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An Approach to the De Novo Synthesis of Life

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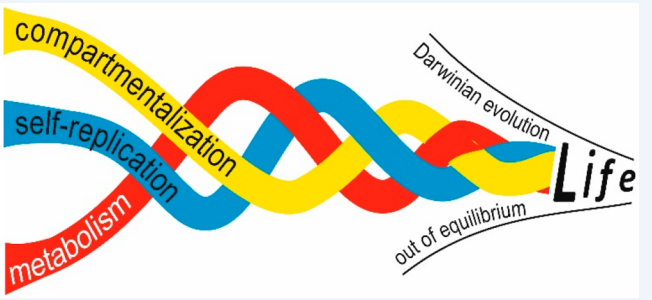
CONSPECTUS: As the remit of chemistry expands beyond molecules to systems, new synthetic targets appear on the horizon. Among these, life represents perhaps the ultimate synthetic challenge. Building on an increasingly detailed understanding of the inner workings of living systems and advances in organic synthesis and supramolecular chemistry, the de novo synthesis of life (i.e., the construction of a new form of life based on completely synthetic components) is coming within reach. This Account presents our first steps in the journey toward this long-term goal. The synthesis of life requires the functional integration of different subsystems that harbor the different characteristics that are deemed essential to life. The most important of these are self-replication, metabolism, and compartmentalization. Integrating these features into a single system, maintaining this system out of equilibrium, and allowing it to undergo Darwinian evolution should ideally result in the emergence of life. Our journey toward de novo life started with the serendipitous discovery of a new mechanism of self-replication. We found that self-assembly in a mixture of interconverting oligomers is a general way of achieving self-replication, where the assembly process drives the synthesis of the very molecules that assemble. Mechanically induced breakage of the growing replicating assemblies resulted in their exponential growth, which is an important enabler for achieving Darwinian evolution. Through this mechanism, the self-replication of compounds containing peptides, nucleobases, and fully synthetic molecules was achieved. Several examples of evolutionary dynamics have been observed in these systems, including the spontaneous diversification of replicators allowing them to specialize on different food sets, history dependence of replicator composition, and the spontaneous emergence of parasitic behavior. Peptide-based replicator assemblies were found to organize their peptide units in space in a manner that, inadvertently, gives rise to microenvironments that are capable of catalysis of chemical reactions or binding-induced activation of cofactors. Among the reactions that can be catalyzed by the replicators are ones that produce the precursors from which these replicators grow, amounting to the first examples of the assimilation of a proto-metabolism. Operating these replicators in a chemically fueled out-of-equilibrium replication-destruction regime was found to promote an increase in their molecular complexity. Fueling counteracts the inherent tendency of replicators to evolve toward lower complexity (caused by the fact that smaller replicators tend to replicate faster). Among the remaining steps on the road to de novo life are now to assimilate compartmentalization and achieve open-ended evolution of the resulting system. Success in the synthesis of de novo life, once obtained, will have far-reaching implications for our understanding of what life is, for the search for extraterrestrial life, for how life may have originated on earth, and for every-day life by opening up new vistas in the form living technology and materials.



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- Yang, S.; Schaeffer, G.; Mattia, E.; Markovitch, O.; Liu, K.; Hussain, A. S.; Ottelé, J.; Sood, A.; Otto, S. Chemical fueling enables molecular complexification of self-replicators. *Angew. Chem., Int. Ed.* **2021**, *60*, 11344−11349.[4] *Two competing self-replicators were placed in a chemically fueled out-of-equilibrium replication-destruction regime. While under equilibrium conditions, the faster and more stable small replicator prevails, chemical fueling allows the slower, molecularly more complex replicator to win the competition. This work demonstrates a concept that is important in the context of evolution: that fueling can drive replicator complexification.*

INTRODUCTION

Chemistry focuses on creating new entities (molecules, materials, systems), more so than perhaps any other science. Over the centuries, chemists have learned how to synthesize molecules of impressive complexity. With the advent of Systems Chemistry,[5−8] the remit of chemistry has expanded to also include the design and synthesis of *systems* of molecules, that harbor systems-level properties that go well beyond the sum of their parts (i.e., emergent properties). Among the most intriguing and challenging of such properties is life. Life represents a huge synthetic challenge,[9−11] deemed intractable for many years. Yet with recent developments in synthetic methodology, supramolecular chemistry, and analytical tools and an improved understanding of biochemistry and evolution, opportunities are opening up to create new forms of life, that are not necessarily constituted of the types of biomolecules that are found in extant life (i.e., proteins, nucleic acids etc.). Approaches to de novo life differ from efforts to create synthetic cells[12,13] by aiming to build life from scratch, utilizing man-made building blocks rather than molecules obtained from current life. Efforts directed toward the synthesis of life will also inform on the possible origins of life on Earth.[14−20] Even though the development of de novo life is not necessarily guided or constrained by issues of prebiotic relevance and geochemical considerations, the general principles and concepts discovered in the process may well extend to current life's origin.

Addressing the challenge of the de novo synthesis of life starts with defining the target. While a generally accepted definition of life does not exist,[21,22] a pragmatic approach to defining this target is to list the key (functional) characteristics that any form of life should encompass.[11] These characteristics are summarized in Figure 1 and include:

(i) Self-replication, which is the ability of a system to autonomously catalyze the formation of copies of itself;

(ii) Metabolism, which is the ability of a system to form its constituents from precursors and connect the internal maintenance of the system to an external energy source;

(iii) Compartmentalization, which is the means by which a systems prevents the uncontrolled spreading of its components into its environment.

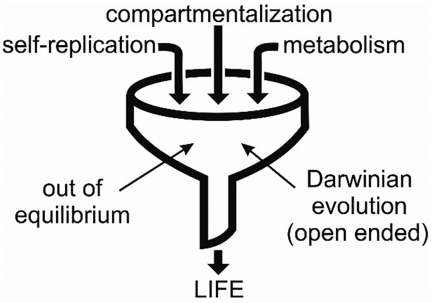

Figure 1. Emergence of life requires the integration of functional subsystems that are responsible for self-replication, compartmentalization, and metabolism under conditions that keep the system far from equilibrium and enable its open-ended Darwinian evolution. Adapted with permission from ref 11. Copyright 2020, Springer Nature.

(iv) Out-of-equilibrium state; life requires a continuous input of energy to keep it from collapsing into a lifeless thermodynamic minimum.

(v) Darwinian evolution: the process of natural selection among mutants produced when replication is accompanied by variability (i.e., mutation).

These characteristics are in part overlapping; i.e., practical implementation of Darwinian evolution requires a reproduction/destruction regime, which is inherently out of equilibrium and the energy harvesting part of metabolism also contributes to maintaining systems out of equilibrium.

With this list of characteristics, a stepwise approach to the synthesis of a minimal form of life presents itself. As a first step, a system may be developed that implements only one of these characteristics and additional features may then be integrated in a stepwise manner.

Considerable progress has been made on the development of systems that capture one of the first four of the five characteristics listed above: Self-replicating molecules have been made based on many different molecular designs;[23−26] reaction networks have been identified that enable chemical complexity to be built up;[27] many different forms of compartmentalization have been investigated,[28,29] including bilayer vesicles,[30,31] microdroplets,[32] coacervates,[33−35] and even absorption on surfaces.[36,37] Finally, out-of-equilibrium chemical systems are attracting renewed attention, particularly in the area of self-assembly.[38−43] Only the implementation of Darwinian evolution in chemical systems outside the realm of biology/biomolecules has not yet received much attention.[44] The current frontier in the de novo synthesis of life encompasses the binary integration of different subsystems; the first reports combining replication (mostly enzyme-mediated) with compartmentalization[45−47] or replication with metabolism[3,48,49] have appeared. Furthermore, methodology has been developed to maintain compartments[50−54] and replicators[4,55−58] out-of-equilibrium.

While the stepwise integration of subsystems is a worthwhile and logical approach, we should not dismiss the possibility that systems that integrate several of life's characteristics can emerge in a single event. Indeed, as we will show below, protometabolic capabilities can arise in systems that were primarily selected for their ability to self-replicate, in a single joined emergence step.

In this Account, I review the progress that my research group made toward the de novo synthesis of life, starting from the serendipitous discovery of self-replicating molecules and then showing how these systems can exhibit dynamics that are relevant to evolutionary scenarios. I will discuss how the

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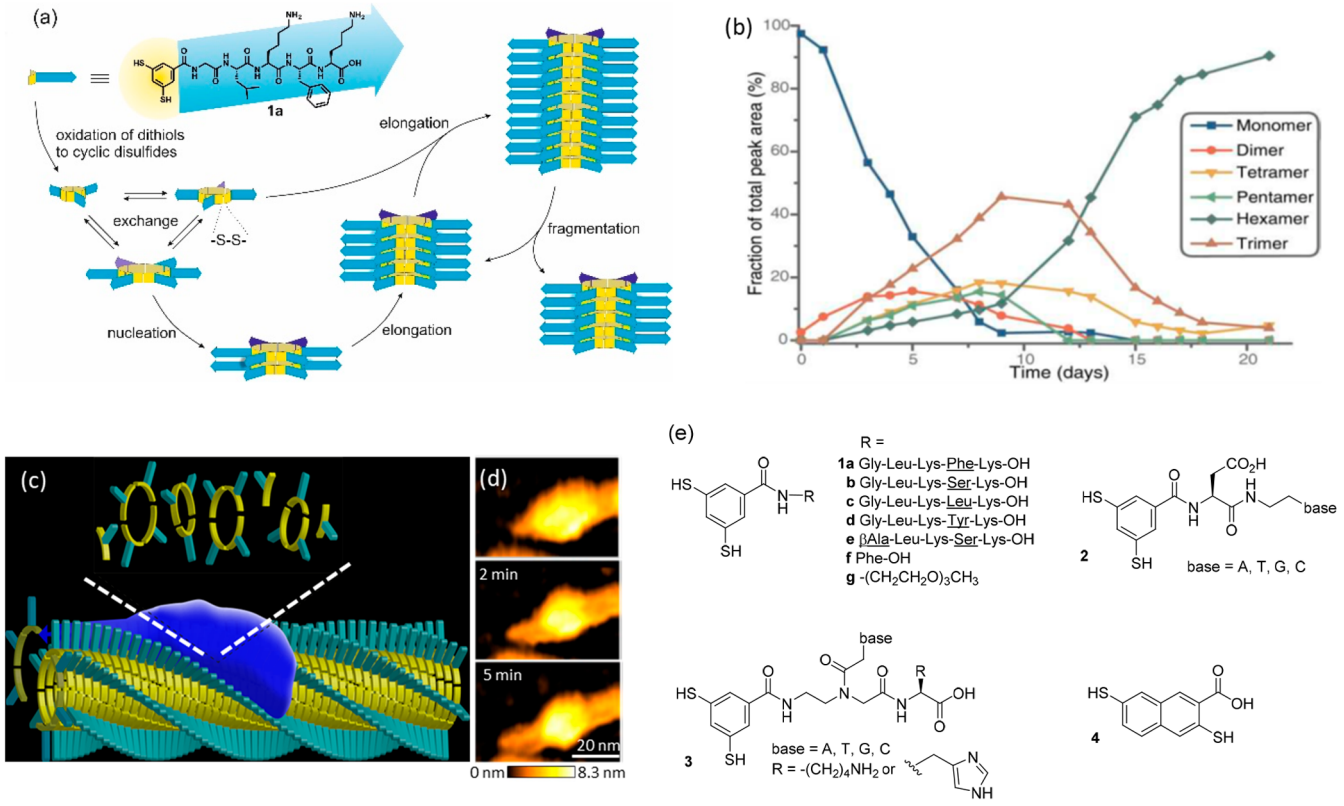

Figure 2. (a) Simplified mechanism by which self-assembly can drive self-replication. (b) Kinetics of formation of different oligomers of **1a** showing a distinct lag phase in the formation of replicator $(\mathbf{1a})_6$.[2] (c) Assembly of precursors on the sides of the fibers promotes self-replication as evident from (d) high-speed AFM images of a fiber growing from a bound precursor aggregate at $t = 0$, 2, and 5 min (data taken from ref 64). (e) Building blocks with which self-assembly driven self-replication has been observed include peptide derivatives **1a**–**f**, but also amino-acid nucleic-acid chimeras **2** and **3** as well as molecules **1g** and **4** lacking any of life's current building blocks.

replicating systems can acquire metabolic activity and how imposing an out-of-equilibrium replication/destruction regime can, in principle, enable their molecular complexification. The Account closes with an inventory of the steps that still need to be made on the path to de novo life and a brief discussion of the implication of reaching the end of this path.

SELF-REPLICATION

Our approach to the de novo synthesis of life started with the serendipitous discovery of self-replicating molecules.[1] So the first step in our approach involved acting on an opportunity that arose, as opposed to being a rational choice. At the time, we worked on dynamic combinatorial libraries[59] and had designed building blocks that we hoped would form folded molecules. The idea was that noncovalent interactions between building blocks within the same library member would shift the composition of the mixture of interconverting molecules toward those that adopt well-defined and stable conformations. We designed building blocks to contain short peptide units, alternating hydrophobic and hydrophilic amino acids. Such a motif is known to fold into β-sheet structures.[60,61] And indeed, β-sheets were found, but, surprisingly, not within but between the molecules formed from the building blocks. Thus, instead of foldamers, we obtained self-assembling stacks of macrocycles.[1] As shown in Figure 2, this stacking process drives the synthesis of the very macrocycles that stack, amounting to their self-replication. The mechanism of self-replication starts with the oxidation of the thiol moieties of the building block (such as **1a**) to give rise to a mixture of macrocyclic disulfides that exchange building blocks with each other through reversible thiol–disulfide chemistry.[62] Stacking of the central aromatic rings, together with β-sheet formation between the peptide side groups, allows a specific macrocycle to assemble (in the example in Figure 2a this is the hexamer). The growth of these replicators usually exhibits a pronounced lag phase, typical for autocatalytic systems, as shown for the emergence of $(\mathbf{1a})_6$ in Figure 2b, The 1-D assemblies grow from their ends (evident from experiments using isotopically labeled material),[63] and, when subjected to mechanical energy through agitating the sample, the stacks break, exposing additional stack ends. This growth-breakage mechanism enables exponential growth of the fibers and the self-replicating macrocycles from which they are constituted.[2]

Detailed investigation of the mechanism of self-replication by high-speed AFM and MD simulations on hexamer replicators made from **1a** revealed that fiber growth involves the recruitment of precursors that bind as aggregates to the sides of the fibers (Figure 2c,d).[64] This material diffuses along the grooves on the fibers to the fiber ends, where fiber growth takes place. This way of guided assembly represents an interesting new mechanism for supramolecular polymerization, simplifying a 3-D search problem (where monomers have to find the fiber ends in solution) into a 1-D search problem (where diffusion along the fiber surface allows those ends to be found faster).

While several peptide-based self-replicators[65,66] and replicator networks[67] have been reported, these typically involve α-helices that interact through helix-bundle formation and replicate through a different mechanism.

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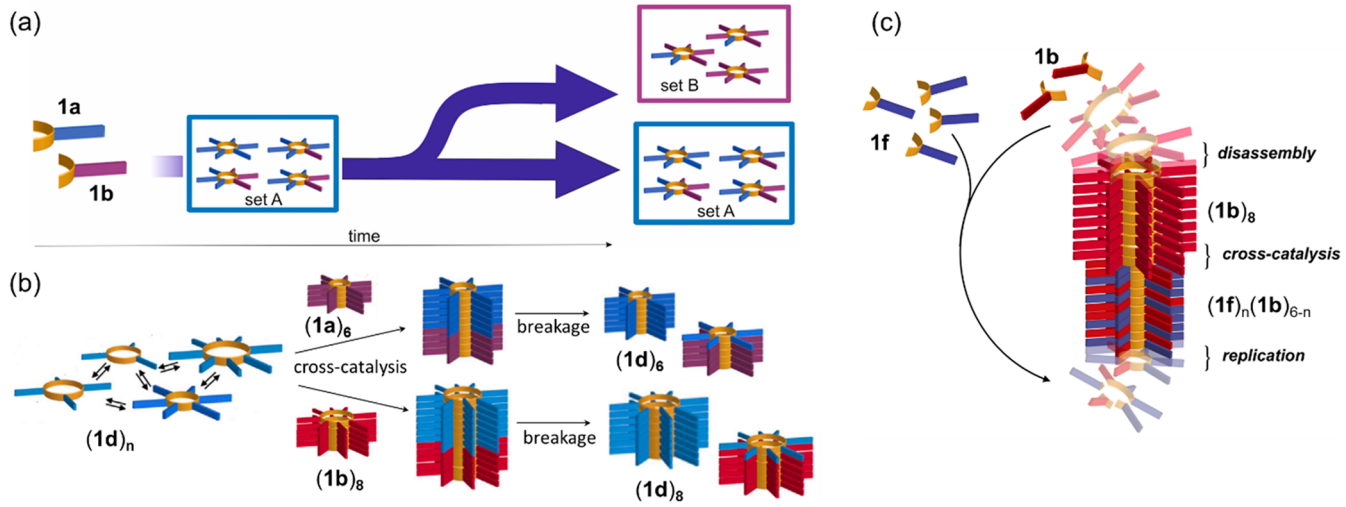

Figure 3. (a) Diversification of self-replicating molecules. Oxidizing a mixture of building blocks **1a** and **1b** leads to two separate sets of replicators that emerge at different times. The first set of hexamers rich in **1a** induces the formation of a second set of hexamers that specialize on **1b**. (b) Sample history dictates replicator composition. Whether building block **1d** gives rise to hexamer or octamer replicator depends on whether the sample was exposed to independently prepared hexameric or octameric replicators, which cross-catalyze the formation of the replicator of **1d** with the corresponding ring size. (c) Parasitic/predatory behavior in which replicator $(\mathbf{1b})_8$ cross-catalyzes the formation of $(\mathbf{1f})_n(\mathbf{1b})_{6-n}$ which subsequently consumes the original replicator $(\mathbf{1b})_8$. Adapted with permission from ref 87. Copyright 2018, John Wiley & Sons, Inc.

The exponential replication mediated by the growth-breakage mechanism solves a problem that has thwarted the replicator field for decades: the inhibition of replication resulting from the tendency of replicators to remain associated with each other.[68] Most other systems of self-replicators involve the ligations of two precursor molecules on a template to produce a dimer of the template, which needs to dissociate before further replication can take place. Dissociation is normally difficult, resulting in parabolic growth, as opposed to exponential growth (termed the "square root law" of replication by von Kiedrowski).[68] Szathmáry showed that parabolic replicators tend to co-exist indefinitely, while exponential replication leads to survival of the fittest and extinction of the weakest replicators.[69] Thus, parabolic replicators cannot normally undergo Darwinian evolution, while exponential replicators can.

Intriguing parallels exist between the replication mechanism shown in Figure 2a and amyloid assembly[70] (implicated in prion diseases, but also suggested to have played a role in the origin of life[71]). Both processes are autocatalytic, exhibit a growth-breakage mechanism, may give rise to different strains and feature roles (albeit different in nature) of fiber sides.

The mechanism shown in Figure 2a appears general. Many different peptides sequences have been used, included remarkably short one such as **1f**,[72] giving rise to replicators with different ring size (the more strongly interacting peptides yield smaller rings, in line with a minimal interaction strength and associated degree of multivalency needed for assembly).[73] In select cases, competition between replicators of different ring sizes occurs with environmental conditions determining the winner. For example, a hexamer of building block **1c** prevailed when shaking, while a heptamer forms under stirring[1] or either a hexamer or octamer of **1b** prevailing, depending on the solvent environment.[74] We have shown that also chimeric building blocks featuring an amino acid and a nucleobase (**2** and **3** in Figure 2e) can give rise to exponentially growing replicators.[75] Even building blocks lacking any peptide and even lacking any similarity to the building blocks of current life (i.e., not featuring any amino acids or nucleobases) can give rise to self-replicators, as evident from the behavior of samples made from oligo-ethylene oxide substituted building block **1g**[76,77] or dimercaptonaphthalene **4**.[78] In contrast to the one-dimensional fibrous assemblies formed from oligomers of **1a**−**g**, **2**, and **3**, building block **1g** gives rise to cyclic hexamers that autocatalytically assemble into sheets, while **4** gives rise to cyclic tetramers of which one particular isomer autocatalytically forms sheetlike aggregates. Thus, the growth-breakage replication mechanism works for 1-D as well as 2-D assemblies. The mechanism of self-replication is also not limited to disulfide chemistry, as Ashkenasy and co-workers demonstrated a similar replication behavior involving native chemical ligation.[79,80]

Note that, some 10 years after the discovery of the self-replicators in the course of aiming for the formation of foldamers, we did succeed in obtaining folded molecules by shortening the peptide sequence, with which β-sheet formation is less feasible,[81] or by introducing nucleobase residues.[82] We recently also explored systems at the boundary of self-replication and folding, describing self-sorting between the two assembly modes[83] as well as the conversion of foldamers into replicators.[84] We conclude that these two modes of assembly are two sides of the same coin. Whether a systems folds or forms self-replicators is difficult to control and depends on whether assembly processes occur intra- or intermolecularly, respectively.[84] In fact, even after millions of years of evolution, the competition between assembly and folding in biology is, in some instances, still poorly controlled, as evident from prion diseases that are caused by the autocatalytic assembly of proteins into β-sheets as opposed to folding.

EVOLUTIONARY DYNAMICS

The examples discussed above featured systems prepared from only a single block which constrains the diversity of products formed. Including a second building block was found to lead to much richer dynamics and revealed behavior that starts to resemble aspects of evolutionary dynamics that we know from living systems. A first example is the spontaneous diversification of self-replicating molecules into two sets observed in dynamic

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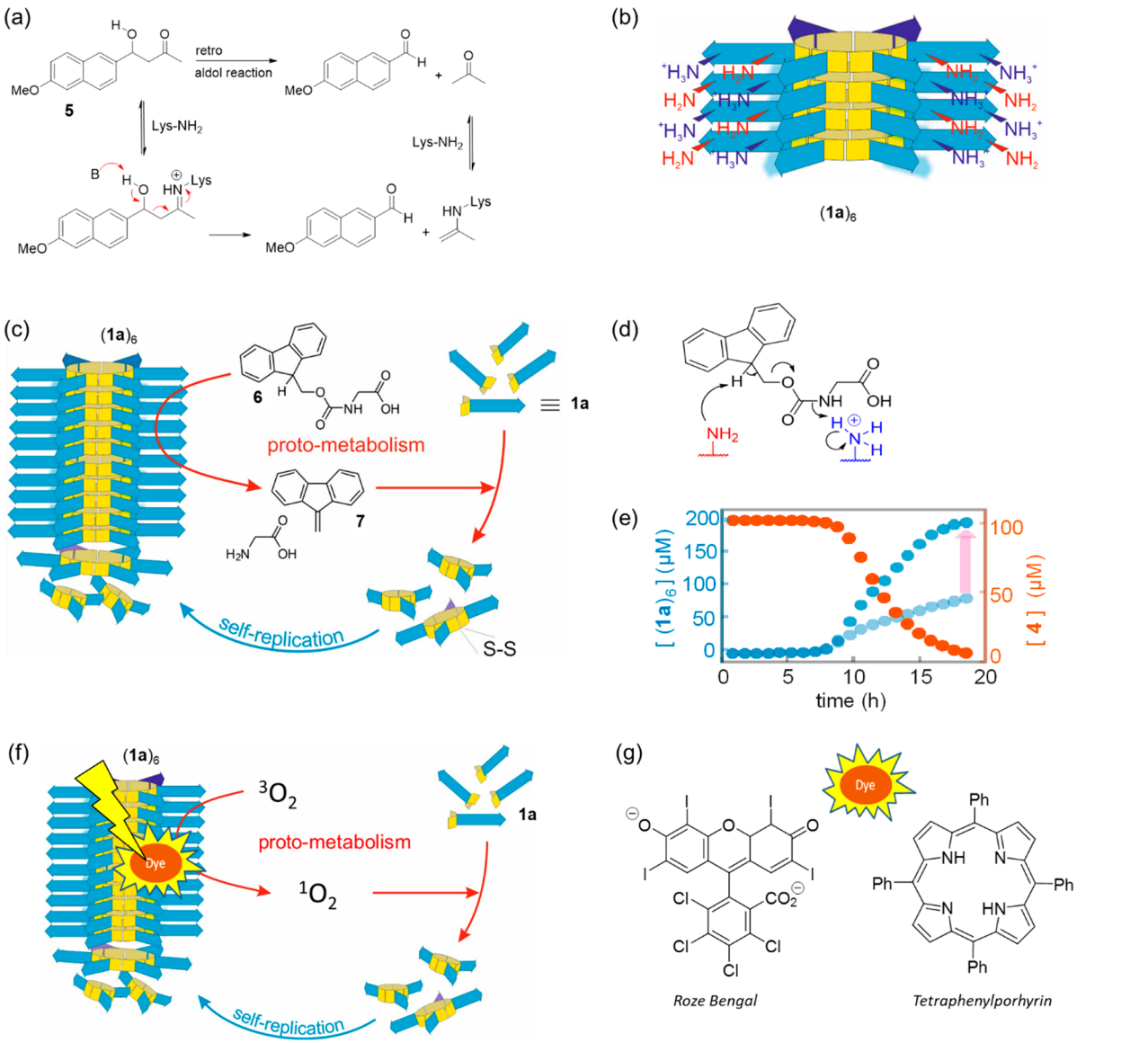

Figure 4. (a) Replicator $(\mathbf{1a})_6$ catalyzes the retro-aldol reaction of methodol **5** involving imine formation between the nonprotonated lysine residues and **5**. (b) The close proximity of many lysine side groups in the assemblies of replicator $(\mathbf{1a})_6$ perturbs the pK_A of the lysine groups resulting in the presence of nonprotonated lysines at neutral pH. (c) Proto-metabolism arising from replicator $(\mathbf{1a})_6$ catalyzing the cleavage of FMOC-glycine (**6**) to yield dibenzofulvene (**7**) which accelerates the oxidation of building block **1a** into the small-ring precursors from which the replicator grows. (d) Postulated mechanism through which $(\mathbf{1a})_6$ catalyzes the cleavage of FMOC-glycine, relying on the simultaneous presence of protonated and nonprotonated lysine amine groups. (e) In an agitated sample prepared from dithiol building block **1a** (200 μM) and FMOC-glycine **6** (100 μM) the emergence of $(\mathbf{1a})_6$ (dark blue circles) coincides with the onset of FMOC cleavage (red circles). Upon repeating the experiment in the absence of FMOC-glycine, replicator $(\mathbf{1a})_6$ emerges at the same time, but grew significantly slower (light blue circles). Adapted with permission from ref 3. Copyright 2020, Springer Nature. (f) Proto-metabolism arising through binding of dyes to replicator $(\mathbf{1a})_6$ which enhances the conversion of triplet to singlet oxygen, accelerating the production of replicator precursor. (g) Dyes used as cofactors for photomediated singlet-oxygen production.

mixtures prepared from building blocks **1a** and **1b**, differing in only a single amino acid residue.[85] In isolation, the two building blocks give rise to hexamer and octamer replicators, respectively.[73] However, upon mixing, only hexamers emerge. First a series of hexamer mutants rich in **1a** appears, followed later by the emergence of another set of hexamers, rich in the remaining building block **1b** (Figure 3a). Seeding experiments showed that the first set of hexamers cross-catalyzes the formation of the second set, indicative of an ancestral relationship. This behavior resembles the process by which species form in biology. Interestingly, upon repeating the experiment, mixed hexamer $(\mathbf{1a})_3(\mathbf{1b})_3$ sometimes emerges as part of the first set and sometimes as part of the second set. Such stochastic behavior is rare in chemistry, but more common in evolutionary biology.

Another mixed building blocks system showed another feature that is important for evolutionary scenarios: history dependence. Starting from building block **1d**, hexamer replicators formed when the mixture was exposed to preformed hexamer replicators (separately prepared from **1a**), while preformed octamer replicators (made from **1b**) funneled the building block into octamers (Figure 3b).[86] Thus, the nature of the self-replicating molecules that form is dictated by the interactions with self-replicators that were already present,

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Acc. Chem. Res. 2022, 55, 145−155

overriding preferences innate to the structure of the building blocks. A similar situation is found for life, which is a state of matter that derives its organization from previous forms of life and this organization is very different from the thermodynamically most stable arrangement of its constituents.

A final example of interesting evolutionary dynamics that was found upon mixing building blocks shows similarities to parasitism and predation. This system features building block **1f**, which differs from the peptide building blocks discussed so far by containing an additional methylene unit in the backbone of the first amino acid (β-alanine instead of glycine). Dynamic mixtures formed upon oxidizing this building block are sluggish at producing any self-replicators. However, in the presence of previously formed octamer replicator $(\mathbf{1b})_8$ a hexamer replicator is formed rapidly which incorporated both building block **1f** and **1b**.[87] Once formed, the new replicator consumes the original replicators to which it owes its existence. These results show that parasitism is to be reckoned with already at the very early stages of the emergence of life.

INTEGRATING SELF-REPLICATION AND METABOLISM

The synthesis of life requires the integration of the different functional subsystems (Figure 1). We recently succeeded in integrating self-replication with a proto-metabolism by making use of the proven potential of peptide assemblies to catalyze chemical reactions.[88−90] Following a number of not very fruitful attempts at engineering catalytic sites into our self-replicators, we discovered that the already existing systems already exhibited impressive catalytic activity for several different chemical reactions, without needing any structural alterations. Specifically, hexamer replicators made from building block **1a** (but not **1a** itself, nor the nonfibrous small ring macrocycles it forms upon oxidation) were able to catalyze a retro-aldol and an FMOC cleavage reaction (Figure 4a−e).[3]

The catalysis of the retro-aldol reaction of methodol **5** proceeds through imine formation between **5** and unprotonated lysine residues of $(\mathbf{1a})_6$ (Figure 4a) which exist at neutral pH as their pK_A is lowered due to the close proximity of other protonated lysines in the assemblies (Figure 4b). Interestingly, even without any optimization, the catalytic activity of the replicator is similar to the best designer enzymes that have been developed for this reaction.[91−94]

The catalysis of the cleavage of FMOC-glycine (**6**; Figure 4c) also relies on the simultaneous presence of protonated and deprotonated lysines (Figure 4d). The latter reaction produces a dibenzofulvene product (**7**) that enhances the rate at which dithiol building blocks **1a** oxidizes to give rise to the mixture of 3- and 4-membered macrocycles that are the precursors of replicator $(\mathbf{1a})_6$. By increasing the rate at which its own precursors are produced, also the rate of replication increases (blue arrow in Figure 4e). Thus, the replicator is able to catalyze a reaction that promotes the formation of its own precursors, amounting to proto-metabolism. This behavior falls short of full-fledged metabolism in that it does not tap into an energy source.

Using another strategy we were able to design a light-driven proto-metabolism relying on replicator $(\mathbf{1a})_6$ binding and activating a cofactor capable of photoredox catalysis.[49] Simply mixing this cofactor (Rose Bengal or tetraphenylporphyrin; Figure 4g) with building block **1a** led to the emergence of replicator $(\mathbf{1a})_6$, which then binds the cofactor enhancing the rate at which it photochemically converts triplet into singlet oxygen (Figure 4f). The latter then accelerates the oxidation of building blocks **1a** yielding the small-ring precursors from which the replicator grows. So, similar to the FMOC cleavage reaction, the replicator enhances the rate at which its own precursors get produced.

In all these reactions, the replicator is far superior at catalysis and cofactor activation compared to its building block or its small-ring precursors as only the replicator assembly provides the microenvironment that results in a perturbed lysine p*K*a and only the assembly provides the hydrophobic binding pockets for cofactors and substrates. Thus, catalytic and proto-metabolic activities are emergent properties of the system.

The fact that structures that were selected solely on their ability to self-replicate also exhibit additional and promiscuous catalytic activity is significant. Such chance emergence of function resembles a mechanism of evolutionary invention called co-option, where a feature that emerged as it provided a certain function was also capable of fulfilling a completely unrelated one (a famous example in evolutionary biology are feathers, which are believed to have originated as they improved temperature control, but were then co-opted to facilitate flight). In the present system of replicators β-sheets arise in the assembly process that drives self-replication and inadvertently organize amino acids in space to yield catalytically active sites or pockets for cofactor binding. The spontaneous occurrence of inventions of this type is highly encouraging as it bodes well for achieving one of the most challenging aspects in the synthesis of life: open-ended evolution (see below).

OPERATING SELF-REPLICATION OUT OF EQUILIBRIUM

Life needs to be maintained out of equilibrium and also the process of Darwinian evolution relies on an input of energy to maintain the replication-destruction cycles that enable evolution. In living systems, metabolic processes maintain the internal organization away from equilibrium. While the norm in biology, in chemistry out-of-equilibrium systems have, historically, received only little attention (perhaps with the exception of, for example, oscillating reactions[95]). However, this situation is now changing and out-of-equilibrium systems are increasingly in the spotlight, particularly in the area of self-assembly.[38−43]

One of the simplest ways of maintaining self-replicating systems out of equilibrium is by placing them in a flow reactor, in which precursors are continuously supplied and part of the reaction mixture is removed. In such setup outflow means death. For homogeneous systems, death though outflow is nonselective (i.e., each replicator has the same probability of being removed in a given time span) and any selection is therefore solely based on the efficiency of replication. We recently implemented a replication-destruction regime in which death is mediated chemically and is therefore potentially selective (i.e., different replicators may exhibit different levels of resilience against chemical decomposition).[4] In such systems, replicator persistence depends on a combination of replication efficiency and resilience to destruction. We have shown that in this regime molecularly more complex replicators may outcompete simpler ones, which is a desirable but not trivial (see below) evolutionary outcome.

We set up a competition between two replicators that differ in molecular complexity: our workhorse replicator $(\mathbf{1a})_6$ and the smaller replicator $(\mathbf{1a})_3$ which forms from the same building blocks **1a** in the presence of guanidinium chloride.[4] In the presence of this salt, the trimer replicates faster than the hexamer, consistent with the notion that simpler molecules can

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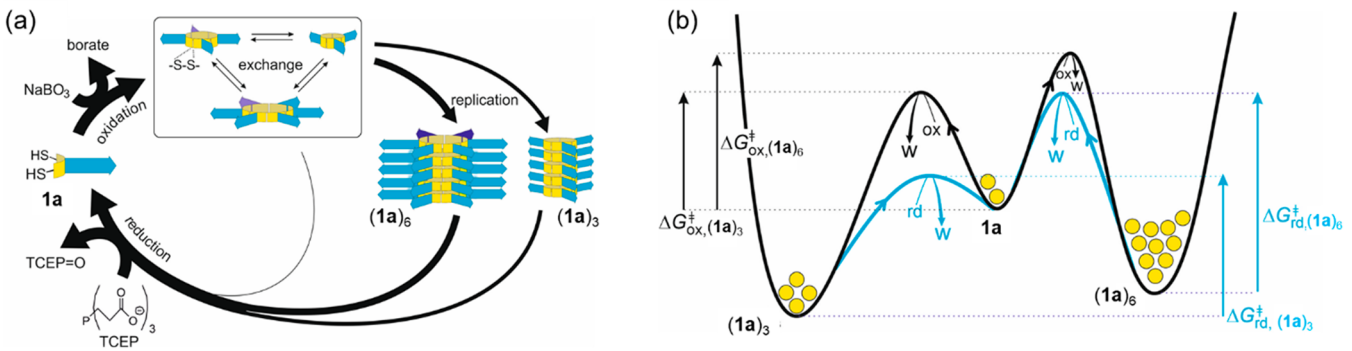

Figure 5. (a) Continuous supply of $NaBO_3$ as oxidant and TCEP as reductant results in a chemically fueled replication-destruction regime in which the slow replicator $(\mathbf{1a})_6$ is able to outcompete the faster, more stable replicator $(\mathbf{1a})_3$ by virtue of being more resilient to chemical destruction.[4] The thickness of the lines represent the magnitude of the flux of material through the different reaction paths (based on a kinetic model parametrized with mostly experimentally determined rate constants). (b) Qualitative Gibbs energy landscape showing the activation barriers ($\Delta G^{\ddagger}$) for the interconversion between dithiol building block **1a** and disulfide replicators $(\mathbf{1a})_3$ and $(\mathbf{1a})_6$ with disulfide formation in black and disulfide cleavage in blue. Abbreviations: rd = reductant; ox = oxidant; w = waste product.

be replicated faster than more complex ones. Furthermore, various experiments also suggest that the trimer is most likely the most stable state of the system (trimer grows at the expense of hexamer when a mixture of the two is stirred), consistent with the notion that simpler molecules are entropically more favorable than more complex ones. Nevertheless, it turned out that the hexamer replicator was able to outcompete the trimer upon exposing it to a replication-destruction regime.

This regime was implemented by continuous and simultaneous addition of oxidant (sodium perborate; $NaBO_3$) and reductant (TCEP; Figure 5a). The perborate mediates the oxidation of thiols to disulfides, while TCEP induces the reverse reaction. Since both redox reagents are present at a low stationary concentration, their direct short-circuiting reaction is insignificant relative to the reaction with thiols and disulfides, which are present at much higher concentrations. Thus, the redox reagents cause a continuous flux of building block through the two competing replicators.

The observation that, in this chemically fueled replication-destruction regime, the molecularly more complex and slower hexamer replicator was able to outcompete the simpler and faster trimer was attributed to the hexamer being more resilient to destruction than the trimer (as confirmed in competitive reduction control experiments; most likely a consequence of steric hindrance of approach of the reductant). These results can be rationalized using the Gibbs energy diagram shown in Figure 5b, which features separate pathways for perborate mediated replication and TCEP mediated destruction. The fact that detailed balance is broken (i.e., disulfide bond formation and breakage take place through separate pathways, each coupled to the conversion of a different high-energy reactant) is essential to escape from thermodynamic control and outcompete the faster but simpler replicator. These results represent one of the first manifestations of selection of a synthetic self-replicator based on its dynamic kinetic stability.[16,96]

Even though the difference in molecular complexity between trimer and hexamer replicator may not be very large, these results are nevertheless conceptually important as they address a problem in early evolution that has become known as the "Spiegelman monster". In the 1960s Spiegelman showed that replicase-mediated in vitro evolutionary experiments on RNA resulted in the dramatic shortening of the RNA sequence.[97] In these experiments serial transfer was used to implement a replication-destruction regime (where not being transferred to the next experiment amounts to death). As all replicators have the same probability of being transferred, death is not selective and selection occurs only based on the speed of replication. Short RNA sequences are replicated faster than long ones and therefore have a competitive advantage in such setting. Thus, there is an inherent tendency to evolve toward reduced complexity. Yet, the emergence and early evolution of life is likely to require an increase in complexity. Our experiments demonstrate an obvious solution to this problem: make sure that complex replicators die slower. In our system, this feature is achieved chemically. In previous work by Braun et al., a similar result is achieved by selective retention of more complex molecules in a flow reactor featuring a temperature gradient enabling selective thermophoretic trapping.[98]

THE NEXT STEPS

In little more than a decade of effort by our lab, substantial progress toward the long-term goal of de novo life has been made and the path ahead is becoming increasingly clear. Self-replication and its integration with a proto-metabolism have been achieved,[3,49] and the resulting binary system can be operated in out-of-equilibrium replication-destruction regimes where replication is accompanied by mutation and selection.[85] The last main ingredient of life that still needs to be integrated is compartmentalization, including a mechanism for compartment division.[99] Integrating this feature would clear an important evolutionary hurdle. It would allow for further development of metabolically active replicators through Darwinian evolution. Without compartments, evolutionary selection for metabolically relevant catalytic activity is challenging as the products of the catalytic activity will benefit not only the replicator that generated them but also all other replicators in the sample, irrespective of whether they contributed to catalysis. When a replicator and the products it generates through catalysis are confined within a compartment, then only the replicator that is responsible for catalysis benefits and can thereby be selected based on its catalytic proficiency. Thus, compartmentalization provides protection against kleptoparasites. In fact, various studies on RNA-based systems have shown that compartments may also protect against other forms of parasitism.[46,47] In addition compartments can enhance the rate of evolutionary adaptation in directed evolution experiments.[100] We already

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Acc. Chem. Res. 2022, 55, 145−155

witnessed in our own experiments that parasites may emerge at early stages in the development of life (see above).[87]

Once compartmentalization has been achieved, the next and possibly final challenge will be to achieve Darwinian evolution of the resulting system in a meaningful way. This step may well represent the biggest challenge of all, requiring the systems to be sufficiently robust to withstand death by entropy (i.e., mutating at a speed that any information it may have acquired is lost again). Yet at the same time the system should have a large enough chemical/structural space available to explore to ensure that evolutionary inventions can be made without limits, making evolution open-ended.[25,101,102] It is essential that the exploration of this space should occur in a restrained manner, where evolution dictates which very small part of the very large space the system occupies at any one time (Figure 6). Such behavior

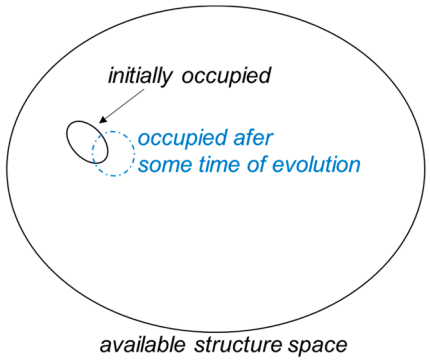

Figure 6. Open-ended Darwinian evolution requires a huge structure space to be available to allow for continuous evolutionary inventions to be made. At any given time, an evolving system must only occupy a tiny subset of this space, putting demands on replication fidelity. In the process of evolution, the location of the occupied subset changes gradually. Note that the occupied and available structure spaces are not drawn to scale; the former is so much smaller than the latter that it would not be visible otherwise.

places high demands on the fidelity of replication, particularly when, in the course of evolution, the information content of the system increases. Related to this issue is the notion of evolvability, which is an aspect that has received hardly any attention in experimental work on synthetic self-replicating systems. The challenge here is to be receptive to aspects of heredity and evolvability that may well differ from the way we are used to thinking about these phenomena, i.e., different from the way nucleic acid sequences evolve. For example, in our systems of replicators, the copying of information appears to occur at the fiber ends (where the fiber end acts as the template for the next ring that is attached). Yet, also the fiber sides play a role in catalyzing the formation of precursors for the replicator (Figure 3c and f) and in channeling this material to the fiber ends (Figure 2c,d). The exact amino-acid composition at the sides of the fiber will therefore impact on the rate of growth of this fiber and if this happens in a way that biases amino-acid incorporation to favor production of the most active amino-acid compositions then such compositions will be heritable. This represents a mechanism of heredity that is conceptually different from the template-based replication of nucleic acids but bears some similarity to the GARD model developed by Lancet et al. based on a lipid-world scenario.[103] Indeed, if our systems are to evolve toward open-endedness, then their information content will eventually have to exceed what is possible based on the limited number of permutations allowed by varying ring size and composition. Once fiber compositions (i.e., the sequence of rings along the fiber) start to become heritable, orders of magnitude more information may be stored and passed on to next generations. Weak heredity of sequence information may arise through the mechanism discussed above. Information transfer through templating by a pre-existing fiber, for example, through base-pairing, would be another mechanism capable of stronger heredity. We have shown that nucleobases can be incorporated into our replicators, albeit without any indication for base-pairing.[75] Work by Hud et al. has shown that base-pairing interactions can occur in self-assembled materials, although these systems cannot self-replicate.[104] So challenges remain.

IMPLICATIONS

If, one day, humans will be able to synthesize life de novo, this will have several implications. First, it will help us with understanding what life is. Having more than a single biochemistry should assist in identifying and generalizing life's distinguishing features. Such knowledge would also help defining the target(s) in our search for extraterrestrial forms of life.

The process of making life will also inform on the path that may have been traveled in the emergence of life on Earth. Even though efforts of synthesizing life are not necessarily directed by current biochemistry or prebiotic geochemistry, having one (or more) synthetic path(s) connecting chemistry to biology might assist in identifying an analogous route that is compatible with conditions on early Earth and that converges on extant biochemistry. Some potentially useful insights on the prebiotic emergence of autocatalytic systems may already be obtained from the work described in this Account. For example, we found that autocatalytic systems emerge spontaneously and readily in mixtures where monomers oligomerize reversibly, provided that these oligomers are capable of self-assembly. This observation suggests that autocatalysis may be easier to achieve than previously thought, given that reversible oligomerization and self-assembly are quite general and widespread phenomena. Our work has also shown that this mechanism may lead to the autocatalytic formation of one-dimensional arrays of nucleic acids.[75] Such arrays may be a stepping stone toward systems in which nucleic acid sequences within such arrays are replicated.

Finally, it is not unreasonable to expect that the ability to synthesize life may have an impact that is at least similar to the impact made by the ability to synthesize organic molecules. It is tempting to draw a parallel between these two developments. It was long thought that organic molecules could only be produced by living organisms. The idea that a "life force" was needed was eventually refuted by demonstrating that such molecules could also be obtained synthetically (a famous example is the synthesis of urea by Wöhler in 1828).[105] These demonstrations gave rise to the field of organic chemistry which has made a tremendous impact in areas ranging from medicine to materials. We are now getting closer to being able to synthesize life (and demonstrating that it is not only a product of existing forms of life). Just like urea was not exactly the most impressive or useful example of an organic molecule, the first form of synthetic life is equally unlikely to impress, when compared to even the simplest currently living organism. Yet, just like the many human-made organic molecules that followed the synthesis of urea, the subsequent forms of human-made life (living technology) are

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Acc. Chem. Res. 2022, 55, 145−155

likely be at least similarly impactful, but in ways that may currently be difficult to predict.

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Notes

The author declares no competing financial interest.

Biography

Sijbren Otto received his M.Sc. (1994) and Ph.D. (1998) degrees cum laude from the University of Groningen in The Netherlands. Following postdoctoral work in the United States with Prof. Steven L. Regen (Lehigh University) investigating synthetic systems mediating ion transport through lipid bilayers, he received a Marie Curie Fellowship and moved to the University of Cambridge, UK, in 1999 where he worked for 2 years with Prof. Jeremy K. M. Sanders on dynamic combinatorial libraries. He started his independent research career in 2001 as a Royal Society University Research Fellow at the University of Cambridge. He moved to the University of Groningen in 2009 where he is now Full Professor. He is founding coeditor-in-chief of the relaunched Journal of Systems Chemistry and has chaired two COST Actions on the subject of Systems Chemistry uniting more than 95 European research groups.

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Acc. Chem. Res. 2022, 55, 145−155