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PERSPECTIVE

Assembly driven protection from hydrolysis as key selective force during chemical evolution

Rotem Edri[1,2] Sarah Fisher[1,2] Cesar Menor-Salvan[3] Loren Dean Williams[4,5] and Moran Frenkel-Pinter[1,2,5]

1 Institute of Chemistry, The Hebrew University of Jerusalem, Israel
2 The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Israel
3 Department of Biología de Sistemas, Universidad de Alcalá, Madrid, Spain
4 School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA
5 Center for the Origins of Life, Georgia Institute of Technology, Atlanta, GA, USA

Correspondence
M. Frenkel-Pinter, The Center for Nanoscience and Nanotechnology, Institute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Jerusalem 9190401, Israel
Tel: (972) 2 6584171
E-mail: moran.fp@mail.huji.ac.il
and
L. D. Williams, School of Chemistry and Biochemistry, Georgia Institute of Technology, 315 Ferst Drive NW, Atlanta, GA 30332-0400, USA
Tel: (1) (404) 385 6258
E-mail: loren.williams@chemistry.gatech.edu

Rotem Edri and Sarah Fisher contributed equally to this article

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The origins of biopolymers pose fascinating questions in prebiotic chemistry. The marvelous assembly proficiencies of biopolymers suggest they are winners of a competitive evolutionary process. Sophisticated molecular assembly is ubiquitous in life where it is often emergent upon polymerization. We focus on the influence of molecular assembly on hydrolysis rates in aqueous media and suggest that assembly was crucial for biopolymer selection. In this model, incremental enrichment of some molecular species during chemical evolution was partially driven by the interplay of kinetics of synthesis and hydrolysis. We document a general attenuation of hydrolysis by assembly (i.e., recalcitrance) for all universal biopolymers and highlight the likely role of assembly in the survival of the 'fittest molecules during chemical evolution.

Keywords: abiotic chemistry; biopolymers; chemical evolution; molecular evolution; origins of life; prebiotic chemistry; Recalcitrance; self-assembly

The origins of biopolymers present some of the most fascinating questions in prebiotic chemistry and in chemical sciences in general. Chemical and geological processes on the prebiotic Earth resulted in an increase in the complexity and diversity of organic molecules, ultimately leading to the molecules of life [1 24]. We suggest that an interplay between the kinetics of synthesis by condensation-dehydration and kinetics of degradation by hydrolysis led to incremental enrichment of some molecular species over others during chemical evolution on the Hadean Earth.

Abbreviations
DNA, Deoxyribonucleic acid; ds, double-stranded; PrP, prion protein; RNA, Ribonucleic acid; ss, single-stranded.

In this review, we focus on the influence of molecular assembly on rates of hydrolysis (i.e., on recalcitrance, as defined below). In the first part of the manuscript we discuss the assembly of biological polymers, and in the second part we discuss prominent demonstrations of attenuation of hydrolysis rates by assembly. We focus primarily on examples related to biopolymers but include several examples of this phenomenon for non-biological molecules, to show the universality of this principle. We suggest that recalcitrance constituted a crucial driving force in the selection of biopolymers. In the context of this review, an 'assembly' is a three-dimensional arrangement of atoms within and between molecules to form secondary, tertiary, or ternary structures through non-covalent interactions. An assembly can be intermolecular or intramolecular, homomolecular or heteromolecular, and can arise *via* annealing, folding, binding, aggregation, or crystallization. Biopolymer assemblies are characterized by both self- and non-self-complementary interactions.

We propose that biopolymers are the products of prolonged chemical evolution driven in part by the selection for assembly during wet-dry cycling [12,25 31]. Here, we include membrane forming amphiphiles in our discussion even though they are not formally polymers. In our model, ancestral chemical species on prebiotic Earth formed chemical linkages that were more prone to hydrolysis and were less influenced by conditions than linkages in current biology. Probable ancestors are short, easily synthesized, easily hydrolyzed heterogenous oligomers that are not generally competent to assemble. However, a small fraction of these species did assemble, which attenuated rates of hydrolysis and increased molecular persistence. In this model, proto-biopolymers with low intrinsic proficiency in assembly and limited control over rates of hydrolysis went extinct. Extant biopolymers, with highly sophisticated assembly proficiencies, are the winners of an intense chemical competition. Here, we document the tendency of assembly to inhibit hydrolysis of biopolymers and suggest assembly contributed to the persistence of 'fit' molecules during chemical evolution.

Assembly of biopolymers

Our focus here is universal biopolymers: DNA, RNA, polypeptide, polysaccharide, as well as amphiphiles. Biopolymers form an astonishing variety of assemblies, which rely primarily on interactions among four major types of building blocks nucleotides, amino acids, monosaccharides, and amphiphiles. Polymerization of building blocks into polypeptide, polynucleotide, or polysaccharide promotes assembly [18]. Single-stranded (ss) DNA anneals to double-stranded (ds) DNA. RNA anneals to DNA to form heteroduplexes. tRNA folds to the canonical L-shape. Proteins fold to globular structures, fibers, pores, and aggregates. Proteins and RNA fold and assemble to form the ribosome. Polyglucose anneals to helices and crystalline structures. Some amphiphile monomers can spontaneously assemble to form membranes.

Nucleic acid assembly

Nucleotides, the building blocks of the informational polymers (RNA and DNA), assemble after polymerization to form long double-stranded (ds) helices in the case of DNA, or stems and loops and more complex and polymorphic structures in the case of RNA. Nucleobases assemble by hydrogen bonding and base stacking. Nucleotides form base pairs when polymerized but not generally on the level of nucleotide monomers [12]. Monomeric or polymeric guanosine can assemble into G-quartets [32]. The driving force for the assembly of biopolymers is so acute that even random polynucleotide sequences form assemblies [33]. PolyU and polydT are the only nucleic acid sequences that do not spontaneously self-assemble. The functions of RNA and DNA are dictated by differences in their assemblies. DNA carries the genetic code in long monotonous B-form helices that store, protect and transfer genetic information. By contrast, RNA, which is involved in transcription and translation and is found in the ribosome and other ribozymes, forms short A-form helices, loops, pseudoknots and kink-turns [34]. Short dsDNA and dsRNA can assemble into complex liquid crystalline states [35 38], and even mononucleotides can assemble into complex liquid crystalline states at very high concentrations [38]. As the number of repeating units increases, the ordering of the liquid crystalline structure increases. This type of assembly might be of significance in the context of the origins of life because ordered and oriented liquid crystal phases can potentially promote non-enzymatic autocatalysis of ligation by placing the nucleic acid fragments in the ideal geometrical position for the phosphodiester bond formation [39].

Polypeptide assembly

The polypeptide backbone is a master of self-assembly (Fig. 1) [18,27,31,40]. Peptides and proteins can self-organize into various structures that are stabilized by complementary hydrogen bonds between backbone carbonyl oxygens and amide hydrogens. The polypeptide backbone has equal numbers of hydrogen bond donors

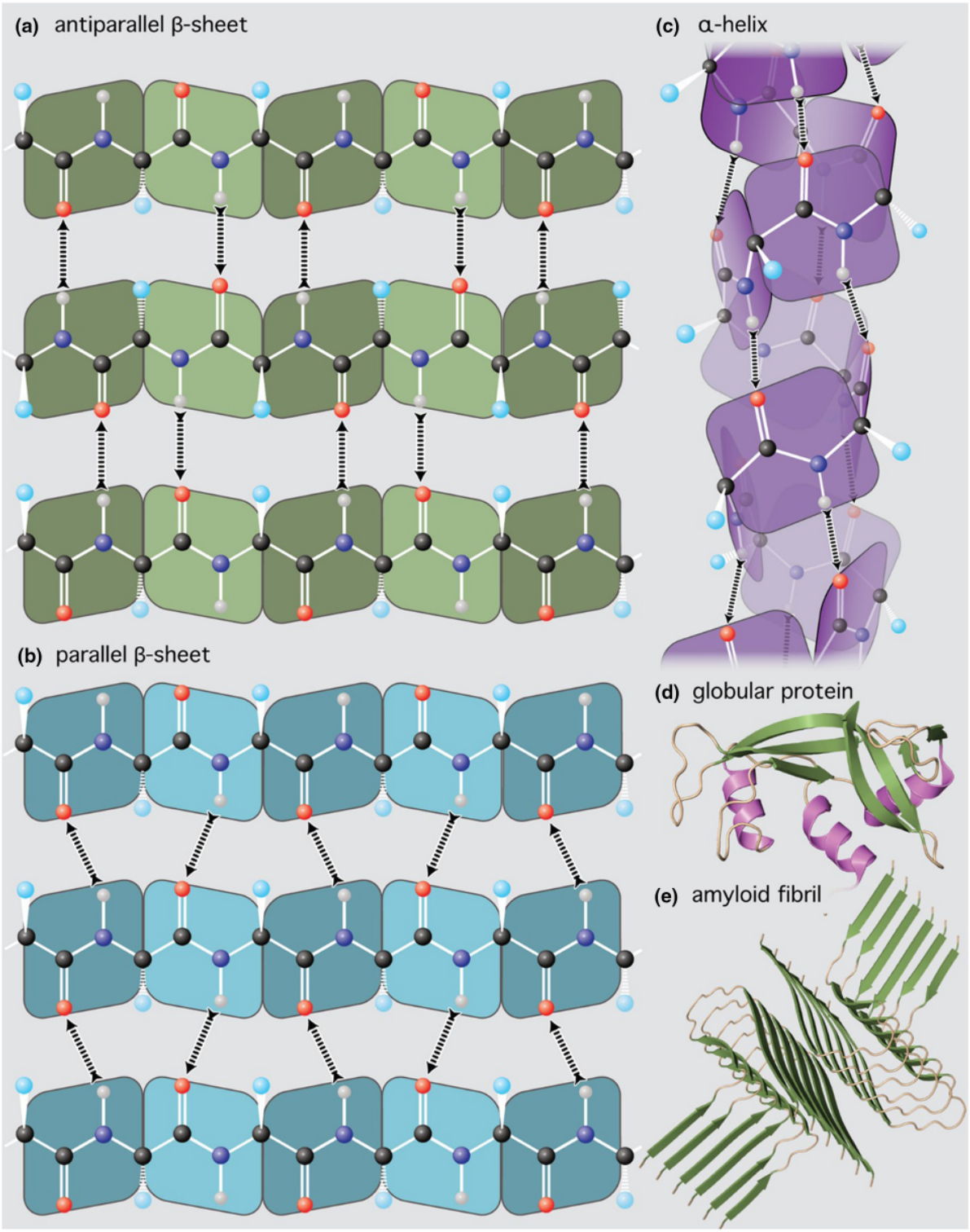

Fig. 1. Polypeptide can form a variety of structures stabilized by hydrogen bonding complementarity. (a) Antiparallel -sheet. (b) Parallel -sheet. (c) α-Helix. (d) A globular protein showing α-helices (violet) and -sheets (green). (e) An amyloid fibril showing -sheets. Hydrogen bonding polarities are indicated by arrows. Reprinted with permission from ref. [18]. Copyright© 2018 Springer Nature.

and acceptors and can adopt multiple conformations in which all backbone donors and acceptors are geometrically matched. In an α-helix, hydrogen bonds form between every fourth amino acid. In parallel or antiparallel -sheets, hydrogen bonds form between peptide linkages of adjacent -strands. Proteins can fold further to tertiary structures, stabilized by additional hydrogen bonds, and hydrophobic, dipolar, and ionic interactions [41]. Enzymes carry active catalytic pockets that are precisely evolved to fit transition states and can catalyze specific chemical reactions [42 45]. In some cases, quaternary structures that involve homo- or heterocomplexation of multiple proteins can be formed. Hierarchical assembly is key to protein function in enzymes, transporters, fibers, and motors.

Simple peptides and even single amino acids can assemble [46 63]. Peptides can assemble to membrane-like structures [64,65] or drive the formation of coacervate droplets to compartmentalize organic molecules [66]. Assembled membrane-forming peptide amphiphiles, can play a role in the graded autocatalysis replication domain (GARD) model [67,68], which is otherwise associated with lipid amphiphiles.

Polysaccharide assembly

Polysaccharides, also known as glycans, serve to store energy (e.g., glycogen and starch) and as structural components (e.g., cellulose and chitin), and play key roles in the immune system, neurodegeneration, and more. Glycans are abundant in biological systems as conjugates with other biopolymers, forming glycolipids, glycoproteins, and proteoglycans, but also as the non-conjugated polysaccharide polymers providing mechanical properties crucial for cell walls or in connective tissues.

Polysaccharides are remarkably diverse and are found as homopolymers, heteropolymers, and oligomers, that are either linear or branched, with a broad variety of neutral or charged modifications. Polysaccharides can assemble into single, double, or triple helices, spheres, capsids, cell walls, and amorphous aggregates [69 71]. Cellulose, the most abundant biopolymer on Earth, is a homopolysaccharide of glucose exhibiting different levels of crystallinity that contribute to its mechanical properties and impart varying profiles of degradability [72]. All hydrogen bonding functionalities of each glucose moiety are involved in cellulose structure. Cellulose is a durable polymer used by plants for defense, structure, and scaffolding (Fig. 2). Chitin is a durable polymer of amide-modified glucose used by arthropods and fungi for defense, structure, and scaffolding. Cellulose and chitin form stable intra-chain interfaces secured by large complementary arrays of hydrogen bond donors and acceptors and hydrophobic surfaces [73,74]. Cellulose derivatives possessing a relatively stiff backbone and flexible side-chain substituents form, under varying temperatures and in the presence of water as well as organic solvents, both thermotropic and

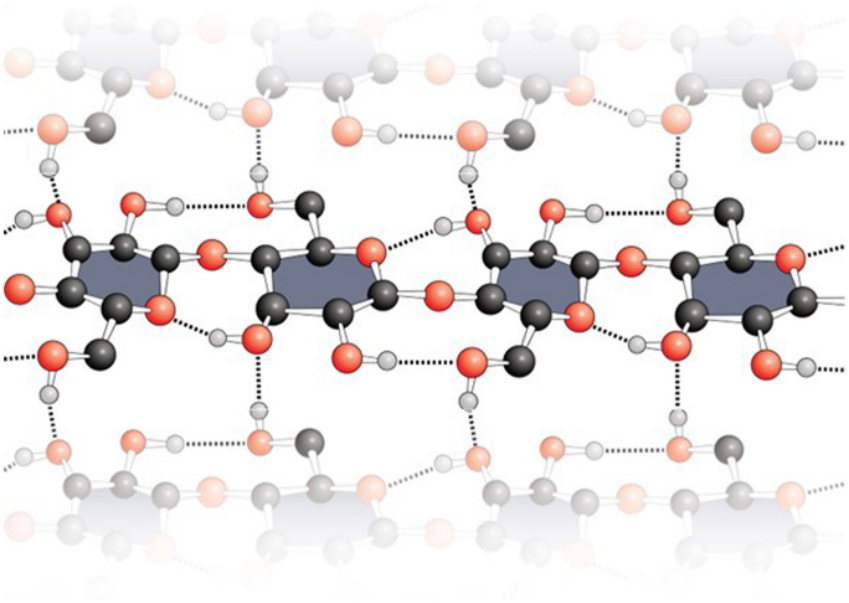

Fig. 2. Cellulose forms ordered fibers. Cellulose contains matched arrays of hydrogen bond donors and acceptors that stabilize the folding of the polymerized glucose into homogeneous fibers. Reprinted with permission from ref. [18]. Copyright© 2018 Springer Nature.

lyotropic liquid crystals, in particular cholesteric liquid crystals [75 78]. With an appropriate solvent, cellulose, which is insoluble in water and various organic solvents, can also form liquid crystalline mesophase [79]. The formation of the liquid crystalline phase as well as spherulites and other structural motifs, such as single and double helices, have been reported for additional polysaccharides, such as amylose, xanthan gum, and debranch starch [80 82]. The anomeric carbon linkage dictates self-complementarity: the -anomer (such as cellulose and chitin), but not the α-anomer (such as glycogen and amylose), facilitates complementary glucose glucose interactions at the polymer level [18].

Amphiphile assembly

Unlike the building blocks of proteins, polysaccharides, and nucleic acids, amphiphiles readily assemble in the monomeric state. Amphiphiles in the form of phospholipids, sphingolipids, glycolipids, sterols, and isoprenoids ether-linked to glycerol phosphate are the primary constituents of cell membranes [83]. The predominant force driving lipid amphiphile self-assembly is the hydrophobic effect, which causes aggregation of the hydrophobic tails *via* the exclusion of water molecules [84,85]. Amphiphiles form various self-assemblies, such as micelles, vesicles, and liquid crystals, including lyotropic and thermotropic liquid crystals as well as amyloid-like structures [86 93]. Amphiphiles can assemble into spheres, rods, discs, hexagons, lamellae, and cubic mesophases, as dictated by both geometrical and thermodynamic considerations [84,94 96]. Liquid crystals are formed by amphiphiles *in vivo*. A pronounced demonstration of naturally occurring liquid crystals is cell membranes formed by phospholipids. Phospholipids self-assemble into a lyotropic lamellar phase, L_α, which is held by the hydrophobic interactions of the phospholipids' tails as well as the hydrogen bonding at the outer and inner surfaces [97]. There is also evidence of other structures formed; for instance, mitochondrial membranes adopt cubic morphology [98].

Recalcitrance: protection of polymers by assembly

Hydrolysis of biopolymers in aqueous media is spontaneous [99 103] and is thus thermodynamically favorable. Given sufficient time, DNA, RNA, polypeptide, polysaccharide, and phospholipids degrade in water into small monomeric species. This negative free energy of hydrolysis is illustrated in Fig. 3. Biopolymers persist in cells because they are kinetically trapped. Building blocks are linked by bonds that have high intrinsic chemical activation energies of hydrolysis indicated by $\Delta G_{(crhskip\ 8f)}$(int) in Fig. 3. Kinetic trapping is a characteristic of phosphodiester, peptide, and glycosidic bonds [104 106].

Assembly enables biopolymers to persist, in both organisms and in the environment, for extended periods without undergoing thermodynamically favored hydrolysis. Assembly contributes to kinetic trapping and biopolymer persistence *via* a phenomenon known as recalcitrance. Recalcitrance is assembly-mediated increase in chemical lifetimes beyond lifetimes predicted by intrinsic chemical activation energies of hydrolysis. Recalcitrance enables biopolymers to chemically persist in living organisms and in the environment for extended

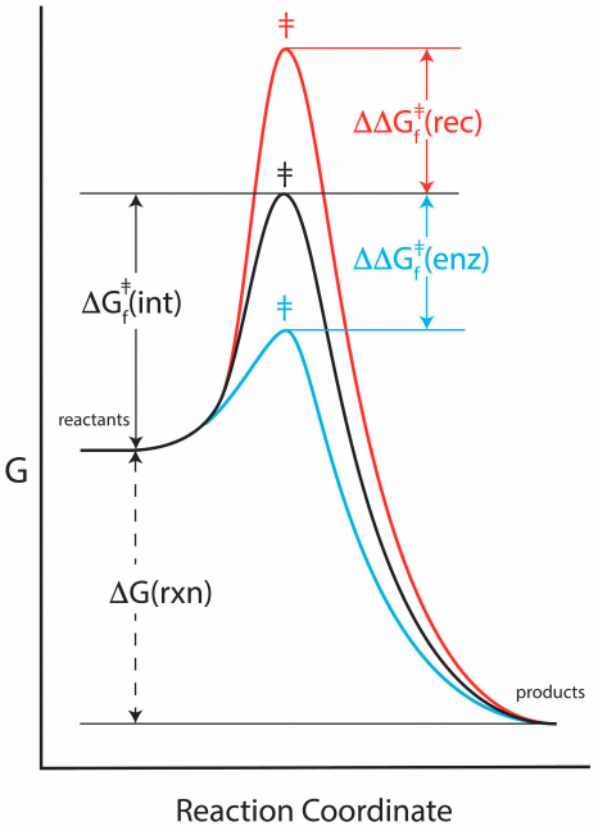

Fig. 3. A generalized reaction coordinate for biopolymer hydrolysis demonstrating how biological systems can either stabilize or destabilize transition states, and by that dynamically increase or decrease reaction rates. Assembly increases activation energies of hydrolysis. Enzymes decrease activation energies for hydrolysis. For simplicity, free energies of all reactants are arbitrarily set to a common value. In fact, both the nature of the biopolymer, its microenvironment, and its assembly state affect the reactant free energy.

periods, far longer than predicted by intrinsic chemical lifetimes [18]. Recalcitrance increases the forward activation energies by $\Delta\Delta G_{(f)}$(rec) (Fig. 3). By contrast, enzymes decrease forward activation energies by $\Delta\Delta G_{(f)}$(enz). In sum, kinetic trapping is mediated by folding and assembly, which sterically excludes access by reactive species such as water and inhibits conformational fluctuations such as those approaching transition states [18]. Biological systems have evolved incredible control of chemical systems by employing on demand mechanisms to either increase or decrease specific reaction rates. A variety of studies that will be discussed herein have demonstrated that resistance to biopolymer hydrolysis is generally greater in assembled than in unassembled states [18,107]. In our model of the origins of life, fine control of rates of hydrolysis contributed to the survival of the 'fittest' polymers during chemical evolution (Fig. 4).

Protection of assembled nucleic acids

Assembled dsDNA has been preserved under environmental conditions for 1 2 millions of years [108]. It was suggested that the chemical basis for selection of the 3 5 backbone linkage of RNA was due to hydrolysis rates in the double-stranded helical state [109 111]. Specifically, it was demonstrated that the hydrolysis rate of 2 5 linkage within double helix is at least 50 100-fold faster than that of a corresponding 3 5 linkage. Moreover, hydrolysis rates of 3 5 backbone linkage of helical dsRNA were at least 5-fold slower than that of a corresponding non-helical ssRNA [110].

The recalcitrance of nucleic acids has been demonstrated in a variety of ways. The discovery and characterization of riboswitches by Breaker and others is based largely on increased recalcitrance of RNA upon ligand binding [112]. Experiments and simulations demonstrate that RNA Goldilocks landscapes of protection depend on assembly [113]. That study focused on RNA degradation *via* an in-line cleavage, which involves a two-step mechanism; first a cleavage through an intramolecular transesterification, followed by a second step of hydrolysis. RNAs that fold can occupy Goldilocks regions of protection. Non-folding RNAs cannot access Goldilocks regions of protection. The widely used method of nucleic acid footprinting is another manifestation of recalcitrance [114,115]. Hydroxyl radicals and other reactive species cleave the polynucleotide backbone. Cleavage is inhibited where DNA or RNA assembles with proteins, resulting in a 'footprint'.

Protection of assembled peptides

Proteins can adopt a variety of recalcitrant assemblies. Unfolded proteins and intrinsically disordered regions of proteins are much more susceptible to proteolysis than folded proteins [116 119]. For example, the rate of hydrolysis of a particular peptide bond in the ribonuclease T1 enzyme decreases by 1700-fold upon folding [119]. Protein assemblies can be preserved under environmental conditions for tens of millions of years [120]. Amyloids, which are assemblies characterized by a fibrillar morphology composed of -sheet, are

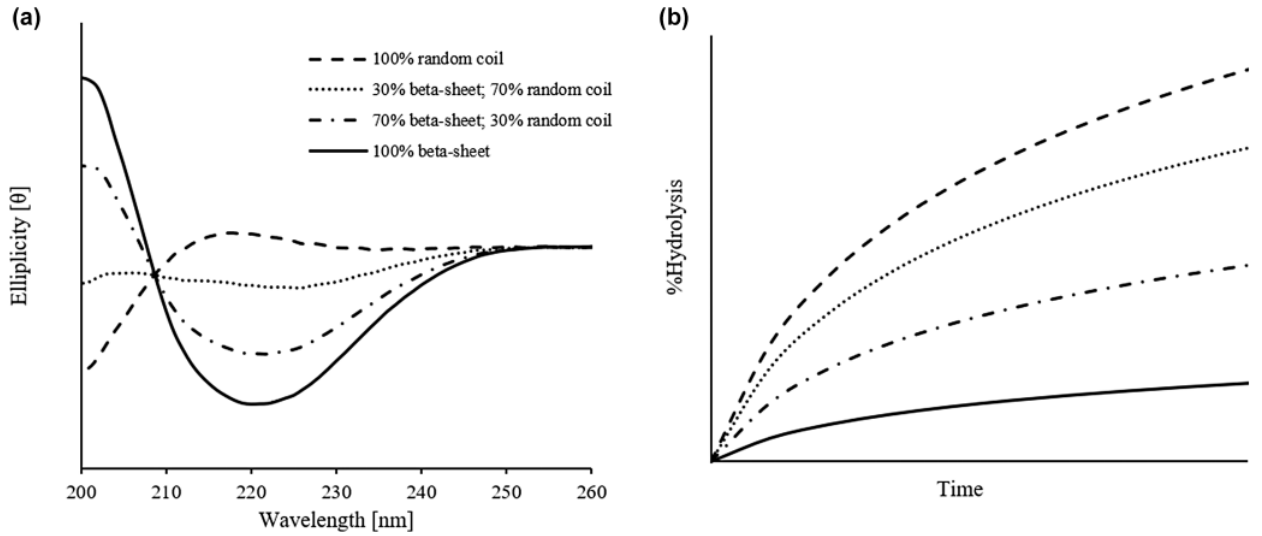

Fig. 4. Hydrolysis is inhibited by assembly. An illustration of (a) Circular dichroism spectra of assembled peptide with varying contents of -sheet and random coil secondary structures. (b) Hydrolysis rate of the corresponding peptides. Peptides capable of forming -sheets undergo hydrolysis at lower rates compared to peptides that form random coil structures.

associated with various diseases and are known to be highly resistant to hydrolysis [107,121 123]. The assembly of the protein PrP leads to amyloid structures that exhibit highly reduced rates of hydrolysis by proteases [107,124 126]. PrP is much more susceptible to hydrolysis when disassembled than when assembled. An amyloid hypothesis of the origins of life [57,127 130] suggests that an early step in chemical evolution was the generation of amyloidogenic peptides that could have self-replicated.

The first direct experimental demonstration of polypeptide recalcitrance was conducted by Brack [131]. Rates of hydrolysis of proteinaceous polypeptides (i.e., containing amino acids found in coded protein) were compared with rates of hydrolysis of non-proteinaceous polypeptides. Proteinaceous polypeptides included alternating Leucine-Lysine [poly(Leu-Lys)] and alternating Valine-Lysine [poly(Val-Lys)]. Non-proteinaceous polypeptides included alternating α-aminobutyric acid and lysine [poly(α-Abu-Lys)] and alternating norvaline and lysine [poly(Norval-Lys)]. Proteinaceous poly(Leu-Lys) and poly(Val-Lys) were shown to be more chemically persistent, with lower rates of hydrolysis, than the non-proteinaceous poly(α-Abu-Lys) and poly(Norval-Lys). The non-proteinaceous polypeptides degraded 15 times faster than the proteinaceous polypeptides under similar conditions [131]. Brack attributed this increased persistence to assembly. The proteinaceous polypeptides assembled into stable -sheets, whereas the non-proteinaceous polypeptides formed random coil or unstable α-helices.

Sequence-dependent recalcitrance of peptides has been reported. Collier designed depsipeptides based on the known Q11 peptide (QQKFQFQFEQQ) except that phenylalanine in the sixth position was replaced by various alpha-hydroxy acids to form analogous ester derivatives of glycine, alanine, leucine, and phenylalanine [132]. All these depsipeptides self-assembled into nanofibers. The rate of hydrolysis of the nanofiber assemblies was dependent on the hydrophobicity of the hydroxy acid sidechain. Faster hydrolysis was observed for glycine and alanine analogs, and slower hydrolysis was observed for the phenylalanine analog, which also formed a more stable assembly. These depsipeptides were shown to form hydrogels. Depsi-L (the depsipeptide containing the hydroxy acid analog of leucine) adopted a -sheet secondary structure, which resulted in the formation of stiffer gels compared to the original Q11 peptide and L-Q11 (a Q11 peptide derivative in which phenylalanine was replaced by leucine). However, the depsi-L hydrogel became softer upon storage due to the readily hydrolyzable ester bond in its backbone.

Zhang *et al.* described sequence-mediated recalcitrance using the EAK16 peptide [(Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys)$_2$] [133,134]. This 16-amino acid peptide has an alanine at every other position interleaved either by negatively charged glutamic acid or positively charged lysine. This peptide can self-assemble into ordered -sheet structures that are stable over a wide range of pH (1.5 11), temperature (25 90 °C), and denaturing conditions (SDS, guanidine, and urea). -sheet structures were detected at very low concentrations (0.6 20 M), as indicated by circular dichroism. This peptide self-assembled into a stable membrane-like structure upon addition of salt even in the presence of SDS [134]. Exceptional stability is achieved through electrostatic interactions between Lys and Glu. These assemblies are resistant to hydrolysis by proteases, such as trypsin and protease K [133,134].

Selection by recalcitrance is predicted in non-replicative systems. Abkevich *et al.* [135] developed a computational model in which random amino acid sequences are generated. A small fraction of sequences form assemblies that were hydrolyzed more slowly than random sequences [57,127 130]. In this simulation, the population gradually shifts to recalcitrant sequences. This simulation demonstrates selection based on recalcitrance, in the absence of replication.

Various potentially prebiotic peptide-analogs demonstrate recalcitrance [136 139]. For instance, drying hydroxy acids leads to synthesis of polyesters that form microdroplets [137,138,140,141]. Peptide analogues such as thiodepsipeptides, which contain both thioester and amide bonds, are of particular interest due to their relevance to prebiotic chemistry and low activation energies for condensation and hydrolysis [142,143]. The Ashkenasy group studied coiled-coils of 32-mer thiodepsipeptides and evaluated their assembly and hydrolysis [144]. Assembly into coiled-coils correlated with the decreased rates of hydrolysis. Template peptides in the presence of reactant electrophiles and nucleophiles in complex dynamic networks formed heterogenous and homogenous assemblies *via* auto- and cross- catalysis with the selection of thiodepsipeptides with well-defined structures [145 148]. Hud and colleagues demonstrated the formation of depsipeptide nucleic acid analogs in dried conditions and showed that upon complementary self-assembly in aqueous solutions, these nucleic acid analogs exhibit slower hydrolysis rates in the assembled state [149].

Protection of assembled polysaccharides

Recalcitrance of cellulose has been extensively investigated due to its importance as a renewable energy

source. Recalcitrance of cellulose has been quantitated; resistance to hydrolysis is determined in part by the free energy of disassembly [150]. It has been demonstrated that assembly of cellulose can completely abolish hydrolysis; assembly can lower the rate of hydrolysis of [(1 4) -D-glucose]$_n$ to practically zero. Various types of assembled polyglucose are among the most persistent and durable biopolymers. Cellulose [151,152] and chitin [153] can achieve lifetimes in the envirorment of up to 250 million years. The persistence of cellulose is greater than that of related hemicelluloses, due to greater recalcitrance.

Protection of assembled amphiphiles

Membranes in archaea, bacteria, and eukaryotes are composed predominantly of amphiphiles, such as phospholipids or ether lipids. Assembly of phospholipids into membranes slows rates of hydrolysis [154]. Assembly of membranes with cholesterol increases the recalcitrance of phospholipids even further [155].

Simpler amphiphile molecules such as fatty acids have been detected in carbonaceous meteorite and could have evolved into phospholipids [156]. Archaeal amphiphiles differ in composition and stereochemistry from those of bacteria and eukarya. It has been postulated that the last common universal ancestor (LUCA) could have had a mixture of both lipid types. Consequently, the biological evolution of membrane lipids does not rule out the possibility of a prebiotic origin for ancestral phospholipids [157]. The Sutherland group explored a mechanism by which assembly-competent phospholipids can be selected, based on an interplay between synthesis and hydrolysis rates [158]. Glycerol-2-phosphate was reacted with activated imidazole-derived fatty acids of up to 10 carbons to form mono- and bis-acylglycerol-2-phosphate. During acylation-hydrolysis cycles, amphiphiles formed, assembled into membranes, and partially degraded. Upon iterative cycles, amphiphiles of C9 and C10 carboxylic acids progressively accumulated at the expense of shorter-chain carboxylic acids (Fig. 5). The

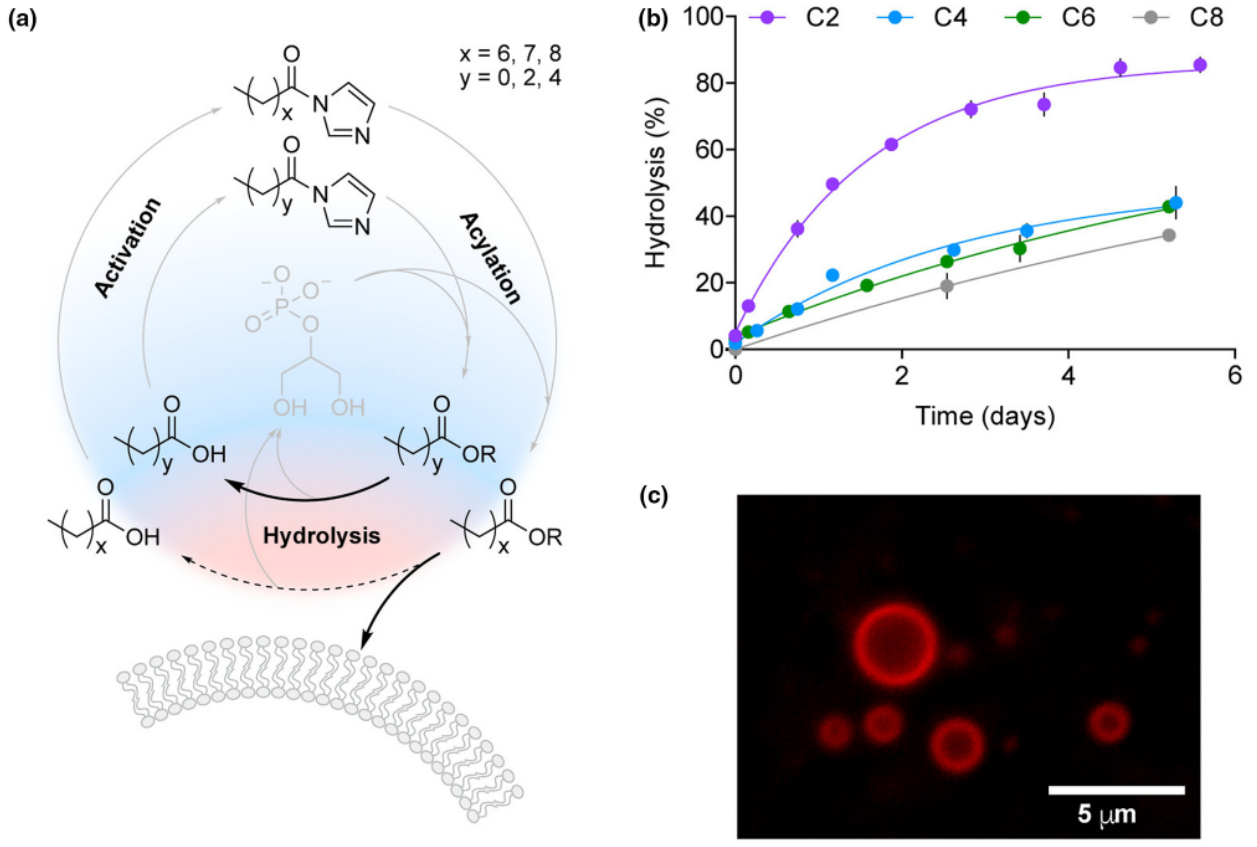

Fig. 5. Selective accumulation of prebiotic phospholipids *via* cycling. (a) Scheme of the acylation hydrolysis cycles. (b) Hydrolysis rates of studied phospholipids. (c) Membranes made of mixed acylglycerol-2-phosphates from the second acylation hydrolysis round were observed. Reprinted with permission from ref. [158]. Copyright© 2019 American Chemical Society.

assembly of amphiphiles into membranes imparted protection from hydrolysis, selecting phospholipids of longer chains.

Protection by heteromolecular assembly

Protein sequences have been tuned by eons of biological evolution [159]. We and others propose that the polypeptide backbone is the product of chemical evolution that occurred prior to biological evolution [31,160]. Depsipeptides, containing mixtures of ester and amide linkages, are probable ancestors of polypeptides. Given that electrostatics are key elements of protein-RNA and protein-DNA interactions in extant life, it was hypothesized that cationic side chains incorporated into proto-peptides could have served in a variety of functions with ancestral nucleic acid polymers in the early stages of life. In support of this notion, it was demonstrated that cationic proto-peptides (depsipeptides and polyesters), either produced as mixtures from plausibly prebiotic dry-down reactions or synthetically prepared in pure form, can engage in direct interactions with RNA, resulting in mutual stabilization [161]. Cationic proto-peptides significantly increased the thermal stability of folded RNA structures. In turn, RNA increased the lifetime of a depsipeptide by reducing the rate of hydrolysis of backbone ester bonds by > 30-fold (Fig. 6). Depsipeptides containing the proteinaceous amino acids lysine, arginine, or histidine adjacent to ester bonds generally promoted RNA duplex thermal stability to a greater magnitude than analogous sequences containing non-proteinaceous residues. These findings support a model in which tightly intertwined biological dependencies of RNA and protein reflect a long co-evolutionary history that began with rudimentary, mutually stabilizing interactions at early stages of polypeptide and nucleic acid co-existence, which perhaps served as a basis for further chemical evolution. On a more general level, complexes and co-assemblies may have been critical for the mutual survival of the interacting molecules during origins of life. Co-assemblies of peptides with nucleic acids and lipids have also been reported [162 165]. The co-assemblies obtained by the non-covalent interactions are sometimes accompanied by structural alterations, as was shown in the case of the introduction of decanoic acid to peptide amphiphiles [162] or the co-aggregation of amyloidogenic peptides and fatty acids [164].

Mutual protection between depsipeptides and RNA is an example of a wider concept of molecular mutualism, in which constructive interactions between 'non-self' interactors benefits both [18,166]. Mutualistic interactions can occur on diverse scales, from the molecular level up to the living organism and ecosystems. At all levels, mutualism can promote co-evolution and impart survival and fitness, which can manifest in persistence [18]. On the molecular level, the formation of complexes *via* interactions between different classes of molecules could have played important roles and lowered hydrolysis rates of

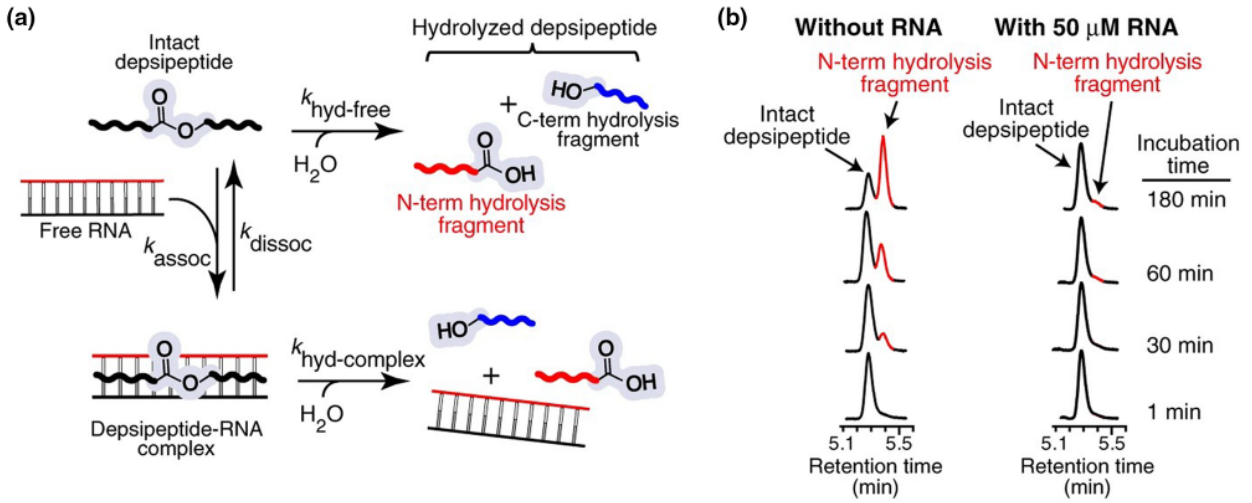

Fig. 6. Stabilizing interactions between proto-peptides and nucleic acids. (a) A schematic of depsipeptide-RNA interactions leading to increased depsipeptide lifetimes. Under conditions wherein complex formation is favorable, the presence of RNA increases the depsipeptide lifetime. (b) HPLC traces showing hydrolysis of depsipeptide at various time points in the presence or absence of an RNA duplex. Reprinted with permission from ref. [161]. Copyright 2020 Springer Nature.

the interacting molecules, as was demonstrated for mixtures of RNA and cationic depsipeptides (Fig. 6) [161]. In another example, Jia *et al*. [39] suggested that the coacervate assembly formation of negatively charged nucleic acids and cationic peptides can serve as a primitive membraneless compartment, or protocell, in which local conditions suitable for chemical reactions can be preserved.

The stability of assemblies influences hydrolysis rates and allows fine tuning *via* conformational isoforms. For example, Ryu and Park investigated the thermal, chemical, and proteolytic stability of diphenylalanine-based nanowires and nanotubes. While the nanowires remained intact even at 200 °C, the nanotubes began to degrade at 100 °C. Circular dichroism and FTIR measurements revealed that the nanotubes adopted -turn-like secondary structures, which further transitioned upon heating at 200 °C. The nanowires displayed high-thermal stability with -sheet secondary structure. Remarkably, the nanotubes exhibited structural transition toward the nanowires upon heating above 150 °C, resulting in a similar crystalline structure. The nanowires exhibited high structural and hydrolytic resistance to the harsh chemical environment, while in the case of the nanotubes, the assessment of their resistance was problematic because the nanotubes readily disintegrated from their solid surface. When exposed to enzymatic proteolysis, the nanowires remained intact while the nanotubes rapidly degraded. This study clearly demonstrated the superiority of nanowires over nanotubes in terms of thermal, chemical, and proteolytic stability. Moreover, it indicated how, by controlling the structure of certain peptides, one can improve peptide durability [167].

Protection of non-biological molecules by assembly

The effect of assembly on hydrolysis rates has also been demonstrated for non-biological chemical systems. For example, Boekhoven and colleagues [168] studied how dissipative assemblies affect anhydride formation and hydrolysis. In these systems, two conjugated reactions take place and feed each other: (a) a non-active precursor is activated through irreversible consumption of a high energy molecule (fuel) to form an anhydride, and (b) the active anhydride-containing molecule (product) is spontaneously deactivated (hydrolyzed) and returns to its original state. While in its active state, the product can spontaneously self-assemble into colloidal particles that inhibit the reverse deactivation (hydrolysis) upon fuel consumption. This dynamic system is under non-equilibrium state driven by the kinetics of the activation and deactivation reactions. Specifically, the conversion of four dicarboxylate precursors, derivatives of aspartic acid and glutamic acid, into the active anhydride upon addition of carbodiimide as fuel was studied. The water insoluble Fmoc-protected products, Fmoc-E and Fmoc-GD anhydrides, formed colloids in the aqueous environment that protected and slowed down hydrolysis rates of their building blocks back to the dicarboxylate form. In a follow-up work [169], the researchers studied chemically fueled self-assembly of the anhydride product of an additional Fmoc-protected peptide precursor under kinetic trapping (Fig. 7). The peptide precursors were terminated with an aspartic acid that forms the anhydride product. The authors elucidated the kinetic-trapping mechanism and demonstrated that the anhydride product forms fibers of -sheets that persist even upon regeneration of the peptide precursor.

Assembly chirality and protection

Recalcitrance provides a mechanism for selection of homochirality. To understand relationships between homochirality and recalcitrance, Brack and Spach [170] characterized -sheet formation of both heterochiral and homochiral peptides. They determined that assembly into -sheets and low rates of hydrolysis (recalcitrance) correlate with homochirality.

Chirality is critical to stabilities of assemblies. For example, Basak *et al*. [171] studied how the chirality of the tripeptide Phe-Phe-Leu affected the properties of the self-assembled organogel fibers (Fig. 8). They labeled all eight possible stereoisomers with a fluorescent ferrocene (Fc) group and the homochiral SSS with the fluorescence quencher group pyrene (Py). When present alone in the solution only isomers SSS, SRR, RRR, RSS, and SSS (Py-labeled) formed stable organogels. The Py-labeled SSS peptide was incubated with the various peptide isomers and fluorescence levels were monitored. When mixed hetero-fibrils were formed, fluorescence quenching was observed. By contrast, the self-sorting of these peptides into two distinct homofiber populations, one of which was the Py-labeled SSS peptide, exhibited fluorescence signals. They inferred that homochiral peptides are favorable in the context of the origins of life because they form more mechanically stable gels, compared to the heterochiral peptides, with uniform morphology. Chiral-dependent gel formation and mechanical properties were also demonstrated by Marchesan *et al*. [172], where sequence-dependent chiral effects were shown for various heterochiral tripeptide stereoisomers of the Val-Phe-Phe peptide. The

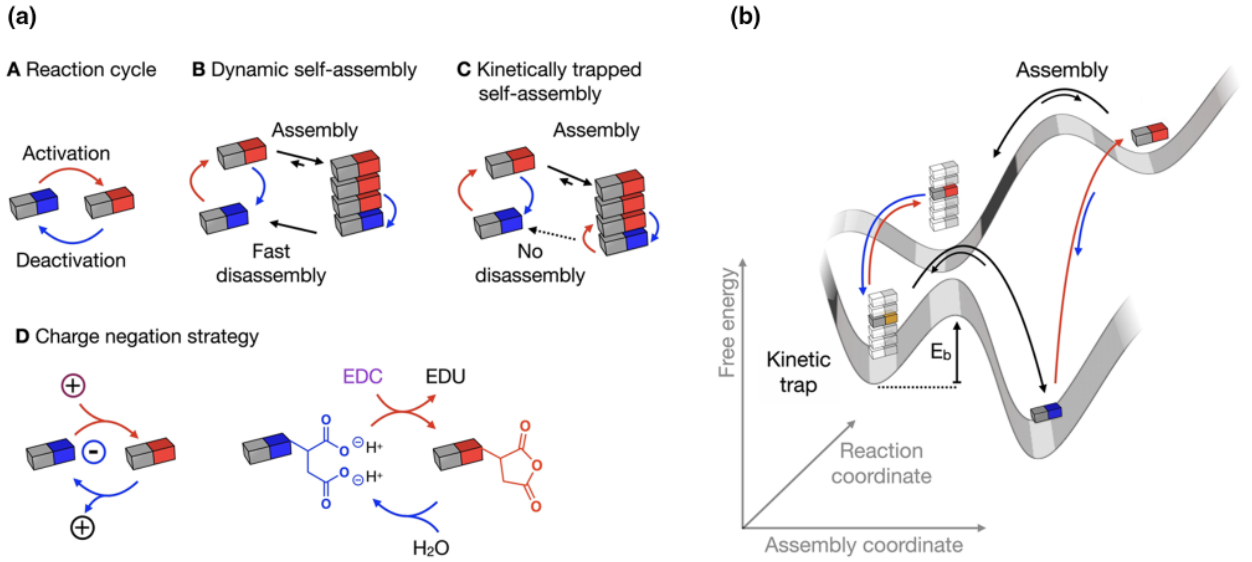

Fig. 7. Kinetic-trapping in fuel-driven reaction cycle. A schematic representation of (a) activation-deactivation reactions involving dynamic self assembly (B) and kinetically trapped self-assembly (C). (b) Energy diagram of the reaction landscape when kinetic-trapping takes place. Reprinted with permission from ref. [169]. Copyright© 2022 Wiley-VCH GmbH under CC BY 4.0 license.

effect of chirality on gel stability was also demonstrated for single amino acids. Specifically, Singh *et al.* [173] found that even a slight addition (8) of D-phenylalanine to L-phenylalanine solution prevented amyloid formation.

Origins of biopolymers

It seems likely that small molecules and short hetero-oligomers were continuously synthesized and degraded by wet-dry cycling on the prebiotic Earth prior to emergence of large and more sophisticated biopolymers. We suggest that incremental enrichment of some molecular species over others during chemical evolution was driven in part by interplay of kinetics of synthesis and hydrolysis. Molecules that are made more quickly and degraded slowly will increase in population relative to other molecules. We use the term 'selection' to describe mechanisms that lead to differences in populations.

Even relatively simple and heterogeneous molecules can assemble. Therefore, it is reasonable to propose a prebiotic scenario in which interactions among short, prebiotically-plausible oligomers led a range of assemblies with variable physical properties and functions. Such assemblies would in turn have been subjected to environmental pressures that could lead to selection. Assemblies would have decreased hydrolysis rates and allowed molecular survival of the fittest molecules. Emergence of assemblies, along with numerous other functions that can be exhibited by short oligomers, could have provided selective advantages to a variety of molecular networks containing different cooperative molecules [164,174 177].

It is reasonable to assume that during chemical evolution, assembly modulates local water activity, with implications for evolution of catalytic function. The formation of local pockets with low water-activity in bulk aqueous media can promote catalysis.

We suggest that selection is intrinsic to evolution, both chemical and biological. In this model, selection does not require replication. During early chemical evolution, molecules were selected on varying combinations of (a) solubility in water, (b) ability to link by condensation-dehydration, (c) chemical transitions into kinetically trapped (persistent) condensates, such as ester-amide exchange, (d) resistance to hydrolysis by assembly (recalcitrance), and (e) catalytic and autocatalytic synthesis of precursors. In this model, intense and mutable non-Darwinian selection sparked the genesis of biopolymers. The amazing assembly proficiencies of biopolymers are the hallmarks of chemical evolution.

In this paper, we focus on attenuation of hydrolysis by assembly. However, recalcitrance is a general phenomena and applies to additional types of chemistry of degradation. In addition to hydrolysis, polymer degradation can occur also through free radical reactions, photo-oxidation, exchange reactions (such as transesterification), and more. For example, as mentioned, nucleic acid footprinting [114,115] is a manifestation of

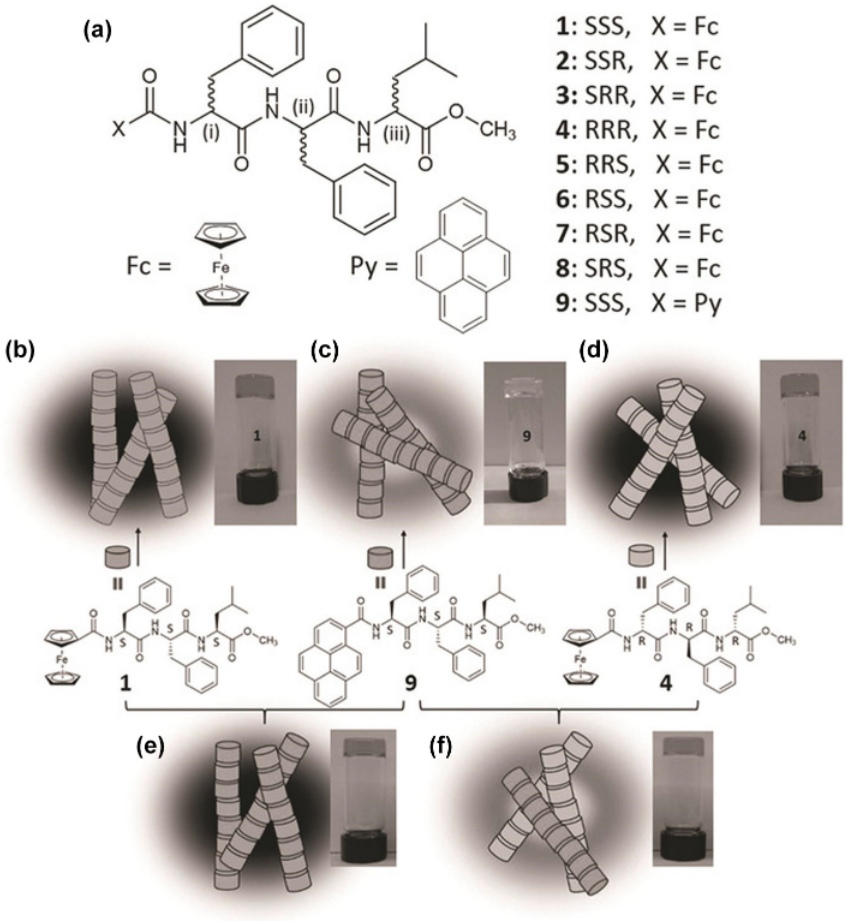

Fig. 8. Chirality dictates assembly propensity. (a) Chemical structures of stereoisomers of ferrocene- and pyrene-based tri-peptides (1 8 and 9, respectively). (b f) Self-assembly of homochiral organogelators into gel nano-fibers. Homochiral Fc-based gelator 1 and 4 form non-fluorescent fibers, whereas 9 forms fluorescent fibers (top panel). Mixing of peptides 9 and 1 produced non-fluorescent fibers, indicating a mixed stacking of peptide chains. However, mixing of peptides 4 and 9 results in fluorescent fibers, indicating self-sorted fiber formation. Reprinted with permission from ref. [171]. Copyright© 2017 John Wiley and Sons.

recalcitrance. In this method, nucleic acid degradation is often facilitated *via* hydroxyl radical and other reactive species that can cleave the polynucleotide backbone.

Summary

To conclude, here we have documented recalcitrance in all universal biopolymers. Recalcitrance is dependent on assembly. Assembly is a spontaneous process that is ubiquitous in life and in abiotic systems and is often emergent upon polymerization. We propose a model in which chemical evolution was gradual and included processes in which monomers were condensed to form oligomers, hydrolyzed partially back to monomers, and ultimately converted into kinetically trapped polymers within organized networks. Molecular complexification and sophisticated assembly increased in concert. Based on the strong relationship between structure and function, as well as the importance of water as a solvent and a biochemical participant [178], we suggest that chemical evolution drove selection of increasingly deep but dynamic kinetic traps.

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