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Enhanced transcription and translation in clay hydrogel and implications for early life evolution

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In most contemporary life forms, the confinement of cell membranes provides localized concentration and protection for biomolecules, leading to efficient biochemical reactions. Similarly, confinement may have also played an important role for prebiotic compartmentalization in early life evolution when the cell membrane had not yet formed. It remains an open question how biochemical reactions developed without the confinement of cell membranes. Here we mimic the confinement function of cells by creating a hydrogel made from geological clay minerals, which provides an efficient confinement environment for biomolecules. We also show that nucleic acids were concentrated in the clay hydrogel and were protected against nuclease, and that transcription and translation reactions were consistently enhanced. Taken together, our results support the importance of localized concentration and protection of biomolecules in early life evolution, and also implicate a clay hydrogel environment for biochemical reactions during early life evolution.

In modern cell-based life forms, the confinement by cell membranes offers localized concentration and protection for biomolecules such as nucleic acids, leading to efficient biochemical reactions such as transcription and translation[1,2]. In addition to its importance in modern cells, this confinement function was also very important for prebiotic compartmentalization in early life evolution prior to the emergence of the last universal common ancestor (LUCA)[3–7]. Many different environments have been considered for this pre-cellular evolutionary stage, including near hydrothermal vents[8], in ocean water[4,9], within hydrogels[10], and around mineral microenvironments near the Earth's surface[11,12]. In defining the pre-cellular environment, it is important to address the following questions: 1) How did biomolecules encounter each other and maintain sufficient proximity to perform complicated biochemical reactions? 2) How did the biomolecules survive in the environment without any protection? To address these issues, several artificial compartmentalization structures have been proposed, such as lipid vesicles[13], fatty-acid vesicles[14], peptide-nucleotide droplets[15], and polymeric droplets[16,17].

Clay minerals have been proposed as a likely candidate among solid materials to play roles for life evolution, due to their wide distribution, historical prevalence throughout the timeline of geological and biological events on Earth (Supplemental Fig. S1), and their affinity for organic molecules[12,14,18–22]. For example, clay has been demonstrated to be capable of catalyzing the polymerization of RNA[12] and accelerating the formation of fatty-acid vesicles (a protocell model)[14]. On the other hand, as an alternative hypothesis, hydrogel microenvironments for life evolution have also been proposed[10]. Both experimental and theoretical studies have indicated that cytoplasm behaves like a poroelastic hydrogel[23,24], and hydrogels microenvironments may provide stability, confinement, and concentration of dilute solutes.

In this work, combining the concepts of clay minerals and hydrogel microenvironments for life evolution, we proposed that clay hydrogel on early Earth provided a confinement function for biomolecules and biochemical reactions. Considering transcription and translation as the central biochemical reactions in biological systems, we employed transcription and translation in a cell-free fashion (cell-free protein expression) as model biochemical reactions (Figure 1a).
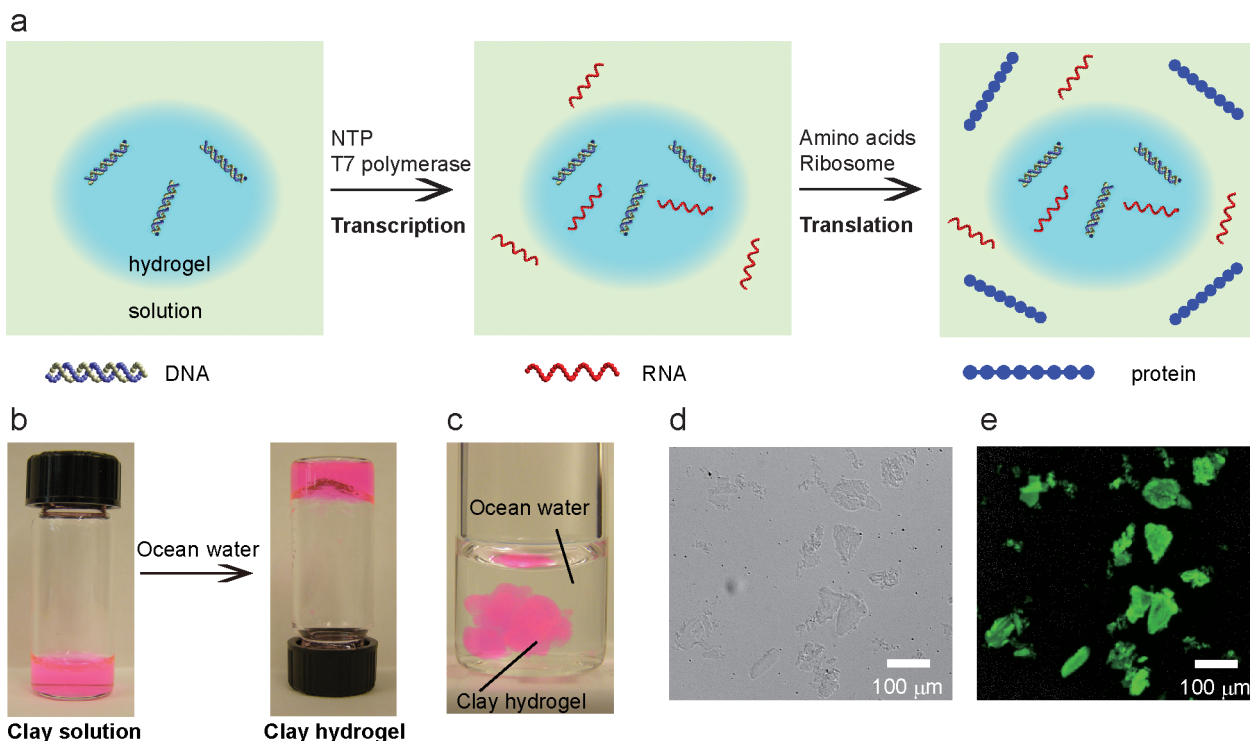
Figure 1 | Schematic diagram of clay hydrogel model and the formation of clay hydrogel in ocean water. (a) Scheme of transcription and translation reactions that occurred in the clay hydrogel environment. (b) The formation of clay hydrogel with ocean water (the volume ratio of clay solution to ocean water is 1 : 1). (c) The formation of clay hydrogel in a large amount of ocean water reservoir (the volume ratio of clay solution to ocean water is 1 : 10). (d and e) Bright field and fluorescence microscopy images of clay hydrogel particles formed in ocean water, respectively. The clay hydrogel was stained with SYBR green.

Results

We discovered that clay forms a hydrogel in ocean water. This is important because clay and ocean water coexisted on and dominated the early Earth surface. After mixing clay with ocean water (volume ratio was 1 : 1), clay spontaneously and instantaneously formed a bulk hydrogel (Figure 1b). We further showed that clay hydrogel also formed even in a much larger reservoir of ocean water (volume ratio of ocean water to clay ranged from 10 : 1 even to 10^6 : 1, Figure 1c), which was more realistic to the scenario on early Earth. In addition, the bulk-scale clay hydrogel was easily broken down by shear forces into micro-particles, which acted as the confinement for biomolecules and biochemical reactions (Figure 1d and 1e).

To understand the mechanism for the formation of clay hydrogel in ocean water, we studied the structures of clay by characterizing the morphology, size and crystal structures via transmission electron microscopy (TEM), dynamic light scattering (DLS) and X-ray diffraction (XRD), respectively (Supplementary Fig. S2–S4). TEM images revealed the disk shape of clay, and DLS data confirmed that the average size of clay was approximately 30 nm, consistent with TEM data. From XRD, clay maintained its crystal structures in the hydrogel format. The mechanism for the formation of clay hydrogel was thus attributed to the overall structure of clay. More specifically, the clay possessed a disk-like structure with an inhomogeneous charge distribution: negative charges on the disk surface and positive charges on the rim[25,26]. This special structure endowed clay with unique properties: in deionized water, the clay disks were homogeneously distributed with an exfoliated structure; whereas, in the presence of ionic species (e.g., ions in ocean water), the clay disks packed together to generate a "house-of-cards" structure which formed a hydrogel (Supplementary Fig. S5).

We note that clay minerals can be found in a range of geological contexts[27,28], such as within the ocean. In addition, the conditions of modern seawater were probably quite different from those of the ancient seawater[29–33]. To explore the formation of clay hydrogel under different environmental conditions, we tested the effect of various conditions, including a range of Fe concentrations, pH, temperatures, and CO_2 contents (Supplementary Fig. S6). The consistent formation of the clay hydrogel suggested that clay hydrogel micro-environments were easily formed in many ancient geological and environmental settings, such as inside marine sediments or in the seawater column at the seafloor. We believe that, in these environments, the clay hydrogel lifetime could be greatly extended, potentially in line with that of evolution.

Next, we tested whether the clay hydrogel provided an effective confinement environment for the nucleic acids. The immobilization of nucleic acids on clay hydrogel was characterized by measuring the attachment efficiency. For DNA (double-stranded), almost 100% molecules were attached onto the clay hydrogel in a wide range of DNA/clay mass ratios; for RNA (single-stranded), the attachment efficiency varied from 10% to 60% (Figure 2a). The attachment of nucleic acids onto the clay hydrogel was ascribed to their electrostatic interactions with the clay hydrogel. The difference in attachment efficiencies between DNA and RNA was due to their different charge densities (double-stranded DNA has approximately twice the charge density of single-stranded RNA).

More importantly, clay hydrogel provided a protective environment for nucleic acids. In order to test this hypothesis, we used a nuclease degradation experiment as a model. As expected, DNA and RNA without the protection of clay hydrogel were easily digested by the DNase and RNase, respectively (Figure 2b, Lane 2, 3 and 2c, Lane 2, 3). Unexpectedly, in the clay hydrogel environment, both DNA and RNA were effectively protected from DNase and RNase digestion (Figure 2b, Lane 6, 7 and 2c, Lane 6, 7). It was noted that DNA and RNA were detached from clay hydrogel in the electric field (electrodesorption) for the agarose electrophoresis analysis, and clay remained in the wells (Figure 2b&2c). We further tested whether the
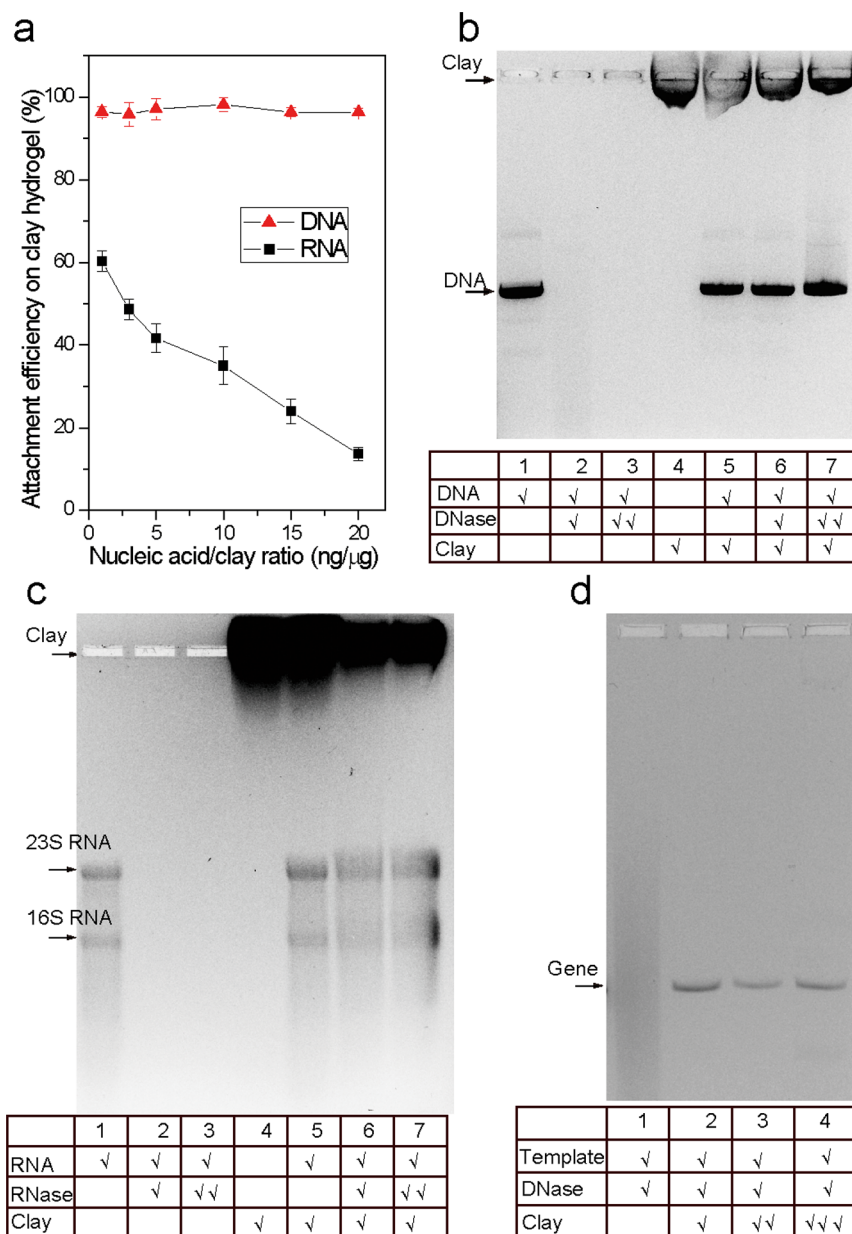
Figure 2 | Localized concentration and protection effects of nucleic acids in clay hydrogel. (a) Attachment efficiency of DNA/RNA on clay hydrogel. (b) Protection of DNA in clay hydrogel environment against DNase. Lane 1: DNA; Lane 2 and 3: DNA plus 0.01 u and 0.05 u DNase, respectively; Lane 4: clay; Lane 5: DNA plus clay; Lane 6 and 7: DNA and clay plus 0.01 u and 0.05 u DNase, respectively. (c) Protection of RNA in clay hydrogel environment against RNase. Lane 1: RNA; Lane 2 and 3: RNA plus 2 u and 4 u RNase, respectively; Lane 4: clay; Lane 5: RNA plus clay; Lane 6 and 7: RNA and clay plus 2 u and 4 u RNase, respectively. (d) The function test of DNA as the template of PCR with the protection of clay hydrogel. Lane 1: without the protection of clay hydrogel; Lane 2–4: with the protection of clay hydrogel, with a variety of clay amount: 200, 300, and 500 ng clay/1 ng template.

functions of nucleic acids can be preserved with the protection of clay hydrogel. We tested the activity of DNA as the template for Polymerase Chain Reaction (PCR). As expected, following the DNase treatment, the DNA molecules in solution were totally digested and were no longer able to function as the template (Figure 2d, Lane 1). Remarkably, in the clay hydrogel environment, DNA could still serve as the template for PCR and was efficiently amplified, indicating that the DNA function was preserved in the clay hydrogel environment (Figure 2d, Lanes 2, 3, and 4).

The above results confirmed that the confinement by clay hydrogel provided an effective environment for localized concentration and protection of nucleic acids. Next, we investigated the effects of clay hydrogel environment on transcription and translation reactions. The plasmid DNA encoded with a reporter gene was

constructed first; with incubation with cell lysates, DNA was transcribed to RNA, which was afterwards translated to reporter proteins[34–37]. Here Renilla luciferase protein (Rluc) was employed as the model reporter protein (Supplementary Fig. S7). To evaluate the efficiency and yield of protein expression in clay hydrogel environment, the solution phase system (SPS) of cell-free protein expression was used as a benchmark, in which genes were homogeneously distributed in the solution.

We surprisingly found that the coupled transcription/translation reaction (an extremely complex process involving more than 30 enzymatic reactions[38]) was not only preserved but was also consistently enhanced in the clay hydrogel environment. We varied the concentration of clay with a fixed gene concentration of 20 ng/μl. As a control, the same amount of plasmid was used in SPS, but without

clay hydrogel in the reaction. Compared with the SPS control, the protein expression yield was consistently enhanced in the clay hydrogel system (Figure 3a). In particular, the clay hydrogel system with clay concentration of 4 μg/μl produced 160 μg luciferase in a 50 μl reaction volume within 8 h, which was equivalent to an expression efficiency of 160 μg of protein per microgram of plasmid and an expression yield of 3.2 mg/ml, representing a 6.6-fold enhancement over SPS. We also fixed the clay concentration at 4 μg/μl and found that, under a wide range of gene concentrations, the clay hydrogel system also exhibited consistently higher yields in comparison with SPS (Figure 3b). We noticed that, with increase of clay and gene concentrations, the protein yields increased first, reached a peak and then decreased (Figure 3a&3b). This phenomenon, as previously reported, is due to the crowding effect[36,39]. In the scenarios of early life evolution, the confinement of biomolecules and biochemical reactions within the clay hydrogel enabled a positive crowding effect, favoring an increase in protein expression. On the other hand, without the clay hydrogel, it would have been very difficult to achieve confinement in a vast quantity of ocean water; therefore the concentration of genes would have been essentially zero, resulting in no protein expression (Figure 3b). In addition, the clay hydrogel system showed a similar kinetic trend as SPS, but with a faster expression rate (approximately 4.4 times greater, proportional to the slope of the linear stage), and with a longer duration of expression before saturation (Figure 3c).

We further isolated transcription from the coupled cell-free protein expression system and measured the concentration of mRNA via quantitative real time PCR (qPCR). The results showed that at every time point the concentration of mRNA in clay hydrogel system was higher than that of SPS (Figure 3d). SDS-PAGE (Figure 3e), luminescence image (Figure 3f) and western blot (Supplementary Fig. S8) all clearly confirmed that more Rluc proteins were produced from the clay hydrogel system than SPS. Several additional experiments verified that the enhancement of protein expression could be exclusively attributed to the clay hydrogel environment (Supplementary Discussion S1).

To test the general role of clay hydrogel in biochemical reactions, we furthermore carried out the reactions of DNA ligation and DNA polymerization in clay hydrogel environment. The results showed that both DNA ligation by T4 ligase and DNA polymerization via Phi29 polymerase were preserved in the clay hydrogel environment (Supplementary Fig. S9).

Discussion

We noticed that the clay hydrogel inhibited nuclease activities (Figure 2b) but did not inhibit the transcription/translation enzymatic reactions (Figure 3). While the exact mechanisms remain to be delineated, we speculate that evolution might have already selected certain enzymes to be much more functional in the clay hydrogel environment than others. In other word, clay selectively protected
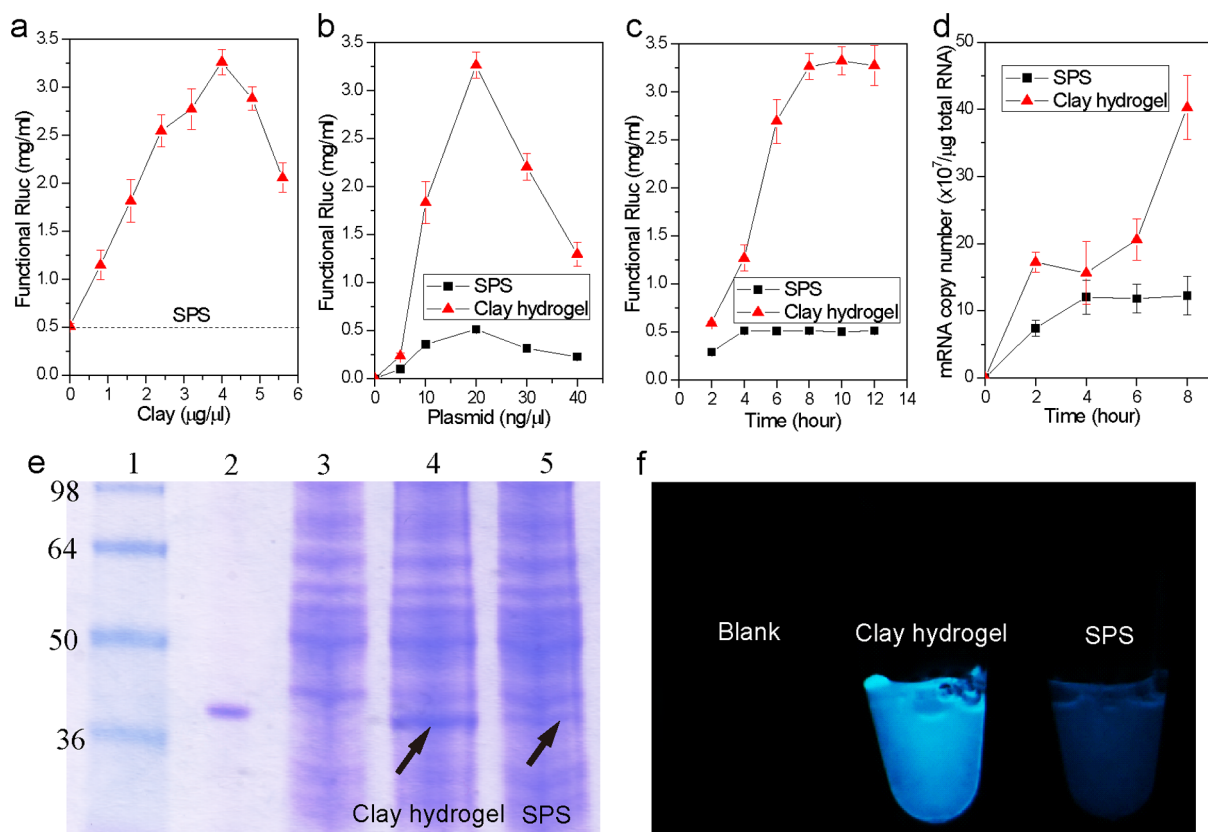
Figure 3 | Transcription and translation in the clay hydrogel environment. (a) Effect of clay concentration on active Rluc protein expression, with fixed plasmid concentration of 20 ng/μl. (b) Effect of gene concentration on active Rluc protein expression of clay hydrogel (red triangles) over SPS controls (black squares), with fixed clay concentration of 4 μg/μl. (c) Time course of active Rluc protein expression produced from clay hydrogel (red triangles) and SPS (black squares). (d) Time course of mRNA expression from clay hydrogel (red triangles) and SPS (black squares). Error bars represent standard deviations from three measurements. (e) SDS-polyacrylamide gel electrophoresis assays of Rluc proteins. Lane 1 corresponds to the pre-stained protein standard (SeeBlue Plus2 Invitrogen, unit is kDa). Pure standard Rluc is in Lane 2 (with molecular weight of 36,113 Dalton). Lane 3 is the lysate control. Lane 4, 5 are Rluc proteins expressed from clay hydrogel and SPS, respectively (molecular weight was 35,991 Dalton). The Rluc bands in Lane 4 and 5 migrated slightly faster than Lane 2 due to slightly lower molecular weight. (f) Comparison of luminescence images of active Rluc protein expressed from clay hydrogel and SPS. Blank is the lysate control.

molecules that were conducive to the RNA and protein synthesis, such as DNA, RNA, transcriptional and translational machinery. These protection mechanisms are under further investigation. Similarly, evolution in general and current enzymes in particular might have been directed by the clay hydrogel environment to favor the organization of a variety of related enzymatic reactions in close proximity. Indeed, current life relies on the confinement by cell membranes to enclose biochemical reactions; several recent reports have already suggested that inside membrane-enclosed cells the properties of cytoplasm are essentially the same to those of poroelastic hydrogels[23,24]. This enclosure ensures localized concentration and protection of the involved biomolecules. However, prior to the LUCA, before the formation of this enclosure, it is an open question as to in what kind of confinement environment the biochemical reactions occurred. Our clay hydrogel environment provided an effective confinement for the biomolecules and the biochemical reactions.

Our data also showed that, in the clay hydrogel environment, RNA was protected and RNA synthesis was enhanced (Fig. 2c and Fig. 3d). Thus, we speculated that many RNA-associated processes might have occurred in the clay hydrogel environment. In turn, this could imply that the clay hydrogel provided a favorable environment for pre-cellular evolution in an "RNA world". In addition, our hydrogel offered a link among ocean water, ancient clay and gel-like cytosol. In the clay hydrogel, nucleic acids were concentrated and protected, and the central biochemical reactions, transcription and translation, were not only preserved but also consistently enhanced in the clay hydrogel environment. In conclusion, we demonstrated the importance of confinement for biomolecules and biochemical reactions in early life evolution and that the early life evolution may have occurred in a clay hydrogel environment.

Methods

Clay hydrogel formation. A commercially available clay (Laponite XLG, Southern Clay Products, Inc.) was used to prepare the clay hydrogel. The formula of Laponite XLG was $LiMgNaO_6Si_2$. Laponite XLG clay solution was prepared in deionized water. After adding ocean water (Aquil) in the clay solution (1 : 1 volume ratio), the bulk hydrogel formed. Alternatively, clay solution was added in a large amount of ocean water (1 : 10 volume ratio), in which clay hydrogel formed in the reservoir. The clay hydrogel was broken into smaller hydrogel micro-particles by pipetting up and down. Fluorescence optical microscopy was employed to observe the morphology of clay hydrogel. The clay hydrogel particles were stained by SYBR Green.

Localized concentration effect. The localized concentration effect of nucleic acid in clay hydrogel environment was characterized by measuring the attachment efficiency of DNA/RNA in the clay hydrogel. The DNA and RNA used here were pIVEX2.4RL plasmid and total E. coli RNA, respectively. Clay/nucleic acid hydrogel particles formed in ocean water reservoir. The mixture was then centrifuged at 15,000 rpm for 30 min. Finally the concentration of nucleic acid in the supernatant was measured using a NanoDrop 1000 spectrophotometer. The amount of nucleic acid adsorbed in the clay hydrogel was calculated by the following formula: $S = (C_0 - C_s)/C_0*100$. Where S is the adsorption efficiency of nucleic acid in the clay hydrogel (%), C_0 represents the nucleic acid concentration of initial clay/nucleic acid solution and C_s is the DNA concentration of nucleic acid in the supernatant.

Protection effect. For the protection test of DNA, the samples were incubated with DNase I (New England Biolabs) at 37°C for 1 hour, followed by incubation at 75°C for 5 min to deactivate DNase. The samples were run on a 3% agarose gel at 100 V for 1 hour. For the protection test of RNA, the samples were incubated with RNase I (Promega) at 37°C for 2 hours, followed by incubation at 65°C for 15 minutes to denature the samples. The samples were run on a 1.5% denaturing agarose gel at 100 V for 1.5 hours.

Construction of plasmid. The Rluc gene was amplified using PCR from the pRL-Null vector (Promega) using two primers: 5′-ATG CCA TGG CTT CGA AAG TTT ATG ATC CAG-3′ and 5′-TAC CCC GGG TTA TTG TTC ATT TTT GAG AAC TCG C-3′. After amplification, the Rluc gene was inserted into the Nco I and Sma I sites of the expression vector pIVEX2.4WG (5 Prime) to generate pIVEX2.4RL vector, which was then propagated by transformation into E. coli cells.

Protein expression and characterization. Cell-free protein expression was carried out in 96-well plates or micro-tubes. Clay/gene hydrogel in situ formed in lysate. Lysate and clay/gene hydrogel micro-particles were incubated in a shaker (Roche) at 24°C and 900 rpm. The active Rluc protein concentration was evaluated by measuring the luminescence following the protocol of a commercial Luciferase Assay kit (Promega) on a plate reader (Biotek Gene5). The SDS-polyacrylamide gel electrophoresis and western blot were employed to characterize the expressed proteins (See details in Supplementary Method S2).

1. Zimmerman, S. B. & Minton, A. P. Macromolecular crowding: biochemical, biophysical, and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.* **22**, 27–65 (1993).
2. Zhou, H. X., Rivas, G. N. & Minton, A. P. Macromolecular crowding and confinement: Biochemical, biophysical, and potential physiological consequences. *Annu. Rev. Biophys.* **37**, 375–397 (2008).
3. Budin, I. & Szostak, J. W. Expanding Roles for Diverse Physical Phenomena During the Origin of Life. *Annu. Rev. Biophys.* **39**, 245–263 (2010).
4. Pace, N. R. Origin of Life - Facing up to the Physical Setting. *Cell* **65**, 531–533 (1991).
5. Bernal, J. D. The physical basis of life. (Routledge & K. Paul London; 1951).
6. Mann, S. The origins of life: old problems, new chemistries. *Angew. Chem. Int. Ed.* **52**, 155–162 (2013).
7. Seckbach, J. Genesis - In The Beginning: Precursors of Life, Chemical Models and Early Biological Evolution. (Springer London; 2012).
8. Martin, W., Baross, J., Kelley, D. & Russell, M. J. Hydrothermal vents and the origin of life. *Nat. Rev. Microbiol.* **6**, 805–814 (2008).
9. Joyce, G. F. The antiquity of RNA-based evolution. *Nature* **418**, 214–221 (2002).
10. Trevors, J. T. & Pollack, G. H. Hypothesis: the origin of life in a hydrogel environment. *Prog. Biophys. Mol. Biol.* **89**, 1–8 (2005).
11. Hansma, H. G. Possible origin of life between mica sheets. *J. Theor. Biol.* **266**, 175–188 (2010).
12. Ferris, J. P., Hill, A. R., Liu, R. H. & Orgel, L. E. Synthesis of long prebiotic oligomers on mineral surfaces. *Nature* **381**, 59–61 (1996).
13. Meierhenrich, U. J., Filippi, J. J., Meinert, C., Vierling, P. & Dworkin, J. P. On the Origin of Primitive Cells: From Nutrient Intake to Elongation of Encapsulated Nucleotides. *Angew. Chem. Int. Ed.* **49**, 3738–3750 (2010).
14. Hanczyc, M. M., Fujikawa, S. M. & Szostak, J. W. Experimental models of primitive cellular compartments: Encapsulation, growth, and division. *Science* **302**, 618–622 (2003).
15. Koga, S., Williams, D. S., Perriman, A. W. & Mann, S. Peptide-nucleotide microdroplets as a step towards a membrane-free protocell model. *Nat. Chem.* **3**, 720–724 (2011).
16. Strulson, C. A., Molden, R. C., Keating, C. D. & Bevilacqua, P. C. RNA catalysis through compartmentalization. *Nat. Chem.* **4**, 941–946 (2012).
17. Martino, C. et al. Protein Expression, Aggregation, and Triggered Release from Polymersomes as Artificial Cell-like Structures. *Angew. Chem. Int. Ed.* **51**, 6416–6420 (2012).
18. Ponnamperuma, C., Shimoyama, A. & Friebele, E. Clay and the Origin of Life. *Orig. Life Evol. Biosph.* **12**, 9–40 (1982).
19. Novoselov, A. A. et al. From Cytoplasm to Environment: The Inorganic Ingredients for the Origin of Life. *Astrobiology* **13**, 294–302 (2013).
20. Sami, F. & Tewari, B. B. Interaction of Amino Acids with Clay Minerals and Their Relevance to Chemical Evolution and the Origins of Life. *Orig. Life Evol. Biosph.* **39**, 348–349 (2009).
21. Ito, M., Yamaoka, K., Masuda, H., Kawahata, H. & Gupta, L. P. Thermal stability of amino acids in biogenic sediments and aqueous solutions at seafloor hydrothermal temperatures. *Geochem. J.* **43**, 331–341 (2009).
22. Cairns-Smith, A. G. & Hartman, H. Clay Minerals and the Origin of Life. (Cambridge University Press, Cambridge; 1986).
23. Fels, J., Orlov, S. N. & Grygorczyk, R. The Hydrogel Nature of Mammalian Cytoplasm Contributes to Osmosensing and Extracellular pH Sensing. *Biophys. J.* **96**, 4276–4285 (2009).
24. Moeendarbary, E. et al. The cytoplasm of living cells behaves as a poroelastic material. *Nat. Mater.* **12**, 253–261 (2013).
25. Ruzicka, B. et al. Observation of empty liquids and equilibrium gels in a colloidal clay. *Nat. Mater.* **10**, 56–60 (2011).
26. Wang, J. F., Lin, L., Cheng, Q. F. & Jiang, L. A Strong Bio-Inspired Layered PNIPAM-Clay Nanocomposite Hydrogel. *Angew. Chem. Int. Ed.* **51**, 4676–4680 (2012).
27. Griffin, J. J., Windom, H. & Goldberg, E. D. The distribution of clay minerals in the World Ocean. *Deep-Sea Res. Oceanogr. Abstr.* **15**, 433–459 (1968).
28. Rateev, M. A., Sadchikova, T. A. & Shabrova, V. P. Clay minerals in recent sediments of the World Ocean and their relation to types of lithogenesis. *Lithol. Miner. Resour.* **43**, 125–135 (2008).
29. Kester, D. R., Duedall, I. W., Connors, D. N. & Pytkowic, Rm. Preparation of Artificial Seawater. *Limnol. Oceanogr.* **12**, 176–& (1967).
30. Miller, S. L. & Bada, J. L. Submarine Hot Springs and the Origin of Life. *Nature* **334**, 609–611 (1988).
31. Knauth, L. P. Salinity history of the Earth's early ocean. *Nature* **395**, 554–555 (1998).
32. Holland, H. D. Sea level, sediments and the composition of seawater. *Am. J. Sci.* **305**, 220–239 (2005).
33. Hren, M. T., Tice, M. M. & Chamberlain, C. P. Oxygen and hydrogen isotope evidence for a temperate climate 3.42 billion years ago. *Nature* **462**, 205–208 (2009).

34. Swartz, J. R. Advances in Escherichia coli production of therapeutic proteins. *Curr. Opin. Biotechnol.* **12**, 195–201 (2001).
35. Katzen, F., Chang, G. & Kudlicki, W. The past, present and future of cell-free protein synthesis. *Trends Biotechnol.* **23**, 150–156 (2005).
36. Park, N., Um, S. H., Funabashi, H., Xu, J. F. & Luo, D. A cell-free protein-producing gel. *Nat. Mater.* **8**, 432–437 (2009).
37. Timm, C. & Niemeyer, C. M. On-chip protein biosynthesis. *Angew. Chem. Int. Ed.* **52**, 2652–2654 (2013).
38. Shimizu, Y. *et al.* Cell-free translation reconstituted with purified components. *Nat. Biotechnol.* **19**, 751–755 (2001).
39. Ge, X. M., Luo, D. & Xu, J. F. Cell-Free Protein Expression under Macromolecular Crowding Conditions. *PLoS One* **6** (2011).

Acknowledgments

Author contributions

D.Y. and D.L. conceived and designed the experiments. D.Y., S.P., M.R.H., T.G., E.J.R., A.K.C., Z.G. performed the experiments. D.Y., S.P., M.R.H., T.G., E.J.R., Z.G. and D.L. analyzed the data. All co-wrote the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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