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Can coacervation unify disparate hypotheses in the origin of cellular life?

Basusree Ghosh[1,a], Rudrarup Bose[1,a] and T-Y. Dora Tang[1,2]

Abstract

Here, we review the recent progress in the characterisation and utilisation of coacervates as protocell models in the origin of life studies. We provide evidence that coacervation could have played a unique role during the origin of life, based on its ability to form from a range of different prebiotically relevant molecules; partition solutes; support and alter RNA catalysis and readily deform its shape. We discuss how these properties could have been important for the formation of the first membrane-bound cells, supporting RNA-peptide evolution and primitive metabolism, and in replicating and proliferating by growth and division processes.

Addresses

[1] Max Plank Institute for Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307, Dresden, Germany

[2] Cluster of Excellence, Physics of Life, TU Dresden, 01602, Dresden, Germany

Corresponding author: Tang, T-Y. Dora (tang@mpi-cbg.de)

[a] These two authors contributed equally.

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Keywords

Coacervates, Origin of Life, Partitioning, RNA-peptide world, Lipid vesicles, Protocell, Protometabolism, Evolution, Multiphase droplets, Enzyme reactions.

Introduction

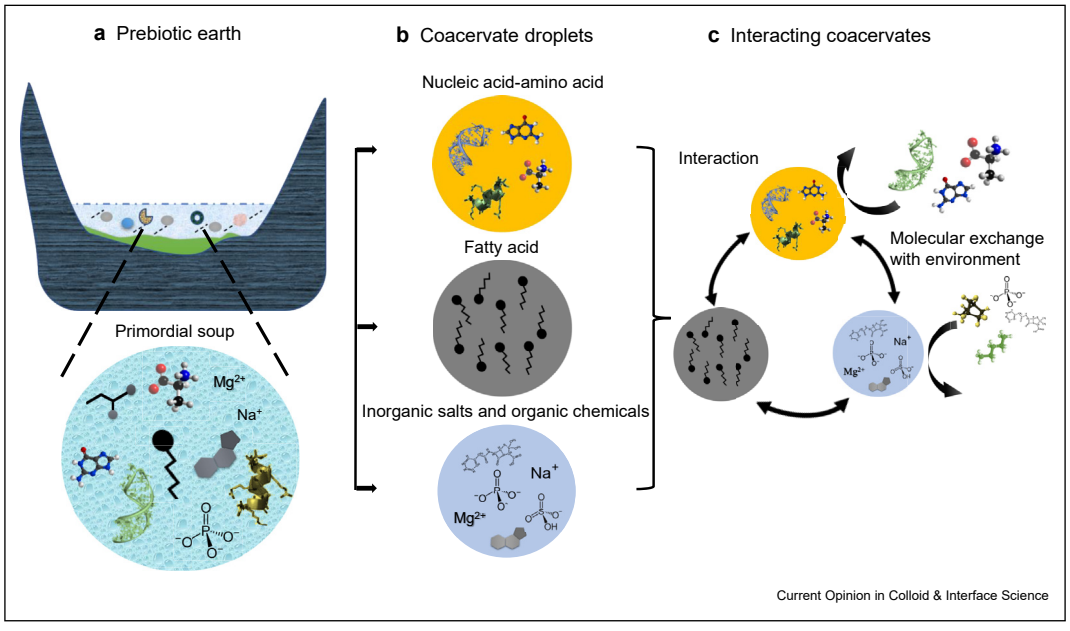
How cellular life originated on prebiotic Earth remains a deep, fundamental, but unanswered question. In particular, how a chemical world of simple molecules would have evolved to complex molecules capable of Darwinian evolution remains a mystery. To answer these questions, one can take a top-down or a bottom-up approach. The top-down approach takes the modern cell and traces it's lineage. This has been effective in providing specific sequences of proteins conserved back to the Last Universal Common Ancestor (LUCA). While being an exceedingly elegant route to tracing life's history, it is likely that there would have been many steps of evolution between the first molecules and cells to the LUCA. The top-down approach provides information about the type of molecules which are important for biological systems such as lipids, nucleotides and proteins. The bottom-up approach considers how the first molecules would have self-assembled together to generate structurally complex and functionally active molecules. By using these biological molecules as a starting point, a number of disparate hypotheses describing the transition from a chemical world to a biological world have been described. One hypothesis being that these molecules could have self-assembled to form protocells. Protocells are defined here as compartments, which could have represented the first reaction chambers or the first cells on the early Earth. In this context, a lipid world would have provided boundary conditions in the form of a primitive compartment to concentrate and localise molecular reactions [1–3]. Alternatively, an RNA world hypothesis is attractive as an origin of life scenario as it would have provided the molecules with genetic information and catalytic activity at the beginning of life [4]. However, the instability of nucleobases to hydrolysis, their limited self-replication and catalytic activities to long RNA, and the relative complexity of the molecule suggest that there are some drawbacks to the RNA world hypothesis [5,6]. An alternative to an RNA world hypothesis is an "RNA-peptide world" that dictates the emergence of RNA and peptide together as a self-replicating system [7]. A metabolism first world would have provided a route to use available energy from the environment for sustaining simple reaction networks. These would have evolved to form more complex reaction networks for energy conversion [8]. In the 1970s, Ganti [9] first conceptualised that a minimal system of life, the chemoton, would have 'functioned under a directed programme'; undergone self-replication and separated itself from the environment possibly via compartmentalisation providing a protocellular environment. In principle, this theory can support the lipid, RNA, peptide and metabolism 'worlds' simultaneously.

In the 1920s, Oparin [10,11] hypothesised that life would have originated inside tiny phase-separated

droplets formed by coacervation [Figure 1]. Complex coacervation is a liquid–liquid phase separation process between two different oppositely charged components in a dilute solution. The polycation and polyanion are attracted to each other by electrostatic interactions and droplet formation is driven by an entropic release from water and counterions from the polymers. This provides a thermodynamically favourable route to droplet-based compartmentalisation on early Earth. The resulting droplets are membrane-free, chemically enriched and in dynamic equilibrium with a polymer-poor outer aqueous phase. Coacervate droplets are intriguing models as protocells as they form between prebiotically relevant molecules such as peptides, RNA, lipids and nucleotides. It has been suggested that coacervate microdroplets are not suitable as protocell models due to their lack of membrane. It was proposed that this would prevent the generation of a chemical gradient generated between the inside of the droplet and the outside [12]. However, it is known that coacervate microdroplets will concentrate reactants (which provides a route to chemical gradient formation); support enzyme reactions; allow the exchange of ions and small molecules with the surrounding media; select for different length of RNA and protect incorporated components from degradation [13–17]. Moreover, their capability to support complex biochemical reactions [16,18] suggests that probing and understanding the physical properties of coacervates could reveal how this general phenomena would have facilitated the onset of cellular life on prebiotic Earth.

Over the last decade, progress in different laboratories has reignited Oparin's ideas that coacervates could have been critical during the origin of life by playing a role in facilitating a transition from a chemical world to a biological world. In this review, we do not advocate for a "coacervate first world' but provide evidence that coacervation can synergise different hypotheses for the origin of cellular life on Earth based on their physicochemical properties. In the first part of the review, we summarise new insights, provided by theory and new technologies; for the physical characterisation of coacervate; the molecular grammar that drives single and multiphase coacervate formation; coacervate material properties; and partition coefficients. We then focus on the possible role of coacervation in different origin of life scenarios such as the lipid world by facilitating membrane-bound compartmentalisation; the RNA-peptide world through RNA catalysis and peptide evolution; the metabolic world and cellular evolution which would have been important for driving the transition from a chemical world to a biological world. Finally, we include concluding remarks.

Figure 1



Coacervate formation on prebiotic Earth. (a) A primordial soup on prebiotic Earth may have been contained biomolecular precursors. **(b)** Coacervation, a phase separation process, could have enriched the biomolecular precursors into membrane-free coacervate droplets to facilitate reactions by up concentration **(c)** Coacervates can concentrate reactants, support enzyme reactions and allow the exchange of ions and small molecules with surrounding media and other compartments.

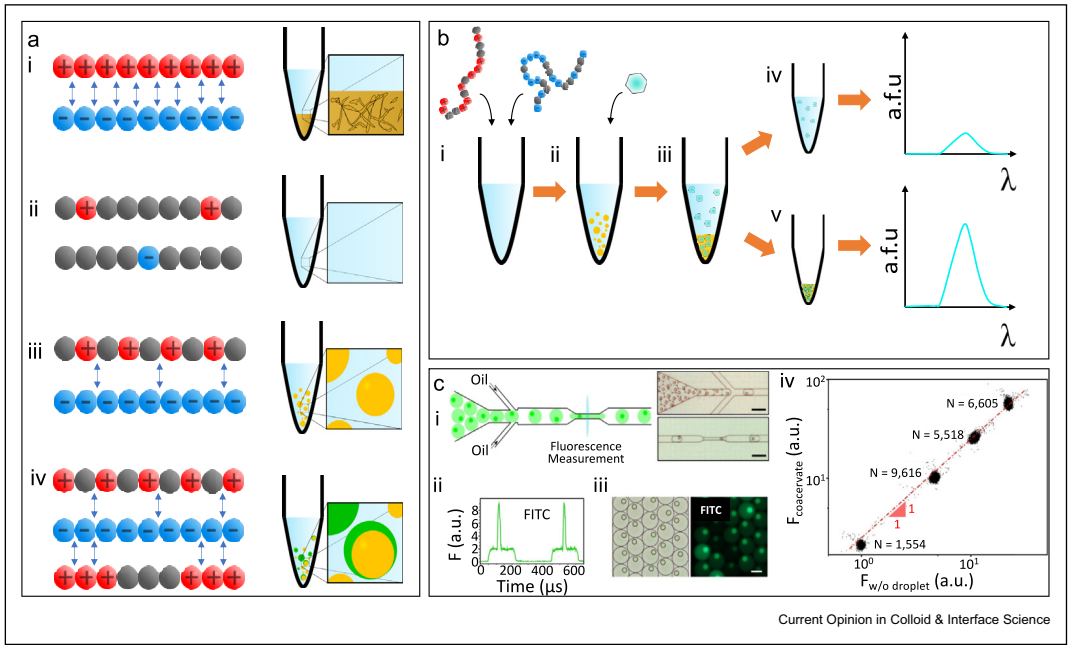
Recent progress in understanding the physical properties of coacervates

Chemical determinants of coacervate formation and material properties

Theoretical studies by Voorn and Overbeek in the 1950s [19,20] showed that complex coacervation was predominantly driven by the charge and length of the polymer chain. Recent studies have shown that coacervate formation is also dependent on the charge distribution on the polyelectrolytes that phase separate to form a coacervate (Figure 2a). Experimental studies, supported by theoretical simulations, showed that the phase diagrams of coacervates formed from 50-mers of negatively charged polyglutamic acid and positively charged poly-lysine-glycine varied depending on the charge distribution on the polycation [21]. Specifically, when the charge distribution was more 'patchy' compared to an even distribution of charges, the phase region of coacervate formation was larger with increased salt conditions. This was commensurate with a broader concentration range of coacervate stability. This is most likely attributed to an increased charge density from the accumulated patches, which strengthens the electrostatic interactions between the polycation and polyanion and stabilises the coacervate droplets.

Not only does the charge distribution affect the properties of the coacervate, but it also appears that the charge density and the flexibility of the polyelectrolyte chains significantly affect the material properties of the coacervates (Figure 2a). Vierreg et al. [22] showed that coacervates formed from double-stranded DNA (between 22 and 88 nt) produce solid precipitates with polylysine (30–100 aa). These precipitates become liquid-like to form droplets with salt concentrations above 500 mM. Jia et al. [23] showed that shorter double-stranded DNA (12 nt) and polylysine will form precipitates or liquid crystalline phases depending on

Figure 2



Coacervate formation and characterisation. (a) Chemical determinants of coacervate formation. The strength of electrostatic interaction between the oppositely charged polyelectrolytes govern whether coacervates can be formed or not. (i) If there is a very strong attraction, precipitates will form. (ii) If the interactions are too weak, the polyelectrolytes will not phase-separate. (iii) Coacervates are formed when the strength of their interactions are optimal. (iv) When there are multiple polyelectrolyte species present in the solution, it is possible to form multiphasic coacervates depending on the critical salt concentrations of the coacervate forming pairs (**b,c) Determination of partition coefficients**. (**b**) Schematic representation of a bulk experimental methodology to measure the partition coefficient. (i) Coacervates are formed by mixing oppositely charged polyelectrolytes. (ii-iii) A molecule of interest is added, and the mixture is centrifuged, leading to the formation of a bulk coacervate phase. (iv) The dilute aqueous phase and (v) the dense bulk coacervate phase are then separated, and the concentration of the molecule of interest is measured using spectroscopy. The partition coefficient (K) is then obtained from the relation $K = [solute]_{coac}/[solute]_{dilute}$. (**c**) shows a recently developed microfluidics-based alternate methodology for obtaining partition coefficients. (i) Coacervates are prepared with the solute of interest using microfluidics and then flowed through a narrow channel to obtain the fluorescence intensity through the droplet as a time sequence (iii) microscopy images of prepared droplets with different concentrations of solute (Scale bar 30 μm). (iv) Partition coefficients obtained from the plots of the fluorescence intensity inside and outside of the droplet for thousands of droplets for different solute concentrations. Reprinted with permission from Ref. [25].

the salt concentration. At comparatively low salt concentrations (≤700 mM NaCl) strong electrostatic interactions between double-stranded DNA and polylysine will favour precipitate formation. Upon further increase of salt (800–900 mM), the interactions are mediated such that liquid-crystalline droplets form.

These studies have been undertaken in simplified and well-controlled systems; however, during the origin of life, it was likely that there would have been mixtures of different molecules within a prebiotic soup (Figure 1). Recent studies have shown that increasing the molecular complexity, within a controlled, laboratory environment can lead to the spontaneous formation of multiphase droplets (Figure 2a). Elastin-like polypeptides (ELPs) with repeated pentamer units of VPG*X*G (V=Valine, P=Proline and G = Glycine) where the amino acid residue *X* was either composed of Valine (V) or Alanine (A) can form multiphase droplets when mixed together, depending on their composition and length [24].

The combination of two types of ELPs with 80 repeating units of the VPG*X*G pentamer composed of $V_{50\%}$ $A_{50\%}$ and A, respectively, would form multiphase droplets. In comparison, mixtures of $V_{50\%}$ $A_{50\%}$ and $V_{80\%}$ $A_{20\%}$ did not form multiphase droplets. This was attributed to differences in the lower critical solution temperatures of each of the individual peptides, i.e., at the minimum salt concentration beyond which coacervates never form. This can be a proxy for the macromolecular density and interfacial tension of the coacervate. Moreover, the length of the peptide was an important factor. For example, 40 repeats of VPGVG did not form multiphase droplets with peptides of VPG*X*G where *X* is $V_{50\%}$ $A_{50\%}$ while longer peptides of 160 repeats did. Multiphasic complex coacervate droplets have also been shown to form as a generic phenomenon arising from differences in critical salt concentrations of the polymers [26]. In addition, multiphase droplets have also been shown to form via dissociative phase separation within coacervate droplets [27]. These studies and others [28] indicate that tuning the chemistries of the coacervate components, their ratios, salt content, and the chemical composition could alter the material properties and their surface tension such that droplets with multi-order structuration can form. These properties are driven by physical interactions between different molecules and as such would have been plausible in the origin of life scenarios.

Multiphase droplets can impact the distribution of biomolecules, and thus, the biochemical reactions contained within them. Results from recent work have reported differential partitioning of fluorescently labelled oligomers of RNA and peptide into the different phases of 2-phase complex coacervate droplets. These were formed from combinations of peptides RRASLRRASL (R = Arginine, A = Alanine, S = Serine and L = Leucine), polyuridylic acid, polylysine, polyglutamic acid, polyaspartic acid, polyacrylic acid, polyallylamine hydrochloride and protamine sulphate [29]. A two-phase complex coacervate, system composed of a strong polyelectrolyte pair, polydiallydimethylammonium chloride (PDDA) and DNA in the core and a weak electrolyte pair, CM-Dextran and DEAE (diethylaminoethyl) dextran on the outside, localised the glucose oxidase (GOx), horseradish peroxidase (HRP) and catalase enzyme cascade. Spatial localisation of the catalase in the outer phase led to an increase in the rate of product formation compared to when all of the enzymes were localised within the inner core [30]. Furthermore, dextranase was shown to partition into and was active in a tetraethylene glycol (TEG) rich outer coacervate phase in multiphase droplets formed by dissociative phase separation within PDDA/ATP coacervate microdroplets [27]. It is unclear whether the molecules in these studies, such as PDDA, CM-Dextran or high molecular weight polyelectrolytes, are prebiotically relevant, as there is no fossil evidence of the chemical composition of the prebiotic soup. In addition, it is highly improbable that evolved enzymes such as glucose oxidase, horseradish peroxidase and catalase would not have been present on prebiotic Earth. Despite this, these studies can still provide insights into the general physical properties of coacervate microdroplets and how they may interact with additional molecules during prebiotic earth without considering specific chemistries.

Differences in chemical, material and structural properties can tune the partitioning of molecules, and thus, any biochemical reactions contained within them. Therefore, connecting the material properties of coacervates to their underlying chemistry and droplet functionality could be critical in understanding how molecular interactions can be regulated within the coacervate. Here, biophysical characterisation of coacervate droplets such as Fluorescence Recovery after Photobleaching (FRAP) [17,31–33] has been effective in obtaining diffusion coefficients and viscosities in droplets. However, the models used for extracting these parameters are based on a non-crowded environment. As the internal environment of the coacervate is crowded this can lead to some limitations in the biophysical analysis and quantification of coacervate droplets. Therefore, new physical models should be established for coacervate environments, which are viscous, crowded and non-dilute. To this end, Jawerth et al. [34] recently developed a novel active microrheology method to determine the surface tension and shear modules of protein/polyelectrolyte-rich droplets via a combination of experiments with theoretical modelling. Further development between theory, experiments and engineering will increase the precision and range of biophysical tools for characterising

coacervate microdroplets that can be important for the physicochemical characterisation in the origin of life studies.

Selective partitioning within membrane-free coacervate droplets

An important prerequisite of compartmentalisation is not only the ability to contain molecules but also the capability to allow the flux of molecules across the interface. For lipid vesicles, the lipid membrane retains molecules within their interior while having the properties to maintain a concentration gradient. The permeability of the membrane is dependent on the lipid composition. As coacervates do not have a membrane, their method of containing and generating concentration gradients occur via a different physical methodology. For example, Jia et al. [35] showed how polyester droplets exhibited different coalescence and molecular uptake properties based on the chemical composition of the droplet. Dehydration of α-hydroxy acids polymerise to form polyesters, which transform to a liquid droplet, from a gel, upon hydration. They showed that RNA was partitioned into phenyllactic acid droplets but were excluded from polylactic acid droplets. Different molecular uptake properties were attributed to the hydrophobicity/hydrophilicity differences within the droplet that arise from it''s chemical composition.

Solutes in the presence of coacervate droplets will spontaneously partition and concentrate into the droplet to reach chemical equilibrium. This can be measured at a steady state by quantifying the partition coefficient. This is the concentration of solutes inside the coacervate phase vs the concentration of solutes outside (Figure 2b). It has been well established that most solutes, including dyes, proteins and RNA, partition into coacervate droplets [13,36,37]. Furthermore, it has been demonstrated that coacervate droplets prepared from CM-Dextran/polylysine exhibit different levels of selectivity for different lengths of molecules. For example, longer length RNAs (39-mer) having a higher partition coefficient ($K = 9600 \pm 5600$) compared to shorter length RNA (12-mer) ($K = 3000 \pm 2000$) [17]. This has important implications in enriching molecules of certain length inside the protocells and provides a means to generate a chemical gradient. For nucleotides, this could allow for the preferential selection for certain lengths from a random pool of oligonucleotides and that can regulate reactions within the coacervate interior (see RNA-peptide world).

Typically, the partition coefficient of a solute is measured by spectroscopic methods (Figure 2b). The concentration of solute in the coacervate phase compared to the supernatant phase is determined by varying the total solute concentration. However, due to the requirement of large amounts of sample, it is often costly and difficult to obtain accurate partition coefficients. Application of other methodologies, such as microfluidics combined with fluorescence microscopy, has enabled the high throughput measurement of partition coefficients by encapsulating different concentrations of solutes with coacervate forming components in water-oil emulsions [25] (Figure 2c). This allows for the screening of a large number of droplets, permitting for the first-time statistical averaging of data with the capability to screen for different coacervate chemistries and solutes for partitioning. The results showed that the chemistry of the coacervate droplet could have a significant effect on the partitioning of a particular solute. For example, the partitioning of NADH into polylysine/ATP coacervate droplets was more than two times compared to CM-Dextran/PDDA droplets. Despite this, deconvoluting the exact parameters that dictate the partitioning of solutes into coacervates remains a challenge. The difficulty in obtaining a set of rules for molecular partitioning of molecules is attributed to a wide range of interactions, which contribute to the partition coefficient, including electrostatic, hydrophobic, van der Waals and π- π interactions. As this method relies on fluorescence methodologies, errors in the partition coefficient may arise from differences in quantum yield of the fluorophores in different environments. This can be overcome by careful characterisation of the fluorophores by Fluorescence Lifetime Imaging (FLIM) to obtain true quantification of the partition coefficients. For now, this approach provides a semi-quantitative, high-throughput route to obtaining partition coefficients.

The role of coacervates in hypothetical origin of life scenarios

From membrane-free compartmentalisation to membrane-bound compartmentalisation

Modern biological cells with phospholipid membranes are a primary indicator that lipid-based membrane-bound compartmentalisation would have been a key evolutionary feature during the origin of cellular life. The cell membrane permits the separation of the intracellular and extracellular reaction space while allowing molecular exchange between the two spaces. In coacervate droplets, the molecular exchange can be passively facilitated by tuning the partition coefficients. However, coacervate droplets lack a structural membrane. Modern engineering methodologies have demonstrated the ability to generate coacervates in water-oil emulsions [25,38] or lipid vesicles [39–43] by microfluidic techniques showing that hybrid protocells with combined features of membrane-bound and membrane-free compartmentalisation can be produced within the laboratory. However, within an origin of life context, it is unlikely that specialised flow methodologies would have driven compartment formation on prebiotic Earth. Therefore, investigating phospholipid and

coacervate interactions can lead to new insights into how membrane-bound lipid compartments could be associated with membrane-free compartments by physical interactions alone.

Studies that were undertaken to characterise the interaction between phospholipid vesicles and coacervate droplets show that phospholipid vesicles will be uptaken by the coacervate or coat the coacervate surface depending on the charge interactions between the vesicle and coacervate (Figure 3Ai). The surface charge and it's strength can be tuned by changing the molar ratio of the coacervate forming components or changing the chemical composition of the polyelectrolytes [44].

Lin et al. [45] showed that positively charged or negatively charged phospholipid liposomes (100 nm) would either form a fibre network within the coacervate or coat the surface of coacervate droplets prepared with polylysine and ss-oligonucleotide depending on the charge. Liposomes with the same charge as the coacervate would typically coat the coacervate droplet. In comparison, phospholipid liposomes that are oppositely charged compared to the coacervate, would be uptaken into the droplet and form a fibre network. Pir Cakmak et al. [44], showed that phospholipid vesicles either formed uniform vesicle layers or aggregates at the coacervate surface depending on the chemical composition and the charge ratios of the coacervate forming components. The difference in the distribution of the lipid vesicles was attributed to the mediation of electrostatic interactions between the lipid vesicle and coacervate, as well as the presence of polycations in the supernatant, which can cause vesicle aggregation prior to interaction with the coacervate surface.

As phospholipids have been proposed to be comparatively structurally complex, it is unlikely that they were present on prebiotic Earth. In comparison, fatty acids have been proposed to be plausible prebiotic surfactants for the formation of vesicles [2,46–48]. Over the last few decades, a number of basic protocellular properties from fatty acid vesicles have been demonstrated. Some examples include growth and division [49], encapsulating and supporting RNA catalysis [50] and the generation of transmembrane proton gradients [51]. Despite this, there are some drawbacks to fatty acids as the sole component to generate the first compartments. This is mostly attributed to the fact that very specific chemical conditions (pH, salt and fatty acid concentration) are needed for the production of fatty acid vesicles. This reduces the chances of fatty acid vesicles forming on prebiotic Earth. In comparison, coacervate droplets will form in a wide range of chemical conditions with very little chemical identity. Recently, it has been shown that fatty acid plays an active role in coacervate formation and partitions readily into coacervates. These structures provide precursor states to lipid vesicles that readily transform upon a pH switch or are driven by the hydrophobic effect at critical lipid concentrations. Therefore, considering fatty acids concomitantly with coacervate droplets could provide a route to membrane-bound compartmentalisation from membrane-free compartmentalisation, which relies on the general partitioning of fatty acid into preformed coacervate droplets. To this end, Tang et al. [37], showed how negatively charged fatty acid monomers are attracted to positively charged coacervate droplets by electrostatic interactions and up taken into positively charged coacervate droplets. Upon sequential addition of fatty acid monomers, a critical concentration is reached, and the internalised fatty acids preferentially form continuous bilayers on the surface of the coacervate droplet. This latter process is driven by the hydrophobic effect. The addition of salt to the dispersion of hybrid protocells leads to the dissolution of the internalised coacervate matrix and the formation of multilayer vesicles.

The ability for fatty acids such as myristic acid to form complex coacervates with NaOH and guanidine hydrochloride offers a simple route to concentrate fatty acids within a dilute soup and drive a transition from membrane-free compartmentalisation to membrane-bound compartmentalisation by pH changes [14] (Figure 3Aii). Additionally, this study showed that YFP (yellow fluorescent protein) partitioned into the coacervate droplet and was encapsulated into the lipid vesicle upon a pH switch where it was protected from protease degradation.

Studies that have investigated lipid vesicle and fatty acid interactions with coacervate droplets highlight the diverse range of structures that can form based on physical interactions alone. As these processes take place spontaneously, they may have been plausible in origin of life scenarios. What is compelling is that a number of self-assembled structures from lipids and polyelectrolytes will form, showing that there may have been a rich diversity of compartment structures within the prebiotic soup on early Earth.

RNA-peptide world

It has been hypothesised that an RNA-peptide symbiosis [7] could have facilitated the transition from a chemical world to a biological world capable of Darwinian evolution. Since Oparin showed that RNA and peptides would form coacervate microdroplets [52], there are a growing number of examples of RNA/peptides/polyamines forming coacervate microdroplets. Typically, polyuracil and polyadenosyl have been used as RNA mimics to form coacervate with polyamines (spermine and spermidine), poly(allyl)amine, polyarginine, polylysine [53]. Conversely, the designed cationic peptide sequence, 'RRASL' (R = Arginine, A = Alanine, S = Serine and L = Leucine), and it''s

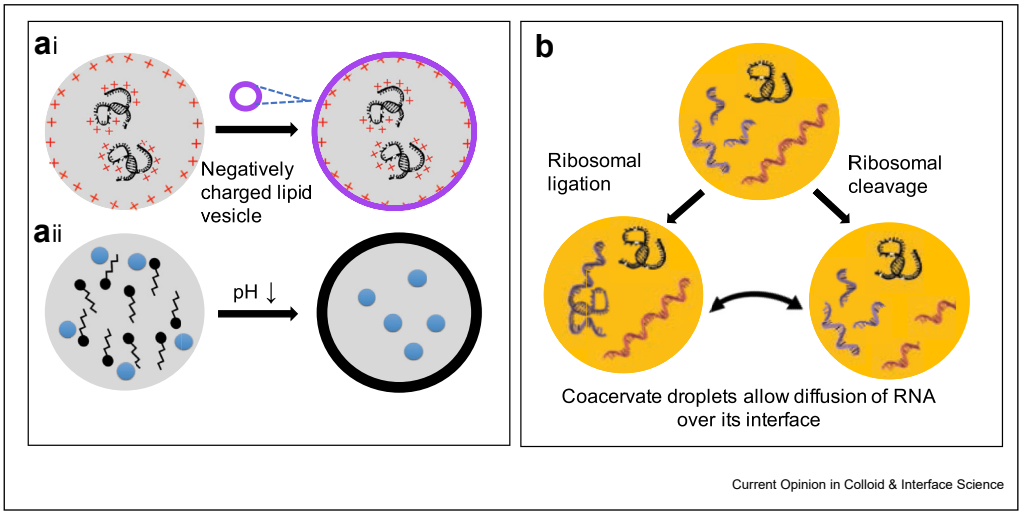
repeats have been used to form coacervates with poly-uridylic acid [54]. Seven-residue peptides containing mixtures of hydrophobic and cationic residues 'KVRVRVK' (K = Lysine, V = Valine and R = Arginine) form stable coacervate droplets with equimolar ATP. In comparison, its orthologue 'VVVRRKK' did not form coacervates, indicating that the sequence, as well as the charge of the peptides, play an important role in driving coacervation [55].

From the RNA world perspective, Tagami et al. [56] showed that peptides could replace the requirement of Mg^{2+} in RNA reactions. An oligo-lysine of five amino acid units enhances RNA polymerase ribozyme function at low Mg^{2+} concentration (2–50 mM). In addition, they showed that the presence of the peptide accelerated ribozyme evolution and enabled peptide-mediated RNA polymerisation within fatty acid vesicles.

Over the last few years, there has been some focus on reconciling the RNA world with membrane-free compartmentalisation, in particular in investigating the function of primitive ribozymes (Hammerhead and hairpin ribozyme) inside complex coacervates [17,35,53,57] (Figure 3b). Typically, ribozymes are partitioned into preformed coacervates where the coacervates have been shown to support ribozyme function. It was shown that coacervate micro-droplets could affect the thermodynamics and kinetics of the ribozyme activity [17]. An up-concentration of ribozyme and substrate within the coacervate phase will increase the rate of reaction. However, at the same RNA concentrations in coacervate and in buffer solution, the ribozyme cleavage was 60 times slower in the coacervate phase compared to the solution. This decrease in activity was attributed to alterations in ribozyme secondary structure by interactions between the ribozyme and the coacervate matrix. Other studies showed that the cleavage of RNA was slightly lower in polyester droplets compared to its' equivalent buffer solution [35]. Although in this study, it could not be ruled out that the reaction was taking place outside of the droplet, followed by product partitioning into the droplet [35].

It has been reported that coacervates prepared with certain poly-cations, such as oligo-arginine or oligo-lysine, can inhibit template-directed RNA polymerisation, while some polycations, such as poly-diallyldimethylammonium (PDDA), can enhance the reaction at suboptimal Mg^{2+} levels [53]. Specifically, poly(allyl)amine, polyarginine and polylysine will interact with the phosphate backbone of RNA by electrostatic interactions and with the nucleotides by H-bonding to impact RNA folding and flexibility [53,57]. As a positive ion source, polyarginine is a good candidate for generating prebiotically relevant membrane-free compartments, but when it comes to supporting RNA activity inside peptide-anion coacervates, it falls behind its lysine analogue. This is probably due to the higher charge density of polyarginine compared to polylysine,

Figure 3



The role of coacervation in hypothetical origin of life scenarios. (a) Coacervation and the lipid world. (i) Nucleic acid-peptide coacervate with net positive surface charge attract negatively charged lipid vesicle (purple) to form a layer of lipid vesicles on the surface of the coacervate droplet. (ii) Coacervates prepared from fatty acids (black), guanidine hydrochloride and NaOH will undergo a spontaneous rearrangement upon a pH drop to form a vesicle with lipid bilayers. Proteins (blue) included within the coacervate droplets are encapsulated inside the lipid vesicle after the pH switch and protected against degradation. (**b) Coacervates and the RNA world.** Functional RNAs (black) within coacervate droplets can ligate or cleave substrate RNAs to longer or shorter nucleotides, respectively. As the partitioning coefficient of RNA is dependent on the length, coacervates can facilitate nucleotide-shuffling between droplets.

which leads to a stronger interaction with negatively charged RNA. This can restrict the flexibility of RNA and affect it's activity [53]. In comparison, the quaternary amine of PDDA has an aprotic positive charge, which has a weaker interaction with RNA compared to polyarginine or polylysine, supporting ribozyme activity better [53].

From a peptide world perspective, early polypeptides developed from a limited number of prebiotic amino acids, were likely to be short with no or with limited structural folds. It has been hypothesised that ligation of short peptides would have enabled a transition to longer peptides with the ability to fold and impart catalytic activity such as nucleotide binding or metal binding. From the top-down approach, Tawfik et al. [58], through maximum likelihood methods, obtained an evolutionarily conserved precursor-domain of helix-hairpin-helix (HhH) protein family, which has been proposed to be a nucleotide binding motif. Non-prebiotic amino acids such as histidine, phenylalanine and tyrosine were either removed or substituted for arginine in order to obtain a 29-mer precursor domain and a 60-mer repeated precursor domain consisting of only prebiotic amino acids. Furthermore, arginine in this domain was exchanged with ornithine, a widely considered precursor to arginine. It was found that the 60-mer repeat domain with both ornithine and arginine could bind DNA, while the single domain showed no DNA binding functionality. However, the single precursor domain (arginine and ornithine) formed coacervate microdroplets with polyuracil demonstrating a direct connection between the peptide world and coacervation. Interestingly, polypeptides with scrambled sequences of the same 29-mer domain showed no coacervation. This supports the growing evidence that coacervation is dependent on the sequence of the peptide [21]. Tawfik's study is the first study that directly connects peptide sequences deduced from LUCA to prebiotic peptides and coacervation. Therefore, coacervation offers a strategy for bringing peptides together for ligation to evolve structure and function.

It is now evident that RNA and peptides readily interact to form coacervates, thus providing a mechanism and route to reconciling the RNA and peptide worlds. It has been shown that the coacervate environment can regulate primitive RNA activity either through up-concentration or by changing the thermodynamics, indicating that it is plausible that RNA and peptides may have co-evolved via coacervation. The coacervate can provide an environment that alters the mechanistic and energetic pathways for primitive RNA and peptide reactions that are not achievable in dilute solution alone. Current evidence shows that the sequence of the peptide will affect the coacervation properties and its structuration, indicating that particular peptide sequences are critical for both the onset of coacervation and peptide function. These studies, taken together, show that a hypothetical RNA-peptide world could have been reconciled via coacervation by chemical enrichment and the provision of a reaction space for RNA cleavage and ligation processes.

Protometabolism

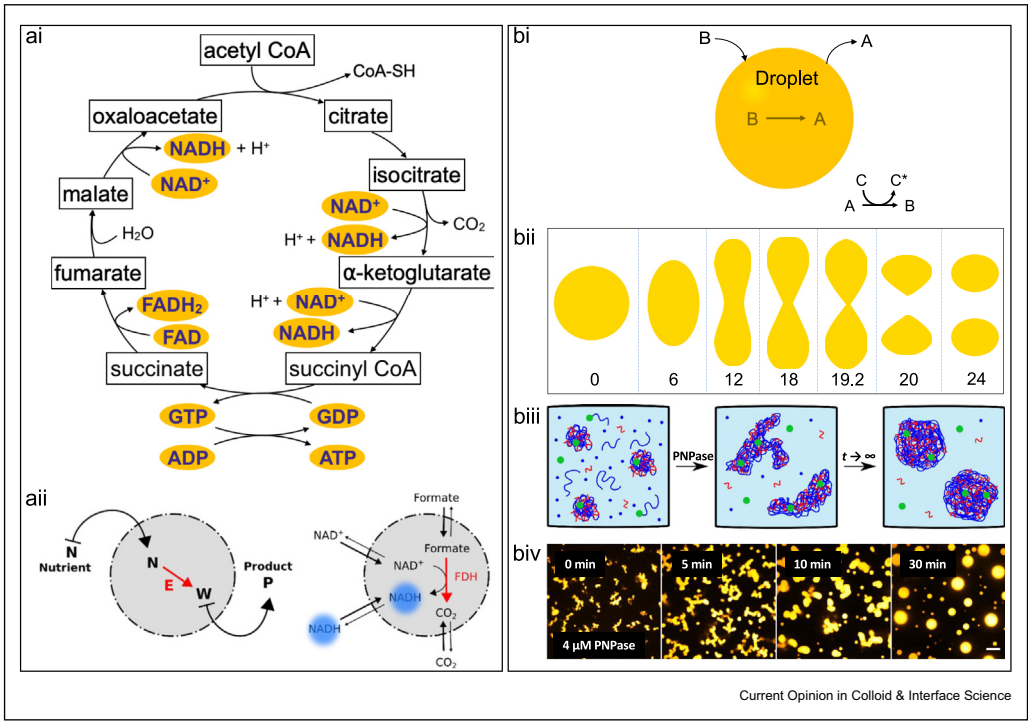
Metabolism is crucial for all forms of life to convert resources into useable energy that enables self-sustenance through reaction networks. As metabolic processes are compartmentalised in modern biological systems, compartmentalisation could have played a role in tuning metabolic processes during the origin of life. It has been proposed that primitive metabolism in the form of autocatalytic sets (self-sustained networks that can catalyse their own reactions) would have provided the framework for the evolution of biological molecules capable of Darwinian evolution on early Earth.

Modern metabolic networks work together with co-factors, which are either metal ions or molecules that bind to enzymes to assist in catalysis. It has been hypothesised that co-factors may have been part of non-enzymatic metabolic networks before the evolution of functional enzymes. Recent approaches in the search for protometabolic networks have used modern cellular pathways as the starting point to trace evolutionarily conserved networks. Xavier et al. [61] found that cofactors such as H_2O; ATP, ADP and AMP; proteins; phosphate; CO_2; $NAD^{+;}$ pyruvate; ammonia; diphosphate; coenzyme A; NADH; pyridine dinucleotides; glyceraldehyde-3-phosphate; acetyl-CoA; amino acids and nucleosides; UTP and CTP; metal and metal-sulphur cluster catalysts; and thiamine diphosphate would have been important cofactors for food generating networks. In particular, NAD^+ would have been crucial as a co-factor, as the number of reflexive autocatalytic food-generated self-sustaining networks (RAF) dramatically reduced when NAD^+ were removed from the network. Interestingly, despite ATP being a key energy source for modern biological systems due to the high energy phosphate bonds, ATP did not have an impact on the sizes of RAF. This was attributed to the presence of molecules that provide an alternative source of energy to ATP. Considering the structure of cofactors such as NAD, FAD, ATP and their similarity to nucleotides, it has been hypothesised that these types of molecules would have co-evolved. Interestingly, phosphate-based small molecules such as ATP, ADP, AMP, GTP, UTP will form coacervates with positively charged components such as small peptides [13]. This suggests that the ubiquitousness of coacervation with cofactors has the

potential to bring together prebiotic metabolism and coacervation (Figure 4a).

A defining feature of modern living systems is their ability to maintain an out-of-equilibrium state. Therefore, an ideal protocell model needs to maintain an out-of-equilibrium state. This requires the inclusion of fuel-driven behaviour and the ability to uptake and utilise that fuel from its' surrounding. Therefore, coacervates, with an energy source that keeps the system away from equilibrium, are important considerations for the origin of life. A general property of protocellular coacervate droplets is the ability to maintain a chemical equilibrium constant for molecules by their partition coefficient. As already described, different molecules will exhibit different intrinsic partition coefficients. Therefore, coacervates represent a facile route to exploit resources from their environment to drive metabolic reactions by maintaining their partition coefficient. This principle has been demonstrated by the encapsulation of the formate dehydrogenase reaction within a coacervate droplet housed within a water-oil emulsion [25]. Formate dehydrogenase converts formate to carbon dioxide with the concomitant transformation of NAD^+ to NADH. To maintain the equilibrium constant of NAD^+ within the coacervate, it will continually uptake NAD^+ from its environment as the molecule is being consumed by the reaction. Additionally, as NADH is produced within the coacervate, it will preferentially diffuse out of the coacervate into its surroundings to maintain its' partition coefficient. As a consequence of the diffusion of NAD^+ and NADH into and out of the reaction centre, the rates of reaction were accelerated at least two-fold compared to when the reaction took place in the coacervate phase alone. These results demonstrate that coacervate droplets can take up resources from their exterior and release products from their interior, thus providing a continual supply of energy. The results show

Figure 4



The role of coacervates in protometabolism and as active droplets. (**a) Coacervates and metabolism.** (i) Schematic representation of the Kreb's cycle, a modern biological metabolic pathway. Many of the co-factors of this metabolic pathway (highlighted in yellow) have been shown to form complex coacervates with other molecules. (ii) (left) Illustration showing how membrane-free coacervate droplets can take up nutrients from their environment and expel waste products based on their partition coefficient. This would have been important for primitive metabolic cycles. (right) Experimental realisation of this based on formate dehydrogenase reactions within coacervate droplets. Reprinted with permission from Ref. [25]. (**b) Active droplets.** (i) A simple theoretical model showing droplets formed from material B where A is a soluble component that is being formed inside the droplet. C is a chemical fuel that drives the reaction from A to B while getting itself transformed to C^*. (ii) Events occurring during droplet division with time due to the processes described in (i). Here the droplet deforms and becomes stretched to eventually pinch and bud out. Reproduced from Ref. [59] (iii) Schematic representation for localisation of PNPase in active poly(U)/spermine coacervates. Its' polymerisation activity leads to shape deformities. (iv) False-coloured time-lapse images of the scheme described in (iii) shows the transition of the droplets from non-spherical shapes to spherical droplets. Scale bar indicates 10 μm. Reprinted with permission from Ref. [60].

that this will alter the kinetics of encapsulated reactions without complex and additional machinery.

Active droplets, i.e., droplets that form from in-situ reactions, can also impact the properties of the droplets themselves. Recent studies have shown how coacervation can be regulated by pH [39] or enzymes [15,62]. As the charge on a polymer is an important driving force for complex coacervation, altering the phosphorylation state, and therefore, the charge can drive the formation or dissolution of droplets. For example, a system containing Lambda protein phosphatase (LPP) and protein kinase A (PKA) dephosphorylates and phosphorylates the serines, respectively, on the peptide sequence RRASLRRASL, leading to the formation and dissolution of coacervate droplets [15]. The overall charge is neutralised, leading to the dissolution of coacervates. In comparison, the phosphorylation state of ATP/ADP can be altered with kinases within peptide/nucleotide coacervates. Sequential addition of phosphatases and kinases leads to a periodic formation and dissolution of droplets by sequential addition of the enzymes [62]. Such work presents scope for the tuneable generation and dissolution of coacervates, which can provide insights on the effect of dynamic assembly and disassembly on supported enzyme behaviour [39]. From an origin of life perspective, this dynamic phase-behaviour of the system can alter topologies in a chemical reaction network and play a critical role in the emergence and evolution of metabolism during prebiotic Earth.

Traditionally, compartmentalisation and metabolism have been treated independently within the origin of life studies, resulting in a debate on which emerged earlier. However, the properties of coacervates indicate that these two routes to the origin of life could be symbiotic rather than independent. Therefore, investigating the interactions of the two may lead to new alternative scenarios for the origin of life.

Evolution through growth and division

A key feature of cellular life is the ability for cells to evolve by growth and division to allow propagation of information to daughter cells. Therefore, this would have been an important step for the evolution of information within protocells. In part, coacervates can circumvent this by the selective partitioning of products and substrates depending on their chemistry. Alternatively, coacervates could have evolved to lipid vesicles and then further grown and divided from there. Recent theoretical work has shown that active coacervates, where droplet material is degraded inside and produced outside of the droplet, can generate shape instabilities that eventually lead to the budding of droplets [59] (Figure 4b). Such dynamic systems, differ from droplets in equilibrium, had been imagined, long ago, by Oparin [52], to have aided in the origin of cellular life.

Recent studies with active RNA/spermine coacervate droplets have demonstrated that under the influence of an enzymatic polymerisation of RNA by polynucleotide phosphorylase, coacervate droplets will become non-spherical, which relax to spherical droplets once the reaction has reached a steady state [60]. In addition, chemically fuelled active droplets provide an interesting lead for self-division where growth is a requirement. Donau et al., have shown that the forward and reverse EDC reaction (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) can convert precursor peptides from a 1+ state to a 3+ state. The 3+ state favours the formation of droplets with RNA, which can grow and shrink as a consequence of the formation and degradation of the product. This takes place on the order of several minutes, compared to the much faster fusion of passive droplets taking place on the order of a few seconds [63]. This system is maintained in an out-of-equilibrium state by the formation and degradation of the product and is a feature required for the growth and division of droplets. While experimentally realising a dividing droplet is an ongoing endeavour these studies show how active droplets can drive growth and shape deformations of droplets as a consequence of *in-situ* reactions.

Conclusion

The fact that coacervate microdroplets can form from a wide range of diverse starting materials such as RNA, peptides, lipids, nucleotides; can selectively partition different solutes; can support and alter reactions housed within them; can themselves be altered by pH or chemical reactions make them intriguing protocellular models during the origin of cellular life. In this review, we have summarised recent progress in the development of new methodologies to characterise the physicochemical properties of coacervate droplets. A broadening understanding of the formation and physiochemical properties suggests that coacervation can be important for unifying disparate hypothesises for the origin of life. Herein, we have highlighted recent examples that provide either direct or indirect evidence that coacervates could have; facilitated a transition from membrane-free to membrane-bound compartmentalisation; provided a route to bring together RNA and peptides for their co-evolution; spatially localised prebiotic metabolites to kick start primitive metabolism; been capable of evolving through growth and division by maintaining an out of equilibrium state. Considering that there may have been a multitude of coincident routes to the origin of life, this hypothesis provides an alternative route to the transition of a chemical world to a biological world by unifying disparate hypotheses. It is time to perform experiments that test and challenge this hypothesis.

In this review, we have not considered how prebiotic environments [64–67], for example, thermophoretic pores, warm shallow pools of water, wet-dry or day–night cycles,

would affect the formation and properties of coacervates. Or indeed how coacervate droplets would tune primitive reaction networks and vice versa by the regulating for the production of materials. All of these are important considerations for investigating the role of coacervation as a critical phenomenon during the origin of life. Consideration of how the first molecules would have formed on the earth [68–71] is a critical additional step in probing the transition from a chemical world to a biological world. As it can be determined whether these prebiotic chemistries would have been sufficient to drive coacervate formation to concentrate and localise reactions. In addition, the application of theoretical approaches such as the graded autocatalysis replication domain (GARD) [72] of lipid micelles or vesicles could be applied to predict the evolution of coacervate droplets.

While the hypothetical role of coacervate microdroplets has been studied for nearly a century, it has only been in recent years that coacervation has been considered an important phenomenon in modern biological systems. It is now generally recognised that coacervation [73,74] contributes to the formation of liquid–liquid phase-separated droplets such as p-granules and stress granules in modern and natural cells. Within the field of cell biology, there have been a number of studies that have probed the contribution of different peptide sequences and amino acids on phase separation properties [75,76]. Therefore, results from biological studies can also provide insights as to how the chemistry of peptides dictates coacervate formation. While it will always be difficult to know what exactly the prebiotic scenarios would have been, a symbiotic approach within the origin of life field with bridges to molecular cell biology, biophysics and synthetic biology could provide new answers to how cellular life originated on prebiotic Earth by considering plausible physicochemical scenarios.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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