for a templating effect of aluminum substitutions in the hydrotalcite lattice, imposing on the amino acids an orientation that favors the condensation of their C-termini. However, amino acids also condense on surfaces that cannot induce this kind of structuring, such as amorphous silica. If the authors'suggestion is correct, condensation should occur more easily on/in hydrotalcites than on silica. Are there experimental results to support this prediction? The authors certainly suggest that there is something special to hydrotalcites: cf. on p.13 "unlike previously observed…(this mechanism has) a strong resemblance to ribosome-catalyzed peptide bond formation". This is a very intriguing idea, but also highly speculative until corroborated experimentally.
The specific treatment of amino acids mobility on p.10 is also an interesting feature. This question is central to the study of bimolecular reactions and is generally overlooked in studies of biomolecules surface condensation. However, the velocity units must be explained – Å nm-1 is not a velocity.
Peristaltic undulations (p.7) of hydrotalcite layers also constitute a rather new observation, even though less related to the central topic of the manuscript.
It should be noted that all of these observations are made possible by the large scale of the modeling, as opposed to other studies of similar systems that only use DFT or similar methods.
The authors have also carried out a valuable and original study of wetting-and-drying cycles that is only cursorily commented in the main text. A separate publication of this part might be justified.

On the down side, I think the paper is less palatable to the general chemist because some questions that (s)he would naturally asked are not treated explicitly. The authors should definitely include in the main text a few sentences to clarify the following questions:
1. How is the layer charge compensated? The "online methods" state that "amino acids… are deprotonated to represent pH 9.5, and in the majority carry a negative charge that counterbalances the positive the positive LDH charge". This is not clear. Was the proportion of deprotonated amino acids chosen on the basis of the corresponding pH? Or of the LDH charge compensation? I assume no other charge compensating ions, such as carbonates, were introduced.
2. Connected to the previous question, what is the acido-basic speciation of amino acids and peptides? They are mostly anions, with one carboxylate and one amine group (cf. "in the majority" above), but are there any zwitterions (or neutral amino acids)? And do proton transfers occur in the various stages of modeling? Also on p.12 "Formation of a peptide bond leads to the loss of a charged group. »: how then is the conservation of charge assured? By the formation of a hydroxide anion?
And on p.8 "the amine side chain is strongly positive" (p. 8): does it mean that it is protonated to an ammonium?
3. What is the criterion for determining through which moiety the amino acids and peptides are adsorbed? (e.g. "adsorption via the backbone" or "adsorption via the side chain" of the Asp molecules.) Is is energetic, based on spatial proximity? Some answers can be found in the supplementary information, but the matter should be clearly stated before the first mention of adsorption mechanism.

Some other points of less general significance:

p. 3, last § « The idea of using hydrated mineral surfaces » - why hydrated ? this is somewhat contradictory with considering “hydrophobic” surfaces

p. 4, 1st §: “Some of these surfaces may have very high enthalpies of hydration, providing a driving force for condensation reaction”: This statement should be qualified. Actually, if adsorption of biomonomers occurs from a water solution, the surfaces are already hydrated, and their hydration cannot provide a thermodynamic driving force for condensation.

p.6: a remarkable paucity of simulation data for peptide-mineral interactions:
for peptide-clays, one can mention
Aquino, A. J. A.; Tunega, D.; Gerzabek, M. H.; Lischka, H., Modeling Catalytic Effects of Clay Mineral Surfaces on Peptide Bond Formation. J. Phys. Chem. B 2004, 108, 10120-10130
or even a first attempt, now largely superseded:
Collins, J. R.; Loew, G. H.; Luke, B. T.; White, D. H., Theoretical investigation of the role of clay edges in prebiotic peptide bond formation. Orig. Life Evol. Biosph. 1988, 18, 107-119
Slightly outdated, but addressing an important problem.

p.7, “Irrespective of the identity of the amino acid, the LDH interlayer dehydrates similarly » : I do not understand what exactly the authors mean by « similarly » ; this contention needs to be developed further.

p.10: “peptide bond formation is endergonic with a free energy…comparable to that of the system’s rehydration, thus providing a driving force for the polymerization”: this sentence must be rephrased. It can be read as meaning that the endergonicity of the reaction provides a driving force, which is contrary to common sense: I suppose the authors actually mean that when it is coupled to the interlayers’ rehydration, the global reaction becomes exergonic.

p.11: “Building upon the attractive hypothesis of Russell and Martin …» : what hypothesis actually ? In the previous text, they are mentioned only as providing a geochemical setting for prebiotic chemistry (alkaline hydrothermal systems), so how is the present work building upon it?

p.12: “…the SIPF theory”: it is not so much a theory as a type of reaction, “Salt-Induced Peptide Formation”. Give the meaning of the acronym for the general reader.

p.13, “unlike previously observed23,24, » : ref. 24 is missing.

Reviewer #2 (Remarks to the Author):

MANUSCRIPT NUMBER: 144565-0

TITLE: Mineral surface chemistry control for origin of prebiotic peptides

Authors: Valentina Erastova, Matteo T. Degiacomi, Donald Fraser, H. Chris Greenwell

General comments: In my opinion, this paper has important results for the understanding of the adsorption and polymerization of amino acids on mineral surfaces and consequently for the

prebiotic chemistry. However, there is lack between the results of this paper and adsorption/polymerization of amino acids in the context of the prebiotic chemistry. Montmorillonite is one the most studied mineral in prebiotic chemistry. The pHpzc of this clay is about 2.0 meaning that at pH above 2.0, it is negatively charged. Thus, it will adsorb positively charged molecules. The authors used a mineral whose net charge is positive. Thus, it adsorbs molecules negatively charged. Indeed, in general, minerals adsorb charged molecules (D.A.M. Zaia, A review of adsorption of amino acids on minerals: was it important for origin of life? Amino Acids 27, 113-118, 2004). In the introduction, the authors should discuss these differences. In addition, because montmorillonite is negatively charged, it has a preference to adsorb amino acids such as lysine, arginine and histidine. However the authors supposed that histidine and lysine adsorbed onto [Mg3Al(OH)8]+, it should be noticed that at pH 9.5 lysine has a positive charge from side chain (pKa3 = 10.5). What is this positive charge effect on the adsorption? The authors could pointed out that in hydrothermal vents pH could reach to pH 11.0 (W. Martin et al., Hydrothermal vents and the origin of life, Nature Reviews/Microbiology, 6, 805-814, 2008). However, what is the effect of this high pH on [Mg3Al(OH)8]+? Could it be decomposed? Also, was [Mg3Al(OH)8]+ a common mineral in prebiotic Earth (R.M. Hazen et al., Mineral evolution, American Mineralogist, 93, 1693-1720, 2008)? I also have a few suggestions as below.

Q.1. RESULTS, SECTION Intercalation of amino acids affects LDH layers “Irrespective of the identity of…..charge on amino acids (Figure S2a)”.

Comment: Why did not aspartic acid follow this trend?

Q.2. RESULTS, SECTION Amino acids and peptides adsorb on LDHs via their C-terminal, “Here the amine side-chain , reducing the adsorption on the LDH surface”.

Comment: If the simulation was carried out at pH higher than pKa (10.5) of lysine could the adsorption increase?

Q.3 RESULTS, SECTION LDHs promote amino acids polymerization “Alanine shows the highest per unit volume (due its double charge)”.

Comment: This result is very interesting for prebiotic chemistry because minerals usually adsorb more amino acids with side-chain charged than amino acids with side-chain uncharged. However, proteins of living being have more amino acids side-chain uncharged than side-chain charged (M.H. Klapper, Independent distribution of amino acids near neighbor pairs into polypeptides. Biochemistry and Biophysics Research Communications, 78, 1018-1024, 1977; I.K. Jordan et al., A universal trend of amino acid gain and loss in protein evolution, Nature, 433, 633-638, 2005). Besides experiments, simulating the prebiotic Earth or interstellar environments showed high amount of amino acid with uncharged side chain, their adsorption onto mineral is low (D.A.M. Zaia et al., Which amino acids should be used in prebiotic chemistry studies? Origins of Life and Evolution of the Biosphere 38, 469-488, 2008). Thus in experiments with wetting/drying cycles could produce peptides with high amount of amino acids like alanine. Thus, the primordial peptides could be more like the proteins of living beings of today. This result could give glue what happen in the prebiotic Earth.

Q.4 Discussion and conclusion

Comment: Reference 24 is cited in the text, but it did not appear in references section

Reviewer #3 (Remarks to the Author):

The submitted manuscript entitled “Mineral Surface Chemistry Control for Origin of Prebiotic

Peptides" is devoted to molecular dynamics (MD) investigation of possible mechanism of peptides synthesis from amino acids within interlayer region of anionic clays – layered double hydroxides (LDHs). The research has scientific novelty and was carried out at a sufficiently high scientific level. Presented results may be of interest to biological community as well as specialists within Earth sciences, chemistry and physics, which makes manuscript a good candidate to be published in Nature Communications. However, the manuscript requires some insignificant corrections and/or additions (as discussed below) before publication.

There are several MD studies devoted to the interaction of amino acids with LDHs (see references below [1-4]), but only studies on the interaction of LDH with nucleic acids / RNA are mentioned by authors in the introduction. In particular, Newman et al. [1] considered the interaction of Phe and Tyr amino acids with Mg3/Al-LDH, having similar stoichiometry as in manuscript. Kalinichev et al. studied systems with deprotonated Glu anions (1-, 2-) intercalated into Mg2/Al-LDH [2]. The interaction of anionic amino acids (Asp, Glu) with Mg2/Al-LDH and the formation of multimolecular hybrid complexes on the LDH surface were investigated in [3]. Interaction / adsorption of cationic Arg amino acid onto Mg2/Al-LDH surface was studied in [4].

Word "no" in Table S1, meaning "number", should be replaced by "N" or "#" (or something third) for better understanding.

Black arrow (axis) on Fig.S2,c is directed to the right, whereas the number of water molecules per amino acid decreases from 20 to zero. It would be more natural way to arrange snapshots (below arrow) in reverse order or change axis label to "dehydration…".

As a note, in the further development of the proposed idea, it would be interesting to perform MD simulations with an explicit calculation of chemical reactions (peptide bonds formation at different pH, T, hydration, etc. conditions), using, for example, ReaxFF-like approach [5].

References

1. Newman S. P., Cristina T. D., Coveney V. and Jones W. Molecular dynamics simulation of cationic and anionic clays containing amino acids. Langmuir 18, 2933–2939 (2002).
2. Kalinichev A. G., Padma Kumar P., and James Kirkpatrick R. (2010). Molecular dynamics computer simulations of the effects of hydrogen bonding on the properties of layered double hydroxides intercalated with organic acids. Philosophical Magazine, 90(17-18), 2475-2488.
3. Tsukanov A. A., and Psakhie S. G. (2016). Energy and structure of bonds in the interaction of organic anions with layered double hydroxide nanosheets: A molecular dynamics study. Scientific reports, 6, 19986.
4. Tsukanov A. A., and Psakhie S. G. (2016). Adhesion effects within the hard matter–soft matter interface: Molecular dynamics. Facta Universitatis, Series: Mechanical Engineering, 14(3), 269-280.
5. Chenoweth K., Van Duin A. C., and Goddard III W. A. (2008). ReaxFF reactive force field for molecular dynamics simulations of hydrocarbon oxidation. Journal of Physical Chemistry A, 112(5), 1040-1053.

Response to Reviewers

We greatly appreciate the reviewers' careful scrutiny of our manuscript, and are delighted with their broadly positive in their assessment of the work. We welcome the opportunity to revise the manuscript in light of their suggestions. Below we provide a detailed description of the adjustments made in response to the reviewers' concerns.

Reviewer #1:

A first original contribution is the evidence for a templating effect of aluminum substitutions in the hydrotalcite lattice, imposing on the amino acids an orientation that favors the condensation of their C-termini. However, amino acids also condense on surfaces that cannot induce this kind of structuring, such as amorphous silica. If the authors' suggestion is correct, condensation should occur more easily on/in hydrotalcites than on silica. Are there experimental results to support this prediction? The authors certainly suggest that there is something special to hydrotalcites: cf. on p.13 "unlike previously observed…(this mechanism has) a strong resemblance to ribosome-catalyzed peptide bond formation". This is a very intriguing idea, but also highly speculative until corroborated experimentally.

In this work we show that amino acids polymerization is possible on hydrotalcites. This phenomenon has been already well studied on silicates. The known drawback of silicates is that adsorbed amino acids must feature charged side chains, and that the release of formed peptides is hindered (see ref. 3). In this work we propose a different adsorption mechanism (via the deprotonated C-terminal), enabling adsorption on any type of amino acid. During polymerization, the growing chain remains attached mainly via its C-terminal, making peptide release feasible. A key feature of our proposed mechanism is that long peptide chains should be obtained by multiple repopulation cycles. The aim of this paper is to bring forward this mechanism as a hypothesis to be tested experimentally. Initial research has been undertaken on Aspartate LDHs (unpublished), which showed evidence of small oligomers forming. This work needs to be built on and the results better verified to ensure reproducibility.

The specific treatment of amino acids mobility on p.10 is also an interesting feature. This question is central to the study of bimolecular reactions and is generally overlooked in studies of biomolecules surface condensation. However, the velocity units must be explained – Å nm-1 is not a velocity.

We thank the reviewer for having noted this. This was a typo, velocities were measured in Å ns-1. We have corrected the text accordingly.

How is the layer charge compensated? The "online methods" state that "amino acids… are deprotonated to represent pH 9.5, and in the majority carry a negative charge that counterbalances the positive the positive LDH charge". This is not clear. Was the proportion of deprotonated amino acids chosen on the basis of the corresponding pH? Or of the LDH

charge compensation? I assume no other charge compensating ions, such as carbonates, were introduced. Connected to the previous question, what is the acido-basic speciation of amino acids and peptides? They are mostly anions, with one carboxylate and one amine group (cf. "in the majority" above), but are there any zwitterions (or neutral amino acids)?

We agree that our explanation was not sufficiently clear, and we have provided further details in Methods section. Supplementary Table 1 reports the total charge of each amino acid, and the amount and nature of counterbalancing ions (Cl- or Na+) used to neutralize the total charge of each simulation box. We have not used carbonates because in early earth conditions atmospheric carbonate concentrations were much lower than current ones.

In our simulations we deprotonated all groups according to their pKa values. Each group having a pKa lower than 9.5 was deprotonated. For example, at pH 9.5 lysine is zwitterionic, carrying a negative charge on the backbone, and a positive -NH3+ on the side chain.

[…] do proton transfers occur in the various stages of modeling? Also on p.12 "Formation of a peptide bond leads to the loss of a charged group. »: how then is the conservation of charge assured? By the formation of a hydroxide anion?

The reviewer correctly notes that a system containing, e.g., 2 amino acids, may have a different total charge thank a system featuring a dipeptide.

We carried out simulation of systems containing single amino acids, peptides and mixtures separately. As such, each system was individually charge-balanced. Full details about the charge of each simulation are provided in Supplementary Table 1.

And on p.8 "the amine side chain is strongly positive" (p. 8): does it mean that it is protonated to an ammonium?

The reviewer is correct, at pH 9.5 lysine will contain an ammonium group on its side chain. We have now corrected this sentence.

What is the criterion for determining through which moiety the amino acids and peptides are adsorbed? (e.g. "adsorption via the backbone" or "adsorption via the side chain" of the Asp molecules.) Is is energetic, based on spatial proximity? Some answers can be found in the supplementary information, but the matter should be clearly stated before the first mention of adsorption mechanism.

Adsorption was determined using a distance cut-off of 2.5 Å, corresponding to the distance of the first hydration layer of the LDH, and is a typical distance for H-bond analysis. We have added this additional information in Methods section, as well as in the caption of Figure 2.

p. 3, last § « The idea of using hydrated mineral surfaces » - why hydrated ? this is somewhat contradictory with considering "hydrophobic" surfaces

Thank you for bringing this to our attention. Hydrophilic mineral surfaces are suitable for

biological catalysis involving polar molecules such as amino acids. We realized that our later mention of hydrophobic/hydrophilic domains may confuse the reader. As this does not provide any information useful to further understand the context of our work, we have decided to remove it.

p. 4, 1st §: "Some of these surfaces may have very high enthalpies of hydration, providing a driving force for condensation reaction": This statement should be qualified. Actually, if adsorption of biomonomers occurs from a water solution, the surfaces are already hydrated, and their hydration cannot provide a thermodynamic driving force for condensation.

Adsorption of biomonomers does indeed occur from a water solution. Layers with adsorbed species can however subsequently dehydrate because of physical phenomena such as heat or tides. We realize this sentence was unclear, and substituted "hydration" for "rehydration".

p.6: a remarkable paucity of simulation data for peptide-mineral interactions: for peptide-clays, one can mention

Aquino, A. J. A.; Tunega, D.; Gerzabek, M. H.; Lischka, H., Modeling Catalytic Effects of Clay Mineral Surfaces on Peptide Bond Formation. J. Phys. Chem. B 2004, 108, 10120-10130
or even a first attempt, now largely superseded:

Collins, J. R.; Loew, G. H.; Luke, B. T.; White, D. H., Theoretical investigation of the role of clay edges in prebiotic peptide bond formation. Orig. Life Evol. Biosph. 1988, 18, 107-119 Slightly outdated, but addressing an important problem.

Our wording was poorly chosen, and we have amended it to "*peptide-LDH interactions*". We thank however the reviewer for having indicated these references, focussing on works about peptides-silicate clays interactions. At p.5, we mention "*Many studies have been carried out on silicate clays to study their potential role in the formation of protobiomolecules*", and provide four references. The suggested references are very pertinent to this statement, and have therefore decided to add the more recent of the two.

p.7, "Irrespective of the identity of the amino acid, the LDH interlayer dehydrates similarly » : I do not understand what exactly the authors mean by « similarly » ; this contention needs to be developed further.

We have replaced "*similarly*" with "*with the same trend*". This is then qualified in the second part of the sentence, stating "*indicating that the basal d-spacing is proportional to the number of atoms (organic load) present in the interlayer […]*".

p.10: "peptide bond formation is endergonic with a free energy…comparable to that of the system's rehydration, thus providing a driving force for the polymerization": this sentence

must be rephrased. It can be read as meaning that the endergonicity of the reaction provides a driving force, which is contrary to common sense: I suppose the authors actually mean that when it is coupled to the interlayers' rehydration, the global reaction becomes exergonic.

The reviewer is right, we are sorry for the confusion. We have rephrased this sentence as follows: "*The formation of a peptide bond releases a molecule of water, thus contributing to the rehydration of the interlayer. We note that peptide bond formation is endergonic with free energy change of 10-20 kJ* mol^{-1}*. This is comparable to that of system's rehydration, that provides a driving force for the polymerization reaction.*".

p.11: "Building upon the attractive hypothesis of Russell and Martin …» : what hypothesis actually ? In the previous text, they are mentioned only as providing a geochemical setting for prebiotic chemistry (alkaline hydrothermal systems), so how is the present work building upon it?

Russell and Martin suggest that alkaline hydrothermal vents, and the mineral assemblages and microstructures found there, may provide a geochemical environment where prebiotic chemistry may have been favoured. We agree with the reviewer that the link between Russell and Martin's work and our own is not as direct as this statement reads. Therefore we have removed an explicit mention of their work in Discussion.

p.12: "…the SIPF theory": it is not so much a theory as a type of reaction, "Salt-Induced Peptide Formation". Give the meaning of the acronym for the general reader.

We have now added the definition of this acronym.

p.13, "unlike previously observed23,24, » : ref. 24 is missing.

We are sorry for this oversight; the references have now been updated.

Reviewer #2:

[…] there is lack between the results of this paper and adsorption/polymerization of amino acids in the context of the prebiotic chemistry. Montmorillonite is one the most studied mineral in prebiotic chemistry. The pHpzc of this clay is about 2.0 meaning that at pH above 2.0, it is negatively charged. Thus, it will adsorb positively charged molecules. The authors used a mineral whose net charge is positive. Thus, it adsorbs molecules negatively charged. Indeed, in general, minerals adsorb charged molecules (D.A.M. Zaia, A review of adsorption of amino acids on minerals: was it important for origin of life? Amino Acids 27, 113-118, 2004). In the introduction, the authors should discuss these differences.

We thank the reviewer for the suggested reference; we have added it to the main text. Unfortunately size limitations in the introduction section did not allow us to further discuss this point without sacrificing others.

In addition, because montmorillonite is negatively charged, it has a preference to adsorb amino acids such as lysine, arginine and histidine. However the authors supposed that histidine and lysine adsorbed onto [Mg3Al(OH)8]+, it should be noticed that at pH 9.5 lysine has a positive charge from side chain (pKa3 = 10.5). What is this positive charge effect on the adsorption?

Although upon dehydration lysine does adsorb on the LDH surface, it does so to a lesser extent than all other negatively charged amino acids (see Figure 2a). Lysine adsorbs via its backbone as all other amino acids, and when adsorbed it follows their same behaviour in terms of adsorption times and velocities (see Figure 4).

The authors could pointed out that in hydrothermal vents pH could reach to pH 11.0 (W. Martin et al., Hydrothermal vents and the origin of life, Nature Reviews/Microbiology, 6, 805-814, 2008). However, what is the effect of this high pH on [Mg3Al(OH)8]+? Could it be decomposed?

LDH are synthesized at high pH (>8), and are stable even at extremely high pH (>12). See for instance:

- J. W. Boclair and P. S. Braterman, "Layered Double Hydroxide Stability. 1. Relative Stabilities of Layered Double Hydroxides and Their Simple Counterparts", Chem. Mater., 1999
- Seron and F. Delorme, "Synthesis of layered double hydroxides (LDHs) with varying pH: A valuable contribution to the study of Mg/Al LDH formation mechanism", Journal of Physics and Chemistry of Solids, 2008

This property enables them to exist in hydrothermal vents.

Also, was [Mg3Al(OH)8]+ a common mineral in prebiotic Earth (R.M. Hazen et al., Mineral

evolution, American Mineralogist, 93, 1693-1720, 2008)?

We thank the reviewer for having pointed this out. We have added this reference in the introduction, along with a mention that such surface was indeed common in early earth.

Q.1. RESULTS, SECTION Intercalation of amino acids affects LDH layers "Irrespective of the identity of…..charge on amino acids (Figure S2a)". Comment: Why did not aspartic acid follow this trend?

Aspartate is deprotonated at both side chain and backbone, leading to a total charge of -2. For this reason, to compensate the LDH charge, for aspartate systems we have used half the concentration of all other amino acids tested in this work. As a consequence, the number of atoms intercalated in the interlayer was smaller in aspartate systems, leading to smaller *d*-spacings (Supplementary Figure 2a). We should also notice that the strong negative charge of aspartate makes it more adsorbing than all other amino acids, as shown in Figure 2.

Q.2. RESULTS, SECTION Amino acids and peptides adsorb on LDHs via their C-terminal, "Here the amine side-chain , reducing the adsorption on the LDH surface". Comment: If the simulation was carried out at pH higher than pKa (10.5) of lysine could the adsorption increase?

At such a pH the lysine side chain would deprotonate. On the basis of results for other amino acids, we expect that lysine adsorption should increase as a result.

Q.3 RESULTS, SECTION LDHs promote amino acids polymerization "Alanine shows the highest per unit volume (due its double charge)". Comment: This result is very interesting for prebiotic chemistry because minerals usually adsorb more amino acids with side-chain charged than amino acids with side-chain uncharged. However, proteins of living being have more amino acids side-chain uncharged than side-chain charged (M.H. Klapper, Independent distribution of amino acids near neighbor pairs into polypeptides. Biochemistry and Biophysics Research Communications, 78, 1018-1024, 1977; I.K. Jordan et al., A universal trend of amino acid gain and loss in protein evolution, Nature, 433, 633-638, 2005). Besides experiments, simulating the prebiotic Earth or interstellar environments showed high amount of amino acid with uncharged side chain, their adsorption onto mineral is low (D.A.M. Zaia et al., Which amino acids should be used in prebiotic chemistry studies? Origins of Life and Evolution of the Biosphere 38, 469-488, 2008). Thus in experiments with wetting/drying cycles could produce peptides with high amount of amino acids like alanine. Thus, the primordial peptides could be more like the proteins of living beings of today. This result could give glue what happen in the prebiotic Earth.

Indeed, in this work we look at the interactions in Early Earth conditions (hydrothermal vent-like, high pH). In these conditions amino acids adsorb via their deprotonated carboxylic group of the backbone on the positive LDHs. So, amino acids with neutral side chain will readily adsorb and form reactive pairs. This in principle allows the uptake of any amino acid from the

environment. Therefore the composition of the peptides produced via the method suggested in this work will be primarily dictated by the availability of the amino acids. This is not the case for negative silicate clays such as montmorillonite, where the amino acids adsorb mainly via their charged side chains.

Q.4 Discussion and conclusion. Comment: Reference 24 is cited in the text, but it did not appear in references section

We are sorry for this oversight; the references have now been updated.

Reviewer #3:

There are several MD studies devoted to the interaction of amino acids with LDHs (see references below [1-4]), but only studies on the interaction of LDH with nucleic acids / RNA are mentioned by authors in the introduction. In particular, Newman et al. [1] considered the interaction of Phe and Tyr amino acids with Mg3/Al-LDH, having similar stoichiometry as in manuscript. Kalinichev et al. studied systems with deprotonated Glu anions (1-, 2-) intercalated into Mg2/Al-LDH [2]. The interaction of anionic amino acids (Asp, Glu) with Mg2/Al-LDH and the formation of multimolecular hybrid complexes on the LDH surface were investigated in [3]. Interaction / adsorption of cationic Arg amino acid onto Mg2/Al-LDH surface was studied in [4]. References:

1. Newman S. P., Cristina T. D., Coveney V. and Jones W. Molecular dynamics simulation of cationic and anionic clays containing amino acids. Langmuir 18, 2933–2939 (2002). 2. Kalinichev A. G., Padma Kumar P., and James Kirkpatrick R. (2010). Molecular dynamics computer simulations of the effects of hydrogen bonding on the properties of layered double hydroxides intercalated with organic acids. Philosophical Magazine, 90(17-18), 2475-2488. 3. Tsukanov A. A., and Psakhie S. G. (2016). Energy and structure of bonds in the interaction of organic anions with layered double hydroxide nanosheets: A molecular dynamics study. Scientific reports, 6, 19986.

4. Tsukanov A. A., and Psakhie S. G. (2016). Adhesion effects within the hard matter–soft matter interface: Molecular dynamics. Facta Universitatis, Series: Mechanical Engineering, 14(3), 269-280.

5. Chenoweth K., Van Duin A. C., and Goddard III W. A. (2008). ReaxFF reactive force field for molecular dynamics simulations of hydrocarbon oxidation. Journal of Physical Chemistry A, 112(5), 1040-1053.

We thank the reviewer for having pointed these references out, and have added the second suggested reference in the introduction. Although the other references are pertinent in the context of amino acid adsorption on mineral surfaces, we have chosen not to add them as they are not addressing the topic of formation of proto-biomolecules or origins of life.

Word "no" in Table S1, meaning "number", should be replaced by "N" or "#" (or something third) for better understanding.

We have now substituted "*No*" with "#".

Black arrow (axis) on Fig.S2,c is directed to the right, whereas the number of water molecules per amino acid decreases from 20 to zero. It would be more natural way to arrange snapshots (below arrow) in reverse order or change axis label to "dehydration…".

We agree with the reviewer, the direction of the arrow could have confused the reader. We have relabelled the figure to clarify our intent, i.e. that the arrow indicates the direction of our simulation protocol, stepwise dehydrating the interlayers.

As a note, in the further development of the proposed idea, it would be interesting to perform MD simulations with an explicit calculation of chemical reactions (peptide bonds formation at different pH, T, hydration, etc. conditions), using, for example, ReaxFF-like approach [5].

We thank the reviewer for the suggestion. Indeed, observing peptide bonds formation in simulation and testing their dependence on different conditions would definitely be a very exciting project continuation. We have recently performed preliminary testing of ReaxFF for our systems, though we are at a too early stage to draw any conclusion. Using QM/MM methods would be suitable for such a study, and should be considered for future modelling work.